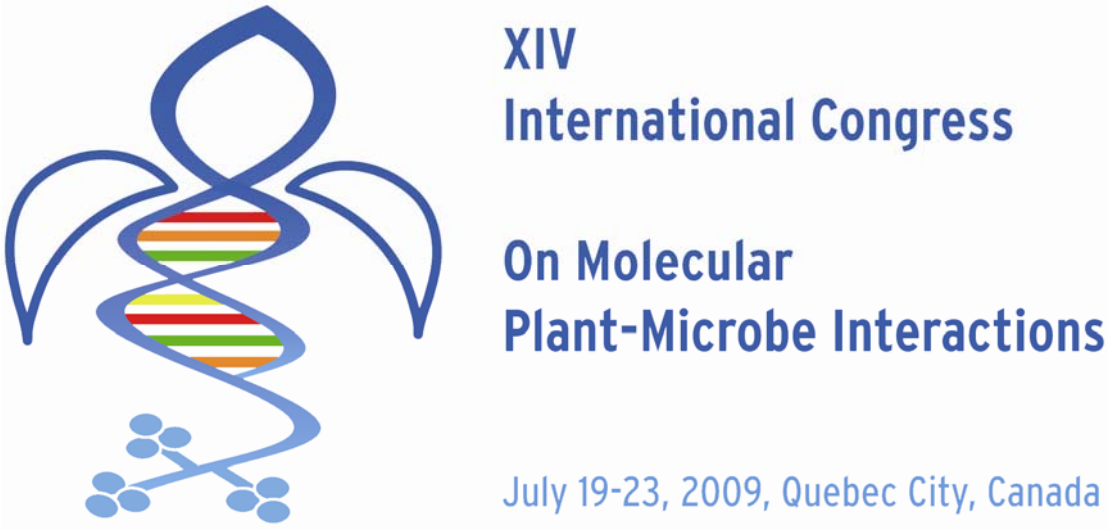

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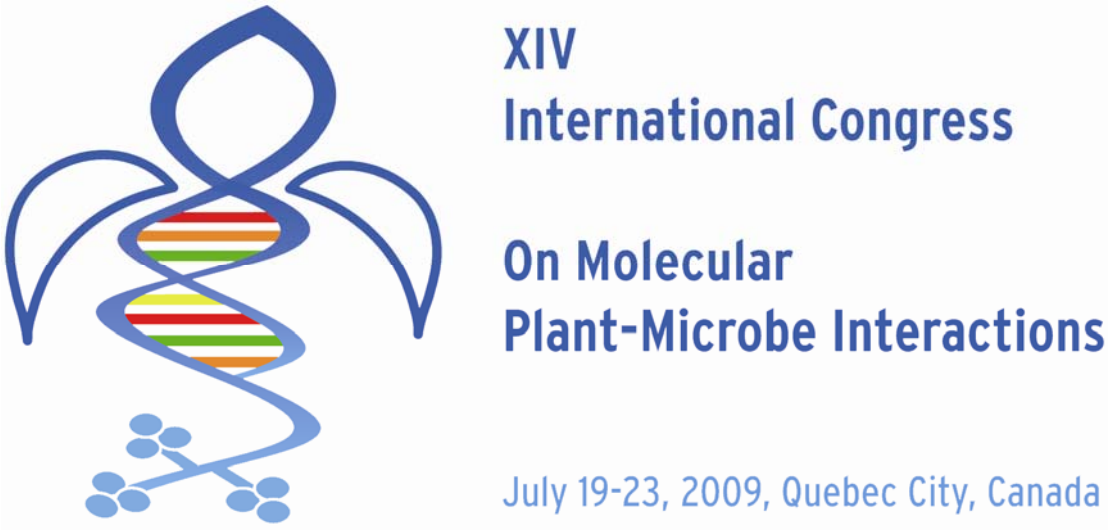

IS-MPMI 2009 XIV International Congress on Molecular Plant-Microbe Interactions

Abstract of Opening Lecture

Facing the issues in agricultural biotechnology

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IS-MPMI 2009 XIV Congress 1

The IS-MPMI is a highly successful organization and its journal, MPMI, publishes high quality research, as evidenced by the fact that its impact factor, among plant journals, has risen steadily over the years. As a plan pathologist, however, my concern is that this significant progress in our understanding of host-parasite interactions has not translated into new and effective means to control plant diseases. There is a danger the MPMI may become an ivory tower of great research on genomics and coordinated gene action, but without connection to the needs of agriculture worldwide. There is a sense of urgency, as some of our major crops are facing new and devastating diseases (such as citrus greening) and we cannot provide effective control measures. Just as our medical colleagues have determined that there is a need for "translational research" to apply research on human diseases more rapidly to the development of better diagnostics and control procedures, our Society should lead in the development of translational research on plant diseases. In addition, MPMI should publish articles that deal with issues in agricultural biotechnology (such as patenting, the naming of "select" agents, the reduction in research support, etc.) that have had negative effects in our efforts to meet the nutritional needs of people in developing countries.

Abstract of Award Lecture

NB-LRR proteins and type III effectors

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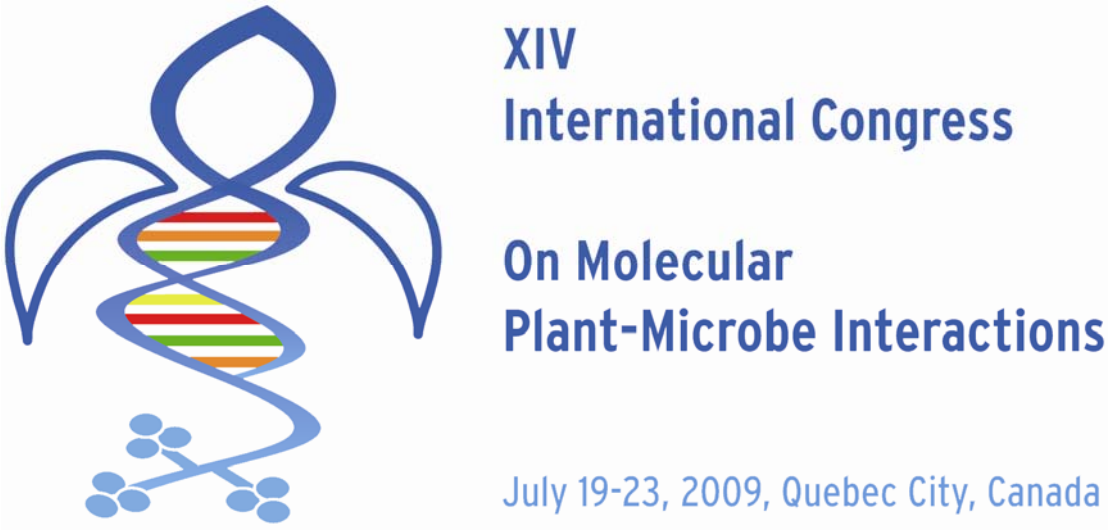
IS-MPMI 2009 XIV Congress 1

Our overall goal is to understand how particular *P. syringae* type III effector proteins have evolved to manipulate plant signaling machinery, and how these manipulations are recognized by the NB-LRR receptors of the plant immune system. Our rationale is that by understanding how a collection of pathogen virulence factors act inside the host cell, we will better understand the normal, defense relevant function of their targets. We study how three sequence unrelated type III effectors, AvrRpm1, AvrB, and AvrRpt2 manipulate RIN4 to trigger RPM1 and RPS2 function. We identified and analyzed new loci required for RPM1 function using 'second generation' forward genetics screens. A combination of genetics (forward and reverse), biochemistry and cell biology is necessary to understand how NB-LRR proteins are assembled into a pre-activation, signal competent state and to define how they are specifically activated after infection. RIN4, a key player in disease resistance mediated by both RPM1 and another NB-LRR protein called RPS2. A few additional genes in Arabidopsis encode proteins related to RIN4 only by their plant-specific NOI domains of ~30aa. They encode proteins of no known function, though the NOI domain contains an AvrRpt2 protease cleavage site (the RCS) and the AvrB binding site (the BBS). We focus on NOI4 and NOI5 which are each required for full RPM1-mediated disease resistance. We have sequenced ~40 *P. syringae* strains for both type III effector discovery and analysis of the rules determining host range.



IS-MPMI 2009 XIV International Congress on Molecular Plant-Microbe Interactions Abstracts of Plenary Session Presentations

Abstracts submitted for presentation at the Plenary Sessions at the XIV International Congress on Molecular Plant-Microbe Interactions in Quebec City, Canada, July 19–23, 2009. The abstracts are arranged in alphabetical order by the last name of the first author. Abstracts are published as submitted. They were formatted but not edited at the IS-MPMI headquarters office.

Biocontrol agents and plant-pathogen interactions: A vision in the third dimension

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IS-MPMI 2009 XIV Congress 2

The way a biocontrol agent (BCA) can reduce disease incidence has been defined mostly on the basis of competition, parasitism or antibiosis directed against the pathogen. With the possible exception of Trichoderma, only rarely has the interaction between the plant and the pathogen been considered to influence the behavior of a BCA. This is rather surprising considering that entomologists have long known that the behavior of a natural enemy will be greatly influenced by the plant substrate on which an insect feeds. This apparent disregard of the interactions between a plant, a pathogen and a BCA at the tritrophic level may have hindered practical implementation of biocontrol of plant diseases. For example, the epiphyte, Pseudozyma flocculosa, is often found in close association with powdery mildews and will rapidly destroy the pathogen colonies following inundative applications. This antagonistic behavior seems to be directed against all but only members of the Erysiphales and to be mediated by the release of an antifungal glycolipid, flocculosin. However other closely related Pseudozyma have recently been shown to produce a similar glycolipid with antifungal activity and yet they do not seem capable to attack powdery mildews. Further, the release of flocculosin does not appear to be synchronized with the colonization of powdery mildew colonies by P. flocculosa, while the latter will not grow on powdery mildew structures excised from their host. These observations suggest that the biocontrol activity of P. flocculosa and its specificity toward powdery mildews are intimately linked with factors released during the interaction between the plant and the biotroph. Identification of these factors would help optimize the activity of the BCA at negating plant-powdery mildew interactions.

Molecular response of *Casuarina* upon infection by *Frankia*

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IS-MPMI 2009 XIV Congress 2

Nitrogen-fixing root nodulation is confined to four plant orders, and encompasses two distinct types of associations - the interaction of legumes (Fabales) with rhizobia bacteria - and actinorhizal symbioses, where the symbionts are actinobacteria of the genus *Frankia*. In contrast to legume, actinorhizal plants being mostly trees or shrubs are not amenable to genetic analysis and are recalcitrant to molecular-biology techniques. However, progress in gene isolation, development of genetic transformation and gene silencing procedures for actinorhizal trees of the *Casuarinaceae* family have opened new avenues for the study of the molecular bases of this non-legume symbiotic interaction. In recent years, we isolated and characterized several plant genes acting in the earliest events of *Casuarina-Frankia* recognition and infection. This includes a subtilase, Cg12 and homologs of the auxin influx carriers (*AUX1-LAX3*) specifically expressed during plant cell infection by *Frankia* (Péret et al., 2007). Furthermore, we have demonstrated that common molecular mechanisms are shared between rhizobial and actinorhizal symbioses: *Casuarina* orthologs of legume genes that are part of the NOD factor signaling pathway like *CgSymRK* (ortholog of *Symrk/DMI2* ; Gherbi et al, 2008) and *CgDMI3* (ortholog of *CCaMK/DMI3*) are essential for actinorhizal symbioses and the regulation of the widely used marker of Legume symbiotic interaction pEnod11 is conserved during the infection by *Frankia*. To acquire a global view of the plant genetic programmes and to identify new key plant genes that control Casuarina nodulation, we also have developed transcriptomic resources (Hocher et al, 2006; Hocher et al, in preparation) that are providing the first pictures of global changes in gene expression during the establishment of actinorhizal symbiosis.

How *Xanthomonas* manipulates the plant cell

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IS-MPMI 2009 XIV Congress 2

We study the interaction between pepper and tomato and the Gram-negative bacterium *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*), the causal agent of bacterial spot disease on pepper and tomato. Successful interactions of *Xcv* with the plant depend on the plant-inducible type III secretion (T3S) system, a molecular syringe which injects 20–30 effector proteins (termed Avr or Xop = *Xanthomonas* outer protein) into the plant cell cytoplasm. One of the best understood type III effectors is AvrBs3, which functions as transcription

factor in the plant cell nucleus and affects both susceptible and resistant plants. *Xcv* strains expressing AvrBs3 induce the hypersensitive reaction (programmed cell death) in pepper plants carrying the resistance gene *Bs3*. In pepper plants lacking *Bs3* and other solanaceous plants AvrBs3 induces a hypertrophy (cell enlargement) of mesophyll cells that probably helps to dissiminate the bacteria. AvrBs3 activity depends on localization to the plant cell nucleus and the presence of an acidic activation domain in the C-terminal region of AvrBs3. New insights into *UPA* (upregulated by AvrBs3) gene induction will be presented.

Breaching the wall activates a pre-zig-zag signalling mechanism of plant defense

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IS-MPMI 2009 XIV Congress 3

The plant cell wall is the first barrier to the invasion by phytopathogenic microorganisms. In order to break down this barrier fungi produce in a sequential manner an array of cell wall degrading enzymes (CWDEs) which produce simple sugars to support the fungal growth. Pectin-degrading enzymes are produced before other CWDEs and act on those wall components that are critical for cell integrity and cell-cell adhesion. One of these components is the homogalacturonan, which may be broken by fungal polygalacturonases (PGs) and fragmented, in the presence of polygalacturonase-inhibiting proteins (PGIPs), into oligogalacturonides (OGs) that activate plant defenses through a signaling pathway independent o f jasmonic acid, ethylene and salicylic acid. We are studying the perception/transduction mechanism played by OGs with both biochemical and genetic tools. We are also studying the structural features of PGIP that allow the evolution of new recognition specificities.

Can *Cladosporium fulvum* become a type-species for plant pathogenic Dothideomycetes?

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Cladosporium fulvum is a biotrophic pathogen that causes leaf mould of tomato. So far, ten effector proteins have been identified from this fungus including avirulence (Avrs: Avr2, Avr4, Avr4E and Avr9) and extracellular proteins (Ecps: Ecp1, Ecp2, Ecp4, Ecp5, Ecp6 and Ecp7). Also many cognate Cf (for *C. fulvum*) resistance proteins have been identified that mediate recognition of Avrs and Ecps and subsequent defense signaling. Although demonstrated for only a few, all Avrs and Ecps are assumed to be virulence factors. Avr2 is an inhibitor of apoplastic plant cysteine proteases and Avr4 is a chitin-binding protein that protects chitin present in the cell walls of the fungus against the deleterious effects of plant chitinases during infection. Ecp6 contains carbohydrate/chitin-binding LysM domains that are supposed to bind chitin fragments released from fungal cell walls during infection and prevent them to cause chitin receptor-mediated induction of basal defense responses. Recently we have sequenced the genome of race 0-Wag of *C. fulvum* that enabled us to perform comparative genome analyses with other sequenced members of the plant pathogenic Dothideomycetes. Surprisingly, so far the genome of *C. fulvum* is most homologous to *Mycosphaerella fijiensis* the causal agent of Black Sigatoka, a devastating fungal disease of the monocot banana. We have now identified for the first time, homologues of the *C. fulvum* Avr4, Ecp2 and Ecp6 effectors in Dothideomycetes, including *M. fijiensis*, *M. graminicola*, *Cercospora nicotianae* and *C. beticola*. Recently, we have tested whether these proteins are functional homologues of the three *C. fulvum* effectors. We will report on the most recent results that have been obtained from these studies.

Driving innate immunity: Emerging perspectives on the role of the ER, nucleus, and chloroplasts

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Resistance (R) proteins are the best-studied sensors of invading pathogens in plants and are involved in the initiation of innate immune response. R proteins are components of a plant surveillance system that serves to recognize pathogen-encoded effector proteins from organisms including viruses, bacteria, fungi and even nematodes. Plant R proteins share common protein domains with animal innate immunity molecules. These include the TIR (Toll-Interleukin 1 homology region), NB-ARC (Nucleotide binding site typical of Apaf-1, R proteins and CED-4) or NOD (Nucleotide binding oligomerization domain), LRR (Leucine rich repeats) and serine/threonine kinase domains. Despite the structural similarities with animal innate immunity molecules, plant immune receptors recognize specific pathogen effectors while mammalian receptors recognize non-specific microbe associated molecular patterns (MAMPs). Although several *R* genes have been cloned, the mechanisms of resistance remain elusive. Specifically, how do immune receptors recognize pathogen effector molecules and how do host cells initiate, mediate, and terminate signaling in resistance? To this end, we are using N immune receptor that confers resistance to tobacco mosaic virus (TMV) as a model system. I will discuss recent advances on innate immune receptor mediated pathogen recognition mechanisms and activation of defense signaling.

Recognition of secreted effectors of flax rust

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IS-MPMI 2009 XIV Congress 3

Flax rust (*Melampsora lini*) is a biotrophic basidiomycete pathogen that infects flax plants (*Linum usitatissimum*). Nineteen different rust resistance genes have been cloned from flax, including 11 allelic variants of the *L* locus, which all encode cytosolic TIR-NBS-LRR proteins. Four families of Avr genes, *AvrL567*, *AvrM*, *AvrP123* and *AvrP4* have been identified in flax rust and all encode small secreted proteins that are expressed in haustoria. Recognition occurs inside the plant cell and yeast-two-hybrid analyses indicate that, in at least two cases, this is based on direct interaction with the corresponding cytosolic NB-LRR R proteins. Bimolecular Fluorescence Complementation assays show that the AvrL567-L6 interaction takes place in both the cytosol and nucleus of plant cells. This suggests that the Avr proteins are translocated into host cells during infection, and immunolocalisation experiments have detected the AvrM inside host cells during infection. Expression of various GFP-tagged AvrL567 and AvrM mutants in plants suggest that these proteins are taken up into host cells in the absence of the pathogen and that this transport is dependent on sequences in the N terminal region. Although the LRR domain is primarily responsible for determining recognition specificity of the flax R proteins, y2h assays indicate that a functional NBS domain is also required for Avr protein interaction. The TIR domain is not required for recognition and several TIR mutations disrupt HR induction, without affecting recognition. Furthermore, overexpression of the TIR domain alone induces HR, suggesting a primary signaling role for this domain. Direct recognition has led to strong diversifying selection in the rust Avr genes to escape recognition and host resistance.

Genomics-directed dissection of R gene-mediated resistance

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Resistance (R) gene-mediated immunity is a major defense mechanism in plants. Despite years of study, how activation of R proteins renders disease resistance is still largely unknown. To address this question, we took advantage of the recent knowledge that R-mediated resistance may involve gene regulation. Using transcriptional profiling in Arabidopsis, we identified candidate genes based on their early induction by the R gene, RPP4. Detailed functional analysis of the candidate gene mutants led to identification of 22 new components affecting RPP4-mediated resistance against downy mildew. Phenotypic clustering placed these components into two pathways: one leads to programmed cell death and the other results in the formation of chemical/physical barriers. Moreover, many of these components also affect broad-spectrum basal resistance, including microbe-associated molecular pattern-triggered immunity. This study demonstrates genetically that R gene-mediated resistance is orchestrated by accelerated induction of multiple defense mechanisms. Since induction of these immune effecter genes occurs prior to expression of many known immune regulators such as EDS1 and PAD4, they may function upstream from these regulators. This finding also suggests that R gene-mediated transcription regulation is an early event in the immune signaling pathway. Identification of these immune effectors in the R gene-mediated resistance is essential for our understanding of plant immunity and provides the necessary markers for future searches for their upstream regulators and downstream targets.

The science and art of developing and commercializing virus-resistant transgenic papaya

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IS-MPMI 2009 XIV Congress 4

The concept of pathogen-derived resistance, which states that a transgenic plant that expresses a transgene of a pathogen will be resistant to that particular pathogen, is a proven approach for developing virus-resistant crops. The coat protein gene of the target virus is normally used as the transgene to impart resistance to the virus. This approach was used to develop papaya ringspot virus (PRSV)-resistant transgenic papaya that expressed the coat protein gene of PRSV. A confined field trial of the transgenic papaya was started in 1992, the same year that PRSV was discovered in Puna District on the island of Hawaii, where 95% of Hawaii's papaya was being grown. PRSV caused tremendous damage, reducing papaya production by 50% in 1998 compared to the 1992 levels. Concurrently, research and other efforts were done to characterize, field-test, deregulate, and commercialize the transgenic papaya. The transgenic papaya was released to growers in 1998 and reversed the impact of PRSV on the papaya industry. The science and art that was practiced to develop and commercialize the transgenic papaya in a timely manner will be discussed.

Cellular reprogramming for arbuscule formation in AM symbiosis

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Most vascular flowering plants have the capacity to form mutualistic symbioses with arbuscular mycorrhizal (AM) fungi. These associations develop in the roots where the symbionts trade nutrients; the fungus obtains carbon from the plant and delivers phosphorus and in some instances nitrogen to the root. Nutrient exchange occurs at a specialized interface between a branched hypha, called an arbuscule, and the root cell in which it resides. Development of arbuscules requires the cellular differentiation of both symbionts. The AM fungus undergoes a striking alteration in growth, from a simple linear hypha to an extensively branched arbuscule, while the root cell undergoes cellular rearrangements to accommodate the fungus, including the development of a new membrane, the peri-arbuscular membrane (PAM), that surrounds the arbuscule. Within the PAM reside Pi transport proteins essential for symbiotic Pi transfer to the plant cell. Using a legume, *Medicago truncatula*, and an AM fungus, *Glomus versiforme*, we have undertaken forward and reverse genetics screens to identify *M. truncatula* genes required for AM symbiosis. This has enabled the identification of novel proteins essential for arbuscule development and function. In addition, this approach has revealed points at which signaling for AM symbiosis intersects with hormone signaling pathways, and provides insight into the coordination of symbiosis with the plants nutrient status, growth and development.

***Pseudomonas syringae* pathogenesis in Arabidopsis**

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IS-MPMI 2009 XIV Congress 4

Plants have evolved a powerful and multi-layered immune system to defend against infection by microbial organisms. However, successful pathogens, such as *Pseudomonas syringae* pv. *tomato* (*Pst*) strain DC3000, have developed specific virulence factors to overcome host immunity and cause diseases. During infection of Arabidopsis, *Pst* DC3000 produces several virulence factors to engage multiple host cell types and causes global changes in host gene expression. The phytotoxin coronatine, a molecular mimic of the plant hormone jasmonyl isoleucine, contributes to bacterial invasion through stomata, development of disease symptoms, and virulence in local and systemic tissues. The bacterial type III secretion system (TTSS) enables the extracellularly localized *Pst* DC3000 to deliver a battery of "effector proteins" directly into the host cell. The action of these effector proteins is associated with suppression of host defense, development of disease symptoms (necrosis and chlorosis) and, presumably, release of nutrients from host cells. Study of the molecular action of effector proteins and coronatine is revealing a fascinating array of host cellular functions associated with vesicle traffic, stomatal function, jasmonate signaling, and senescence-associated leaf chlorosis.

Molecular mechanisms underlying pathogen recognition

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Plant disease resistance proteins belonging to the nucleotide binding-leucine rich repeat (NB-LRR) class often detect pathogen effector proteins indirectly, via sensing modification of effector targets. We are studying this process in three different NB-LRR proteins, RPS5 from Arabidopsis and Rpg1b and Rpg1r from soybean. RPS5 detects the cysteine protease AvrPphB of *Pseudomonas syringae*, and this detection requires a second Arabidopsis protein, PBS1. Cleavage of PBS1 by AvrPphB is required to activate RPS5. Current work is focused on defining what parts of PBS1 are required for interaction with RPS5, what parts of RPS5 are required for downstream signaling, what other proteins are part of the RPS5 complex prior to activation, and whether subcellular relocalization of RPS5 occurs upon activation. The soybean Rpg1b and Rpg1r proteins mediate recognition of the *P. syringae* effector proteins AvrB and AvrRpm1, respectively. In Arabidopsis, these two effectors are recognized by a single NB-LRR protein, RPM1, and this recognition requires a second Arabidopsis protein, RIN4. The AvrB and AvrRpm1 proteins both induce phosphorylation of RIN4, and it is believed that this phosphorylation event activates RPM1. We are thus investigating whether Rpg1b and Rpg1r employ a similar recognition strategy, and if so, how Rpg1b and Rpg1r distinguish between AvrB and AvrRpm1. We have identified four RIN4 orthologues in soybean and have shown that at least two of these bind to AvrB in a yeast two-hybrid assay. Current work is focused on testing whether any of these soybean RIN4 proteins are phosphorylated in the presence of AvrB and AvrRpm1, and whether any are required for Rpg1b and/or Rpg1r function. The implications of this work in terms of NB-LRR evolution will be discussed.

Endogenous small RNAs and host RNAi machinery added a fundamental layer of regulation in plant immunity

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Small RNAs regulate gene expression in many cellular processes in eukaryotes. Emerging evidence suggests that host endogenous small RNAs play a role in plant immune responses. To identify and characterize pathogen-regulated small RNAs at the whole genome level, we performed high-throughput sequencing of small RNA libraries prepared from bacteria- and fungi-challenged *Arabidopsis*. We identified a diverse set of endogenous small RNAs, including miRNAs, nat-siRNAs, gene-targeting hc-siRNAs, and protein-coding gene-associated siRNAs. The distribution, biogenesis and biological functions of these small RNAs have been studied. Many of these small RNAs are either up- or down-regulated by various pathogens and may subsequently regulate target genes, thus contributing to gene expression reprogramming and fine-tuning in plant immune responses. We also found that some of the RNAi pathway components are important for regulating plant biotic stress responses. Our results suggest that host endogenous small RNAs and host RNA-silencing machinery represent a fundamental layer of control in plant immune responses.

Using pathogen effectors to understand host resistance mechanisms

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Plant pathogens use small molecules and also proteins to render their hosts susceptible. Many bacteria and other pathogens use a specialized secretion system to deliver proteins into host cells that interfere with host defence. We have taken advantage of the bacterial type III secretion system (T3SS) to investigate effectors from filamentous pathogens such as oomycetes. We are using T3SS delivery of oomycete effectors from *Pseudomonas* sp. to investigate the effector complement of the downy mildew pathogen *Hyaloperonospora parasitica (Hpa)*. I will report recent data on *Hpa* effector functions and on the use of the Solexa/Illumina sequencing instrument to advance our understanding of *Hpa* pathogenicity. We are using Illumina paired read sequencing and Velvet software (Zerbino and Birney, Genome Research, 2008) to assemble sequences of multiple races of another oomycete pathogen, *Albugo candida*, which is particularly effective at shutting down host defence. The analysis of its effectors is likely to provide very interesting new insights into host defence mechanisms. In addition, we are using T3SS delivery of oomycete effectors to investigate the molecular basis of pathogen/host specificity and non-host resistance. An update on recent progress will be presented.

The effectors of smut fungi

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The basidiomycete fungus *Ustilago maydis* is a biotrophic maize pathogen that does not use aggressive virulence strategies and needs the living plant

tissue for completion of its life cycle. The *U. maydis* genome codes for a large set of novel secreted effector proteins. A significant percentage of the respective genes are clustered in the genome and are upregulated during pathogenic development (Kaemper et al., 2006). Many of these gene clusters have crucial roles during discrete stages of biotrophic growth. We now show that *U. maydis* is eliciting distinct defense responses when individual clusters or individual genes are deleted. Maize gene expression profiling has allowed us to classify these defense responses and provides leads to where the fungal effectors might interfere. We describe where the crucial secreted effector molecules localize, their interaction partners and speculate how this may suppress the observed plant responses. In addition, we will present insights on effector evolution and function that stems from a comparative genomics approach in which the genomes of the related smut fungi *Sporisorium reilianum* and *U. scitaminea* were sequenced using 454-technology.

He's not just that N to you: A role for the bacterial nitrogen stress response in symbiotic nitrogen fixation

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IS-MPMI 2009 XIV Congress 5

The Rhizobium-legume association is a model of coevolved nutritional complementation. The bacteria elicit the formation of specialized nodules on the roots of the host plant, in which they undergo differentiation into the microaerophilic bacteroid state. Bacteroids reduce (Fix) atmospheric dinitrogen to ammonia, which is supplied to the host plant in exchange for carbon compounds. In order for rhizobia to generate ammonia and release it to the plant the bacteria must uncouple symbiotic N2 fixation from nitrogen stress metabolism. Using 15N2 labeling, we showed that in the symbiosis between Sinorhizobium meliloti and alfalfa, a mutation in GlnD, the major bacterial nitrogen stress response sensor protein, led to a symbiosis in which nitrogen was fixed (Fix+). However, the association did not result in substantially increased plant growth (Eff$^-$) and most fixed 15N was not present in the plant after 24 hr (Yurgel & Kahn. 2008. PNAS. 105:18958-18963). These data suggest that this glnD mutant releases fixed nitrogen into the environment. We speculate that this is the result of a breakdown in communication in the mature nodule, caused by abnormal activity of the GlnD protein. We will discuss the role of GlnD in S. meliloti symbiotic productivity. We will also discuss potential new approaches to generating a Rhizobium-legume association able to produce substantially more nitrogen through fixation.

Evolutionary and functional dynamics of *Phytophthora infestans* effector genes

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IS-MPMI 2009 XIV Congress 5

It is now well established that oomycete plant pathogens secrete effectors that target the apoplast or are translocated inside host plant cells to enable parasitic infection. Apoplastic effectors include several types of inhibitor proteins that interfere with the activities of extracellular plant hydrolases. Host-translocated (cytoplasmic) effectors include the RXLR and Crinkler (CRN) families, which carry conserved motifs that are located downstream of the signal peptide and mediate delivery into host cells. How these effectors perturb plant processes remains poorly understood although some RXLR effectors are known to suppress plant immunity. This presentation will report on the progress we made in unraveling the evolutionary and functional dynamics of effector genes of the potato late blight pathogen *Phytophthora infestans*. More specifically, we will focus on the insights obtained from sequencing the genomes of *P. infestans* and that of four closely related species, and our progress in deciphering the virulence activities of *P. infestans* effectors.

Small signaling molecules in the plant immune response: Strategies to identify targets and molecular mechanisms

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In plants and animals, the first active defense response against microbial invasion is initiated upon recognition of pathogen-associated molecular patterns (PAMPs). These patterns, also referred to as elicitors, include components of fungal cell walls such as chitin, lipopolysaccharides from the outer membrane of Gram-negative bacteria, flagellin, or EF-Tu. The signaling pathways and molecular mechanisms underlying PAMP-triggered immunity are not completely elucidated. Genetic and biochemical evidence points to an intimate link between PAMP-triggered immunity and the initiation of a conserved set of plant defense responses such as production of reactive oxygen species (ROS), deposition of callose, protein phosphorylation, and transcriptional activation of early response genes. Several plant-derived small molecules including salicylic acid, jasmonic acid and the established plant hormones auxin, cytokinin and abscisic acid mediate these responses, whereas pathogen-derived effectors and non-proteinaceous toxins impair the plant immune response and thereby support disease development. By screening various chemical libraries comprising both natural and synthetic compounds, we identified small biologically active molecules that specifically perturb the plant defense system and hence can be used as probes to identify components of defense signaling pathways. Identification of the protein targets of small molecule probes and plant-endogenous compounds is of fundamental importance for understanding the molecular mechanisms of signal transduction and the dynamics of plant defense. The different experimental approaches to achieve this goal will be discussed, in particular the yeast three-hybrid technology, which allows the systematic, genome-wide search for targets of small organic molecules.

Small RNAs, emerging regulators of the *M. truncatula-S. meliloti* symbiotic interaction

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Legume roots are able to develop a *de novo* meristem through the symbiotic interaction with nitrogen-fixing rhizobia. In *Medicago truncatula*, the root nodule, shows a variety of cell types from the apical meristem to a fully differentiated nitrogen-fixing region. Cellular identities of nodule regions may be determined by networks of transcription factors. Post-transcriptional regulation of a variety of mRNA targets (including transcription factors) by small 21 to 24 nt RNAs, comprising the microRNAs (miRNAs) and short-interfering RNAs (siRNAs), is emerging as a novel developmental mechanism. We showed that regulation of *Mt*miR166, which targets type III HD-ZIP transcription factors, is crucial for patterning of root vascular bundles, and formation of lateral roots and nodules. Deep sequencing of small RNAs from nodule and root tips from *Medicago truncatula* allowed us to identify 113 novel candidate miRNAs. These miRNAs are encoded by 278 putative hairpin precursors in the *M. truncatula* genome. Several miRNAs are induced or repressed in nodules when compared to root tips and a large variety of targets could be predicted for these genes. Spatial regulation of miRNA expression may be crucial for posttranscriptional silencing of regulatory genes during root nodule differentiation and/or infection processes.

Regulation of *Sinorhizobium meliloti* genes during early and intermediate stages of nodule formation

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We study the signal exchange between legume plants and their nitrogen fixing symbionts. In the case of *Sinorhizobium meliloti*, an early signal is the mixture of flavonoid compounds produced by the plant, that induces *S. meliloti* nodulation (*nod*) genes via the transcription activator, NodD1. Two other forms of NodD are encoded in the genome of *S. meliloti*. We have characterized the interaction of NodD1 with GroEL-GroES and flavonoids, finding that affinity for DNA binding is increased by interaction of the protein with the flavonoid signal; however, this interaction is not specific to the host plant's most active flavonoid. We are exploring other factors that control and facilitate the transcription of genes at the *nod* gene promoters. Accessory sigma factors that direct RNA polymerase (RNAP) to particular promoter sequences may be important. *S. meliloti* is intriguing in that the genome encodes a standard sigma factor and an N-related factor *rpoN*, plus twelve alternative sigma factors: two *rpoH* genes and ten *rpoE*-like genes. However, it encodes no *rpoS* sigma factor. Using a genetic strategy, we are carrying out a systematic study of the function of accessory sigma factors. We have earlier found that the *relA* gene product, responsible for acculumation of (p)ppGpp, is required for symbiosis, which also suggests that direct effects of the form of RNAP on transcription of symbiosis promoters. In addition to components of RNAP, we are interested in broader regulatory circuits including the ExoR-ExoS-ChvI system that regulates a number of bacterial behaviors including EPS production, motility, and biofilm formation. We are examining specific DNA targets of ChvI binding, to define the molecular basis for co-regulation of the ChvI target genes.

Genomic analysis of secondary metabolite production by *Pseudomonas fluorescens*

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Pseudomonas fluorescens is a diverse bacterial species known for its ubiquity in natural habitats and its production of secondary metabolites. The high degree of ecological and metabolic diversity represented in *P. fluorescens* is reflected in the genomic diversity displayed among strains. Certain strains live in association with plants, protecting them from infection by plant pathogens due, in part, to their production of antibiotics and extracellular enzymes. Nearly 6% of the genome of the biological control bacterium *P. fluorescens* Pf-5 is devoted to the biosynthesis of secondary metabolites. With few exceptions, these biosynthetic gene clusters are present in strain-specific regions of the genome. Many orphan gene clusters, which encode for the biosynthesis of unknown natural products, have also been identified in strain-specific regions of the Pf-5 genome. Through combined bioinformatic and chemical analyses, the products of several orphan gene clusters have been identified and characterized. The novel cyclic lipopeptide orfamide A lyses zoospores produced by phytopathogenic *Phytophthora* spp. The FitD toxin, which contributes to the newly-discovered insecticidal activity of Pf-5, and several analogs of rhizoxin, a macrocyclic lactone with antifungal activity, are also synthesized from gene clusters in strain-specific regions of the Pf-5 genome. To better understand mechanisms of biological control, the transcriptome of Pf-5 grown on seeds has been determined in microarray experiments, highlighting secondary metabolism genes expressed *in situ* by the bacterium. Comparative and functional genomics of *P. fluorescens* have revealed novel secondary metabolites and new insights into the biology of the bacterium.

The complex molecular interactions and technology transfer models of *Trichoderma* biocontrol agents

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Trichoderma spp. are among those microbes that help to feed the world. Many beneficial strains are produced and commercialized as a few hundred bioproducts in about 50 countries of 5 continents. Different models of technology transfer and implementation have been followed, depending on the type of application (biopesticides and/or biofertilizers) and the related legal constrains (registration), but also on the local economical and political conditions. *Trichoderma*-based products have been taken to the market by following either the chemical pesticide, a "cottage industry" or government monopoly models. However, applications are directly related to the level of understanding of the multiple mechanisms of action, activities, effects and interactions of these biocontrol agents. Not only they directly kill or inhibit pathogens, but also activate extensive metabolic changes in treated plants and indirectly alter plant-pathogen interactions. They establish a symbiotic-like relationship with the roots, resulting in growth promotion and increased resistance to both biotic and abiotic stress. We analyzed the "interactome" of the multiple players (*Trichoderma*, plant and pathogen) involved in the complex, three-way molecular cross-talk occurring in the rhizosphere, and identified BAMPs (Biocontrol-Associated Molecular Patterns) plus a few key "effectors". For instance, a *Trichoderma* hydrophobin has been found to be directly antimicrobial, as well as to induce, depending upon the concentration, a multiplicity of effects on the plant. It activates oxidative burst, the antioxidant system, and ISR with the accumulation of defence-related compounds, and further affects the auxin pathways, induces *de novo* rhizogenesis, and causes an epinastic phenotype in transformed tomato plants.

Hormone cross-talk in disease resistance: The role of ABA

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Abscisic acid (ABA) has long been known for its role in abiotic stress tolerance. Only in recent years, evidence has appeared that this plant hormone plays a prominent role in biotic stress as well. First reports suggested that ABA acts as negative regulator of disease resistance. However, subsequent research revealed that ABA can also promote plant disease resistance. The emerging picture today highlights that ABA is involved in a complicated network of synergistic and antagonistic interactions with other defense signals. The role of ABA appears to depend on the type of attacking pathogen, its specific way of gaining entry into the host and, hence, the timing and the type of affected plant tissue. The current knowledge about the physiological impact of ABA on plant resistance is insufficient to provide solid explanations for the sometimes contradictory recent reports. However, amidst this apparent controversy, there is a general pattern that suggests a stimulatory role of ABA in plant defense during early stages of pathogen invasion, but a mostly suppressive influence at later colonization stages. The different facets of the role of ABA in plant disease resistance will be presented and discussed.

Virus resistance induced by NB-LRR proteins involves Argonaute4-dependent translational control

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Active resistance to viruses is afforded by the products of plant disease resistance (*R*) genes encoding NB-LRR proteins. Upon recognition of pathogen-derived elicitors, NB-LRR proteins are thought to initiate a number of signaling pathways that lead to the containment of the pathogen. Little is known however, about the molecular mechanisms that ultimately curtail pathogen accumulation. We have developed a novel experimental system to study how viruses are contained during an NB-LRR-induced anti-viral response. Co-expression, in *Nicotiana benthamiana*, of the tobacco NB-LRR protein N, with its cognate elicitor, induces an anti-viral response that targets heterologous viruses but does not induce the cell death response commonly associated with *R* genes. Using potato virus X (PVX) as a reporter construct, we show that the targeting of PVX during the anti-viral response depends on viral *cis* elements. During this induced anti-viral response PVX RNAs are generated but virus-encoded proteins do not accumulate and viral RNAs do not associate with ribosomes. These results suggest that NB-LRR proteins induce anti-viral mechanisms that specifically inhibit the translation of viral transcripts, which in turn would prevent subsequent replication and movement of the virus. Dissection of the anti-viral response with viral suppressors of RNA silencing indicated the involvement of Argonaute proteins. Indeed, the induced anti-viral response was compromised by the down-regulation of *Argonaute4*-like, but not *Argonaute1*-like genes, as was resistance to PVX in *Rx2* transgenic plants. Our results suggest that specific Argonaute proteins are directly or indirectly involved in mediating the translational control of viral transcripts in NB-LRR-mediated virus resistance.

***Arabidopsis* immune responses to host-adapted pathogens**

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Plants and pathogens are in a continual evolutionary battle to gain control of defence activation thresholds. Host-adapted biotrophic pathogens can overcome initial plant barriers to infection mediated by plasma membrane-resident Pattern Recognition Receptors (PRRs). These virulent pathogen strains encounter further barriers that impede colonization of host tissues (post-invasion basal defences) and enable recognition of specific pathogen effectors by intracellular immune receptors. Molecular processes of plant receptor activation and signaling leading to resistance are beginning to be unraveled. One outstanding question is how perception of various pathogen effectors by different receptors is integrated inside the host cell. A major convergence point appears to be at the level of transcriptional reprogramming, leading to derepression of basal defences. Resistance mediated by intracellular TIR-NB-LRR receptors depends on the EDS1 family of nucleo-cytoplasmic basal defence regulators. We have been examining the sequence of events between activation of the *Arabidopsis* TIR-NB-LRR receptor RPS4 (recognizing a bacterial effector, AvrRps4) and induction of resistance and localised plant cell death. Although most RPS4 accumulates outside the nucleus, a small nuclear pool is necessary for triggering EDS1-dependent resistance and changes in defence gene expression. We find that nuclear accumulation of EDS1 increases early after pathogen infection and is important for basal and RPS4-triggered resistance. We are now testing whether the nuclear pools of RPS4 and/or EDS1 complexes associate directly or indirectly with the DNA since this may reveal how plant cells integrate resistance signals and fine-tune responses to a particular pathogen type or environmental stress.

Integrated immune responses to microbial infection

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Plants evolved surface Pattern Recognition Receptors (PRRs) to detect the presence of highly conserved microbe-associated molecular patterns (MAMPs). We identified a novel Arabidopsis PRR, *At*GSRK (glucan-sensing receptor kinase) that is required for post-invasive oomycete/fungal defense responses. *At*GSRK directly and specifically interacts with b-glucan through its extracellular C-type lectin domain. The conservation of single copy *GSRK* homologs in Arabidopsis, rice, and poplar is consistent with its proposed role in glucan sensing across plant species. Different cytoplasmic signaling domains in plant GSRK and the vertebrate glucan receptor Dectin-1 imply a convergent evolution of glucan sensing in plant and animal innate immune systems. Plants mount MAMP-triggered immunity at the cost of other physiological processes such as growth and abiotic stress responses. We describe Arabidopsis "*priority in sweet life*" (*psl*) mutants that show de-repressed sucrose-induced flavonoid accumulation in the presence of elf18, a bacterial MAMP detected by the receptor-like kinase EFR. We have isolated several *PSL* genes and describe their role in different layers of innate immunity.

Recognition of pathogen effector proteins by NB-LRR immune receptors: Strategies for the molecular breeding of durable disease resistance

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It has now well established that all classes of pathogens are able to deliver effector proteins directly to host plants often via specialized infection structures. Pathogen effector proteins are involved with the suppression or modulation of plant innate immunity and fundamentally control plant pathogenesis. Interestingly, the same proteins that modulate pathogen virulence are also involved in triggering genotype-specific plant disease resistance. In this presentation, I will highlight the original approaches that led to the discovery of pathogen effectors and how this information has shaped our current understanding of the "dual" role of effectors in both plant pathogenesis and the activation of disease resistance signalling pathways. Furthermore, I will provide recent data from my laboratory in our attempts to employ pathogen effector proteins as molecular probes to identify host targets controlling plant innate immunity. Finally, I will present our recent data on elucidating the molecular events that are involved in effector recognition and the activation of plant disease resistance and how this knowledge can be employed to molecularly breed for durable resistance in agricultural crops.

Common and not so common keys to symbiotic entry

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One of the most exciting discoveries in the area of symbiotic plant-microbe interactions has been the identification of evolutionarily conserved plant genetic determinants underlying arbuscular mycorrhizal (AM) and nitrogen fixing root nodule symbioses (RNS). In the model legume, *Lotus japonicus*, these determinants define the so called "common symbiosis pathway", which is comprised of seven distinct genes. These genes act downstream of microsymbiont-specific perception to mediate entry of both fungi and bacteria into the host root. Rapidly accumulating new data indicate, however, that the initial uptake and subsequent intracellular colonization of roots by bacteria also necessitate plant functions that are dispensable to the fungal symbiosis. Thus, in addition to recruitment of genes that sustain more ancient AM symbiosis, unique signalling mechanisms must have been established to accommodate the bacterial partner. In my presentation, I will discuss selected aspects of these signalling events in the context of plant functions that mediate symbiotic admittance.

Defining the regulatory mechanism that controls infection-related development by the rice blast fungus *Magnaporthe oryzae*

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Magnaporthe oryzae is the causal agent of rice blast, one of the most serious economic problems affecting rice production. During plant infection, *M. oryzae* develops a differentiated infection structure called an appressorium. This unicellular, dome-shaped structure generates cellular turgor, that is translated into mechanical force to cause rupture of the rice cuticle and entry into plant tissue. My research group is interested in determining the molecular basis of appressorium development and understanding the genetic regulation of the infection process by the rice blast fungus. By generation of a range of temperature-sensitive mutants governing cell cycle control points, we have shown that development of a functional appressorium is linked to the control of nuclear division. Initiation of appressorium development is controlled by entry into S-phase because genetic perturbation of DNA replication prevents germ tubes differentiating into appressoria. Entry into mitosis is both necessary and sufficient for appressorium generation, while mitotic exit is necessary for re-polarisation of the appressorium to form a penetration hypha. Following mitosis, conidia undergo cell collapse and programmed autophagic cell death. The absence of non-selective autophagic cell machinery in *M. oryzae* is sufficient to prevent the fungus from being able to cause disease. These findings indicate that appressorium morphogenesis requires completion of mitosis and initiation of autophagic recycling of the contents of the fungal spore to the appressorium. Appressorium formation is also associated with an oxidative burst that requires NADPH oxidases that are virulence determinants of *M. oryzae*. To study appressorium physiology and function in greater detail we have used proteomics to define the major changes in protein abundance associated with plant infection by *M. oryzae* and metabolite fingerprinting to define major metabolic changes in both the fungus and its host during the onset of rice blast disease. This is linked to our study of the physiology of turgor generation and the role of glycerol, trehalose and glycogen metabolism to the production of infection-competent appressoria. Collectively, we are attempting to gain an understanding of the genetic and metabolic control mechanisms that govern the successful infection of rice by *M. oryzae*.

Small RNA pathways and their interference by pathogens in eukaryotes

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RNA silencing is a pan-eukaryotic gene regulation process whereby small interfering (si)RNAs and micro (mi)RNAs produced by Dicer-like enzymes repress gene expression through partial or complete base-pairing to target DNA or RNA. Besides their roles in developmental patterning and maintenance of genome integrity, small RNAs are also integral components of eukaryotic responses to adverse environmental conditions, including biotic stress. Until recently, antiviral RNA silencing was considered a paradigm of the interactions linking RNA silencing to pathogens: Virus-derived sRNAs silence viral gene expression and, accordingly, viruses produce suppressor proteins that target the silencing mechanism. However, increasing evidence shows that endogenous, rather than pathogen-derived, sRNAs also have broad functions in regulating plant responses to various microbes. In turn, microbes have evolved ways to inhibit, avoid, or usurp cellular silencing pathways, thereby prompting the deployment of countercounterdefensive measures by plants, a compelling illustration of the never ending molecular arms race between hosts and parasites. Several original illustrations of these various aspects will be provided, using examples from ongoing studies in our laboratory.

Auxin - at the root of nodule development?

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We investigated the role of auxin in the regulation of nodule and lateral roots organogenesis in *Medicago truncatula*. Auxin is known to be a crucial regulator of lateral root development, a process that shares features with nodule formation. In both cases, a new root meristem is initiated, differentiates and emerges from the root. However, lateral roots initiate in the pericycle and indeterminate nodules initiate in pericycle and inner cortex cells. Our hypothesis is that, among other factors, different requirements for auxin (transport) determine which and how many root organs are formed. First, we found that local auxin transport inhibition is required for nodule initiation and is mediated by the action of flavonoids. In contrast, flavonoids are not required for auxin transport regulation during lateral root development. Second, we altered the auxin sensitivity in the root by silencing several auxin response genes. This led to inhibition of nodule but not lateral root initiation, suggesting that auxin sensitivity partly determines which organ is formed. Third, we investigated shoot-to-root long distance auxin transport, which was

positively correlated with nodule numbers. Autoregulation mutants that are unable to repress excessive nodule formation were unable to regulate long distance auxin transport. Fourth, we tested whether auxin transport is under regulation of external nitrogen at concentrations that inhibit nodulation. Nitrate interfered with both short and long distance auxin transport, and this was dependent on the action of the autoregulation gene in *M. truncatula*. Our results suggest that auxin transport regulation is necessary for nodule initiation and control of nodule numbers, and that auxin requirements differ between lateral root and nodule formation.

Negative regulators of innate immunity in cereal-fungal interactions

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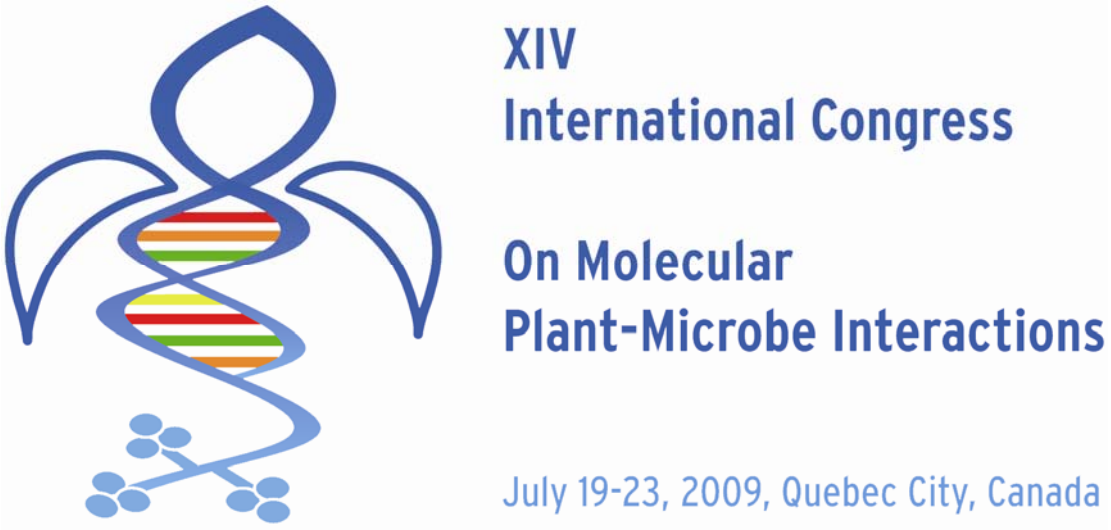
IS-MPMI 2009 XIV Congress 8

Plants have evolved complex regulatory mechanisms to control the defense response against microbial attack. Both temporal and spatial gene expression are tightly regulated in response to pathogen ingress, modulating both positive and negative control of defense. BLUFENSIN1 (BLN1), a small peptide belonging to a novel family of proteins in barley, wheat, and rice, is highly induced by attack from the obligate biotrophic fungus, *Blumeria graminis* f. sp. *hordei*, causal agent of powdery mildew disease. BLN1 negatively impacts plant defense, is predicted to be secreted, and contains both structural and sequence similarities to knottins, small disulfide-rich proteins characterized by a unique disulfide through disulfide knot. To discern regulatory targets of BLN1, we conducted Barley1 GeneChip analysis of *Bln1*-silenced plants via *Barley stripe mosaic virus*-induced gene silencing (BSMV-VIGS). Sixty GeneChip hybridizations were performed, based on 5 replications of 12 BSMV-VIGS/host-pathogen interactions. Mixed linear model analysis revealed 36 significant new genes ($p < 0.0001$; FDR < 5%) that are suppressed together with *Bln1* (Contig12219_at; $p=2.31^{E-07}$), or induced when we compare BSMV:$Bln1_{248}$ silenced plants to the BSMV:00 control. These candidates appear to have a role in *R*-gene mediated and innate immunity networks, thus, the functional identification of their precise roles will be a key step in understanding plant defense.



IS-MPMI 2009 XIV International Congress on Molecular Plant-Microbe Interactions Abstracts of Concurrent Session Presentations

Abstracts submitted for presentation at the Concurrent Sessions at the XIV International Congress on Molecular Plant-Microbe Interactions in Quebec City, Canada, July 19–23, 2009. The abstracts are arranged in alphabetical order by the last name of the first author. Abstracts are published as submitted. They were formatted but not edited at the IS-MPMI headquarters office.

Identification and profiling of American elm genes involved in a compatible interaction with the fungal pathogen *Ophiostoma novo-ulmi*
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Dutch elm disease (DED) is a pandemic tree disease that has killed millions of elm trees, especially in North America and Europe. The molecular bases of this disease are still poorly understood. In order to identify genes involved in the interaction between the susceptible *Ulmus americana* and *Ophiostoma novo-ulmi*, an *in vitro* system using callus cultures was developed. Cytochemical tests were carried out to validate the use of this system for genomic analyses of the interaction. A cDNA library using suppression subtractive hybridization was constructed from infected elm callus tissue 72 hours post inoculation (hpi). A total of 535 expressed sequence tags were generated and grouped into 314 unisequences, the majority corresponding to elm genes identified during the interaction. Fifty-three unisequences representing genes involved in different pathways associated with plant defense were selected by differential screening and were shown to be upregulated in the infected tissues. The expression profiles in mock and infected elm callus culture of a subset of 18 elm genes were analyzed in more detail by quantitative reverse transcriptase polymerase chain reaction. They confirmed upregulation and constitutive expression of selected genes during the infection process and showed that the earliest time point for induction in expression was 48 hpi. This study provides, for the first time, a genome-wide resource for the elm and furthermore identifies molecular mechanisms likely involved during the interaction between *U. americana* and the DED pathogen.

Riboflavin synthesis participates in plant immune responses in *Nicotiana benthamiana*
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Rapid production of nitric oxide (NO) and reactive oxygen species (ROS), called NO burst and oxidative burst, respectively, is characterized during resistance to biotic and abiotic stresses. Our previous studies showed that MAPK cascades regulate NO and oxidative bursts induced by INF1, which is produced by the oomycete pathogen *Phytophthora infestans*, and that the radical burst appears to have distinct effects on disease resistance against diverse pathogens according to their infection strategies (Asai et al., 2008 Plant Cell; Asai and Yoshioka, 2009 Mol. Plant-Microbe Interact.). We have screened candidates related to cell death, designated *CELL DEATH ASSOCIATED* (*CDA*) genes, in randomly genes-silenced plants by VIGS. *CDA1* encodes the gene for riboflavin synthesis. Riboflavin converts to FAD and FMN known as coenzyme of NADPH oxidase, NO synthase and nitrate reductase, indicating that CDA1 might be responsible for NO and ROS productions. Silencing *CDA1* compromised not only HR cell death but also radical burst induced by INF1 and MEK2^{DD}, a constitutively active mutant of MEK2, and also affected disease resistance to *P. infestans* and *Colletotrichum orbiculare* in *N. benthamiana*. Furthermore, administration of riboflavin rescued the compromised the radical burst and HR cell death induced by INF1 in *CDA1*-silenced plants. These results indicated that riboflavin synthesis is involved in regulation of NO and ROS productions and HR cell death.

Functional genomics of proteases in chili pepper (*Capsicum annuum*)
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Plant genome encodes hundreds of proteases, which break peptide bond of proteins. Proteases play key role in regulation of biological processes in plantswhich include plant metabolism, physiology, growth and defense. Proteases are classified into 5 families: Cystein proteases (CysPro), Serine proteases (SerPro), Threonine proteases (ThrPro), Metalloproteases (MetalloPro), and Aspartic protease (AspPro) based on the nucleophile and oxyanion stabilizer. We have selected 940 putative proteases from EST of *Capsicum annuum* using protease domain from MEROPS database through blastX, hmmpfam and hmmsmart. They were classified into 227 CysPro, 359 SerPro, 36 ThrPro, 156 MetalloPro, 161 AspPro and 1 unknown proteases, respectively. To identify function of pepper proteases, we have cloned

proteases into TRV-LIC vector to perform virus-induced gene silencing. Some of the protease-silenced phenotypes such as deformation of upper leaves, loss of pigmentation, severe stunting or modifications in pathogen defense will be presented in the meeting.

Deciphering PAMP-triggered immunity in Arabidopsis

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IS-MPMI 2009 XIV Congress 10

In plant innate immunity, the first line of microbial recognition relies on the perception of pathogen-associated molecular patterns (PAMPs) by pattern-recognition receptors (PRRs) leading to PAMP-triggered immunity (PTI). In Arabidopsis, the leucine-rich repeat receptor kinases EFR and FLS2, in association with BAK1, mediate recognition of the bacterial PAMPs EF-Tu and flagellin, or their peptide surrogates flg22 and elf18 respectively. Despite the critical role of PRRs in anti-microbial immunity, only limited knowledge exists on how they function at the molecular level and on their downstream signalling events. By forward genetic, we have identified 160 *elf18-isensitive* (*elfin*) mutants; 57 corresponding to efr mutants. The identification and characterisation of six *ELFIN* genes shed light on how EFR protein expression is controlled, as well as on immediate downstream signalling. We describe an unexpected sub-specialisation of the ER quality control (ER-QC) machinery for innate immunity, and the first demonstration of a physiological role of the ER-QC in trans-membrane receptor function in plants. In addition, we identified a new *bak1* allele that impacts differentially the requirement of BAK1 in PTI and brassinosteroid signalling. We are using a combination of genetic, cell biology and biochemistry to unravel the molecular mechanisms underlying this puzzling phenotype. This study should reveal how BAK1 intricates with different signalling pathways in plants. Our quest to decipher PTI signalling is complemented by the search and characterisation of EFR-interacting proteins (EIPs) in yeast and *in planta*. We are also describing the feasibility of trans-family heterologous expression of PRRs to engineer broad-spectrum disease resistance in plants.

Signatures of adaptation to obligate biotrophy in the *H. arabidopsidis* genome

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Many plant pathogens extract nutrients exclusively from living plant tissue and cannot grow apart from their hosts (termed "obligate biotrophy"). We have obtained a draft genome sequence of the oomycete *Hyaloperonospora arabidopsidis*, a natural downy mildew pathogen of *Arabidopsis thaliana* and a model for obligate biotrophy. Although downy mildews are uniformly obligate, they are closely related to *Phythophthora* spp. and other oomycetes that can exist as saprobes and destroy plant tissue after they have invaded their hosts. Thus, comparison of the *H. arabidopsidis* genomes with recently sequenced *Phytophthora* genomes provides an opportunity to understand how an obligate biotroph has evolved from a free-living ancestor that employed a very different pathogenicity strategy. Our comparisons revealed two striking themes. First, gene families encoding proteins with potential to damage host cells or otherwise trigger defense responses (cell wall-degrading enzymes, elicitins, necrosis-inducing proteins, RxLR effectors, and others) were dramatically reduced in *H. arabidopsidis* compared to *Phytophthora*, indicative of optimization for stealth inside the host. Second, genes involved in several metabolic pathways were absent from *H. arabidopsidis*, suggestive of metabolic dependency on the host. Some features of *H. arabidopsidis* gene space (maintenance of large numbers of secreted effectors, reduction in cell wall-degrading enzymes) are paralleled in the genomes of non-obligate, biotrophic fungi (the plant pathogen *Ustilago maydis* and the symbiont *Laccaria bicolor*), demonstrating that oomycetes and fungi have evolved similar molecular adaptations to a biotrophic lifestyle even though these lineages evolved independently from one another.

Nitric oxide signalling in plant defence: Interplays with Ca^{2+} and iron homeostasis

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We previously reported that nitric oxide (NO) is rapidly produced in plant cells challenged by elicitors of defence responses. NO synthesis is required as a step in the pathways leading to the rise in cytosolic ($[Ca^{2+}]_{cyt}$) concentration. NO enhances $[Ca^{2+}]_{cyt}$ by promoting phosphorylation-dependent events. This process might involve NtOSAK, a protein kinase belonging to the SnRK2 family. We provided evidence that NO does not S-nitrosylate NtOSAK but induces its activation by promoting the phosphorylation of two serine residues located within the active site of the enzymes. Our work also highlights a tight relationship between NO and iron homeostasis. Indeed, we recently identified *NAS4* as a NO target gene in *A. thaliana* plants challenged by (a)biotic stresses. Nicotianamine synthases (NAS) catalyse the formation of nicotianamine (NA), a non-proteogenic amino acid playing a major role in iron homeostasis in graminaceous and non-graminaceous plants. We showed that *atnas4*, a mutant carrying a T-DNA insertion in *AtNAS4* displays a half-reduced content of NA in leaves. *atnas4* exhibits hypersensitivity to iron deficiency, characterized by the development of intercostal chloroses in young leaves, and by a strong reduction of root growth. Interestingly, the *atnas4* mutant shows a highest sensitivity to the bacterial pathogen *Erwinia chrysanthemi*, highlighting a key role for NA in plant resistance.

Localisation and function of *Phytophthora infestans* RXLR effectors and their host targets

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Eukaryotic plant pathogens, like their better-characterised prokaryotic counterparts, secrete an array of effector proteins that may suppress or modulate host innate immunity to establish infection. Deciphering the biochemical activities of effectors to understand how pathogens successfully colonize and reproduce on their host plants has become a driving focus of research in the fields of fungal and oomycete pathology. AVR3a, the first effector characterized from the oomycete pathogen of potato and tomato, *Phytophthora infestans*, was found to contain an N-terminal RxLR and dEER motif required for its translocation across the host plasma membrane. Developing genomic resources have allowed large-scale computational screening for this conserved motif to reveal approximately 500 *P. infestans* RXLR-EER effectors. We are cloning *RXLR* effector genes to investigate their roles in virulence and their localisation in plant cells. Yeast-2-hybrid and bimolecular fluorescence complementation are being used to investigate effector-target protein interactions, and to localize these during infection. I will present our progress in the investigation of pathogenicity functions of the AVR2 and AVR3a RXLR effectors, including images visualizing their translocation from haustoria and data showing that these effectors interact with different host proteins to establish infection. A range of approaches, including virus-induced genes silencing, are being used to determine the roles of host targets in defence.

Symbiotic exception: The Nod-independent interaction between the Aeschynomene legumes and photosynthetic Bradyrhizobia

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There was a long-held dogma that the synthetised of Nod factors (NF) by rhizobia was absolutely required to trigger nodule organogenesis in legume. However, the universality of the NF paradigm was recently overturned, by the finding that the genome of two photosynthetic Bradyrhizobium strains (BTAi1 and ORS278) that elicit root- and stem-nodules on a particular group of Aeschynomene plants do not contain the canonical nodABC genes necessary for the synthesis of NF (Giraud et al. 2007). To obtain further insight into the bacterial genes involved during the early steps of this NF-independant symbiosis, we have screened a large Tn5 mutant library of the Bradyrhizobium ORS278 strain for their inability to induce nodules on Aeschynomene. No strict nodulation deficient mutants were found. However, several mutants affected in the different steps of the purine biosynthesis pathway were severely impaired in nodulation. This suggests that a bacterial

purine derivative such as cytokinin, may play a key role in triggering nodule formation on Aeschynomene. A number of independent observations support such a hypothesis. First, we have found that photosynthetic bradyrhizobia are able to synthesize cytokinins and, second, we observed that the exogenous addition of certain pure cytokinins could induce the formation of nodule-like structures. At the plant level, experiments are in progress to determine whether the NF-independent signaling pathway in Aeschynomene plants share common elements with the NF-dependant symbiosis as described in the model legumes. In my talk I will give an overview and present recent advances in the Nod-independent symbiosis between photosynthetic bradyrhizobia and Aeschynomene.

Identifying genes involved in *Grosmannia clavigera*'s response to pine tree defenses

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The current mountain pine beetle (MPB) epidemic in British Columbia is a prime example of the devastating immediate and long-term effects of insect/fungal diseases on Canada's forests. Such epidemics involve specific interactions between a host tree, insect vector and fungal pathogens. Bark beetle-vectored fungal pathogens like *Grosmannia clavigera* that can bypass or overcome a tree's physical and chemical defenses can rapidly colonize the phloem and sapwood, killing the tree. To characterize the mechanisms involved in pine colonization and the molecular processes that allow G. clavigera to tolerate terpenoid and phenolic host defenses, we have generated 1) an annotated draft genome using a combination of several different sequencing technologies, 2) gene expression profiles for this fungus when exposed to selected tree defense associated metabolites, and 3) a preliminary genome wide map of nucleotide variation in protein coding genes. We will highlight features of the draft genome sequence that may support the fungal pathogen's tolerance towards tree chemical defenses and compare the stress effects of terpenoids and phenolics on gene expression.

Map-based cloning of the *Eucalyptus grandis* genomic region that encompass the rust resistance gene *Ppr-1*

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Eucalyptus is the most widely planted hardwood crop in the tropical and subtropical world because of its superior growth, broad adaptability and multipurpose high-quality wood biomass for several industrial applications. *Eucalyptus* rust caused by *Puccinia psidii* is the most important eucalypt disease in Brazil and it is considered a potential threat to eucalypt plantations worldwide. In a previous work, a major gene for *Puccinia psidii* rust resistance (*Ppr-1*) was found and mapped in *E. grandis* (Junghans et al., 2003). A linkage group encompassing RAPD and microsatellite markers was built around *Ppr-1*. The RAPD marker AT9/917 co-segregated with *Ppr-1* without a single recombination in 994 meioses. Based on the size of the segregating population, the maximum estimated possible genetic distance between AT9/917 and *Ppr-1* was estimated to be 0.462 cM (a=0.01), which may corresponds to a physical distance of ~200 kb. This estimated physical distance makes feasible the positional cloning of *Ppr-1*. With this goal in mind, we have constructed a *E. grandis* BAC library with average insert size of ~150 kb. By using a SCAR marker derived from RAPD AT9/917 we have successfully isolated a BAC clone of ~140kb that contains the AT9/917 marker. By using PCR markers derived from the ends of this initial BAC clone additional overlapping BAC clones were identified. Altogether the isolated clones span a genomic region of ~400 kb that contains several NBS-LRR resistance gene analogs. A detailed high resolution genetic mapping using microsatellite derived from this BAC contig is being executed to pinpoint the location of *Ppr-1*. Complementation studies using the NBS-LRR analogs are also underway.

Symbiotic use of pathogenic strategies: Rhizobial protein secretion systems

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Rhizobia — a diverse group of soil bacteria — induce the formation of nitrogen-fixing nodules on the roots of legumes. Nodulation begins when the roots initiate a molecular dialogue with compatible rhizobia present in the soil. Most rhizobia reply by secreting lipo-chito-oligo-saccharidic Nod-factors that enable entry into the legume. A molecular exchange continues that in compatible interactions permits rhizobia to invade root cortical cells, differentiate into bacteroids and fix nitrogen. Additional molecular signals utilised by rhizobia are secreted proteins or surface polysaccharides. One group of proteins secreted by rhizobia have homologues in bacterial pathogens and may have been co-opted by rhizobia for symbiotic purposes.

Signaling in arbuscular mycorrhizal phosphate transport

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The arbuscular mycorrhizal (AM) symbiosis represents the most widely distributed mutualistic root symbiosis. We reported that root extracts of mycorrhizal plants contain a lipophilic signal capable of inducing the phosphate transporter genes *StPT3* and *StPT4* of potato (*Solanum tuberosum* L.), genes that are specifically induced in roots colonized by AM fungi. The same signal caused rapid extracellular alkalinization in suspension-cultured tomato (*Solanum lycopersicum* L.) cells and induction of the mycorrhiza-specific phosphate transporter gene *LePT4* in these cells. The active principle was characterized as the lysolipid lysophosphatidylcholine (LPC) via a combination of gene expression studies, alkalinization assays in cell cultures, and chromatographic and mass spectrometric analyses. The same genes are required for operation of the mycorrhizal phosphate uptake pathway via AM fungal hyphae into colonized root. A combined approach including physiological analysis using ^{33}P as a radiotracer and phosphate transporter gene expression studies in mycorrhizal and non-mycorrhizal plants allowed us to propose a model which integrates lysophospholipids as signals in the AM symbiosis and the plant P status controlling the mycorrhizal phosphate uptake pathway in solanaceous species.

Modulation of viral replication through the control of viral RNA-dependent RNA polymerase degradation by the ubiquitin-dependent proteasome pathway

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Replication of positive-strand RNA viruses is initiated by the viral RNA-dependent RNA polymerase (RdRp). We previously reported that the RdRp of *Turnip yellow mosaic virus* (TYMV) is phosphorylated in plant cells, a modification that plays a key role in the regulation of viral replication. We now have evidence that TYMV RdRp is ubiquitylated and degraded by the 26S proteasome pathway in plant cells. The degradation machinery targets the N-terminal non-catalytic domain of the RdRp, and the phosphorylation status of the protein was shown to impact its turnover. In addition, we mapped several lysine residues that constitute target sites for ubiquitylation, whose mutations led to RdRp stabilization. Finally, we report that TYMV RdRp is degraded by the 26S proteasome pathway during viral infection and that its stabilization improved viral RNA accumulation, supporting yet another fundamental level of regulation of viral replication through the control of RdRp stability.

Regulatory systems controlling gall formation by the bacterium *Pantoea agglomerans*

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Pantoea agglomerans pv. *gypsophilae* (*Pag*) has evolved from an epiphytic bacterium into *hrp*-dependent tumorigenic pathogen on gypsophila. Gall formation by *Pag* has been shown to be affected by *hrp/hrc* genes, phytohormones and the quorum-sensing (QS) regulatory system. The ultimate goal of this study is to understand the interactions between global regulatory systems, auxin and cytokinin signaling and the *hrp* regulatory genes in relation to gall formation. Disruption of the QS genes *pagI*, *pagR* and deletion of both substantially reduced the transcription levels of the *hrp* regulatory genes *hrpXY*, *hrpS* and *hrpL*, as determined by qRT-PCR. Expression of *hrpL in planta* was inhibited by addition of 20 μM or higher concentrations of the QS signal C_4-HSL. Transcription of *pagI*, *pagR*, *hrpL* and *hrpS in planta* were substantially reduced in *iaaH* mutant (disrupted in IAA biosynthesis *via* the

indole-3-acetamide pathway), and *etz* mutant (disrupted in cytokinin biosynthesis) whereas, the *ipdC* mutant (disrupted in IAA biosynthesis *via* the indole-3-pyruvate pathway) substantially increased expression of these genes. The global regulatory systems, GacS/GacA and the posttranscriptional regulators RsmA-*rsmB* are currently investigated by studying their effect on the expression of various regulatory genes as well as their hierarchy by means of qRT-PCR and gel retardation. Results presented suggest the involvement of IAA and cytokinins in regulation of the QS system and *hrp* regulatory genes.

Development of RNAi technology for plant protection

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RNA interference (RNAi) plays important roles in plant defense against invading viruses and other pathogens. Viral RNAs are found to be degraded by RNAi pathway in resistant plants, on the other hand, many viral proteins act as gene silencing suppressors and are required for infection. Recently, we developed a plant-based RNAi technology to dampen the insect detoxification system. Cotton bollworm (Helicoverpa armigera) has adapted to live on cotton plants that accumulate high concentrations of gossypol and related sesquiterpene aldehydes which are generally toxic. In animals, cytochrome P450 monooxygenases are key enzymes in xenobiotics metabolism. We isolated a gossypol-inducible P450 monooxygenase gene, CYP6AE14, from H. armigera. CYP6AE14 expression level in midgut was correlated with larval growth in the presence of gossypol. Transgenic Arabidopsis, tobacco and cotton plants expressing a hairpin RNA construct of CYP6AE14 (dsCYP6AE14) were generated. When the transgenic plant tissues were used for feeding bollworms, the CYP6AE14 transcript abundance in midgut was substantially decreased. In a small scale assay, the transgenic cotton plants showed enhanced resistance to H. armigera, and growth of the bollworm larvae were drastically reduced. Further experiments with a glutathione-S-transferase gene (GST1) showed that the RNAi was also effective. In insect RNAi experiments, injection has been the mostly used method to deliver dsRNA molecules into insect tissues. However, injection is inapplicable in the field. Using transgenic plants to induce insect RNAi has a great application potential in agricultural pest control. Further investigation of the processing and traverse of the silencing signal will provide new insights into plant-insect interactions.

Functional analysis of candidate genes involved in disease resistance in lettuce

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EST sequencing by the Compositae Genome Project (CGP) has resulted in the identification of more than 724 candidate genes potentially involved in pathogen recognition, signal transduction pathways or defense responses in lettuce. These resistance gene candidates (RGCs) are distributed across all nine chromosomes, often in clusters. Thirty-six genes for resistance to seven different diseases have been mapped relative to 113 RGCs using various approaches. RNAi is being used to test the functional connection between RGCs and resistant phenotypes. A subset of RGCs, primarily NBS-LRR encoding genes, have been selected for functional analysis based upon their co-localization with a resistant phenotype, the class and the gene family they belong to and the presence of characteristic motifs they encode. Generation of a comprehensive library of RNAi tester stocks for RGCs is underway. So far, we have generated 26 tester lines and tested 14 of them for a total of 17 resistance phenotypes. RNAi has resulted in abrogation of the seven resistance phenotypes conferred by genes in the *Dm3* and *Dm5/8* clusters. RNAi is also being employed to determine which signal transduction cascades are downstream of different resistance genes in lettuce; RNAi lines for several key components of resistance signal transduction pathways are being generated. For the signaling genes, RNAi is controlled by a dexamethasone-inducible promoter, since constitutive RNAi of several genes is lethal in lettuce. Together these data will provide a mechanistic understanding of the genes involved in resistance to multiple diverse diseases in lettuce.

Identification and characterization of the RIN4 protein complex

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In order to resist infection, plants rely on their innate immune system to both inhibit pathogen entry and multiplication. We investigated the function of the *Arabidopsis* protein RIN4, which acts as a negative regulator of plant innate immunity. We have identified six novel RIN4 associated proteins using immunoaffinity chromatography and mass spectrometry. Recent data on the biochemical and genetic characterization of these RIN4 associated proteins will be presented. The association between RIN4 and the plasma membrane H+-ATPase AHA1 will be described in detail. Our results indicate that RIN4 interacts with the C-terminus of AHA1 and can regulate its activity. Our results are consistent with a model in which RIN4 and AHA1 act in concert to regulate leaf stomata during the innate immune response, thus blocking the entry of bacterial pathogens into the leaf interior.

Strategies of plant viruses to counteract RNA silencing based antiviral plant response

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Viruses are inducers, as well as targets, of RNA silencing-based antiviral defence. Replication intermediates or folded viral RNAs activate RNA silencing, generating small interfering RNAs (siRNAs), which are the key players in the antiviral response. Viruses are able to counteract RNA silencing by expressing silencing-suppressor proteins. It has been shown that many of the identified silencing-suppressor proteins bind long double-stranded RNA or siRNAs and thereby prevent assembly of the RNA-induced silencing complex (RISC), which targets the corresponding viral RNA for degradation. However, recently identified suppressor proteins have been shown to interact with the protein components of silencing machinery such as Beet western yellows virus P0 that targets Argonaute1 (AGO1) protein, the slicer component of RNA Induced Silencing Complex (RISC) for degradation (Bortolamiol et al., 2007, Baumberger et al., 2007). We have further explored the molecular bases of P0 mediated silencing suppression demonstrating that the expression of P0 in plant tissues is not able to interfere with the slicer activity pre-programmed siRNA/miRNA-Argonaute1 (AGO1) complexes. Strikingly we have demonstrated that AGO1 protein is part of a higher molecular weight RNA silencing related complex, which development was arrested by the presence of P0 protein. The strategy of P0 is to target AGO1 only before recruitment of siRNA/miRNA, provides a delayed effect on AGO1-mediated endogenous silencing, while it could efficiently prevent the new antiviral RISCs formation facilitating the establishment of virus infection.

RIN4 is a multifunctional regulator of Arabidopsis immune defense against *Pseudomonas syringae*

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The plant innate immune system consists of two main branches. Perception of pathogen-associated molecular patterns (PAMPs) activates PAMP-triggered immunity (PTI). Pathogenic bacteria deploy virulence factors to suppress PTI, including translocated type III effector proteins. In addition to suppressing PTI, type III effectors also activate the second branch of plant innate immunity, effector-triggered immunity (ETI) via activation of NB-LRR disease resistance proteins. ETI can be indirectly activated by PTI-suppressing effectors, and the idea that ETI reinforces PTI may help explain the robust nature of innate immunity in plants. Support for this model comes from Arabidopsis RIN4, which (1) negatively regulates PTI, (2) is targeted by three sequence unrelated type III effectors that suppress PTI, and (3) mediates recognition of these effectors via NB-LRR proteins. RIN4 is perturbed by AvrRpm1, AvrB, and AvrRpt2, which are PTI-suppressing type III effector proteins from *P. syringae*. RIN4 interacts *in planta* with two NB-LRR-proteins, RPM1 and RPS2, and perturbation of RIN4 elicits their response to AvrRpm1, AvrB and AvrRpt2. Structure-function analysis of RIN4 has revealed that (1) RIN4 has multiple domains capable of negatively regulating PTI, (2) one of these domains is critical for regulation of RPM1, and (3) membrane targeting of RIN4 differentially affects its ability to regulate PTI and ETI. Further, mutagenesis of putative and known phosphorylation sites within RIN4 will reveal the importance of post-translational modification in regulation of both PTI and ETI. Collectively, our results indicate that RIN4 is multifunctional with separable activities involved in regulation of these two central branches of plant immunity.

Role of biosurfactants and phenazines in Pseudomonas-mediated biological control of soilborne fungal pathogens

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Previously, we showed that *Pseudomonas aeruginosa* PNA1 suppresses *Pythium myriotylum* root rot on cocoyam (*Xanthosoma sagittifolium*) by the

synergistic action of phenazine antibiotics and rhamnolipid biosurfactants. Since *P. aeruginosa* is an opportunistic human pathogen, we started an aimed screening for alternative pseudomonads producing both phenazines and biosurfactants that could replace PNA1. Pseudomonas strains were isolated from the rhizosphere of healthy white and red cocoyam plants appearing in naturally infested fields in Cameroon. Antagonistic strains could only be retrieved from the rhizosphere of red cocoyam, which shows field resistance against the root rot disease. Among the antagonistic strains, *Pseudomonas* CMR12a was selected because of its combined production of phenazines and cyclic lipopeptide (CLP) biosurfactants and its excellent *in vivo* biocontrol of *P. myriotylum*. Site specific mutants of CMR12a in phenazine and CLP biosynthesis were constructed and used in infection experiments. The phenazine mutant still provided biocontrol towards *P. myriotylum*, while the CLP mutant lost its biocontrol capacity indicating that the ability of CMR12a to suppress *P. myriotylum* can mainly be attributed to CLP production. Consequently, the biocontrol mechanism appears to be different from that of *P. aeruginosa* PNA1. CMR12a and its mutants were also used to control different anastomosis groups of *Rhizoctonia solani* on bean. Both the phenazine and CLP mutant still provided biocontrol towards *R. solani* on bean, albeit to a lower extent as the wild type strain CMR12a, while a mutant deficient in both phenazines and biosurfactants, completely lost its biocontrol activity. It appears that phenazines and biosurfactants have an additive effect in this pathosystem.

Identification of key genes involved in the establishment of symbiotic and pathogenic plant-microorganism interactions

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In compatible interaction between plants and biotrophic microorganisms, neoformation of organs occurs to ensure an efficient relationship between both partners. During the interaction between *Medicago truncatula* and *Sinorhizobium meliloti*, bacteria induce the development of root nodule with a permanent meristem, and chronically infect plant cells from zone II before differentiating into atmospheric nitrogen fixing bacteroids. *M. truncatula* is also plant host for root-knot nematodes, such as *Meloidogyne incognita*. During this compatible pathogenic interaction, root-knot nematodes induce redifferenciation of root cells from the vascular cylinder into specialized feeding cells called "giant cells". Hyperplasia and hypertrophy of the surrounding cells lead to the formation of typical root galls. This phenomenon invokes host pathways in common with those necessary for nitrogen-nodule formation, including induction of specific transcription regulators, early nodulin genes and cytokinin-response pathways. This suggests that nematodes and *rhizobia* exploit a relative common strategy of plant cell infection at the cellular and molecular level. In order to highlight key genes involved in gall and nodule developments, parallel laser microdissection of giant cells from galls and cells from zone II of nodules were performed. The RNA pools were extracted from these cells, amplified and used for transcriptomic studies with *M. truncatula* Affymetrix® DNA chips. A comparative analysis of results will be presented and discussed. This approach enables targeted enrichment of specific cell types, thereby increasing the selectivity of our functional investigation of genes important for the establishment and development of the interaction between plant and symbiotic or pathogenic microorganisms.

Effector proteins of phytoparasitic nematodes

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Effector proteins of the microscopic root-parasitic cyst (*Heterodera* and *Gobodera* spp.) and root-knot (*Meloidogyne* spp.) nematodes are synthesized in specialized esophageal gland cells and secreted through the stylet (hollow oral spear) to facilitate plant root penetration and subsequent transformation of selected root cells into enlarged, multinucleate feeding sites. A relatively large suite of developmentally-expressed nematode parasitism genes that encode secretory effector proteins have been identified that include cell wall modifying proteins, mimics of plant CLE peptides and annexins, ligands of plant regulatory proteins, potential modulators of the host proteasome and defense response, enzymes of secondary metabolism, proteins that can localize to the plant cell nucleus, and many novel putative effector proteins of unknown function. Protein-protein interactions have been demonstrated between nematode effectors and plant transcription factors, cell wall proteins, and plant proteins involved in defense and stress response. Complementation of *Arabidopsis thaliana* mutants and plant host-derived RNA interference (RNAi) have been used successfully to assess the function of different phytoparasitic nematode effector proteins and their importance in plant parasitism.

Stochastic actin dynamics and disease resistance signaling: The AvrPphB-actin connection

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The actin cytoskeleton plays important roles in development, reproduction, senescence and disease resistance. In plants, localized reorganization of the actin cytoskeleton has been implicated in plant defenses against pathogenic fungi and oomycetes with limited, indirect evidence. To date, there are no reports linking actin organization or function with resistance against phytopathogenic bacteria. Here, we demonstrate that actin dynamics play a role in the activation of gene-for-gene resistance in *Arabidopsis thaliana* following inoculation with the phytopathogenic bacterium *Pseudomonas syringae* pv. tomato. Using a reverse genetic approach we explored the roles of *Arabidopsis* Actin Depolymerizing Factors (ADFs) in plant defenses. AtADF4 was identified as being specifically required for resistance triggered by the effector AvrPphB, but not AvrRpt2 or AvrB. Recombinant AtADF4 bound to monomeric actin (G-actin) with a marked preference for the ADP-loaded form, and inhibited the rate of nucleotide exchange on Gactin, indicating that AtADF4 is a *bona fide* actin depolymerizing factor. Exogenous application of the actin disrupting agent cytochalasin D partially rescued the *Atadf4* mutant in the AvrPphB-mediated hypersensitive response, demonstrating that AtADF4 mediates defense signaling through modification of the actin cytoskeleton. Unlike the mechanism by which the actin cytoskeleton confers resistance against fungi and oomycetes, AtADF4 is not involved in penetration resistance, further suggesting a role for the actin cytoskeleton in mediating intracellular defense signaling. The use of variable angle epifluorescence microscopy (VAEM) to image real-time actin filament dynamics during pathogen response and defense signaling will be discussed.

Improvement of virus induced gene silencing (VIGS) in rice through *Agrobacterium* infiltration

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Virus induced gene silencing (VIGS) is a method to determine gene function in plants through a mechanism that targets and destroys specific RNA sequences in cells. One important advantage of VIGS over most other functional genomic methods is that it allows analysis of the functions of many genes within a short period of time. We previously produced a clone of a fescue strain of Brome mosaic virus (C-BMV) from which infectious transcripts could be synthesized to induce gene silencing in several monocotyledonous species, including rice. Rice has the smallest genome among the model cereal crops, its sequence is known and there are many mutant lines making it an ideal plant for both molecular and biochemical studies to determine cereal gene functions. Improving gene function determination technologies (e.g. VIGS) will further aid the worth of rice as a model monocotyledon species. To provide more successful infections and better VIGS in rice, we have constructed a binary vector where a double CaMV 35S promoter drives expression of the C-BMV and target host gene sequences. The modified binary vectors were transformed individually into *A. tumefaciens* strain C58C1 and Eha105. We determined that vacuum infiltrating *A. tumefaciens* C58C1 containing the modified VIGS vector into young rice plants resulted in approximately 80% of the infiltrated plants being infected. Seedlings infiltrated with *A. tumefaciens* harboring the C-BMV containing a 250 bp rice phytoene desaturase gene insert showed stronger and more durable photobleaching phenotypes than those observed in plants mechanically inoculated with RNA transcripts of the original vector, indicating that the modified vector has enhanced potential for rice reverse genomics studies.

Abscisic acid regulation of nodulation

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Nodulation is tightly regulated in legumes to ensure appropriate levels of nitrogen fixation without excessive depletion of carbon reserves. This balance is maintained by intimately linking nodulation and its regulation with plant hormones. It has previously been shown that ethylene and jasmonic acid are able to regulate nodulation and Nod factor signal transduction. Here we characterize the nature of abscisic acid (ABA) regulation of nodulation and its

coordination with cytokinin signalling that is an essential component of nodule organogenesis. ABA acts in a similar manner as JA and ethylene, regulating Nod factor signaling and impacting on the nature of Nod factor induced calcium spiking. We show that genetic inhibition of ABA signaling through the use of a dominant-negative allele of *ABI1* leads to a hypernodulation phenotype. ABA can suppress Nod factor signal transduction in the epidermis and can regulate nodulation-associated cytokinin signaling in the cortex (Ding et al., 2008). Rhizobial inoculation leads to a localised accumulation of ABA as evidenced by the analysis of *RD22* induction, a marker for ABA perception. The enhanced levels of ABA corresponds both spatially and temporally with rhizobial induction of cytokinin signalling. We propose that the cross talk between Nod factor, ABA and cytokinin is a dynamic process and the interplay between these positive and negative hormonal regulators of nodulation provides a mode for the regulation of nodule formation.

Survival in the land of giants: How do bacterial pathogens make a living in the plant environment?

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Understanding the molecular mechanisms underpinning the ecological success of plant pathogens is critical to develop strategies to control of diseases and to protect crops. Plant pathogenic bacteria are not just interacting with the plant itself, but with a variety of potential predators present in the soil, such as nematodes, insect larvae and amoebae. The aim of this project is to identify the gene systems that contribute to the ecological success of the plant pathogen, *Pseudomonas syringae*, with a specific focus on anti-predation and pathogenicity mechanisms. A Rapid Virulence Annotation (RVA) screening was developed to identify which gene systems allow the pathogen to cope with predators. This assay was developed to identify *P. syringae* cosmids that enhanced *Escherichia coli* survival in the presence of predators. Three cosmid libraries comprising 2000 cosmids were screened against each predator and 400 found that reduced bacterial killing or killed the insect larvae of *Galleria mellonella*. Sample end-sequencing of cosmids was carried out. Bioinformatics analysis was used to identify the genes encoded on each cosmid. We identified genes that could be contributing to anti-predation including T6SS, hemolysin and insecticidal toxins. A comparison of the genes conferring resistance within other *P. syringae* strains showed differences in the organization of these clusters, indicating evolutionary changes. These cosmids will be mutagenized using a transposon and the contribution of each individual gene will be evaluated. Furthermore, gene expression *in vivo* will be identified by IVET (*In vivo expression technology*) screens. These data provide an important understanding of how bacteria cope with biotic pressures and to give insight to the function of the genes and their role in ecological success.

A cellulose synthase-like protein is required for durable disease resistance in barley

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Nonhost resistance of crops plants to pathogens is clearly underexplored, despite the agronomic potential of targeted exploitation of the trait. Here, we describe the results of a transient, high-throughput RNAi-based screening in barley for genes that are required for nonhost resistance to the wheat powdery mildew fungus *Blumeria graminis* f. sp. *tritici* (*Bgt*). Silencing of ten out of 750 tested RNAi targets, most corresponding to pathogen-upregulated genes in the epidermis, resulted in significant breakage of nonhost resistance. One of these RNAi target genes encodes a cellulose synthase-like protein (CSLD), which might be involved in localized cell-wall re-inforcement at sites of attempted fungal penetration. Transient silencing of *CSLD* also induced host hypersusceptibility to the barley powdery mildew *B. graminis* f. sp. *hordei* (*Bgh*). Specificity of silencing was demonstrated by RNAi rescue with a synthetic *CSLD* gene saturated in silent point mutations. Trangenic barley lines that carry an RNAi construct targeting *CSLD* and exhibit strongly reduced *CSLD* transcript levels were found to be hypersusceptible to *Bgh*. Importantly, these plants allowed growth and sporulation of the nonhost fungus *Bgt*. On the other hand, growth and seed set of the transgenic lines was not affected by the RNAi construct, but their epidermal walls were found to be hypersusceptible to degradation by fungal cell-wall degrading enzymes. The results suggest an essential role of epidermal cell walls of barley and their putative re-shaping upon pathogen attack in durable types of disease resistance. Moreover, the data demonstrate the feasibility of high-throughput functional genomics approaches in a *Triticeae* crop for the discovery of genes with an agronomic potential.

Characterisation of gene expression of the arbuscular mycorrhiza-specific blue copper proteins in *Medicago truncatula*

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Medicago truncatula arbuscular mycorrhiza (AM)-specific blue-copper binding proteins (bcps, eg. MtBcp1) have been shown to be up-regulated by arbuscular mycorrhizal colonisation. Their expression seemed to correlate with the number of arbuscules and previous proteomic characterisation of MtBcp1 indicated a potential localisation on the periarbuscular membrane. The expression of MtBcp1 and an other AM-specific blue copper-binding gene in *M. truncatula* A17 plants was monitored during a time-course analysis in 1, 2, 3, 5 and 7-week old plants by real-time quantitative PCR. In parallel, AM colonisation of *M. truncatula* mutants was investigated in Myc^- mutants TRV25 C and D (*dmi3, Mtsym13*), $Myc^{-/+}$ mutants TR25 (*dmi2, Mtsym2*) and TR89 (*dmi2, Mtsym2*), Myc^{++} mutant TRV17 (*sunn, Mtsym12*) and A17 as control. In time-course analysis, colonisation intensity, arbuscule content and expression MtPT4 gene increased continuously during time, similarly to the expression levels of both bcps. Myc^- mutants stopped at the appressoria formation stage showed no expression of the bcp genes. On the other hand, TR25, TR89, A17 and TRV17 showed a gradual increase of bcp expression both in 3 and 7-week old plants, as observed for both colonisation intensity and arbuscule number. By showing strong correlations between AM colonisation and expression of AM-specific blue-copper protein genes, our results suggest a basic role of both bcps in symbiotic functioning and may support the hypothesis that they are linked to the arbuscules. To support this hypothesis, RNAi constructs strategy is underway.

Effector proteins in flax rust, wheat stem rust and *Fusarium oxysporum*

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The study of the interaction between flax and flax rust has elucidated the nature of resistance and avirulence proteins and their interactions during infection. We have also developed a transformation system for flax rust that should be applicable to other biotrophic fungi. We are using these studies as a model for studying less tractable and less genetically defined host-pathogen systems such as the wheat stem rust pathogen *Puccinia graminis tritici* (Pgt). We have prepared and sequenced a cDNA library of gene expressed in haustoria and defined a set of 360 secreted proteins as effector candidates. Bioassay procedures are being developed to screen these candidate effectors for proteins that induce host cell death in a specific stem rust resistance gene dependent manner. Using the tomato-*Fusarium oxysporum* f. sp. *lyocpersici* (Fol) system as model, we are also seeking effector proteins in *Fusarium oxysporum* f. sp. *vasinfectum* (Fov), the cotton wilt pathogen. Using PCR primers and DNA probes we are able to detect only one of 12 known Fol effectors in Fov isolates and this one gene provides a perfect diagnostic marker for Australian Fov. Among a small library of Fov genes expressed during infection we have identified a further two effector candidates in Fov but not Fol. *Fusarium oxysporum* appears to carry a large diversity of effector proteins specific to each lineage of host specific strains (formae speciales) with some effectors present in several but not all formae speciales. This is consistent with inter-strain transfer of effector genes, gene diversification and selection for different effector compliments during pathogen adaption to host plant species.

Plant metacaspases antagonistacally regulate *lsd1* runaway cell death and are involved in disease resistance

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Caspase proteases initiate and execute apoptosis, or programmed cell death, in animals. In plants, caspases per se have not been identified, but so-called

metacaspases (Uren et al., 2000) and caspase-like activities have been described (Lam and del Pozo, 2000). The Arabidopsis genome contains nine genes encoding metacaspases, which can be classified into two groups: type I metacaspases (*AtMC1*, *AtMC2* and *AtMC3*) contain an *LSD1*-like zinc finger prodomain, whereas type II metacaspases (*AtMC4-9*) lack any prodomain. Metacaspases are thought to be positive regulators of cell death, while the zinc finger protein *LSD1* has been shown to negatively regulate cell death and disease resistance (Dietrich et al., 1997; Kaminaka et al., 2006). *LSD1* loss of function mutant plants are characterized by a form of inappropriately triggered cell death designated runaway cell death (rcd). We will present data showing that *AtMC1* and *AtMC2* antagonistically regulate *lsd1* rcd. Moreover, *AtMC1* and *AtMC2* are not only induced during the hypersensitive response, but *atmc1* and *atmc2* mutants show slightly increased disease susceptibility. Additionally, conditional over-expression of *AtMC1* leads to cell death, which is dependent on a caspase-like activity. Thus *AtMC1* and *AtMC2* are likely regulating programmed cell death both in response to pathogens and in the absence of LSD1. Preliminary, indirect data indicates that *AtMC1* and *AtMC2* (and possibly other metacaspases) control cell death by regulating each other.

Building up plant defence by cell wall sensing

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Plant cell walls are at the forefront of plant- microorganism interactions. It has long been known that they play an important role in the establishment and outcome of diseases and symbioses. Along time, they have been first perceived as a passive mechanical barrier that should be crossed to allow pathogen penetration and colonization, then as a dynamic structure endowed with signaling and defence potential, and more recently as a sensing cell surface layer. There is now compelling evidence that changes of the cell wall status, either by mechanical stimuli or by polymer alteration, lead to plant defence against various pathogens. Cellulose is one such polymer. *Phytophthora parasitica nicotianae*, an oomycetal pathogen of tobacco, and more generally *Phytophthora* species, produce a glycoprotein called CBEL that contains two Cellulose Binding Domains (CBDs) and is devoid of enzymatic activity. CBEL is located at the inner and outermost layers of *Phytophthora* cell walls, and allows adhesion to cellulosic substrates and to plant cell walls. It acts as a general elicitor, activating the basal immune system of various plants, notably *Arabidopsis thaliana*, through the SA, JA, and ET pathways. Within CBEL, the CBDs are essential and sufficient to trigger defence responses, hence the notion that CBDs are novel PAMPs. Indeed, defence genes were induced upon infiltration of plant leaves with a synthetic CBD. The mechanisms underlying the perception of CBEL and of the CBDs are under study. Since protoplasts do not present the rapid calcium influx triggered by intact CBEL on plant cells, it is proposed that CBEL and CBDs interfere with a cell wall integrity pathway that remains to be elucidated.

Modulation of quorum-sensing signal by lactonases in *Agrobacterium tumefaciens*

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A. tumefaciens can infect a broad range of dicotyledonous plants and transfer an oncogenic DNA fragment, the T-DNA, from its tumour-inducing (Ti) plasmid to the nuclear genome of the plant hosts. In transformed plant tissues, expression of T-DNA genes leads to uncontrolled synthesis of growth regulators, auxin and cytokinins, resulting in formation of a tumour, a phenomenon known as crown gall disease. T-DNA also encodes for synthesis of opines which are specific growth substrates and signals used by the virulent Agrobacteria. Some opines, called conjugative opines, are required for synthesis of quorum-sensing (QS) signal, 3-oxo-octanoyl-homoserine lactone (OC8-HSL). QS-pathway controls amplification of copy-number and conjugative transfer of Ti plasmid. However, the phytopathogen *A. tumefaciens* C58 expresses two lactonases AttM and AiiB which can cleave ring of the gamma-butyrolactones including OC8-HSL; the lactonases AiiB and AttM may potentially affect QS regulated functions. In this work, the regulation and implication of lactonases in QS were evaluated and compared. Lactonase AttM is encoded by At plasmid and its expression is stimulated in the presence of GABA and GABA-by products. In contrast, lactonase AiiB is encoded by Ti plasmid and its expression is stimulated in the presence of agrocinopines. A constructed *aiiB* mutant accumulated OC8HSL at a level ten-fold higher than that of the wild type strain, and showed an exacerbated expression of a key QS-regulated function, conjugation of Ti plasmid, as well as an increase of the number of emerging tumours on host plant.

Altruistic suicide in a bacterial plant pathogen: Dual functionality of a phage abortive infection (Abi) system with promiscuous host Toxin-Antitoxin (TA) capacity in *Erwinia*

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A plasmid in a strain of *Erwinia carotovora*, subspecies *atroseptica* (Eca) (*Pectobacterium atrosepticum*) carried a gene encoding a protein similar to AbiQ from Gram-positive lactococci. In lactococci, this protein aborts viral infection. Abortive infection (Abi) systems operate by blocking morphogenesis of infecting bacteriophages by a process akin to a programmed cell death. This traps the invading virus in a cell undergoing prokaryotic apoptosis, blocking virus propagation. Thus the process is like an altruistic suicide committed by the infected bacterium to limit infection of clonal siblings - and save the bacterial population. We named the Eca version of the AbiQ protein ToxN when we showed that this system acts as a toxin in *Erwinia*. It is also active in other bacterial genera when transferred horizontally. ToxN is suppressed in Eca via a repetitive RNA product of a gene (*toxI*) 5′ of *toxN*, transcribed in a bicistronic operon – the first example of this new type of protein:RNA,Toxin-Antitoxin (TA) system. The ToxIN system is very active and aborts the infection of multiple *Erwinia* phages (and phages infecting other genera). Using structure-function analysis with spontaneous and site-directed mutagenesis, we have investigated the toxicity of ToxN and the role of ToxI RNA in suppression of ToxN activity. We are studying how viral infection drives the bacterial host into a precocious cell death by interacting, directly or indirectly, with the plasmid-encoded ToxIN system to activate the toxicity. Such information may be exploitable in control of *Erwinia* and could enable development of novel therapeutics inducing programmed cell death in this plant pathogen and in diverse bacterial pathogens of plants and animals.

Virulence and avirulence of the extracellular pathogens *Cladosporium fulvum* and *Verticillium dahliae* on Arabidopsis and tomato

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In our research we exploit tomato and Arabidopsis as hosts for the fungal pathogens *Cladosporium fulvum* and *Verticillium dahliae*. Both species grow strictly extracellular without the formation of feeding structures and are contained by so-called receptor-like proteins, of which the type members are Cf-resistance proteins, in incompatible interactions with tomato. We have recently demonstrated that *Ve1*, but not *Ve2* acts as a functional *Verticillium* resistance gene in tomato, and started to unravel the genetics of *Ve*-mediated resistance signaling. Downstream signaling components required by Ve1 for *Verticillium* resistance only partially overlap with those required for Cf-mediated resistance against *C. fulvum*. Furthermore, we have recently established the first model for RLP-signaling in Arabidopsis, which allows studying the genetics of RLP-mediated disease signaling in this host. Our latest results will be discussed. Our analysis of host-pathogen interactions with the extracellular pathogens *C. fulvum* and *V. dahliae* also concerns the effectors that are secreted by these pathogens while colonizing their hosts. Intriguingly, we recently identified an effector from *C. fulvum*, designated Ecp6, that is conserved between these two phylogenetically unrelated species and which acts as a genuine virulence factor. The role of Ecp6 during host colonization will be discussed.

AT-rich isochores as ecological niches for effectors in the genome of *Leptosphaeria maculans*

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The genome of the ascomycete *Leptosphaeria maculans* shows the unusual characteristics to be organized in isochores. GC-equilibrated isochores

(average 52% GC) are gene-rich whereas AT-rich isochores (40–43% GC) are mostly devoid of active sequences and are made up of mosaics of intermingled and degenerated repeated elements. The three avirulence (*AvrLm*) genes identified so far in this species are isolated in the middle of large AT-rich isochores. Our postulate thus was that AT-rich isochores were specific "ecological niches" for avirulence genes and effectors in *L. maculans*. This was firstly validated by analysis of three genes lying in the same genome environment (*LmCys* genes) and showing the same characteristics as *AvrLm* genes (low GC content, strong overexpression at the onset of plant infection, encoding for small secreted proteins -SSP- often rich in cysteines). Of these, one, *LmCys2*, was shown to act as an effector, probably contributing to suppression of plant defense. A systematic search for SPP as effector candidates was performed using bioinformatics. 455 AT-rich isochores were extracted from the genome data and their repeat content masked using the *L. maculans* repeat database. Non-repeated regions were then investigated with a pipe-line dedicated to the identification of SSP. This provided us with three datasets: 529 SSP-encoding genes in GC-equilibrated isochores, 498 non-SSP- and 122 SSP-encoding genes in AT-rich isochores. Part of this latter set of genes was analyzed for their occurrence in natural populations and expression data in culture and in planta. Finally, the 122 AT-SSP putative proteins showed structural features reminiscent of the *AvrLm* and *LmCys* genes. Possible diversification mechanisms as a function of genome location will be discussed.

The involvement of quorum sensing in biofilm formation in *Sinorhizobium meliloti*

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Sinorhizobium meliloti is a Gram-negative soil bacterium that fixes nitrogen during its symbiotic association with its host legume, *Medicago*, *Melilotus*, or *Trigonella*. Establishment of a successful symbiosis requires the coordinated action of both partners. Bacterial cells coordinate their behavior through cell-density dependent gene regulation or quorum sensing (QS). In *S. meliloti*, SinI produces a series of long-chain *N*-acyl-homoserine lactones (AHL) that act as membrane-permeable signal molecules. At high cell densities, AHLs accumulate in the cell, where they activate LuxR-type transcriptional activators. *S. meliloti* has two LuxR-type transcriptional activators, SinR and ExpR. Previous work has shown that the Sin/ExpR quorum sensing system regulates several processes that are critical for successful infection including motility, chemotaxis, and the production of EPSII and succinoglycan. Here, we assess the role of the Sin/ExpR system in biofilm formation. QS has been implicated in many other bacteria in various aspects of biofilm development including structure, maintenance, stress resistance, and regulated cell removal. We find that mutating *expR* and either of the *sin* genes results in delayed biofilm formation and altered biofilm architecture within 24 hours. A four-fold reduction in biofilms produced by the *sin* mutants relative to the wild-type occurs. However, at 72 hours, biofilm formation by the *sin* mutants is restored, but the *sin* mutants show a profound difference in biofilm architecture. In wild-type biofilms, cells aggregate into a series of towers. In contrast, cells in sin biofilms aggregate into web-like structures, suggesting that the *sin* mutants make different cell-to-cell attachments than the wild-type.

Proteome analysis of lipid rafts associated with rice innate immunity mediated by OsRac1

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OsRac1, a small GTPase in rice, functions as a key regulator of defense signaling in rice. Plasma membrane is known to contain the microdomains, called "lipid raft", which are enriched in sterols and sphingolipids. Theses domains can be fractionated as detergent–resistant membranes (DRM) by their resistance to nonionic detergent extraction at 4°C. Based on the research of the lipid rafts in animal cells, it is indicated that the association between the small GTPase and lipid raft microdomains was an important role in transmembrane signaling. However, information on the lipid raft is still limited in plant cells. The aim of this study is to analyze the components of lipid raft domains associated with innate immunity of rice. At first, we fractionated DRM proteins from rice cultured cells by using Triton-X 100 and sucrose density-gradient ultracentrifugation. The component proteins in the DRM fraction were identified by Orbi-Trap mass spectrometry. Also, we analyzed the DRM fractions isolated from constitutive active OsRac1 (CA-Rac) and dominant negative-OsRac1 (DN-Rac) expressing rice cells. We investigated whether the raft components among wild type, CA-Rac and DN-Rac cells were different and some proteins were identified. Based on results of this analysis, we show the association of the lipid raft and rice innate immunity.

The role of auxin and PIN proteins in the development of nematode feeding sites

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Intercellular transport of auxin is essential for various aspects of plant growth and development and it was shown that it accumulates during the early stages of nematode infection. In situ expression studies using promoter-/GUS/GFP/ fusions revealed temporal and spatial expression patterns of /PIN1/, /PIN2/, /PIN3/, /PIN4/ and /PIN7/ during the early events of NFS establishment. Additionally we infected single and double /pin/ mutants to analyse their role during the nematode infection process. Our results clearly demonstrate that the plant-parasitic nematode /H. schachtii/ manipulates its host auxin distribution network in order to induce NFS and explain for the first time the mechanism behind the dynamic auxin gradients during NFS development.

The role of quorum sensing in the *Sinorhizobium meliloti*-alfalfa symbiosis

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The ExpR/Sin quorum-sensing system of the gram-negative soil bacterium *Sinorhizobium meliloti* plays an important role in the establishment of symbiosis with its host plant *Medicago sativa.* A mutant unable to produce autoinducer signal molecules (*sinI*) is deficient in its ability to invade the host, but paradoxically, a strain lacking the quorum sensing transcriptional regulator ExpR is as efficient as wild type. We compared the whole-genome expression profile of the wild type strain with strains missing one of the quorum sensing regulatory components to identify genes controlled by the ExpR/Sin system throughout the different stages of the free-living bacterial life cycle, as well as *in planta*. Our analysis revealed that ExpR is a highly versatile regulator with a unique ability to show different regulatory capabilities in the presence or absence of autoinducer. In addition, this study provided us with an explanation for the plant invasion defect displayed by the autoinducer mutant. We also discovered that the ExpR/Sin quorum-sensing system is repressed after host invasion. Therefore, quorum sensing plays a crucial role in the regulation of many cell functions that ensure the successful invasion of the host and is inactivated once symbiosis is established.

Natural and induced defense responses against aphids

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The hydroxamic acids (HAs) are board acting defence compounds biosynthesised in wheat. The most biocidal, DIMBOA, is stored in the vacuole as the inactive glucoside and is released upon pathogen attack. We are examining whether the relationship between HA production and resistance can be exploited, either through breeding or genetic engineering, to generate wheat varieties displaying environmentally sustainable resistance. We have studied *BX* (HA biosynthetic) gene expression, and HA accumulation in a range of wheat lines. Gene expression is highest in the coleoptile and root, but levels of DIMBOA (predominantly glucoside) are similar in the root and leaf. Transport or release of the compound from the roots may explain this. There is limited genetic variation of HAs in hexaploid wheats but a diploid B genome wheat produces high and sustained levels in the leaf. Tetraploid wheats contain higher levels than hexaploids and diploid A genome wheats contain no HAs in the foliar tissue. Cereal aphids do not affect *BX* gene expression but cause a rapid localised increase in plant glucosidase gene expression accompanied by conversion of glucoside to free DIMBOA, thereby inducing the release of stored, inactive compound but not *de novo* biosynthesis. Preliminary screening indicates that there is genetic variation in the magnitude and kinetics of this response. DIMDOA levels are regulated in the plant leaf to prevent auto toxicity and attempts to increase DIMBOA concentration by infiltrating the compound to cut leaves results in its immediate glucosylation. Therefore a means to enhance aphid resistance by exploitation of the HA pathway may involve both increase of the storage pool of glucoside and an increase in the inducibility of the glucosidase in response to aphid feeding.

Isoflavonoids participate in hypersensitive cell death during race-specific soybean-Phytophthora interactions

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The isoflavonoid biosynthetic pathway is well defined in soybean. Certain aspects of its coordination and deployment during defense responses and for priming defense potential have been well elucidated in the context of the glucan elicitor (a PAMP or MAMP) from the pathogen *Phytophthora sojae*. Like most secondary metabolites, soybean isoflavonoids have been thought to contribute to general or basal resistance, more in line with a disease lesion limiting function. However, in infection studies following silencing of some biosynthetic genes we unexpectedly found that isoflavonoids were involved in hypersensitive, and perhaps other types of cell death, as well as race specific resistance. The results of *Agrobacterium rhizogenes* mediated silencing of isoflavone synthase, chalcone reductase and the elicitor-releasing β-glucanase genes will be discussed in terms of possible signaling pathways during race-specific soybean-Phytophthora interactions. The molecular implications for co-evolution of Phytophthora-soybean race-cultivar structure will also be addressed.

Stomata modulation by *Xanthomonas campestris* pv. *campestris*

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Xanthomonas campestris pv. *campestris* (*Xcc*) is a bacterial intravascular phytopathogen that is the causal agent of the black rot of crucifers. As with many phytopathogenic bacteria, *Xcc* produces a range of factors that contribute to the ability of the bacterium to parasitize the host. The production of extracellular enzymes and EPS is subject to co-ordinate positive regulation by a cluster of genes, the *rpf* cluster (for regulation of pathogenicity factors). Mutations in *rpf* genes lead to a reduced virulence in host plants. Several of the *rpf* genes, mediate regulation via a small diffusible molecule named DSF (for diffusible signal factor). In this work we attempted to clarify the mechanism of endophytic colonization through stomata of the phytopathogenic bacterium *Xcc*. We found that movement through stomata of *Xcc* depends on a secreted factor which can prevent both ABA and bacterial induced stomatal closure, and whose synthesis is regulated by the *rpf*/DSF system. To evaluate the physiological relevance for infection of the stomata modulating activity of *Xcc*, we performed both *in vitro* and *in vivo* assays. *In vitro*, unlike wt *Xcc*, *rpfF* and *rpfC* mutants have a reduced migration through stomata in isolated Arabidopsis epidermis, as expected from their compromised ability to modulate stomatal apertures. *In vivo*, we found that an *Xcc* extract can restore the virulence of Pst cor- strain on Arabidopsis plants inoculated by dipping, suggesting that the ability of *Xcc* to modulate stomatal activity is relevant for bacterial infection. In addition, we found that Arabidopsis MPK3 is required for inducing promotion of stomatal closure by bacteria, but not by ABA. The presence of this kinase is also required for the inhibition of ABA induced stomatal closure by *Xcc*.

Cellular and transcriptional reprogramming of host plants in the presence of arbuscular mycorrhizal fungi

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Arbuscular mycorrhizal (AM) fungi contribute significantly to soil nutrient uptake in plants, but as obligate biotrophs, they depend on their hosts for carbohydrates. Here, we describe how important cellular and transcriptional reprogramming takes place in host cells in the presence of AM fungi during all the steps of the interaction. Plant cell cultures and roots perceive diffusible fungal signals which elicit short and long term responses, as well as transcriptional changes, detected with a Lotus japonicus Affimetrix GeneChip. The physical contact between the AM fungus and the root surface causes a deep reorganization inside the epidermal cells of Medicago truncatula prior to fungal colonization: in vivo confocal imaging studies have shown the formation of cytoplasmic assemblies consisting of cytoskeleton, endoplasmic reticulum, organelles and membrane vesicles, named the prepenetration apparatus. Such events are mirrored by expression changes in some genes, among which an expansin-related gene. A wide transcriptional reprogramming was observed when the fungus colonizes the L. japonicus cortex, and differentiates the arbuscules, which are the key elements for the symbiotic nutrient exchange. Here, substantial changes in transcripts involved in regulatory networks, transport, cell wall and membrane biogenesis were detected and located in the arbusculated cells, mirroring parallel confocal microscopy and ultrastructural observations in Medicago and carrot roots. Based on our results, we propose that plant cells respond differently to diffusible, mobile signals from AM fungi and to their physical contact, in both cases exerting a direct control on fungal colonization.

Secondary metabolites: Pivotal players in defense, pathogenesis and signaling

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Secondary metabolites play important roles in host-pathogen interactions. For example, antimicrobial secondary metabolites representing diverse chemical families function as phytoalexins and phytoanticipins in plant defense. Furthermore, it is well established that toxic and plant growth altering secondary metabolites from fungal and bacterial pathogens play important roles in disease development. Finally, several plant secondary metabolites are signal molecules regulating host defense. An overview of these roles will be presented as an introduction to this session. In addition, specific examples of how secondary metabolites might function in resistance and induced resistance will be discussed using terpenoid and phenolic compound accumulation of potato and isoflavonoids in soybean as examples. Structural polymers, such as pathogen-induced lignification, are also associated with host defense, and the role of these cell wall alterations will be discussed using results from resistance responses of potato tuber tissue and cucumber seedlings to fungal pathogen.

Enhanced nitrogen use efficiency in wheat using *Trichoderma* seed inoculants

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Trichoderma strains have been known as biocontrol agents as plant disease and also for their abilities to increase plant growth and yield. Many strains can be added as bioinoculant seed treatments and induce beneficial effects for the life of at least an annual crop due to their abilities to become long-lasting plant symbiotic endophytes in roots. Among the beneficial effects noted are increased root development. One important need in plant agriculture is to improve nitrogen fertilizer use efficiency (NUE) especially in monocotyledonous crops. Nitrates and nitrates are important water pollutants, and certain nitrous oxides are important greenhouse gases and both may be derived from plant fertilizers that are not taken up by plants. We demonstrated some years ago that seed treatments with *T. harzianum* T22 could enhance NUE in maize, but there could be significant negative interactions of T22 with certain maize phenotypes. This negative interaction does not appear to occur with wheat. We have now discovered new strains that are highly efficient in enhancement of NUE on wheat; at low N fertilizer levels, the amount of N taken up wheat in the presence of the new strains may be 2x that which occurs without *Trichoderma* seed treatments. Growth of wheat is highly correlated with N levels in our trials. Since there are about 3 billion pounds of N applied to wheat in the USA alone, this finding provides a major opportunity to reduce N fertilizer and to increase yields of wheat. This will enhance farm income and reduce nitrogen fertilizer pollution in the environment using an inexpensive fungal seed inoculant.

Role of secondary metabolites in the *Trichoderma*-host interaction

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Species belonging to the genus *Trichoderma* are mycoparasites used as biological control agents of a wide range of aerial and soil-borne plant pathogens. Mycoparasitism by *Trichoderma* is a complex process, including the formation of specialized structures, secretion of a complex set of cell wall degrading enzymes, and antibiotics. In addition, it has been shown that *Trichoderma* penetrates plant roots, stimulates plant growth, and induces defense responses. It has, therefore, been considered a plant symbiont. Several molecules produced by *Trichoderma* have been proposed to induce such plant responses, including proteins and secondary metabolites. To gain further insight into mycoparasitism and the *Trichoderma*-plant interaction we have

generated mutants that are unable to produce peptaibols and polyketides, by deleting the gene *cfwA*, encoding a phosphopantetheinyl transferase. We have demonstrated that the lack of these two types of secondary metabolites dramatically affects the capacity of *Trichoderma* to antagonize a variety of phytopathogenic fungi. However, some of its host fungi are still antagonized by the Δ*cfwA* mutants. Furthermore, deletion of this gene significantly affects the capacity of the fungus to stimulate plant growth. Finally, we have used *Arabidopsis thaliana* as a plant model to study the effect of the mutation in *cfwA* in the *Trichoderma*-Plant interaction, and activation of gene expression in the plant in response to *Trichoderma*.

Identification and characterization of novel natural alleles of *eIF4E* in barley providing broad-spectrum resistance to *Bymoviruses*

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In breeding for resistance to *Bymoviruses* either *rym4* or *rym5* allelic recessive resistance genes are commonly used. Each of these genes provides resistance only to a subset of virus isolates. Recently we demonstrated that *rym4* and *rym5* encode amino-acid changes bearing variants of the eukaryotic translation initiation factor 4E (eIF4E). Plant eIF4E appears to interact with the VPg protein of poty- and bymoviruses, and this interaction is required for virus multiplication. Mutations in eIF4E are thought to result in inability of this protein to bind viral VPg and, ultimately, in disease resistance. In this study we investigated the global variation in *eIF4E* available in the barley germplasm using a high-resolution melting for PCR amplicons derived from cDNA as a low-cost assay combined with Sanger sequencing of selected amplicons. This study identified > 40 novel *eIF4E* alleles in a diversity collection comprising > 1100 barley accessions. The majority of these alleles contain non-synonymous point mutations, which result in amino-acid changes in the encoded protein. Some of the novel alleles contain a small deletion or insertions. 3D-protein modeling revealed that most of these mutations were located in a specific region near to the cap-binding pocket of eIF4E suggesting a potential role of this region in binding of viral VPg. Importantly, most of the newly identified *eIF4E* alleles direct resistance to at least one and some for all European bymovirus isolates. Interestingly, the highest diversity of *eIF4E* alleles appears to occur in geographic regions with a long history of yellow mosaic disease, suggesting a possible co-evolution race between bymoviruses and their primary host barley.

New insights into mechanisms of effector function and regulation in phytoplasmas

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Phytoplasmas are insect-transmitted plant pathogenic bacteria. They replicate in the plant phloem and throughout their insect vectors. Phytoplasmas are dependent on plants and insects for dispersal, and can influence their hosts in various ways. Aster Yellows phytoplasma strain Witches' Broom (AY-WB) induces clustering of small succulent leaves, leaf serration and leafy flowers, and reduces resistance to insect vectors in Arabidopsis. Hence, we use the AY-WB-Arabidopsis interaction to study how phytoplasmas manipulate plants and plant-insect interactions. AY-WB infection changes transcript levels of many Arabidopsis developmental and defense response genes. However, only five genes are upregulated early in AY-WB infection; these include genes for transcription factors involved in plant development. To determine how AY-WB interferes with plant development, we mined the AY-WB genome for genes encoding secreted proteins (SAPs) that are candidate effectors. Indeed, several SAPs induce phenotypic changes in Arabidopsis. One of these, SAP11, induces severe leaf serration and shoot proliferation, and the induction of these phenotypes correlates with nuclear localization of SAP11. We also found that SAPs, including SAP11, are frequently encoded on regions named potential mobile units (PMUs), which resemble integrative and conjugative elements (ICEs). The SAP11 PMU encoding several SAPs is predominantly expressed in plants. Another ± 20 kb PMU encoding SAPs exists as linear and circular forms. The latter is predominant in insects leading to higher expression of PMU genes in insects relatively to plants. In summary, AY-WB effectors interfere with plant development and many effectors are encoded on PMUs that regulate AY-WB gene expression during plant and insect infection.

Sfp-type 4'-phosphopantetheinyl transferase is indispensable for athogenicity of the corn pathogen *Colletotrichum graminicola*

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In filamentous fungi Sfp-type 4'-phosphopantetheinyl transferases (PPTases) activate different enzymes involved in primary (α-aminoadipate reductase, AAR) and secondary metabolism (polyketide synthases, PKSs, and nonribosomal peptide synthetases, NRPSs). Fungal genomes harbor several PKS and NRPS genes, and members of both gene classes are essential for successful infection in plant pathogenic Ascomycota. Therefore PPTases may not only be required for vegetative development, but also represent central regulators of fungal pathogenicity. We cloned the PPTase gene CgPPT1 of the maize anthracnose fungus Colletotrichum graminicola by complementation of the Saccharomyces cerevisiae Δlys5 mutant, and generated PPTase-deficient mutants (ΔCgppt1) by targeted gene disruption. In vitro ΔCgppt1 strains were auxotrophic for lysine, unable to synthesize toxic secondary metabolites and siderophores, did not grow under iron-limiting conditions and were hypersensitive to reactive oxygen species. Infection-related morphogenesis was drastically affected in ΔCgppt1 strains. Rarely formed appressoria of ΔCgppt1 strains were non-melanized, ruptured on intact plant surfaces and were thus unable to invade the host leaf tissue. When inoculated onto wounded leaves, hyphae of ΔCgppt1 strains colonized maize leaves at rates comparable to those of the wild type (WT) strain but failed to generate necrotic anthracnose disease symptoms. Although initials of acervuli were found, ΔCgppt1 strains were defective in asexual sporulation. Thus, CgPPT1 is indispensable for fungal pathogenicity.

Reduction of disease symptoms caused by fungal pathogens in transgenic wheat plants expressing the polygalacturonase-inhibiting protein 2 (PvPGIP2)

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The plant cell wall represents the main barrier to the colonization of host plant tissue. To overcome this obstacle, most fungal pathogens produce a variety of enzymes that degrade the wall polysaccharides. Endo-polygalacturonase (PG) is secreted during the infection process and the relevance of its activity has been demonstrated in several plant-pathogen interactions, including the cereal pathogens *Claviceps purpurea* and *Fusarium graminearum*. PG activity is controlled by the protein inhibitor PGIP (PG-inhibiting protein). The effectiveness of PGIP in limiting cereal tissue colonization by fungal pathogens has been recently shown in transgenic wheat plants expressing the bean PvPGIP2. These plants showed a significant reduction of symptoms following the infection of *Bipolaris sorokiniana* or *F. graminearum* suggesting that pectin hydrolysis is an important step for fungal penetration of cereal species in spite of a low pectin content in their cell wall. In order to verify better the contribution of PGIP in wheat defence, we have analyzed in more detail the response of wheat transgenic lines expressing PvPGIP2 against *F. graminearum* and we have extended the analysis to different pathogens, including *Claviceps purpurea* and *Puccinia recondita*.

Priming in systemic plant immunity

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Whole plant immunity to diverse pathogens often develops after localized infections. This response is called systemic acquired resistance (SAR). After a primary infection, distal leaves show enhanced accumulation of the defense signal salicylic acid (SA) and SA-dependent responses upon secondary infection. Although SAR requires intact SA signaling, SA is not a long distance mobile signal for establishing SAR. To identify candidate mobile signal molecules for SAR, we used GC-MS analysis to scan for metabolites enriched in vascular sap from Arabidopsis that were induced for SAR versus mock-treated. Azelaic acid ($C_9H_{16}O_2$) accumulated to high levels in vascular sap from SAR-induced leaves, was mobile in plants and conferred local and systemic resistance in a manner dependent on SA signaling. Azelaic acid did not directly induce SA accumulation, but instead primed plants to highly

accumulate SA upon infection. Mutation of the *AZELAIC ACID INDUCED 1* (*AZI1*) gene, which is induced by azelaic acid, resulted in the specific loss of systemic immunity triggered by pathogen or azelaic acid and of the priming of SA induction in plants. Furthermore, the predicted secreted protein AZI1 is also important for generating vascular sap that confers disease resistance. Thus, azelaic acid and AZI1 are SAR components involved in priming defenses.

Structural and functional analysis of the SGT1-RAR1-HSP90 complex required for plant immunity

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SGT1, a cochaperone of HSP90, is required for innate immunity in plants and animals. Understanding the relationship between SGT1 and other cochaperones such as P23, AHA1, or RAR1 is a major step toward the understanding of the HSP90 machinery in immunity processes. NMR spectroscopy and X-ray crystallography combined with mutational analyses of the core Hsp90-Sgt1 complex revealed the nature of its binding with the CS domain of SGT1. Although CS is structurally similar to P23, these domains were found to noncompetitively bind to different regions of HSP90. Yet, unexpectedly, full-length SGT1 could displace P23 from HSP90. Furthermore, acrystal structure of the core HSP90-SGT1-RAR1 complex reveals a novel conformation of HSP90 with the two cochaperones as a hetero hexamer. A unique nature of the hetero hexamer complex will be discussed.

Functional analysis of *Lotus japonicus LjCSLD1* and *LjRHL1 loci* required for root hair development

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In many legumes, susceptible root hairs are the primary sites for the initial physical contact between the host plant and compatible nitrogen-fixing bacteria. This leads to the initiation of root invasion and nodule organogenesis. To clarify the significance of root hairs during the *Lotus japonicus-Mesorhizobium loti* symbiosis, we have performed detailed analysis of previously characterized symbiotic mutant lines which also showed defects in root hair development. *L. japonicus short (Ljsrh1)*, *variable (Ljvrh1-1)* and *roothairless* (*Ljrhl1-1*) mutant lines were characterized. We show here that *Ljsrh1* and *Ljvrh1-1* carried lesions in the same *Cellulose Synthase-Like D* gene (*LjCSLD1*). LjCSLD1 showed the highest homology to Arabidopsis KOJAK (LjCSLD3) and the AtCSLD2 proteins of unknown function. The results of intra-allelic complementation provided a genetic evidence for homodimerization of the LjCSLD1 proteins, which will be discussed in the context of a predicted biochemical function of LjCSLD1. Furthermore, we will show that lack of root hairs in *Ljrhl1-1* mutant line was caused by a lesion in a basic helix-loop-helix (bHLH) transcription factor. The results of cross-species complementation experiments allowed us to identify three *bHLH* genes from Arabidopsis, which act in at lease partially redundant manner to regulate root hair development.

cDNA microarray analysis of *Tobacco mosaic virus*-infected hot pepper plant: Functional study of protein phosphatases

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The interaction between plants and microbes are complex, and several relationships have been described. Protein kinsases and protein phosphatases are known to regulate the numerous cellular processes by post-translational modification of regulators. Both kinases and phosphatases can be found in bacteria, plants and animals. However, even though protein phosphatases have been studied in various signaling precesses including abscisic acid (ABA), pathogen and stress responses as well as development, they have been largely neglected compared to kinases. In this study, to identify and understand fundamental role of protein phosphatases involved in hot pepper and TMV interaction, cDNA microarray was exploited. We identified specifically increased transcripts of serine/threonine phosphatases and tyrosine-specific phosphatases in *Tobacco mosaic virus* (TMV)-inoculated TMV-resistant hot pepper (*Capsium annuum* L. cv. Bugang) plant compared with control plant. To examine the function of protein phosphatases screened from microarray data, we carried out expression analysis by RT-PCR and RNA blot and examined knock-down phenotype using virus-induced gene silencing system (VIGS). In addition, microarray data analysis of protein phosphatases in hot pepper mitogen-activated protein kinase 1(*CaMK1*) knock-down plant was also carried out. Putative data analysis suggests that several phosphatases are involved in TMV-induced defense signaling cascade in hot pepper plant.

Regulation of universal stress proteins by quorum sensing in *Burkholderia glumae*

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Burkholderia glumae has a TofI/TofR quorum sensing system that enables cell to cell communication using of *N*-octanoyl homoserine lactone (C8-HSL) as a signal. TofI of *B. glumae* is the autoinducer synthase, which produces the C8-HSL, and TofR is the cytoplasmic autoinducer receptor. TofR/C8-HSL regulates toxoflavin production and expression of *qsmR*, which is an IclR type transcriptional regulator. We used the two-dimensional gel electrophoresis and mass spectrometry techniques to identify proteins of *B. glumae* important for pathogenicity and quormone responses. Proteome analysis revealed that C8-HSL produced by *B. glumae* significantly induced or reduced the level of different proteins. Among those, universal stress protein (UspA) was of interest. UspA superfamily encompasses a conserved group of proteins that are found in bacteria, archaea and eukaryotes. The *uspA* in *E. coli* is one such general responder to growth arrest and its protein product becomes one of the most abundant proteins in stationary phase cells. *B. glumae* has eleven *uspA* genes which are similar to *uspA* in *E. coli*. We demonstrate that expression of *uspA1* and *uspA2* of *B. glumae* is regulated by QsmR. In contrast to *E. coli uspA* mutants, the *uspA* mutants of *B. glumae* exhibited no increased sensitive to UV light, H_2O_2, osmotic shock, and ethanol. This is the first description that the *uspA* gene expression is regulated by quorum sensing.

Dissecting plant defense signaling by chemical and molecular genetics

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Chemical genetics utilizes small molecules to specifically perturb defined regulatory mechanisms *in vivo* allowing for real-time control of homologous biological processes across species barriers. We use this approach to discover synthetic elicitors targeting components of the plant defense web. Combined with methods of molecular genetics, chemical genetics provides us with novel tools to study the behavior and architecture of the defense network and to discover new components of this regulatory system. We are performing high-throughput screens of diverse chemical libraries for synthetic elicitors that activate in Arabidopsis reporter genes containing promoters from the defense-associated gene clusters *LURP* (late up-regulated in response to Peronospora) and *JEDI* (Jasmonic Acid/Ethylene dependently induced). We also identified multiple pathogen-responsive Arabidopsis enhancer trap lines that are used in elicitor screens. One of the new synthetic elicitors we identified, DCA, triggers defense reactions dependently and independently of the central defense regulator NPR1. Members of the DCA-responsive *ACID* (associated with chemically induced defense) cluster are being examined for their roles in defense. Our goal is to establish a suite of synthetic elicitors with a variety of distinct target specificities. A comprehensive set of synthetic and natural defense elicitors combined with inhibitors and genetic mutants will allow for a detailed dissection of the plant defense network and is likely to serve as a powerful tool for the emerging field of systems biology. Blends of synthetic elicitors identified by our study may also facilitate the design of new pesticides providing protection to plants by stimulating their inherent defense mechanisms.

Inhibitors of microbial serine proteinases in cycads and other gymnosperms
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Proteinase inhibitors (PI) play an important role in the molecular interaction and co-evolution of plants with phytophagous organisms. Serine PIs have been well studied in angiosperms but until recently not identified in gymnosperms. Among the latter, the Cycadales are of particular interest since they represent the most primitive living seed plants and are sometimes considered a "missing link" between vascular non-seed plants and the more advanced seed plants. Seeds of representatives of two of the four major groups of gymnosperms, the Cycadales and the economically important Coniferales, were analysed for inhibitors of the serine proteinases trypsin (T), chymotrypsin (C), subtilisin (S) and elastase using isoelectric focusing combined with gelatin replicas. Inhibitors of S (a typical enzyme of fungi and bacteria) were identified in members of both orders, being particularly active in the Cycadales. In two Cycas species these inhibitors were also active against T and C, proteinases typical of both fungi and animals. Partial sequencing of an isoform from *C. siamensis* seeds (Cs-CSTI) showed similarity to Kunitz-type inhibitors from angiosperms. Analysis of expressed sequence tag (EST) databases confirmed the presence of mRNAs encoding Kunitz-type inhibitors in the Cycadales and Coniferales and also demonstrated their presence in a third major group of gymnosperms, the Ginkgoales. Analysis of EST databases also showed that inhibitors related to the potato inhibitor 1 family, another group of inhibitors of microbial S-like proteinases present in flowering plants, are also present in all four groups of gymnosperms. The results show that gymnosperms and angiosperms contain two similar types of serine PIs which may provide protection against microbial pathogens or limit the activity of symbiotic microorganisms.

Innate immunity in Rhizobium legume symbiosis
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Symbiosis between legume plants and *Rhizobium* soil bacteria results in the formation of nitrogen fixing root nodules. Nodule development is induced by bacterial signal molecules, the Nod factors. However, further signalling events between the host plant and its bacterial partner are needed to make the nodule functional. In *M. truncatula* and closely related species (belonging to the IRL Clade), bacteroids undergo a remarkable differentiation process involving cell enlargement, increased membrane permeability, inability for cell division while they keep an active metabolism (Mergaert et al. 2006). We have shown that plant factors control bacteroid differentiation. We have identified a wide spectrum of nodules specific antimicrobial peptides (nsAMPs) that act in the nodules of *M. truncatula* and closely related species to govern terminal differentiation of bacteroids. These legume plants exploited the potential of innate immunity and evolved an unprecedented diversity of nsAMPs to control bacterial cell number and cell fate in symbiosis.

Physical characteristics of calcium oxalate crystals as determinants in structural defense against chewing insects in *Medicago truncatula*
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The description of calcium-oxalate-defective (*cod*) mutants in the barrel medic, *Medicago truncatula* Gaertn., has provided a valuable tool to examine roles of mineral crystals in defending plants against chewing insects. When compared to wild type *M. truncatula*, leaves of mutant lines containing reduced levels of calcium oxalate are preferred by larvae of the lepidopteran *Spodoptera exigua* Hübner. When provided as the sole diet, mutant leaves lead to lower larval mortality, greater larval and pupal size, and more efficient conversion of leaf material to insect body mass. Commercial preparations of monohydrate calcium oxalate are chemically similar to crystals found in legumes, however at < 2 mm diameter, they are considerably smaller than the 8–10 mm crystals found surrounding *M. truncatula* secondary veins. When added to insect artificial diet at relative levels up to 7.5-fold higher than in leaves, commercial preparations of small crystals have no observed negative impact on insect performance. Unlike the crystals found in leaves, which act as abrasives on insect mandibles, the smaller crystals have no visible effects on *S. exigua* mandibles. The addition of commercial calcium oxalate preparations to artificial diet show that the size and/or structure of crystals are the critical determinants in the negative impact that this mineral has on insect performance. Therefore, genes responsible for controlling size and shape of calcium oxalate deposition might be important tools in regulating the level of insect defense that they impart. Transcriptome analysis of constitutive gene expression of wild type *M. truncatula* and two mutant lines, via Affymetrix gene chips, has been useful in identifying a number of genes that are differentially expressed in *cod* mutants.

Activation of the RPP1B-mediated innate immune response
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Oomycetes include some of the most devastating plant pathogens, yet little is known about the molecular mechanisms controlling pathogen virulence and plant disease resistance. In our laboratory we are using an *Arabidopsis thaliana* / *Hyaloperonospora arabidopsis* pathosystem to analyze the molecular basis of plant innate immunity. Specifically, we are studying the interactions between the Arabidopsis resistance protein RPP1B and the cognate pathogen-derived effector ATR1. Since *H. arabidopsis* cannot be grown in culture, we have established a transient expression system to study ATR1 and RPP1B *in planta*. We have shown that co-expression of ATR1 and RPP1B in *Nicotiana tabacum* results in a rapid hypersensitive response. Employing this assay, we have identified domains of RPP1B necessary for the effector-induced cell death. Interestingly, our deletion analyses also revealed regions of RPP1B that were able to activate cell death in the absence of ATR1. We have monitored cellular localization of RPP1B and its truncated variants. Finally, we identified a functional Nuclear Localization Sequence (NLS) in RPP1B and addressed its role in activation of RPP1B-mediated innate immunity.

A novel RNA-binding peptide regulates the establishment of the *Medicago truncatula* - *Sinorhizobium meliloti* nitrogen-fixing symbiosis
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Plants use a variety of small peptides for cell to cell communication during growth and development. Leguminous plants are characterized by their ability to develop nitrogen-fixing nodules via the interaction with symbiotic bacteria. During nodule organogenesis, several so-called nodulin genes are induced, including large families that encode small peptides. Using a triple hybrid approach in yeast cells, we identified two new small nodulins, MtSNARP1 and MtSNARP2 (for Small Nodulin Acidic RNA-binding Protein) which interact with the RNA of *MtENOD40*, an early induced nodulin gene showing conserved RNA secondary structures. The SNARPs are acidic peptides encoded by a small gene family in *Medicago truncatula*. These peptides exhibit two new conserved motifs and a putative signal peptide that redirects a GFP fusion to the endoplasmic reticulum, suggesting that they are secreted. *MtSNARP2* is expressed in the differentiating region of the nodule, showing co-regulation with several early nodulin genes including *MtENOD40*. *MtSNARP2* RNA interference (RNAi) transgenic roots showed aberrant early senescent nodules. Therefore, nodule invasion and persistence may be regulated by secreted RNA-binding peptides.

Destruction of Arabidopsis microtubule networks by the *Pseudomonas syringae* Type III effector HopZ1a
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The eukaryotic cytoskeleton plays a critical role in intracellular transport and in maintaining cellular structure, and is an important target of numerous

animal pathogens. Despite being implicated in multiple facets of plant immunity, no phytopathogenic effector has yet been demonstrated to specifically target the plant cytoskeleton. In an effort to identify type III effectors that can attack conserved host targets, such as the eukaryotic cytoskeleton, we developed a heterologous *in vivo* assay to tandem-affinity-purify (TAP)-tagged *Pseudomonas syringae* type III effectors in human embryonic kidney (HEK293T) cells. HEK293T cells expressing the type III effector HopZ1a displayed altered cell morphology indicating a possible perturbation of the cytoskeleton. Purification of HopZ1a-associated complexes by tandem affinity purification identified tubulin as a potential interacting target. Further analyses demonstrated that HopZ1a specifically interacts with tubulin heterodimers as well as polymerized microtubules *in vitro*. Using a ^{14}C-labelled acetyl-coenzyme A transferase assay we found that HopZ1a is an acetyltransferase activated by eukaryotic tubulin. Activated HopZ1a acetylates itself as well as tubulin and causes a substantial decrease in the microtubule networks of *Arabidopsis thaliana* seedlings. Our results demonstrate that the eukaryotic cytoskeleton is a target of both animal and plant pathogens, and provides a framework for studying the role of the plant microtubule network in phytopathogen virulence and host defence.

Research involved in expanding sales of the phosphate inoculant JumpStart

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A phosphate inoculant containing the P solubilizing fungus *Penicillium bilaiae* was launched in Western Canada in 1990 and was used on a few thousand hectares of wheat. In 2008, the inoculant JumpStart was sold in the three Prairie Provinces and 10 US states and on 10 crops and hundreds of thousands of hectares. This increase in sales resulted from not only marketing efforts, but from continued research on different crops, improved formulations, and compatibility with on-seed fungicides. Further expansion beyond North America will involve the continuous improvement of the product, which, may include more formulation work and improved strain selection.

Molecular dissection of pathogen-inducible flavonoid pathway in sorghum

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Sorghum is well known for its production of 3-deoxyanthocyanidin phytoalexins following fungal inoculation. Availability of EST and genome sequence datasets has facilitated our efforts to characterize the molecular components of the biosynthesis pathway. Biochemical functions of different flavonoid structural genes were established by complementation analysis in Arabidopsis *tt* mutants. Differential expression of chalcone synthase (CHS), flavonoid 3'-hydroxylase, and dihydroflavonol 4-reductase (DFR) gene family members during light-induced anthocyanin and pathogen-induced 3-deoxyanthocyanidin synthesis was demonstrated. In contrast, flavanone 3-hydroxylase gene expression was activated by light only. Recombinant enzyme assays demonstrated that both the light-specific and pathogen-specific DFR proteins accept flavanone and dihydroflavonols as substrates. Other classes of sorghum defense-related metabolites and the structural genes involved were also investigated. *SbSTS1*, initially identified as a pathogen-induced CHS-like EST, was shown to encode a stilbene synthase. Infected sorghum seedlings were then found to accumulate *trans*-piceid as a major stilbene metabolite. However, *trans*-piceid alone may not be a significant defense component against the anthracnose pathogen *Colletotrichum sublineolum*. On the other hand, accumulation of flavone aglycones was detected in infected seedlings and luteolin was found to be highly inhibitory on spore germination of the fungus. Subsequently, we identified a pathogen-inducible sequence which showed weak homology to dicot flavone synthase II (FNSII) sequences. Transgenic expression in Arabidopsis resulted in the accumulation of flavone glycosides, confirming that the sorghum sequence encodes a functional FNSII enzyme.

Diverse interactions between plant viral RNAs and host translation initiation factors reveal many ways to usurp the host's translation machinery

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Viral mRNAs must take control of the host's protein synthesis machinery. Plant mRNAs initiate recruitment of the translational machinery via their 5′ m^{7}GpppG cap structure that is recognized by the cap-binding factor eIF4E. Simultaneously, eIF4E binds eIF4G, a large scaffolding protein that recruits several factors and ultimately the ribosome. Together eIF4E and eIF4G form the heterodimer eIF4F, a rate-limiting factor in translation initiation. More than half of all known plant viruses have uncapped RNA genomes, yet these RNAs translate efficiently. Many of these viral RNAs harbor a cap-independent translation element (CITE) in the 3′ untranslated region (UTR). Here we investigate how some of these CITEs recruit eIF4F, despite lacking a 5′ cap. We found that Barley yellow dwarf virus-like CITEs (BTEs) exist in at least three different viral genera and display remarkable structural variation. The BTE binds and requires eIF4G, but not eIF4E, for efficient translation initiation (Treder et al., RNA 2008). We have identified key domains within eIF4G that are essential for BTE binding and translation. In contrast, the Pea enation mosaic virus RNA 2 CITE (PTE) has an entirely different structure than the BTE. It binds and requires eIF4E in a manner that depends on an intact cap-binding pocket in eIF4E. To our knowledge this is the first eukaryotic RNA known to bind eIF4E with high affinity in the absence of a m7GpppN cap. We also compared the efficiencies of several cap-dependent and cap-independent translation enhancers in their full viral UTR contexts. They vary greatly in their ability to stimulate translation in vivo. In summary, RNA viruses have evolved and exchanged diverse RNA structures that recruit translation factors in different ways to compete with host mRNAs and initiate infection.

Development of effective agents enhancing plant quality and health based on ecological backgrounds and molecular mode of action

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Enhancing growth performance of plants by means of beneficial microbes represents a serious alternative to conventional, chemical-based pesticides and fertilizers. Problem-oriented development based on ecological knowledge and molecular tools resulted in a selection of microbial agents intended to protect plants against phytopathogenic fungi and/or to promote plant growth and quality. On the one hand, pathogen-specific antagonists were selected and promoted which effectively colonize the plant roots and suppress disease development. Consequently, two products named Rhizostar® and Salavida® based on *Serratia plymuthica* HRO-C48 to control *Verticillium dahliae* and *Pseudomonas trivialis* 3Re2-7 to suppress *Rhizoctonia solani*, respectively, were developed for affected crops. Another strategy based on complementary set of biological control agents consisting of *P. trivialis* RE*1-1-14, *P. fluorescens* L13-6-12, *S. plymuthica* 3Re4-18 and *Trichoderma reesei* G1/8 which is applied to sugar beets threatened by *R. solani*. Secondly, in the recent past, special attention has paid to the desertification and salinization of agricultural areas in arid and semiarid regions. We demonstrated the potential of salt tolerant plant growth promoting bacteria (viz. *Stenotrophomonas rhizophila* P69) to significantly improve growth of crop plants stressed by drought and elevated salinity. Besides the quantity of yield, the quality of agricultural goods is of public relevance. We have shown that Methylobacterium strains applied during flowering stage positively influence the flavour of strawberry fruits. To facilitate product development, our research relies upon comprehensive studies about the mode of action and the regulation thereof, interaction with the plant, and the ecological impact.

Poplar-Melampsora rust interaction: MAPK-interacting proteins at work

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With their long life cycle, trees must have accurate mechanisms of sensing microbial invasion and elaborate signalling networks in order to activate the appropriate defense response. With the availability of its whole genome sequence, ease of growth and clonal propagation, and routine transformation, poplar (Populus spp.) is now considered a model tree species for genomics research. Moreover, genomes of a cortege of associated microorganisms are being sequenced including a tree pathogen, Melampsora poplar rust. With these tools in hand, the poplar-rust pathosystem holds great potential for the study of tree-microbe interaction at the molecular level. We pursued various approaches to identify poplar genes involved in the interaction with the biotrophic Melampsora rust pathogen. We found that MAPK cascades contribute to the establishment of plant disease resistance using several approaches. MAPK gene expression is deregulated during susceptible and resistant interactions with different Melampsora species. We also identified novel MAPK-protein interactions using a yeast two hybrid approach and obtained evidence that poplar MAPKs modulate numerous downstream signalling components associated with the defense response. Experiments are

now underway to functionally analyse specific MAPK-interacting candidates and their potential roles in poplar defense. We will present the molecular analysis of these candidate genes, results from transcript profiling and data from genetic transformation experiments.

Differential accumulation of small RNAs during *Magnaporthe oryzae* growth and development

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Magnaporthe oryzae, the rice blast pathogen, is a filamentous fungus which requires a specialized infection cell called the appressorium to infect rice and results in significant grain losses annually. Fungi, including *M. oryzae*, possess the machinery for RNA silencing. A key step is attributed to small RNAs (sRNAs), ~ 20 – 30 nucleotides (nt) long. In plants and animals, sRNAs such as small interfering RNAs (siRNAs) and microRNAs (miRNAs) play important functional roles in RNA silencing. However, their presence and role in fungi is largely unknown. In this study, we generated 124,024 and 25,832 sequences ≥ 16 nt from mycelia and appressoria, respectively. SRNAs perfectly matching the genome were characterized based on genome features (genes, repetitive DNA, rRNA, tRNA). Surprisingly, about 14.0% of sRNAs from mycelia mapped to repeats such as MAGGY in contrast to only 2.3% in appressoria. Notably, sRNAs mapped to both sense and antisense strands in equal frequency and had a peak length of 22 – 23 nucleotides; characteristics of siRNA. Our results suggest that expression of repetitive elements such as transposons were silenced in mycelia. In addition, we found ~ 19.5% of appressoria sRNAs mapped to tRNA in contrast to ~ 9.4% from mycelia. *M. oryzae* small RNAs exhibited a bias for U enrichment and C suppression at position 1 especially on the antisense strand. Our data, demonstrates that tRNA cleavage occurred at the tRNA anticodon and was tissue dependent suggesting that tRNA processing may regulate protein synthesis. In conclusion, evidence suggests different classes of sRNAs exist in *M. oryzae* and their frequency profiles in different tissues suggest they may play an active role in growth and development.

Discovery of a novel protein, a putative elicitor from a biocontrol *Fusarium oxysporum*, inducing resistance to Fusarium wilt in tomato

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Nonpathogenic *F. oxysporum* strain CS-20 reduces incidence of Fusarium wilt of tomato (*F. oxysporum* f. sp. *lycopersici*) via induction of systemic resistance. Strain CS-20 synthesizes non-fungitoxic high-molecular weight metabolites (HMWM) which protect tomato from wilt and can be isolated from fungal biomass. The goals of this study were to investigate putative pathways of HMWM-induced resistance and to identify CS-20 metabolites with elicitor activity. Using qRT-PCR we found a significant enhancement of *pr-1* expression in leaves of tomato seedlings when roots were exposed to the HMWM. The protective effect of protein and non-protein fractions of HMWM separated on Sephacryl S-400 were tested, revealing this effect was associated with one protein fraction while other fractions, including a non-protein component, were virtually inactive. SDS-PAGE of the active fraction detected proteins in the molecular mass range from below 18.4 to 45 kDa that produced bands of lower molecular weights in gels with 2-ME, suggesting the presence of interhelical disulfide bonds. Two homogeneous polypeptides very specific to the resistance-inducing fraction were isolated using reverse phase HPLC. Their molecular masses measured by MALDI-TOF mass spectrometry were 20215 and 10033 Da. The N-terminal sequence (25 amino acids) of the 10033-Da polypeptide was determined by automated Edman degradation. The sequenced fragment was enriched in basic and cysteine amino acid residues, and contained a unique cysteine motif. BLAST search in the UniProt database revealed no homology of the sequenced fragment with known proteins. These results suggest discovery of a previously unknown protein produced by the biocontrol strain CS-20, which apparently elicits resistance to Fusarium wilt in tomato.

A 14-3-3 protein positively regulates programmed cell death mediated by Pto and MAPKKKα

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Programmed cell death (PCD) is triggered when Pto, a serine-threonine protein kinase recognizes either the AvrPto or AvrPtoB bacterial effectors from *Pseudomonas syringae* pv. *tomato*. This PCD requires MAPKKKα (mitogen-activated protein kinase kinase kinase) protein as a positive regulator in tomato and *Nicotiana benthamiana*. To examine how PCD-eliciting activity of MAPKKKα protein is controlled, we identified MAPKKKα-interacting proteins using a yeast two-hybrid assay with tomato cDNA prey library. From this screening, two tomato 14-3-3 proteins were found to interact with the full length of MAPKKKα protein in yeast. Based on virus-induced gene silencing assay in *N. benthamiana*, one of these 14-3-3 proteins was necessary for both MAPKKKα and Pto/AvrPto-mediated PCD, but not for cell death induced by Bax or NtMEK2^{DD}. Consistently, over-expression of the 14-3-3 with the full length of MAPKKKα in *N. benthamiana* enhanced MAPKKKα-mediated PCD. The 14-3-3 contains a conserved phosphopeptide-binding motif. Amino acid substitutions in this motif abolished the PCD-enhancing activity of 14-3-3. In addition, these substitutions abolished interaction with the full length of MAPKKKα in yeast and in vivo. Our results indicate that a 14-3-3 positively regulates PCD induced by Pto and MAPKKKα.

CS 15-6 Npv41, an aspartic protease specific of root nodules, is involved in root nodule development and plant innate immunity in *Phaseolus vulgaris*

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Unraveling root nodule gene functions will contribute to expand our understanding of the mechanisms underlying root nodule morphogenesis and symbiotic nitrogen fixation. The implementation of different molecular, genetic and genomic approaches has made possible the identification of a vast array of new genes never described before to play a role in symbiosis. In this work, we report the isolation and characterization of a novel nodulin from *Phaseolus vulgaris*, named Nodulin 41 (Npv41) in agreement with its nodule specific pattern of expression and apparent molecular weight of 41 kDa. The most striking characteristic of Npv41 was its ability to bind to denatured proteins, a trait exploited in the purification process through affinity chromatography. Npv41 showed high similarity to plant aspartic proteases involved in resistance to bacterial pathogens in Arabidopsis. Prediction analyses indicate that the signal peptide of Npv41 directs the protein to the secretory pathway. The amino acid sequence of the mature protein indicates that a 50 amino acids-long pro-segment is removed The sub-cellular localization of Npv41 indicates that this protein is indeed accumulated in uninfected cells, probably in the endoplasmic reticulum where it could function as a protease and/or as a chaperone-like molecule. Interestingly, knocking down Npv41 transcript levels by RNAi completely impairs root nodule formation implying the possibility that Npv41 has also a role at early stages of the symbiotic interaction and in the innate immune response during nodule ontology.

Insights into the plant defense mechanisms induced by *Bacillus* lipopeptides

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Cyclic lipopeptides from beneficial rhizobacteria may constitute a novel class of microbial-associated molecular patterns. These compounds can be specifically perceived by plant cells to mount some defensive response against subsequent pathogen ingress. Here, we report on the characterization of host defense mechanisms stimulated by surfactin from biocontrol *Bacillus subtilis* strains. In tobacco suspension cells, surfactin induces defense-related early events such as extracellular medium alkalinization coupled with ion fluxes and reactive oxygen species production. The occurrence of these events is closely related to Ca^{2+} influx, phospholipase activity and dynamic changes in protein phosphorylation. Surfactin also stimulated some typical defense

enzymes and modified the pattern of phenolics produced by the elicited cells. In whole plants, lipopeptide-overproducing *Bacillus* isolates also induces some defense reactions, notably the accumulation of antifungal coumpounds and the activation of the so-called oxylipin pathway leading to the synthesis of a wide array of biologically active secondary metabolites. Key enzymes of this pathway are stimulated concomitantly with the reduction of disease symptoms at the macroscopic level suggesting that they actively contribute to the global defensive response of the host plant lighted following *Bacillus* cLP perception. Some of the defense events induced by surfactin are similar to those stimulated by PAMPs suggesting a convergence of the early cellular reactions. However the plant response to surfactin also retains some specific aspects that will be discussed.

Characterization of an *Arabidopsis thaliana* mutant resistant to thaxtomin A, a phytotoxin produced by *Streptomyces scabies*, the causal agent of potato common scab

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The filamentous bacterium *Streptomyces scabies* is the main causal agent of common scab of potato. This disease is characterized by the development of necrotic and scabby lesions on the surface of potato tubers, thus altering their quality and market value. However, no potato cultivars are completely resistant to common scab. During the infection process, *S. scabies* synthesizes phytotoxins, called thaxtomins, that are essential for the development of disease symptoms. In particular, the phytotoxin thaxtomin A has been shown to inhibit cellulose synthesis and activate a program of cell death in various plant species. Thaxtomin A can also inhibit seed germination and root growth in *Arabidopsis thaliana* seedlings. Based on these characteristics, we have identified an *Arabidopsis thaliana* EMS mutant that is more resistant to thaxtomin A than wild-type plants at the level of germination and root growth. The mutated gene is currently being mapped by positional cloning. Gene expression analyses using the Affymetrix Arabidopsis GeneChip array has revealed that more than 179 genes are down-regulated (> 2.0 fold) in this mutant while 22 genes are up-regulated (> 2.0 fold). The identification of the mechanism of resistance to thaxtomin A in this mutant may provide new tools to develop potato cultivars with increased resistance to common scab.

Signal transduction in plant root symbiosis

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We are interested in unraveling the molecular mechanisms involved in the intracellular accommodation of symbiotic microorganisms by plants. Legumes form symbiosis with phosphate-acquiring arbuscular mycorrhiza symbiosis and nitrogen-fixing rhizobia bacteria. Forward genetics has identified a series of plant genes required for early developmental stages of both symbioses. The predicted protein products of these 'common symbiosis genes' comprise a receptor kinase, nuclear localized ion channels and components of the NUP84 sub-complex of the nuclear pore. These components act upstream of symbiosis induced calcium spiking, which is likely to be decoded by a complex formed by a calcium- and calmodulin-dependent protein kinase and CYCLOPS, a nuclear protein with a coiled-coil domain. Recent progress in analysing the function of individual symbiosis signaling components at the mechanistic level will be presented.

***Datisca glomerata* as an actinorhizal model plant**

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Two root nodule symbioses between higher plants and nitrogen-fixing soil bacteria are known, legume-rhizobia and actinorhizal symbioses. *Datisca glomerata* is the only actinorhizal species with a generation time suited for mutagenesis and genetic analysis. However, *D. glomerata,* coming from a different phylogenetic lineage than all other actinorhizal species examined so far on a molecular level, has an atypical infection mechanism and oxygen protection system. In order to develop *D. glomerata* as a model system, three approaches were followed. (1) To compare gene expression in roots and nodules of *D. glomerata,* SAGE-like cDNA libraries were sequenced from both organs. Functions of newly identified nodule-specific genes were compared with those known for legumes and for other actinorhizal plants. (2) Conservation of infected cell-specific transcription factors from other types of root nodules was examined by expressing promoter-GUS fusions in transgenic hairy roots of *D. glomerata.* (3) Protection of nitrogen fixation from oxygen does not require a symbiotic class II hemoglobin in *D. glomerata* nodules. Class II hemoglobins contribute to the production of reactive oxygen species. In order to compare the demands for antioxidant defense in a hemoglobin-dependent and a hemoglobin-independent system, hydrogen peroxide production and plant antioxidant defense mechanisms were compared in nodules of *D. glomerata* nodules, and in nodules of the well-examined actinorhizal tree *Casuarina glauca*. This work was supported by the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (Formas) and the Swedish Research Council (VR).

The genomes of selected nitrogen fixing Rhizobiaceae analyzed by ultrafast sequencing technologies

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This talk will concentrate on sequencing projects dealing with the genomes of different Rhizobiaceae, namely the *Sinorhizobium meliloti* isolates 1021, SM11 and Rm41 as well as with *Sinorhizobium fredii.* One of the first rhizobial genomes ever sequenced was that of *S. meliloti* 1021. It consists of the chromosome and two megaplasmids. An international consortium collaborated in the sequencing process making use of the classical Sanger technique. It should be mentioned that in a recent publication the annotation of the *S. meliloti* 1021 genome has been updated with post genome data. In the meantime, the introduction of ultrafast sequencing techniques facilitates the sequencing of further *S. meliloti* genomes. The Bielefeld group started to sequence the genome of the *S. meliloti* SM11 isolate by using the GS20 sequencer of the 454 company. Three GS20 runs resulted in altogether 112 Mb which were finally assembled into a chromosome, two megaplasmids and two further medium size plasmids. *S. meliloti* SM11 is an interesting field isolate since it outcompetes at a high degree *S. meliloti* 1021 in the nodulation process. Possible gene regions involved in the enhanced nodulation capacity could be identified in the genome sequence of *S. meliloti* SM11. At the moment two further genomes, namely the genome of S. meliloti RM41 and of S. fredii HH103, are sequenced in Bielefeld using the GS-Flx sequencer. First results on the annotation of the draft genomes will be presented.

***VAPYRIN*: Discovery and characterization of a *Medicago truncatula* gene required for arbuscule development during AM symbiosis**

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The arbuscular mycorrhizal (AM) symbiosis is an ancient, widespread and ecologically important mutualism between plants and fungi. This symbiosis is characterized by fungal penetration of plant roots and transfer of nutrients through fungal structures called arbuscules, which develop within plant cortical cells. While the genetic pathway that allows root penetration is being elucidated, little is known about plant genes that control arbuscule development within cortical cells. We have discovered a gene in the model legume *Medicago truncatula* we named *VAPYRIN*, or *MtVpy*, that is induced in AM roots. RNAi knockdown of this gene impairs root penetration and abolishes arbuscule formation. The promoter and coding sequence were cloned from genomic and cDNA libraries, respectively, and promoter:UidA fusions in transgenic roots show *VAPYRIN* expression overlaps with the area of AM fungal colonization in addition to expression in the stele and root cap. Native promoter translational fusions to GFP reveal VAPYRIN accumulation associated with fungal hyphae. *VAPYRIN* is a member of a plant-specific gene family with orthologs in dicots and monocots, however there is no putative ortholog in Arabidopsis, a non-mycorrhizal plant. The structure is composed of an amino-terminal domain with homology to VAP/MSP proteins and a carboxy-terminal domain with eight ankyrin repeats; both domains are known to mediate protein-protein interactions. We hypothesize this gene may play a scaffolding role during fungal penetration of epidermal and cortical cells by binding multiple proteins. Additional experiments are now underway to further reveal the molecular function of *VAPYRIN* in the AM symbiosis.

An increase of methyl-esterification of pectin reduces the susceptibility of plants to fungal and bacterial infection

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The plant cell wall is a physical barrier against pathogens and its modification is often associated to changes in plant defense responses. Pectin is responsible for mechanical properties of the wall such as porosity, adhesion and rigidity and is the substrate of pectin-degrading enzymes such polygalacturonases (PG) and pectin methylesterases (PME). The action of these enzymes is the pre-requisite for subsequent cell wall degradation by other polysaccharide-degrading enzymes. Pectin is synthesized in a highly methylesterified form and is subsequently de-esterified *in muro* by PME. On the other hand PME activity is finely regulated by the presence of multiple enzyme isoforms and by the action of pectin-metylesterase inhibitors (PMEI). PME knockout and PMEI over-expression have been used to stably increase pectin methylesterification in Arabidopsis plants. We will present results showing that decrease of PME activity and associated increase of pectin methylesterification are correlated to a decreased susceptibility of Arabidopsis to *Pectobacterium carotovorum* and *Botrytis cinerea*.

Molecular mechanisms controlling heterochromatic gene silencing in Arabidopsis

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For genetic dissection of molecular pathways controlling heterochromatic gene silencing in plants we developed a new test system for transcriptional gene silencing (TGS) in Arabidopsis, which is based on T-DNA transgenes containing tandem repeats of the Luciferase reporter gene expressed under the control of the CaMV 35S promotor. Until now more than 30 TGS suppressor mutations identifying 18 different genes could be genetically defined. Genetic and molecular analysis revealed a sequence of interdependent molecular processes controlling TGS. In conjunction with deacetylation and dimethylation reactions initially H3K9 and DNA methylation are established followed by molecularly complex final processes controlling higher order chromatin structure. The final process in TGS is controlled by a series of novel factors. Mutations affecting final establishment of TGS suggest that high levels of H3K9, H3K27 and DNA methylation are not sufficient to manifest gene silencing. Many of the identified functions are implicated in genome wide epigenetic programming and control chromocenter heterochromatin, transgenerational epigenetic effects or genomic imprinting in Arabidopsis. Furthermore manifold effects of TGS suppressor mutations on plant developmental processes demonstrate a wide functional overlap between processes controlling heterochromatin formation and gene activities during plant development. Implication in epigenetic control of pathogen responses will be discussed.

Increase of seed germination and seedling growth by *Microbacterium thalassium* showing a phosphate solubilizing phenotype

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Selected Plant Growth Promoting Rhizobacteria (PGPR) are known to increase seedling development by nitrogen fixation, phosphate solubilization, phytohormones production and several biocontrol functions. In this study, several plant rhizospheres (*Lactuca sativa*, *Theobraoma cacao*, *Brachiaria* spp. and *Iresine herbsttii*) were sampled from different Southwest regions of Venezuela. Isolation, screening and characterization of nine rhizobacteria were carried out by using several biochemical tests and the 16S rDNA molecular sequencing. The strains were classified as *Enterobacter* spp., *Enterobacter cloacae*, *Pseudomonas* sp., *Pantoea stewartii*, *Burkholderia caribensis*, *Microbacterium thalassium*, *Rhizobium radiobacter* and *Bacillus megaterium*. All strains, except *B. megaterium*, showed mineral phosphate solubilizing (MPS) activity when using tricalcium phosphate in solid media. The PGP effect of these rhizobacteria was studied using a *Zea mays* L. with 74% seed germination. The germination rate increased to 91% ($P < 0.05$) when seeds were inoculated with *M. thalassium* strain IR45, followed by 87% ($P < 0.05$) with *B. megaterium* strain 24b2. Also, these two strains increased the number of roots and the epicotyl size of maize without nutrient addition. Moreover, *M. thalassium* strain IR45 increased significantly the germination rate of *Capsicum annum* L. and *Lactuca sativa* L. This is the first report for *M. thalassium* as a PGPR with MPS capabilities, which could be potentially useful for the production of plant nursery.

Cellular dynamics in plant immunity

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Intracellular membrane compartmentalization and trafficking are pivotal for eukaryotic life. Plant defence in response to pathogen infection is tightly associated with reprogramming of vesicle trafficking pathways. These include exocytic/secretory routes for focal accumulation at pathogen penetration sites, and endocytic pathways as shown for induced internalization of the FLS2 receptor, responsible for perception of bacterial flagellin (flg22). Importantly, FLS2 endocytosis appears to be linked with flg22 responses. In an attempt to identify molecular components of FLS2 endocytosis we isolated FIP1 as an FLS2 interacting protein. FIP1 localizes to the plasma membrane and like FLS2 accumulates into endosomes upon flg22 elicitation. We will discuss FIP1 function in FLS2 endocytosis. To further elucidate the role of endocytic vesicle traffic during plant defence we established a genetic screen for endocytosis mutants applying high throughput quantitative confocal laser microscopy (QCLM). We used FYVE-GFP as an endosomal marker and inspected about 10,000 M2 seedlings for altered endosome numbers. To date we identified 11 *fel* mutants (*fel* = FYVE endosome levels) with elevated or reduced vesicle numbers. Interestingly, *fel4* not only displayed increased vesicle numbers but also enlarged vesicles. Initial characterization of *fel4* and *fel5* will be discussed. Taken together, our combined approach of genetic and cell biology should provide novel insights into the role of endocytic traffic in plant immunity or pathogen-triggered disease.

Plant innate immunity and resistance to necrotrophic pathogens: at the cell wall and beyond

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The cell wall provides a passive barrier against pathogens and pests, constitutes a reservoir of antimicrobial compounds and is a source of signalling molecules. During plant development or microbial infection cell-wall perturbations occur that lead to changes in gene expression. The characterization of plant resistance to necrotrophic and vascular pathogens has revealed the relevance of cell wall in the regulation of Arabidopsis thaliana immune responses. Thus, mutants (e.g. ern1/irx1) impaired in Cellulose synthases (CESAs) required for the synthesis of cellulose from secondary cell wall shows a constitutive activation of ABA-signalling pathway and accumulate a diverse set of antimicrobial compounds, which lead to a broad spectrum resistance to pathogens. To determine the relevance of cell wall in plant innate immunity, biased resistance screenings of additional Arabidopsis cell wall mutants were performed, and novel signal transduction pathways and regulatory proteins controlling plant innate immunity were identified. Among these regulatory proteins, several Receptor-Like-Kinases (RLKs), such as ERECTA, and a Mitogen-Activated Protein Kinase Kinas Kinase (MAP3K) were characterized. Plant RLKs have been suggested to be important components of the cell-wall monitoring integrity pathway, and to regulate basal resistance to pathogens by controlling the recognition of microbial patterns. MAP3Ks regulate RLKs-mediated innate immunity response by activating MAP Kinase cascades. The genetic interaction among these novel components of innate immunity as well as the recent data describing the characterization of the specific interconnection between cell wall integrity and activation of plant innate immunity will be presented.

Glucosinolates are an important first layer defence of *Arabidopsis* against *Phytophthora brassicae*

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The phytoalexin (camalexin)-deficient Arabidopsis mutant *pad2* was shown to be highly susceptible to the oomycete pathogen *Phytophthora brassicae*. Camalexin deficiency was not the cause of susceptibility as evidenced by the resistance of other camalexin mutants. Recently *pad2* was found to accumulate less glucosinolates (GS) in response to the generalist insect herbivore *Spodoptera littoralis*. We were asking if GS deficiency might explain the susceptibility of *pad2* to *P. brassicae*. Transcript profiling revealed that GS biosynthetic genes are up-regulated after inoculation with *P. brassicae*. GS deficient mutants showed reduced resistance, but the double mutant *cyp79B2cyp79B3*, compromised in camalexin- and indole GS biosynthesis was found to be highly susceptible to *P. brassicae*. These genetic data are supported by toxicity assays showing that both camalexin and GS inhibit the *in-vitro* growth of *P. brassicae*. Hence, the susceptibility of *pad2* can be explained by the combined deficiency of both classes of secondary metabolites. These compounds act sequentially with an early role for the indole GS in the first layer of defence while camalexin becomes important at later stages. Interestingly, the inhibitory effect of indole GS was independent of cellular destruction, thus suggesting a mode of action different to the 'mustard oil bomb' that depends on tissue disruption.

OsRac1 GTPase is a key regulator of PTI and ETI in rice

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Plants show two immune responses to attack by pathogens. One is the response to pathogen-associated molecular patterns (PAMPs) and termed PAMP-triggered immunity (PTI) and the other is effector-triggered immunity (ETI). We have been studying the role of small GTPase Rac (also called Rop) in rice innate immunity by using several experimental approaches. Our previous studies indicate that OsRac1 is involved in both PTI and ETI in rice. We isolated a number of proteins associated with OsRac1 by proteomic approaches. Based on molecular genetic and biochemical studies we found that a number of proteins which are either novel or well studied in other systems could form a protein complex whose functional core is OsRac1. Proteins include known co-chaperones such as HSP90, HSP70, RAR1 (Thao et al.2007), RACK1 (Nakashima et al., 2008), Hop/Sti1, enzymes such as NADPH oxidase for ROS production (Wong et al., 2007), CCR for lignin biosynthesis (Kawasaki et al., 2006), and MAPK (Lieberherr et al., 2005). Several other proteins are also found. We recently found that OsRac1 is bound and activated by the NB-LRR type R protein Pit for rice blast fungus. The Pit protein is localized at the plasma membrane where it interacts with OsRac1. These results suggest that OsRac1 is involved in ETI. We also found that OsRac1 is rapidly activated by chitin and sphingolipid elicitors, which are blast fungus-derived PAMPs. Taken together, our studies show that OsRac1 is a key regulator of both PTI and ETI in rice.

Resistance to sap-sucking insect pests in a model legume *M. truncatula*

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Aphids and related phloem feeding insects cause severe plant damage, through feeding activities and as vectors of plant viruses. Australian breeders at SARDI have introgressed bluegreen aphid (BGA)) resistance into three popular cultivars and generated three pairs of resistant and susceptible near-isogenic lines in the model legume *M. truncatula*. A major focus of our research has been on resistance to BGA in cultivar Jester, which is near-isogenic with susceptible cultivar Jemalong, the reference genotype for *M. truncatula*. We have identified and mapped a dominant gene conditioning BGA resistance in Jester called *AKR* (*Acyrthosiphon kondoi* resistance). This gene is tightly linked to resistance gene analogs of the NBS-LRR gene family. We are taking a number of approaches to uncover the mechanisms by which this gene confers aphid resistance. These include using targeted approaches to identify signaling pathways involved in plant defense against BGA aphid and genomic approaches involving microarrays and transcription factor profiling. Rapid progress is also being made in identifying and characterizing other aphid resistance genes in *M. truncatula* including the identification of single dominant resistant genes against spotted alfalfa aphid (SAA) and pea aphid. We have also identified a moderate form of SAA resistance that is mediated through multiple QTLs, which we have been able to assign to specific subsets of the overall aphid resistance phenotype. The wealth of molecular and biochemical resources available to the *M. truncatula* community will assist us in elucidating the major factors effecting aphid resistance and the knowledge has potential for aphid protection in crops.

Signals in the underground: Microbial signals and plant productivity

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Plants are the primary source of energy for the biosphere. Some of this is released to other elements of the biosphere while the plants are still alive, in some cases to organisms that benefit the plants. Our group has examined aspects of plant-microbe interactions for the past 20 years. Initial work demonstrated that low spring soil temperatures were slowed the establishment of the nitrogen fixation symbiosis on legumes, and that the greatest effect was through disruption of the initial signal exchange between the two symbiotic partners. When we added appropriate signals to the bacterial symbiont, prior to its inoculation onto the plants, the symbiosis developed more quickly. Flavonoid signals were found to be effective, and more recently jasmonates have been found to be at least as efficacious. Addition of flavonoids or jasmonates causes the bacterial symbionts (rhizobia) to produce nod factors (lipo-chitooligosaccharides), which alert the plant to the presence of the correct types of rhizobia. We found that nod factors themselves can cause direct stimulation of plant growth of legumes non-legumes. We have also been working with a series of plant growth promoting rhizobacteria. We found that some of these stimulate the early growth of soybean plants, leading to accelerated nodulation and nitrogen fixation. We also found that when these bacteria produce diffusible microbe-to-plant growth-stimulating signals. These signals have proven to be proteins. In at least some cases we have also shown that these proteins are bacteriocins. As fossil fuel reserves dwindle there is increasing interest in biofuels; any technology that increases plant productivity has the potential to increase biofuel production and these microbially produced signals can act in this capacity.

Application of signal molecules for better crop production

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LCOs (Lipo-chitooligosaccharides, a.k.a. Nod factors) are signal molecules produced by rhizobia that are essential for the nodulation process in legumes. Plant responses to the addition of LCOs with soybeans in laboratory and greenhouse studies include root hair deformation, enhanced root development, and early plant growth responses. Supplementing LCOs with rhizobia has been researched and developed with the introduction of commercial products for soybeans, peas, peanuts and alfalfa. Further exploration of LCOs with non-legumes in greenhouse and field studies has also provided evidence for LCO activity with non-legumes. Greenhouse trials, harvested after three weeks, with LCOs applied to corn before planting has demonstrated increased growth parameters including shoot and root dry biomass. Examination with the WinRhizo Scanning System demonstrated an increase in leaf area, and also volume, surface area, and diameter of roots. Field trials with LCO on corn provided increased root development, early plant growth, and final grain yield. Experimentation with seed treatment of LCOs on cotton in greenhouse and field trials has also demonstrated positive plant responses to LCOs.

Chitin signaling in Arabidopsis

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Chitin, a polymer of N-acetyl-D-glucosamine, is a component of fungal cell walls. Plant cells are equipped with mechanisms to perceive the chitin signal derived from fungal pathogens to activate plant innate immunity. To investigate chitin signaling in plants, we took advantage of the model plant *Arabidopsis thaliana* due to its tremendous molecular and genetic resources. Our studies have revealed a number of critical components in the chitin-elicited signaling pathway, including potential receptors, MAPK cascade and other components. Interestingly, the chitin signaling pathway appears to

overlap with the phytobacterial flagellin- and EF-Tu-elicited signaling pathways, suggesting that plant cells may perceive different PAMPs from various pathogens via specialized receptors and then utilize a conserved, common downstream pathway to mediate plant innate immunity. We will present an update of our current work which reveals additional complexity in chitin signaling and its relationship to plant fungal resistance.

Increased fungal resistance of sugar beet by pathogen induced expression of super auto activated resistance proteins

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Plant diseases caused by fungal infections result in serious yield and quality losses of crops. We report the pathogen induced over expression of super auto activated resistance genes of the CC-NBS-LRR type as a new strategy to increase the fungal resistance of the crop sugar beet. In order to circumvent the detrimental effect of a constitutive expression of R genes in transgenic plants the R genes were combined with pathogen inducible synthetic promoters. Synthetic pathogen-inducible promoters exclusively containing well defined regulatory elements of PR genes were identified which showed a 1000 fold induction after fungal infection of the crop and a low basic activity in uninfected tissue. The promoters were activated by different fungal pathogens like *Cercospora beticola*, *Rhizoctonia solani* and *Erysiphe betae*, indicating that the promoters are feasible for the development of a broad spectrum disease resistance. The ability of R-genes to trigger a hypersensitive reaction (HR) was further improved either by mutations in the NBS domain or by the singular expression of a new DAE subtype of the CC domain. The induced over expression of the CC domain in transgenic plants was superior to the expression of the full length CC-NBS-LRR gene and resulted in a 50% reduction of diseased leaf areas. Expression of PR-protein genes were earlier and stronger detectable in the transgenic lines compared to the non-transgenic control. Our results demonstrate that the controlled activation of plant defence reactions by genetic elements of plant origin is a promising strategy for a sustainable improvement of disease resistance.

Interactions between necrotrophic fungi and conifers: Genomic and transcriptomic studies of *Heterobasidion annosum s.l.* root rot

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Annosum root rot is one of the most devastating diseases in conifer forests. It is caused by the necrotrophic basidiomycete *Heterobasidion annosum s.l.* consisting of a species complex with partly overlapping geographic distributions and host ranges. Recently, the genome of *H. annosum* has been sequenced and annotated. The analysis of the gene content showed presence of the basic gene sets necessary for wood decomposition but revealed also several protein domain families with significant expansion in *H. annosum* in comparison with other basidiomycetes and plant pathogens that include candidates responsible for the specific life style of the fungus. We have constructed a genetic linkage map, recently transferred to the physical gene map, and identified several QTLs that are associated with e.g. pathogenicity, growth rate and fungal interactions, these also give candidate genes for host interactions. Transcriptome analysis indicate that coping with oxidative stress, producing secondary metabolites, degrading wood components and detoxifying host defence reactions are part of the arsenal activated in contact with living host tissue. Studies are on the way to silence or knock out candidate genes in the fungus to verify the importance of several of the indicated candidate genes. Transcriptome analysis of the host tree *Pinus sylvestris* indicate that response to pathogen presence is building up with time and includes cell death, induction of phenolpropanoid pathway and induction of antimicrobial proteins.

The role of auxin in symptom development upon *Rhodococcus fascians* infection of *Arabidopsis thaliana*

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As a phytopathogenic bacterium, the Gram positive actinomycete *Rhodococcus fascians* interferes with growth and development in a myriad of host plants, typically leading to the formation of multiple shoots. Although this has been attributed mainly to the action of bacterial cytokinins, *R. fascians* was shown to produce auxin via the indole-pyruvic acid pathway. Moreover, biochemical analyses showed a transient increase in the IAA content in infected Arabidopsis plants. The co-localisation of *DR5*::*GUS* expression and bacterial colonisation in leaves spot-inoculated with *R. fascians* indicated that the bacteria were responsible for the elevated auxin levels. However, histochemical staining of *DR5*::*GUS* plants infected in the apical region suggested that *R. fascians* may trigger auxin biosynthesis in the plant as well. Indeed, using Q-PCR, IAA biosynthesis was shown to be upregulated demonstrating that the observed auxin accumulation should be ascribed to both partners. The role of auxin signalling in the plant was tested by infecting a range of Arabidopsis mutants. Although most of them responded as wild-type plants, *slr* and *axr3-1* had a significantly reduced response. Because auxin is known to enhance the competence of plant tissues to respond to cytokinins, we postulate that IAA represents a second essential signal molecule in this interaction. The impaired auxin signal transduction in *slr* and *axr3-1* and the possible loss of the amplification of the cytokinin signalling may well be at the basis of the reduced reaction of these mutants. Our working hypothesis is that bacterial auxin plays a dual role in this pathology -first as a colonisation factor and later as a virulence factor- and that plant-derived auxin is co-responsible for the induced morphological modifications.

Genetic dissection of the infection and organogenic pathways required for Rhizobium-legume symbiosis

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Formation of root nodules in legumes in response to lipochito-oligosaccharides (Nod-factors) signals secreted from rhizobia is an example of inducible organ formation. A genetic program controlling and synchronising two processes running in parallel controls development of the nitrogen fixing root nodules. Nodule primordia are formed from root cortical cells initiating cell divisions and simultaneously a bacterial infection process targets the primordia developing from the cell division foci. Mutations in key symbiotic genes often arrest both processes and it has therefore been difficult to assign genes to pathways. We have now used the Lotus japonicus snf1 and snf2 spontaneous nodulation mutants, uncoupling organogenesis from infection, to assign genes to the separate infection and organogenic pathways. Synthetic mutants combining snf1 or snf2 with loss of function alleles of genes encoding receptors and signal transduction components normally required for infection and/or nodulation were made and the role and position of central symbiotic genes was investigated. The position of the Nod-factor receptors (Nfr1 and Nfr5) the LRR receptor kinase (SymRK), cation channel(s) (Castor and Pollux), nucleoporins (Nup85 and Nup133), the Ca2+/calmodulin dependent protein kinase (CCaMK), Cyclops, the cytokinin receptor (Lhk1) and the transcriptional regulator Nin will be presented and discussed.

Isolation of a novel TAL effector resistance gene via differential transcript profiling

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The Gram-negative bacterium *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) is the causal agent of bacterial spot disease of pepper and tomato. *Xcv* injects via its type III secretion system the transcription factor like (TAL) effector AvrBs3 that induces host cell hypertrophy by binding and activating the promoter of Upa20, a master regulator of cell size. In resistant plants, AvrBs3 also activates the promoter of the pepper *Bs3* gene, a mechanistically and structurally novel type of resistance (*R*) gene that encodes a YUCCA-like flavinmonooxygenase. Similarly, the rice *R* gene *Xa27* is transcriptionally induced by the matching TAL effector AvrXa27. We used transcriptional upregulation via TAL effectors to identify a novel *R* gene from pepper via differential transcript profiling. Recent progress on the identification and analysis of this *R* gene will be presented.

Phytoplasma strain Aster Yellows Witches' Broom (AY-WB) secreted proteins induce morphological changes in *Arabidopsis thaliana*

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Phytoplasmas are bacterial pathogens that are limited to the plant phloem and are introduced into plants by phloem-feeding insects, including leafhoppers.

Phytoplasmas manipulate their plant hosts in a variety of ways. They interfere with plant development leading to witches' broom (shoot proliferation), phyllody (flowers that become leafy), and yellowing symptoms. They also enhance insect fitness and alter plant chemistry to make volatiles that attract insects. Our hypothesis is that phytoplasmas secrete effector proteins to manipulate the plant host. Genome sequencing and subsequent mining for secreted proteins of phytoplasma strain AY-WB, which infects *Arabidopsis* and induces production of succulent and serrated leaves, resulted in the identification of 56 putative effectors named Secreted AY-WB Proteins (SAPs). One of these, SAP11, was shown to target plant nuclei. We aimed to determine the effectors that induce morphological changes in plant and/or increase the fitness of phytoplasma/insects by creating *Arabidopsis* transgenic lines that overexpress 56 SAP genes. So far, we have obtained *Arabidopsis* lines for 36 SAPs. The 35S:SAP11 lines showed severe leaf serration and crinkled siliques. The induction of these phenotypes correlates with the targeting of SAP11 to plant nuclei. Furthermore, over-expression of SAP05 induces the production of elongated leaves with no serration. Thus, so far, SAP11 and SAP05 induce morphological changes in *Arabidopsis*. Investigations of whether SAP11 and SAP05 affect phytoplasma replication rates and insect attractiveness of plants are ongoing.

How to resist tomato resistance proteins?

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Our group studies the molecular basis of disease resistance and susceptibility in plants. We use the interactions of the fungus *Fusarium oxysporum* f. sp. *lycopersici* (Fol) and the root-knot nematode *Meloidogyne incognita* with tomato as model. Resistance of *Fol* races producing Avr2 is mediated by the R protein I-2, whereas resistance to *M. incognita* requires Mi-1.2. Both R proteins are NB-LRR proteins and contain atripartite nucleotide-binding domain fused to a leucine rich repeat domain. As part of our ongoing efforts to unravel R protein function, we perform structure-function analyses of the different (sub) domains in I-2 and Mi-1. Furthermore, we study intramolecular interactions in wild-type Mi-1 and in null- and autoactive-mutants. The results presented support our model of how R proteins function as molecular switches. Upon colonisation of the host, *Fol* secretes many small proteins into the xylem sap. One of these proteins encodes Avr2 and matches I-2. Avr2 has a dual function; it not only confers avirulence but is also required for full virulence on susceptible hosts. Point mutations in Avr2 allow the fungus to avoid I-2-mediated recognition, while maintaining full virulence. Hence avirulence and virulence can be uncoupled, providing the fungus with a means to resist resistance protein I-2.

Group II introns as controllable gene-targeting vectors, contribution to the development of functional genomics in microorganisms and plants

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Group II introns are both catalytic RNAs and mobile retroelements found in organelle (lower eukaryotes and higher plants) and bacterial genomes that splice via a lariat intermediate, in a mechanism similar to that of spliceosomal introns. Group II introns are able to insert into specific DNA target sites. The fact that they are able to carry foreign DNA, even genes; that they can be targeted to insert into different DNA sites in the absence of homologous recombination, simply by modifying the intron RNA, combined with the high insertion frequency and specificity has made these genetic elements useful tools with many applications involving genetic engineering, functional genomics and gene therapy. We focus our research on the intron RmInt1 discovered by our group in the *Sinorhizobium meliloti* genome in 1998 and our aim is to use it as a controllable gene-targeting vector, and to contribute with this novel system for gene disruption to the progress of functional genomics in microorganisms and plants. We have been able to retarget RmInt1 to get gene-disruptions in bacteria (i.e. *S. meliloti* and *Escherichia coli* cells) and to introduce as cargo foreign DNA sequences and genes within the intron RNA sequences. We will present data about expression and cellular localization of the intron-encoded protein (reverse transcriptase/maturase) in Arabidopsis protopasts that open the possibility of it use for gene disruption in higher eukaryotes.

Functional interacción between the NADPH oxidasa and heterotrimeric G proteins in the pathogen response

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The Rboh genes (*Respiratory burst oxidase homologs*) from the plant NADPH oxidase are responsible for the production of reactive oxygen species (ROS) in response to pathogens and other responses to the environment. In response to ozone, there is also a production of ROS similar to the pathogen response, where the Rboh and heterotrimeric G proteins have been implicated. Our goal is to characterize the functional interaction between the Arabidopsis Atrboh and the heterotrimeric G proteins in the context of the pathogen response. We performed epistasis studies between the NADPH oxidase mutants *atrbohD* and *atrbohF* and mutants in the α (*gpa1-4*) and β (*agb1-2*) subunits of the heterotrimeric G proteins. We observe that *agb1-2* suppresses the mutant phenotypes in the *atrboh* in response to bacteria and the oomycete *Hyaloperonospora Arabidopsis*. This positions the heterotrimeric G proteins downstream of the *Atrboh*. However, *agb1-2* and the *atrboh* show an additive effect on susceptibility to the necrotrophic fungus *Plectosphaerella cucumerina*, which suggest two different pathways. There is, therefore, a complex relationship between the signaling of ROS produced by NADPH oxidase and the heterotrimeric G proteins, where the functional interaction between these regulatory systems varies depending on the context of the pathogen.

A new approach to identify genes potentially involved during mycoparasitism in the tritrophic system *Ampelomyces quisqualis*, powdery mildew and host plant

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Ampelomyces quisqualis is a naturally occurring mycoparasite of several powdery mildew species. The anatomy of the mycoparasitic relationship between *A. quisqualis* and its hosts has been widely investigated, but the interaction at molecular level is poorly understood. After a recognition phase (enhanced germination of *A. quisqualis* conidia and directed growth of germ-tubes toward powdery mildew conidia), *A. quisqualis* actively penetrates the fungal cell wall and invades the cytoplasm causing the death of the cell. *A. quisqualis* never completely kills the fungal host and its aggressiveness in the latest phase of hyperparasitism is commonly reduced. Powdery mildews are obligate parasites of plants, which cannot be grown in artificial media; therefore gene expression during mycoparasitism should be studied in a tritrophic system. To overcome this technical issue we used a new approach in a tritrophic model system (*A. quisqualis*, *Podosphaera xanthii* and *Cucurbita pepo*). Total RNA was extracted from the complex of the three organisms and cDNA sequenced using high throughput sequencing technology (FLX 454). More than 60,000 different contig sequences were obtained. The sequence data set was used to synthesize a DNA microarray chip (CombiMatrix) representing, in different proportions, the transcripts of the three organisms. *A. quisqualis* transcriptome was distinguished by hybridizing the chip with pure genomic DNA of the three organisms. A second chip with the probeset representing only *A. quisqualis* gene transcripts was synthesized and used for the identification of the genes involved in different phases of mycoparasitism (host recognition, active and late stage of parasitism). Strengths and weakness of this genetic approach will be discussed.

Systemic immunity – insights from genetic, transcriptomic and metabolomic approaches

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Establishment of broad-spectrum systemic immunity or systemic acquired resistance (SAR) to normally virulent pathogens provides potential novel opportunities for biotechnological approaches to crop protection. This enhanced resistance/tolerance can extend for a growing season and is dependent upon accumulation of salicylic acid (SA) in the systemic tissues. However, our understanding of how systemic immunity is established, the

nature of the local immunising signal(s), subsequent translocation and perception in naïve responding leaves is fragmentary. Traditionally, research has focussed upon late stages of SAR, but post-genomic technologies now permit integrated studies on the dynamics of establishment of systemic immunity and insights into the signalling networks contributing to the global primed state. This talk will discuss how local perception of bacterial effector proteins from *Pseudomonas syringae* can result in systemic reprogramming of the *Arabidopsis thaliana* transcriptome and metabolome. We highlight a role for bacterial modulation of phytohormones in distal responding tissues as central to establishment of SAR.

How oomycete and fungal effectors enter host cells

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Oomycete and fungal plant pathogens produce effector proteins to condition host tissue for susceptibility. Many of these proteins can enter into the cytoplasm of plant cells, where they interfere with plant defense signaling and may also be recognized by intracellular plant resistance gene products. We have shown previously that conserved RXLR-dEER motifs in the N-terminus of several oomycete effectors enable these proteins to enter plant cells in the absence of any pathogen machinery. However, identification of motifs that fungal effector proteins to enter host plant cells have so far eluded identification. We carried out extensive mutagenesis of the oomycete RXLR-dEER motifs to improve our understanding of the spectrum of amino sequences that can enable host cell entry. Using this information, we have identified variant RXLR sequences in the N-termini of many fungal effectors, and have shown that they can enable host cell entry. Furthermore, we have identified a family of receptor molecules in plant cells that bind both oomycete and fungal N-terminal domains. Both the strength of binding and the specificity of binding are affected by mutations in the RXLR motif. This family of receptor molecules is also conserved in animal cells, and we have shown that the PEXEL motif of Plasmodium effectors ($RXLX^{E}/_{Q}$) can also bind to these receptors. Thus effectors from three different kingdoms of eukaryotic pathogens have evolved, convergently, the ability to target a highly conserved receptor family in eukaryotic hosts. A small molecule capable of blocking effector entry has been identified, opening possibilities for controlling diseases caused by pathogens that depend on effector entry.

Identification of nodule-responsive MicroRNAs from common bean (*Phaseolus vulgaris*) in symbiosis with *Rhizobium tropici* under abiotic stress conditions

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Transcription factors and miRNAs (21-24 nt) are key regulators of plant development, plant adaptive responses to stress and nutrient homeostasis. There is still little knowledge about the regulatory roles of miRNAs in legume plants. In this regard, novel miRNAs from soybean that are expressed in roots infected with *Bradyrhizobium* (Subramanian et al. 2008) have been sequenced, the crucial roles of miR166 and miR169 in *Medicago truncatula* nodule development (Boualem et al. 2008; Combier et al. 2006) and PvmiR399 in phosphate starvation signaling in common bean, have been demonstrated (Valdés-López et al. 2008). In this work we present the identification of miRNAs from common bean, the most important legume for human consumption. We focused in the analysis of bean nodule-responsive miRNAs from plants in symbiosis with *Rhizobium tropici* under abiotic stress conditions (P and Fe deficiency, Mn toxicity and low pH). The approach used included the design of mini-arrays, spotted with synthesized 21-mers from conserved Arabidopsis and novel soybean and common bean miRNAs (Arenas et al. submitted), which were hybridized to radio-labeled miRNA-enriched RNA samples obtained from nodules of bean plants grown under control vs. stress treatments. We found that 18 miRNAs showed changes in accumulation under the different nutritional conditions tested. Differential expression of selected miRNAs in stressed bean nodules was confirmed by Northern blot analysis. Interestingly, two of these miRNAs have a calmodulin and EP2/EREBP as putative targets. Experiments in progress aim to demonstrate the role of selected bean nodule-responsive miRNAs and their target mRNAs in organ development and nutrient stress adaptation.

Mining the active proteome in plant-pathogen interactions

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Although proteomics and transcriptomics have generated a wealth of data to describe plant-pathogen interactions, the activities of proteins during these interactions remain difficult to predict because of post-translational regulations. To reveal the genome-wide activities of proteins during plant-pathogen interactions, we have launched activity-based protein profiling (ABPP) in plant science. ABPP is based on the use of biotinylated or fluorescent small molecules that react with the active site of enzymes in a strictly activity-dependent manner. We have established ABPP to monitor the activity of members of different classes of cysteine proteases and serine hydrolases, including RCR3, RD19, VPE, P69B, GLIP1, and SABP2. Probes for other protein classes such as the proteasome, phosphatases, glucanases and kinases are currently explored. Fluorescent and minitagged probes are used for *in vivo* activity-based imaging and profiling, respectively. We apply ABPP to study infections of Arabidopsis and tomato with various pathogens to 1) find up- or downregulated protein activities during infection, and 2) screen for pathogen-derived proteins that inhibit plant enzymes. This approach revealed e.g. that the fungal Avr2 effector protein from *Cladosporium fulvum* targets diversifying, defense-related tomato proteases. Furthermore, serine hydrolase profiling revealed the activities of Botrytis lipases and cutinases during infection of Arabidopsis. These and other examples illustrate the opportunities and possibilities offered by applying ABPP in plant pathology and show that ABPP is a robust and important new emerging technology.

A plant viral movement protein is an elicitor of ER stress and programmed cell death when expressed from a heterologous virus

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Plant viral synergistic diseases involve two unrelated viruses which cause a more a severe disease than when they occur in single infections. Investigations of the PVX/potyvirus studies clearly show that expression of viral sequences from the genome of a synergistic virus partner can produce compelling new insights into viral protein functions which can transform our understanding of anti-viral defense mechanisms in plants. PVX and *Tobacco mosaic virus* (TMV) represent another synergist disease that produce necrotic symptoms on tobacco plants. Early investigations reported that one or more PVX movement proteins enable TMV infection of resistant tomato carrying the *Tm-2* gene indicating that PVX contributes an activity that alters plant immunity to TMV infection (Taliansky *et al.*, 1982). Because our laboratory studies the function of PVX movement proteins, we decided to express PVX proteins from the TMV genome to gain further insight into PVX protein function. PVX TGBp3 is an ER resident protein. The quality control of TGBp3 involves ER resident chaperones to aid protein retro-translocation from the ER followed by degradation via the proteasome machinery (Ju *et al.*, 2008). This mechanism of ER-associated protein degradation (ERAD) and is also activated by stress conditions that disrupt ER homeostasis. Experiments revealed that PVX TGBp3, when expressed from the TMV genome, triggers programmed cell death (PCD). Key ER resident chaperones (BiP, PDI, calreticulin, and calmodulin) are up regulated prior to ROS production and tissue necrosis, linking ER stress to PCD. *NbSGT1* and *NbSKP1* are involved in SCF-mediated protein degradation and are essential for TGBp3 induced PCD. Experiments revealed components of TGBp3 related cell death overlap with *N*- gene mediated host defense responses.

Surprises in the genome of the mycorrhizal mushroom *Amanita bisporigera*

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Mushrooms in the large (>500 species) genus *Amanita* are mycorrhizal symbionts, and some are deadly poisonous. We sequenced *A. bisporigera* to 2x coverage and analyzed its potential to secrete plant cell-wall-active enzymes and identified the genes for its lethal toxins. *A. bisporigera* is

remarkably deficient in genes for cell wall degrading enzymes. Its genome contains no pectic lyases, a single carbohydrate esterase (CE), and no members of GH families 3, 5-7,10-12, 15, 28, 30, 54, 62, or 74. Overall, *A. bisporigera* has only 45% as many enzymes as the saprophytic mushroom *Coprinopsis cinerea*. The lack of plant cell-wall active enzymes is probably related to the obligate symbiotic niche; enzymes are either not necessary for carbon acquisition, or are detrimental to establishment of the symbiotic state. The genome of *A. bisporigera* also yielded the genes involved in the biosynthesis the amatoxins and phallotoxins, which are bicyclic octa- and heptapeptides. The toxins are synthesized on ribosomes as 35- and 34-amino acid proproteins, respectively. The two proproteins are highly similar at the N and C-termini, including flanking Pro residues, but the central "toxin" regions are dissimilar. A processing enzyme was purified from the phallotoxin-producing mushroom *Conocybe albipes* that could release the mature toxin peptides. The processing enzyme is a member of the prolyl oligopeptidase (POP) family of Pro-specific peptidases.

Cellular targeting and host-specific recognition of cyst nematode CLE proteins requires the variable domain

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Cyst nematodes produce secreted peptide mimics of plant CLAVATA3/ESR (CLE) peptides likely involved in redirecting CLE signaling pathways active in roots to form unique and essential feeding cells. The hallmark structure of plant CLEs, which includes an N-terminal signal peptide (SP), a highly variable domain(VD), and a conserved 14-aa CLE domain at or near the C-terminus, is preserved in nematode CLE proteins. The SP targets plant CLEs to the extracellular space where the preproteins are processed to release bioactive CLE motif peptides of 12-aa. Structure-function studies of nematode CLE proteins determined that the12-aa CLE motif peptide is required, but not sufficient for function *in planta*. Similarly, the variable domain of nematode CLE proteins is necessary for their function in the extracellular space in the absence of a SP, implicating a potential role in protein targeting if nematodes secrete CLEs directly to the cytoplasm of parasitized root cells. Site-directed mutagenesis and swapping of the variable domain sequence immediately N-terminal to the conserved CLE domain determined that nematode CLEs are also subject to host-specific recognition. We propose that this domain is important for host-specific CLE peptide processing which may help explain the molecular mechanism controlling host-range specificity of cyst nematodes.

Analysis of plant protein complexes by immuno-affinity chromatography and quantitative mass spectrometry

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Signal transduction in plant host defense often involves proteins that are expressed at low levels. This presents a technical challenge when purifying native protein complexes involved in signal transduction. A commonly used approach for isolation and analysis of protein complexes involves the use of affinity chromatography, followed by identification of the constituent proteins using mass spectrometry. One problem is that a large number of non-specific proteins are often pulled down together with the specific proteins. To overcome this obstacle, we adopted a quantitative mass spectrometry approach to distinguish the specific from the non-specific interactions. Transgenic plants expressing epitope-tagged target protein were grown on MS plates with ^{15}N as sole N source, whereas nontransgenic control plants were grown on regular MS plates with ^{14}N as N source. After immuno-affinity purification with the mixed samples, the eluted protein mix was digested and subjected to mass spectrometry for peptide sequencing. The sequencing results gave both the peptide identity and the $^{15}N/^{14}N$ ratio that differentiate specific and non-specific binding. An example of using this approach to elucidate the Arabidopsis MOS4-associated Complex (MAC) will be presented.

Many model pathogen strains instead of one: Taking advantage of natural diversity to unravel the basis of *Pseudomonas syringae* host specificity in the post-genomic era

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Pseudomonas syringae pv. *tomato* DC3000 is the *P. syringae* isolate most widely used to investigate the molecular interaction between bacterial pathogens and the model plants *Arabidopsis thaliana* and tomato. However, DC3000 is neither a typical tomato nor a typical Arabidopsis pathogen. It is a representative of typical pathogens of leafy greens in the genus Brassica. Therefore, DC3000 can be assumed to be best adapted to cause disease in that genus - but not in Arabidopsis or tomato. Other *P. syringae* isolates, which are very closely related to DC3000, are typical highly virulent celery, snapdragon, peach, or tomato pathogens. To identify the genes in these strains that either contribute to disease in their hosts and/or interfere with disease in their nonhosts, a comparative evolutionary genomics approach has been applied by: 1. reconstructing the evolution of this group of strains using multilocus sequence analysis, 2. determining host range on five plant species, 3. sequencing the genomes of multiple representative isolates, and 3. identifying conserved gene differences between isolates that have different host specificity. The role of these gene differences in host specificity is now being verified experimentally. This is done by making gene deletions, expressing genes ectopically in isolates in which they are naturally missing, and by comparing plant responses to these isolates. This has revealed that simple gene-for-gene resistance is not sufficient to explain differences in host specificity between isolates. Examples of gene differences that were identified between isolates and experimental verification of their role in host specificity will be presented and discussed.

A cytoplasmic protein kinase required for FLS2-mediated immune responses

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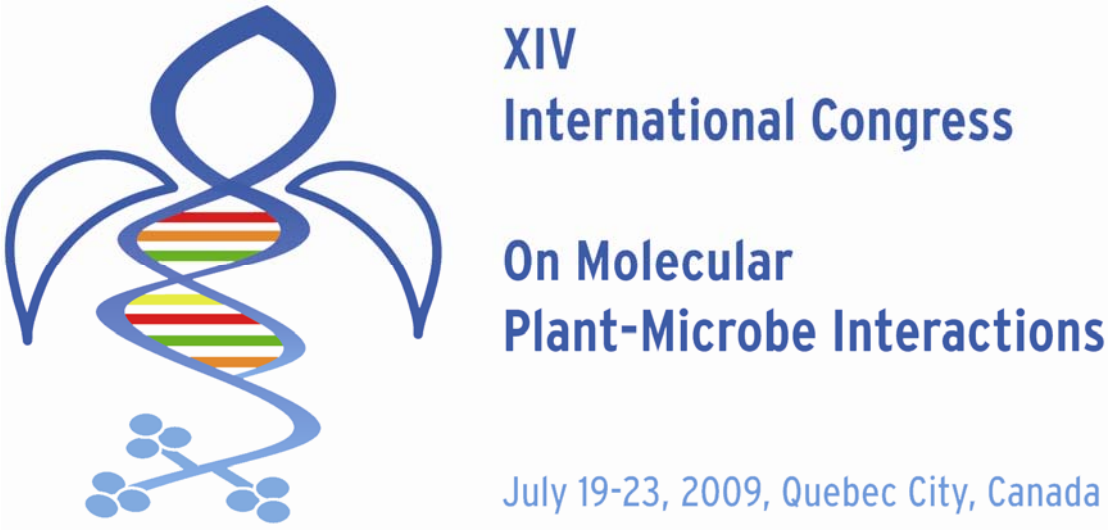
PAMP-triggered immunity is critically important for plants to fend off potential pathogens, but little is known about the PAMP signaling mechanism. For example, the best understood immune receptor FLS2 specifically recognizes the bacterial flagellar peptide flg22 to trigger defense responses. At present, only a few components have been identified for the FLS2-mediated signaling. These include BAK1, a receptor-like kinase that associates with FLS2 upon the recognition of flg22, and several MAP kinases. We identified a cytoplasmic protein kinase termed PBL as a novel component of the PAMP signaling pathway. PBL directly interacted with the kinase domain of FLS2 *in vitro*. The interaction was supported by co-immunoprecipitation experiments when FLS2 and PBL were transiently expressed in protoplasts. The *pbl* mutant plants were significantly compromised in flg22-induced reactive oxidative burst, indicating that PBL is required for FLS2-mediated signaling. Flg22 protection assay showed that the *pbl* mutant was impaired in PAMP-induced resistance to *Pseudomonas syringae* bacteria. The PBL protein was rapidly phosphorylated *in vivo* upon the exposure to flg22. Interestingly, AvrPto, a bacterial effector protein known to target the FLS2 kinase, blocked the flg22-induced phosphorylation of PBL. AvrPto did not interact with PBL nor BAK1. These results are consistent with the notion that AvrPto is a kinase inhibitor that targets the FLS2 kinase to block PAMP signaling and suggest that PBL is a substrate of the FLS2 kinase.



IS-MPMI 2009 XIV International Congress on Molecular Plant-Microbe Interactions Abstracts of Poster Presentations

Abstracts submitted for poster presentations at the Plenary Sessions at the XIV International Congress on Molecular Plant-Microbe Interactions in Quebec City, Canada, July 19–23, 2009. The abstracts are arranged in alphabetical order by the last name of the first author. Abstracts are published as submitted. They were formatted but not edited at the IS-MPMI headquarters office.

The *Meloidogyne incognita* genome: Insights into plant parasitism in nematodes

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IS-MPMI 2009 XIV Congress 30

The Southern root-knot nematode *Meloidogyne incognita* is a mitotic parthenogenetic parasite able to infect roots of almost all cultivated plants, causing important annual losses to world agriculture. The whole genome sequence of *M. incognita* revealed the co-existence of two highly diverged copies for the majority of the genome. Two hypotheses are currently considered to explain this result. One hypothesis proposes that these dissimilar copies represent ancient paternal and maternal haplotypes from a sexual ancestor that lost sexuality a sufficiently long time ago, allowing considerable divergence between ancient "allelic" regions. An alternative hypothesis is that the two observed divergent copies are the result of a hybridization event between two related but distinct species that gave rise to asexual hybrids. Such genetic plasticity could explain the extremely wide host range and geographic distribution of this polyphagous nematode. Another interesting feature of the genome is the spectacular presence of an extensive set of plant cell wall-degrading enzymes in this nematode, which has no equivalent in any animal studied to date. This suite of enzymes likely modifies and subverts the host environment to support nematode growth. Initial analyses show that these enzymes are not found in other metazoan animals and their closest homologs are bacterial, suggesting that these genes were acquired by multiple horizontal gene transfer (HGT) events. As the first whole genome available for plant-parasitic and asexually-reproducing metazoan, *M. incognita* represents an ideal model to analyze the relation between this mode of reproduction and the success of parasitism in an agricultural pest as well as the contribution of horizontally-transferred genes to this lifestyle.

Evaluation of Pseudomonades species phosphate solubilizaing on physiological and morphological characteristics of rice

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Plant Growth Promoting Rhizobacteria (PGPR) are known as groups of rhizospheric bacteria that improve plant growth and development characteristics by differents mechanisms. Amonge of plant growth promoting rhizobacteria psodomonases genus have special importance in soil, due to heigh ability of them in phosphate solubilizing as a direct mechanism for improvement of plants and so as biofertilizer but its important that tested indoginosis starins on different locations and plants. Therefore this research was designed for evaluation of pseudomonades strains that identified as phosphate solubilization with high ability on physioligical and morphological characteristics of rice. In a pot culture experiment used of seeds rice inoculation with 5 isolates (FP1,FP2,FP3,FP4,FP5) + control in 4 replicationon. Tarom variety of rice results showed that use of pseudo-monades species increased growth and rice yield. These increasing were significantly in comparison to control for grain yield, 1000 seeds weight, numberes of panicle, height plant, fresh weight of plant, harvest index and cholorophylla content.

Involvement of the Cytoplasmic inclusion (CI) protein in the overcoming of an eIF4E-mediated resistance against *Lettuce mosaic virus*

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The recessive allelic genes $mo1^1$ and $mo1^2$ in lettuce, corresponding to mutant alleles of the gene encoding the eukaryotic translation initiation factor 4E (eIF4E) (Nicaise et al., 2003), are currently used to protect lettuce crops against the potyvirus *Lettuce mosaic virus* (LMV). LMV-E is a resistance-breaking isolate, while LMV-0 is unable to induce symptoms on cultivars carrying the $mo1^2$ allele (tolerance) and generally does not systemically invade cultivars carrying the $mo1^1$ allele (resistance). The capacity of *Lettuce mosaic virus* to overcome the lettuce *mo1* resistance was analyzed using reverse genetics. Results showed that exchange of the VPg from LMV-E into LMV-0, allowed the overcoming of $mo1^1$ only, while the region coding for the C-terminal portion of the CI and 6K2 allowed the overcoming of both eIF4E alleles. Site-directed mutagenesis pinpointed a key role of amino acid at position 621 in the C-terminal portion of the CI, in the virulence of LMV-E (Abdul-Razzak et al., 2009). Furthermore, interactions between LMV CI-C-ter proteins (wild type and mutant forms) and the 3 lettuce eIF4Es ($4E^0$, $4E^1$ and

4E[2]) were demonstrated. The LMV-E CI-C-ter interacts more strongly than CI-0 C-ter with all the 3 eIF4Es and the mutation at position 621 does not abolish any interaction *in vitro.* This is the first example of a potyvirus CI gene acting as a determinant for eIF4E-mediated recessive resistance breaking.

DNA non-homologous end joining pathway is varied among isolates of *Magnaporthe oryzae*

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Magnaporthe oryzae is the causal agent of rice blast, the most important disease of rice. DNA recombination is reported as an important factor participating in the variability in *M. oryzae*. In eukaryotes, two major systems for DNA recombination, homologous recombination and non-homologous end-joining (NHEJ) are known. Well-known NHEJ pathway involves Ku70/Ku80 heterodimer and DNA ligase IV. In order to evaluate the importance of these genes in the growth and pathogenicity of *M. oryzae*, deletion mutants of these genes were obtained from Japanese isolates Ina168 and Ina86-137, using pDESTR system. The *mgku70*, *mgku80*, *mgku70/mgku80*, and *mglig4* mutants of Ina86-137 were significantly sensitive to methyl methanesulfonate (MMS) than the wild-type isolate. In Ina168, however, the sensitivity of all mutants to MMS was not significant. Gene targeting frequency of these mutants were assessed by *AdeA* gene deletion. All the mutants of Ina86-137 showed the elevation of targeting frequency to more than 75%, but Ina168 mutants did not show any significant elevation of the targeting frequency. These results indicated that *M. oryzae* has multiple pathways of NHEJ, one of those requires Mgku70/Mgku80 heterodimer and Mglig4, and the others do not require them. In addition, the contribution balance between pathways is different among strains; Mgku70/Mgku80-Mglig4 pathway participates mainly in Ina86-137, but acts as an alternative in Ina168.

Intra-host evolution of *Maize dwarf mosaic virus*

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Host plant selection has demonstrated to play a significant role in the structure of natural Maize dwarf mosaic virus (MDMV) population. In this study we evaluate the genetic variation of four MDMV haplotypes subjected to serial passages in susceptible and tolerant maize inbreds. Nucleotide identities among these MDMV haplotypes oscillated between 94–98% at the coat protein (CP) gene and 90–97% at the helper component (HC). Transmission efficiency and symptoms expression were recorded at each passage and direct sequence analysis of CP and HC performed at early, medium and final passages. Data obtained suggest that the emergence of aggressive viral haplotypes results from the use of the tolerant inbred. Genetic changes were detected in early passages and maintained along successive passages. Nucleotides substitutions occurred in fixed sites in both genomic regions. Evidence of inter-haplotype reversion to the wild type haplotypes was detected. Intra-host population of the serial passages were further analysed by cloning and sequencing five clones per passage.

Screening trichothecin resistant yeasts for trichothecene degradation capability

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Trichothecenes are a structurally diverse group of thermostable toxins produced by plant pathogenic fungi, which act as inhibitors of eukaryotic protein biosynthesis. If plant biomass containing trichothecenes (e.g. as a result of *Fusarium* diseases) is used for bioethanol production, the toxin is concentrated in the animal feed byproduct DDGS. Our goal is to identify yeasts with the ability to detoxify trichothecenes by irreversibly altering the core toxin structure. Our working hypothesis is that yeasts may exist in nature, which can detoxify trichothecenes produced by competing fungi. Since the most relevant *Fusarium* mycotoxin deoxynivalenol has low toxicity towards wild-type yeasts, we utilized trichothecin (TTC) in our screen. This toxin is produced by *Trichothecium roseum*, a fungus causing rot of grapes and other plant diseases. In the first stage we screened the BOKU yeast collection (www.acbr-database.at) for TTC resistant candidates. A TTC concentration was chosen for selection that allowed growth of a *S. cerevisiae* strain with a trichodermin resistance mutation (*RPL3-W255C*, formerly designated *tcm1*), but blocked growth of wild-type *S. cerevisiae*. TTC resistant yeasts were then tested in a secondary screen for the ability to reduce the toxicity of TTC present in the spent culture medium. For this test 5 µl of medium was spotted on a lawn of an engineered hypersensitive bakers yeast strain (relevant genotype: *pdr5 pdr10 pdr15 ayt1 ubi4 ubp6*). Candidates showing a clear reduction of the halo size should have resistance mechanisms other than drug efflux or target insensitivity. These strains are now tested with structurally different trichothecenes (e.g. *Fusarium* toxins). Chemical changes of the toxins are analyzed by HPLC-MS/MS.

Analysis of PAMPs-induced OsRac1 activation using FRET biosensor in rice

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Every year world agricultural production suffers loss due to disease caused by a variety of pathogens including bacteria, fungi, and viruses. However, the relationship between a plants and pathogens is unclear. Understanding this at the molecular level will contribute to increasing agricultural production. In plants, perception of pathogen-associated molecule patterns (PAMP) triggers innate immune responses that contribute to disease resistance. Previously, we showed that the small GTPase OsRac1, belonging to Rac/Rop GTPase family, plays key roles in defense signaling of rice. Overexpression of the consititutively active form of OsRac1 enhances reactive oxygen species production in rice cells treated by N-acetylchitooligosaccharide elicitor, a PAMP derived from the rice blast fungus. However, the spatiotemporal dynamics of OsRac1 activation during defense response is unknown. Here we report the development of an intracellular fluorescence resonance energy transfer (FRET) biosensor that facilitates the monitoring of OsRac1 activation by elicitors *in vivo*. The FRET biosensor is composed of OsRac1, the CRIB motif of human PAK1, which binds specifically to the GTP-bound form of Rac, and Venus and SECFP, as FRET donor and acceptor, respectively. We showed that N-acetylchitooligosaccharide and cerebroside A induce OsRac1 activation on the plasma membrane of rice protoplasts within 3 minutes after their treatments using FRET biosensor. Moreover, we identified OsGEF7, which has a PRONE domain, guanine nucleotide exchange factor (GEF) of OsRac1 identified by yeast two hybrid screening. Interestingly, a phosphorylation mimic mutant OsGEF7 induces the activation of OsRac1 *in vivo*, indicating that OsGEF7 is regulated by phosphorylation.

Mapping functional domains in the *Arabidopsis thaliana* receptor kinase EFR

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Higher plants have sensitive perception systems for pathogen associated molecular patterns (PAMPS). Two of these PAMPs recognized by *A. thaliana* are the bacterial proteins flagellin and EF-Tu (Elongation factor Tu). The corresponding receptor proteins in A. thaliana have been identified as the leucine-rich repeat receptor kinases (LRR-RK) FLS2 (flagellin sensing) and EFR (EF-Tu receptor), respectively. The epitope recognized by EFR is the acetylated N-terminus of the EF-Tu protein. Synthetic peptides comprising 18 to 26 aa of this N-terminus, termed elf18 and elf26, are fully active as ligands and stimulate defense response in subnanomolar concentration. In this work we set out to map the sites in the ectodomain of EFR that are responsible for ligand binding and receptor activation. In a first set of experiments we used gene constructs coding for EFR receptors lacking the kinase domain or various parts of the LRR domain. In contrast to wildtype EFR, none of these truncated receptors was functionally active when transiently expressed in *Nicotiana benthamiana*. Also no ligand binding was observed except for a construct that lacked the kinase domain only. In a second approach we constructed chimeric receptors with specific domains of EFR being replaced by corresponding domains from the structurally related, but functionally distinct, receptors FLS2 and ELI3 (EFR-like 3). Testing these constructs for functionality of ligand binding and receptor activation allowed attribution of these functions to specific parts in the 21 LRR consisting ectodomain of EFR.

Analysis of a genomic region in the barley smut fungus, *Ustilago hordei*, containing a cluster of predicted secreted proteins and an avirulence function

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Ustilago hordei is becoming a model for the small grain-infecting smut fungi. *U. hordei* infects barley and oats and genetic analyses have revealed gene-for-gene relationships governing virulence – host susceptibility, and avirulence – host resistance. We recently reported on the presence of an avirulence gene, *UhAvr1*, located within an 80-kb genetic interval. DNA sequencing of 110 kb in this region identified amongst others, 11 small proteins of approximately 200 to 260 amino acids with an N-terminal signal peptide motif indicating that these might be secreted. The region is syntenous to the reported *U. maydis* 19A cluster harbouring at least 23 predicted secreted proteins belonging to at least five groups. The *U. hordei* region has a remarkable reduced complement of these homologs and only one or two copies representative of each group. In addition, the region has many stretches of repetitive DNA including sequences related to transposable elements. The same region in the virulent parent of a mapping population segregating for *UhAvr1* and *Uhavr1*, was sequenced, as were homologs of the predicted effectors in several geographic isolates and races. Comparison revealed point mutations in several ORFs and promoter regions. Results on functional analyses, including a gene knock-out, will be presented. We also generated a new population segregating for *UhAvr6* and at least one new avirulence gene *Avrx* based on pathogenecity tests on a new cv, Sm89010. After testing many AFLP, RAPD and SSR markers we found a RAPD marker possibly loosely linked to *avr6*.

Metabolic fingerprinting of the plant-pathogen pathosystem, *Rhizoctonia solani*-*Solanum tuberosum* using Fourier transform mass spectrometry (FT-ICR/MS)

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Based on the latest developments in analytical instruments and software design for metabolomic analyses, metabonomics and metabonomics have emerged within functional genomics as a new tool for the in-depth understanding of biological systems and interactions. Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR/MS) is a superior MS detector enabling great resolution and mass accuracy lower than 1ppm, therefore representing an excellent platform for metabolomic studies. FT-ICR/MS metabolic fingerprinting was applied to study the plant-pathogen interaction of the pathosystem *Rhizoctonia solani*-potato (*Solanum tuberosum* var. Kennebec). Metabolic profiles of healthy and infected potato sprouts three days post-inoculation were acquired both in positive and negative modes and processed spectra were subjected to the multivariate analyses: principal components analysis (PCA), orthogonal partial least squares-discriminant analysis (OPLS-DA), and orthogonal partial least squares-hierarchical cluster analysis (OPLS-HCA) using the SIMCA-P+ 12.0 software (Umetrics, Umeå, Sweden). There was an excellent discrimination and classification of treatments, with a high predictive ability $Q^2_{(cum)}$ = 0.99 and 0.98 in the positive and negative modes, respectively. In response to *R. solani* infection, there was a significant accumulation of the alkaloids, *a*-solanine, solasonine, solanidine, and solasodine in infected potato sprouts compared to the control. Interestingly, the *de novo* synthesis of the phytoalexin phytuberin was only observed in *R. solani* infected sprouts. These results clearly suggest that plant-fungal interactions can be assessed through the application of metabolomics using FT-ICR/MS metabolic fingerprinting.

Investigating the genetic mechanisms contributing to motility of *Pseudomonas fluorescens* SBW25 over a semi-solid surface

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The plant growth promoting rhizobacterium *Pseudomonas fluorescens* strain SBW25 can improve plant health and growth by disease suppression. Flagella-mediated motility is known as an important factor contributing to *P. fluorescens* bacterial attachment to plant surfaces and for biofilm formation. A *P. fluorescens* SBW25 mutant (SBW25*ΔfleQ*) lacking the *fleQ* master regulator of the flagellum exhibits delayed and altered swarming on semi-solid agar. To identify the reason for this, two thousand IS-Ω-Km/hah transposon mutants of SBW25*ΔfleQ* were screened to identify mutants altered in swarming motility. Twenty-seven mutants were identified with IS-Ω-Km/hah insertions in genes encoding non-ribosomal peptide synthetases, regulatory genes, a putative transglycosylase and hypothetical genes with unknown functions. None of the genes resembled genes that encode other motility systems, such as for twitching motility or adventurous gliding motility. These mutants were differentially affected in swarming, from no movement from the point of inoculation to delayed or altered patterns of movement. Some mutants were unable to move due to loss of viscosin surfactant production. Ectopic expression of FleQ in the SBW25 *fleQ*-viscosin mutant did not complement surfactant production, but did result in motile cells that exhibited surface motility; interestingly, the pattern of movement differed from both wildtype SBW25 and SBW25*ΔfleQ*. We propose that SBW25 expresses two different systems (flagellum and surfactant) that contribute to motility *in vitro*: swarming motility (by flagellum) and sliding motility (by surfactant). Whether sliding motility is involved in motility *in vivo* is yet to be investigated. The contribution of these systems to bacterial ecological success in plant colonization will also be determined.

***In planta* approaches to identify new virulence factors of *Erwinia chrysanthemi* 3937**

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Erwinia chrysanthemi (Ech) is a broad host range soft rotting pathogen that causes severe maceration symptoms on several host plants including *Arabidopsis thaliana* (Ath). During the last decades, several Ech pathogenicity factors were identified, such as cell wall degrading enzymes, oxidative and osmotic stress tolerance systems, iron uptake systems and some regulatory modules. Most of the corresponding genes exhibit a concerted expression synchronized with the onset of the symptomatic phase of the interaction. To identify genes that are important for early plant colonization (asymptomatic phase) and for the switch to the symptomatic phase, a *gfp*-based gene-trapping library was generated and screened for genes that are up-regulated *in planta*. Three classes of transformants were identified after inoculation on chicory leaves. Seven mutants expressed *gfp* specifically *in planta*: three in the maceration zone, one in the surrounding asymptomatic colonization zone and three others in both zones. They all displayed an altered virulence on different host plants, whereas they were able to produce known virulence factors on plates (pectinases, cellulases, proteases, siderophores…). Among them, a citrate regulator mutant came up and its characterization is underway. Additionally, in order to get a dynamic and global view of Ech gene expression in interaction with Ath, we are developing transcriptomic approaches using whole genome microarrays. To tackle bacterial adaptation to the plant environment and dissect the physiological switch from asymptomatic to symptomatic phases of the infection, various strategies of bacterial RNA purification/enrichment from Ath infected plants at different times after inoculation are currently tested.

Regulation of disease responsive genes mediated by epigenetic factors: Interaction *Pseudomonas syringae-Arabidopsis thaliana*

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Genes in eukaryotic organisms function within the context of chromatin, and the mechanisms that modulate the structure of chromatin are defined as epigenetic. Epigenetic regulation of gene expression is mediated in part by posttranslational modifications of histone proteins. This is achieved through the activity of genes belonging to two families of antagonistically acting regulators, proteins of the trithorax group (trxG) and the Polycomb group (PcG), implicated in activating and repressing functions, respectively. Covalent modifications of histone-tail amino acid residues are considered to constitute a 'code' that controls gene activity (Strahl and Allis, 2000). Histone tails can be acetylated, methylated, phosphorylated, or ubiquitinated, creating recognition sites for cellular complexes with activating or repressing roles. The SET domain, identified in the *Drosophila* SU(VAR)3-9, the Pc-G protein E(Z) and the trx-G protein TRITHORAX, is a histone methyltransferase peptide responsible for the methylation of lysine residues on histone H3 and histone H4. Histone H3 can be methylated at lysine 4, 9, 27, 36, and 79. Activity depends on whether the lysine-NH2-groups are monomethylated, dimethylated, or trimethylated. In *Drosophila* absent, small, or homeotic-1 discs (ASH1) is a member of trithorax-group proteins that play essential roles in epigenetic regulation of Hox genes. Homologous of this protein have been identified in plants, and *Arabidopsis* has 4 gene homologs (ASHH1-4) and 3

related genes (ASHR1-3) (Baumbusch *et al*, 2001). We describe here the characterization and functional analyses of the *Arabidopsis* ASH1-homolog protein family and the epigenetic regulation of disease-responsive genes as a result of the interaction plant-pathogen.

Analysis of the specificity of the associative symbiosis between *Azospirillum lipoferum* and rice based on phenolic metabolism profiling and root growth promotion assays

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Rice (*Oryza sativa*) represents a major food crop. In the rhizosphere, plant growth-promoting rhizobacteria (PGPR) can benefit the plant through nitrogen fixation and modulation of the plant hormonal balance (*via* phytohormone production or ACC deaminase activity). However, little information is currently available about the specificity of the interaction between PGPR and plants. To better understand, at a molecular level, the specificity of the interaction between *Azospirillum* and rice, we compared two rice cultivars, Cigalon and Nipponnebar, either non-inoculated or inoculated with the PGPR *A. lipoferum* 4B or B510, the pathogen control *Burkholderia glumae* AU6208 or the commensal control *Escherichia coli*. A significant positive effect on root development was only observed when strain 4B and the Cigalon cultivar were used together. A metabolomic approach was developed on the plant partner, and principal component analysis of chromatographic data revealed significant modifications of metabolomic profiles according to the inoculant and the rice cultivar. These modifications result in the decrease of the quantity of certain secondary metabolites, noticeably in the presence of *A. lipoferum* 4B. Therefore, the success of the associative symbiosis differed according to the *A. lipoferum* strain and the rice cultivar, and this was reflected by changes in plant secondary metabolome.

The role of Skp1/Cul/F-box E3 ubiquitin ligase in *Agrobacterium*-mediated plant transformation

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Successful genetic transformation of plants by *Agrobacterium tumefaciens* requires that the bacterial T-complex (DNA-protein) is actively imported into the hosts' nucleus wherein it's probably uncoated of the cognate proteins prior to integration into the host genome. The essential components of the T-complex include the single stranded T-DNA, virulence proteins (VirD2, VirE2, VirE3 and VirF) and host proteins that facilitate the transfer, import and integration. The removal of the protein complex from the T-complex is likely achieved by targeted proteolysis mediated by VirF and the ubiquitin proteosome complex (UPS). The polyubiquitinization pathway involves several classes of enzymes, the most interesting being the ubiquitin protein ligases (or E3). In this study, we evaluated the involvement of the skp1/culin/F-box (SCF)- E3 ligase complex and its role in plant transformation. Gene silencing, mutant screening and gene expression studies suggested that the Arabidopsis homolog of yeast SKP1 (suppressor of kinetochore protein 1) protein, Ask1 and Ask2 are required for *Agrobacterium*-mediated plant transformation. Additionally, we also identified the role for Sgt1b (suppressor of the G2 allele of Skp1), an accessory protein that associates with SCF-complex and interacts with Ask1 for its role in plant transformation. We also report here identification of specific Skp1-interacting (SKIP) genes which are Ask1 and Ask2 interactors and carry a F-box motif, that differentially responded to *Agrobacterium* infection. Based on our observation we speculate that these *SKIP* genes could likely be the plant specific F-box gene that targets either the host proteins or their bacterial counterparts for polyubiquitination and subsequent degradation by 26S proteosome.

Arabidopsis *MAPK Phosphatase 1* (*MKP1*) negatively regulates MAMP-triggered immune responses

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A primary component of plant defense is the detection of microbe-associated molecular patterns (MAMPs) by plasma-membrane localized receptors. MAMP recognition results in rapid and transient activation of phosphorylation-dependent signaling pathways that lead to a wide array of defense-related responses including extensive changes in gene expression. Although several kinases, including MAPKs, have demonstrated roles as positive regulators of MAMP-activated signaling pathways, the corresponding phosphatases that must down-regulate MAMP-induced signaling remain largely unknown. By taking a candidate gene approach, we found that Arabidopsis *mapk phosphatase 1* (*mkp1)* seedlings display enhanced stress phenotypes, including increased anthocyanins and decreased root length, in response to treatment with the peptide MAMP elf26. In addition, qPCR profiling of a small subset of MAMP-induced genes revealed that some, but not all, are expressed to higher levels in elf26-treated *mkp1* seedlings. We also observed that *mkp1* seedlings are more resistant to infection by *Pseudomonas syringae* pv. tomato DC3000. Interestingly, untreated *mkp1* plants do not show overt stress phenotypes, suggesting that the enhanced resistance is stimulus-dependent rather than constitutive. Given the known role of MKP1 in regulating MAPK activity during genotoxic stress, we hypothesize that elf26-dependent phenotypes in mkp1 plants are the result of increased MAPK signaling. In support of this possibility, we found that the amplitude of MPK3 and MPK6 activation after elf26 treatment is enhanced in mkp1 seedlings. We have now generated *mkp1/mpk6* and *mkp1/mpk3* double mutants to further assess the role of these individual MAPKs in *mkp1* phenotypes. Our progress in characterizing these mutants will be presented.

Conserved oömycete effector proteins suppress programmed cell death

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Many plant pathogens produce effector proteins that are exported to the interior of host cells. Large superfamilies of candidate effector genes, carrying RXLR and EER host targeting motifs, have been identified in genomes from oömycetes such as the downy mildew pathogen *Hyaloperonospora arabidopsidis (Ha*) and various *Phytophthora* species. Although these superfamilies are very diverse, certain effector genes are conserved between *Phytophthora* and *Hyaloperonospora*, suggesting that they have key roles in pathogenicity. We have conducted preliminary functional analysis of nine predicted *Ha* effectors from that have identifiable homologs in *Phytophthora sojae* (*Ps*) and other *Phytophthora* species. From the preliminary characterization, we have selected a pair of homologous effectors, *Ha96* and *Ps163*, for further study. These effectors suppress Bax-induced programmed cell death in soybean, suggesting they function to suppress plant defenses. In addition, these effectors are localized to the nuclei of cells and Ps163 triggers a *SGT1*- and *Hsp90*-dependent hypersensitive response in *Nicotiana benthamiana.* We will present results from the characterization of *Ha96, Ps163* and other effectors. These results are based on transient expression assays in soybean, *Arabidopsis* and *N. benthamiana*, along with analysis of stably transformed *Arabidopsis*. Our data indicate that these effectors are able to suppress diverse cell death elicitors in a specific manner.

Investigation of the role of small regulatory RprA RNA in *Pectobacterium carotovorum*

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The plant pathogenic bacterium *Pectobacterium carotovorum* subsp. *carotovorum* (*Pcc*) produces virulence factors able to degrade plant cell walls (pectinases, cellulase and protease). In addition to genes coding for regulatory proteins, regulatory RNAs can also modulate the expression and activity of proteins needed for complete regulation of virulence genes in *Pcc*. It has been shown that regulatory RNAs (RsmB RNA) can affect the expression of plant cell wall degrading enzyme genes in *Pcc*. RsmB RNA acts by inhibiting the effect of global repressor protein RsmA. In addition to RNAs that affect the activity of proteins, there is a group of RNAs that act by basepairing with target mRNAs. One of the members of this group is small regulatory RprA RNA. RprA RNA positively affects the translation of RpoS by direct interaction with RpoS mRNA in *Escherichia coli* and *Salmonella*. In this study we provide evidence that RprA RNA is involved in the regulation of protease production in *Pcc*. We show that although inactivation of *rprA* does not affect production of protease, overexpression of *rprA* has positive RpoS-independent effect on protease expression in *Pcc.* These results indicate that RprA RNA has an *rpoS*-independent target in plant pathogenic *Pcc*. To isolate the genes

which products mediate the effect of RprA on protease expression we used transposon analysis. The *Pcc* strain SCC3193 carrying the plasmid overexpressing *rprA* was mutagenized with Km-Mu mini-transposon. Transposon containing clones (approx. 6000) were then screened for protease production on milk-plates. We have isolated several genes that have potential to modulate the effect of *rprA* overexpression on protease production in strain SCC3193.

In vivo hydrogen peroxide imaging during _Medicago truncatula_ root development and microbe interactions

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Plants appear to generate Reactive Oxygen Species such as superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) as signaling molecules to control various fundamental processes. Overall, H_2O_2 is a major player in symbiotic and pathogenic interactions. In pathogeny, H_2O_2 leads to programmed cell death in incompatible interactions, but in symbiosis H_2O_2 production must contribute to the control of nodule formation, by exerting a "positive" effect on both partners. Therefore, it is essential to determine the H_2O_2 signature during plant microorganism interactions. For this purpose, we used a new genetic encoded probe specific to H_2O_2. This probe, named HyPer, consists of circularly permuted yellow fluorescent protein (cpYFP) inserted into the regulatory domain of the prokaryotic H_2O_2-sensing protein, OxyR. The cDNA encoding HyPer has been introduced into a binary vector in order to overproduce HyPer in the *M. truncatula* plant model. The capacity of the probe to respond to H_2O_2 has first been assessed: exogenous H_2O_2 induced an increase of fluorescence in *M. truncatula* transgenic roots. More interestingly, *in vivo* signals have been detected during root and root hair development, validating already published data. Secondly, *in vivo* H_2O_2 imaging was then performed during *M. truncatula* interaction with i) *Sinorhizobium meliloti* (symbiosis leading to a new root organ able to reduce atmospheric dinitrogen) and ii) *Meloidogyne incognita* (interaction leading to a root-knot nematode feeding site). New insights in H_2O_2 involvement in *M. truncatula* developmental and microorganisms infection processes will be presented.

Identification of key players in plant-microbe symbiotic signaling through protein interactions

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Nod factors are lipochitooligosaccharides produced by symbiotic rhizobia and able to elicit, in legume hosts, responses similar to those induced by the rhizobia themselves such as nucleus-associated calcium spiking and nodulin gene expression. Several genes involved in Nod factor signaling have been cloned in model legumes through forward genetic approaches. Among them, *NORK* encodes a receptor-like kinase required for calcium spiking and *DMI3* encodes a calcium/calmodulin dependent kinase (CCaMK) that transduces calcium spiking into a phosphorylation cascade. Both genes are required for the establishment of legume nodulation and arbuscular mycorrhization. Using yeast two-hybrid in *Medicago truncatula*, a 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase (MtHMGR1) was identified as interacting with NORK (Kevei *et al.*, 2007). The different HMGR isoforms are key enzymes in the mevalonate biosynthetic pathway that lead to the production of diverse isoprenoid compounds. By testing other members of this HMGR family and other receptor-like kinases, a specific interaction was revealed between NORK and MtHMGR1. Recently, MtHMGR1 was also found to interact specifically with Nod factor receptors. An HMGR inhibitor as well as knocking-down *MtHMGR1* expression in *M. truncatula* transgenic roots by RNA interference drastically decreased nodule development confirming that MtHMGR1 plays a crucial role in this signaling pathway. Similarly, we have identified a novel protein interacting with DMI3 named IPD3 (Interacting Protein of DMI3) (Messinese *et al.*, 2007). Split yellow fluorescent protein assays indicate that IPD3 and DMI3 physically interact in plant nuclei. The analysis of *ipd3/cyclops* mutants in legumes and rice indicates that IPD3 controls symbiotic interactions across angiosperms.

Functional analysis of the symbiosis receptor kinase (SYMRK)

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The Symbiosis Receptor Kinase (SYMRK) is required at the early stages of symbiosis with arbuscular mycorrhiza fungi or rhizobia and the entry of these endosymbionts into plant cells. To study its cellular localization, we fused the SYMRK genomic sequence to fluorescent proteins. In tobacco epidermal cells, SYMRK localized to the plasma membrane. Tagged versions of SYMRK have been introduced into *Lotus japonicus* plants in order to study the spatiotemporal dynamics of SYMRK complex during the symbiotic response. To unravel the mechanism of action of this receptor kinase, we investigated the SYMRK interactome. Several SYMRK interactors were isolated through a yeast split-ubiquitin screen. The interactors were tested in planta using the split YFP (BIFC) system. To investigate the role of the resulting interactors in symbiosis, RNAi constructs targeting the corrsponding genes were introduced into *Lotus japonicus* by hairy root transformation and the symbiotic phenotype was analysed after inoculation with *Mesorhizobium loti*. The analysis of SYMRK complex and SYMRK dynamics will provide insight into symbiotic signalling transduction and plant receptor function.

Priming capacities of potato endophytic bacteria

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Endophytic bacteria are powerful and environmental friendly tool to increase the growth and defense capacities of agriculture crops, while the molecular mechanisms of these phenomena still remain unclear. Two *Pseudomonas* strains and one *Methylobacterium* strain isolated from potato tissues were tested for their ability to enhance growth and pathogen resistance of potato plants. All selected isolates stimulated potato growth, and one *Pseudomonas* and one *Methylobacterium* strain increased resistance, i.e. primed the plants towards *Erwinia carotovora* infection. The priming capacity of the *Methylobacterium* strain was inversely proportional to the size of bacterial inoculum and varied between the four potato cultivars tested. Therefore, we are now in the process of studying the effect of *Methylobacterium* sp. on the innate endophytes population in potato, which may become activated by the *Methylobacterium* sp. and result in priming of the plants for enhanced defense. Analysis of antioxidant enzymes of potato inoculated with endophytes revealed moderate activation of the antioxidant system which was associated with the induced resistance to the pathogen. This can be one of the mechanisms of priming by endophytic bacteria. We demonstrated a significant activation of both salicylic acid and jasmonate/ethylene-dependent defense genes in potato plants treated with endophytes after challenge inoculation with a pathogen. We conclude that endophytic bacteria of potato can activate both basal and inducible defenses in the host plant in a resource-safe manner. Various endophyte strains of potato possess a different mechanisms of interaction with plants, which can depend on the size of bacterial population.

Wounding induced changes in the CuZn *sod* gene expression in unripe avocado fruit

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The superoxide dismutases (SODs) are a family of metalloenzymes that catalyze the disproportionation of superoxide anion radicals (O_2^-) into hydrogen peroxide (H_2O_2) and molecular oxygen. There are three types of SOD, containing Mn, Fe or Cu plus Zn as prosthetic metals. In higher plants, SOD isoenzymes have been localized in different cell compartments such mitochondria, peroxisomes, cytosol, chloroplasts and apoplast. The CuZnSOD isoenzyme could be having a role in the production of species reactive oxygen in unripe avocado fruits in response to wounding. Avocado fruit is important to Mexico as a principal producer in the world. These fruits could be damaged during transport, manipulation or insect attack. Using specific inhibitors two isoenzymes to CuZn SOD and one to Mn SOD have been characterized in previous studies in our lab, from unripe avocado fruits treated by wounding. Specific oligonucleotides to CuZn SOD were designed from conserved domains and used to RT-PCR reactions from mesocarp tissue after wounding. A partial cDNA of 340 bp was amplified (*sodpa*) having homology with plants CuZn *sod* genes. At least three copies were observed by Southern blot analysis. Actually the characterization of gene expression by RT-PCR real time under different stress conditions and the apoplastic CuZn SOD isoenzyme analysis in response to wounding are in progress.

Atg26-enhanced pexophagy is required for host invasion by the plant pathogenic fungus *Colletotrichum orbiculare*

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The number of peroxisomes can change rapidly in response to changing environmental and physiological conditions. A type of selective autophagy, known as pexophagy, is involved in their degradation, although the physiological role of pexophagy remains to be clarified. Here, we report that the cucumber anthracnose fungus *Colletotrichum orbiculare* undergoes peroxisome degradation to infect host plants. A homologue of *ATG26* (*CoATG26*) was identified in a random insertional mutagenesis screen aimed at identifying the genes involved in pathogenesis. The *coatg26* mutant developed appressoria but exhibited a specific defect in the subsequent host invasion step. *ATG26* encodes a sterol glucosyltransferase that enhances pexophagy in the methylotrophic yeast *Pichia pastoris*, implying a relationship between pexophagy and fungal phytopathogenicity. Consistent with this, the peroxisomes are degraded inside vacuoles, accompanied by the formation of autophagosomes during the infection-related morphogenesis of *C. orbiculare*. The autophagic degradation of peroxisomes was significantly delayed in the appressoria of the *coatg26* mutant. Functional domain analysis of CoAtg26 suggested that both the phosphoinositide-binding domain and the catalytic domain are required for pexophagy and pathogenicity. In contrast to the *coatg26* mutant, which is able to form appressoria, the *coatg8* mutant, which is defective in the entire autophagic pathway, cannot form normal appressoria in the earlier steps of morphogenesis. These results indicate a specific function for CoAtg26-enhanced pexophagy during host invasion.

Comparative analysis of functionally analogous disease resistance genes in Arabidopsis and soybean

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Resistance (R) genes able to mediate detection of the Pseudomonas syringae effector proteins AvrB and AvrRpm1 are found in both Arabidopsis and soybean. The Arabidopsis RPM1 gene enables detection of both AvrB and AvrRpm1. In contrast, the tightly linked soybean genes Rpg1b and Rpg1r can distinguish between these two effector proteins and are specific for AvrB and AvrRpm1, respectively. While phylogenetic analyses indicate that RPM1 and Rpg1b evolved the same specificity independently, it is not known whether they use a common strategy to detect the AvrB protein. Neither is it understood how the soybean Rpg1 proteins can distinguish between AvrB and AvrRpm1 while the Arabidopsis RPM1 protein cannot. It has been previously shown that RPM1 function depends on an additional Arabidopsis protein, RIN4, which is phosphorylated in the presence of either AvrB or AvrRpm1. Interestingly, RIN4 is also targeted for cleavage by a third P. syringae effector, AvrRpt2. Our analyses indicate that soybean contains four RIN4 homologues (gmRIN4s). Interestingly, AvrRpt2 blocks Rpg1b/r function in soybean and targets at least 3 of the gmRIN4s consistent with a RIN4 homologue being required for Rpg1 function. Furthermore, one of the gmRIN4s interacts strongly with AvrB in yeast-2-hybrid assays. VIGS is currently being used to determine which, if any, of the gmRIN4s are actually required for AvrB and/or AvrRpm1 recognition in soybean. The Rpg1 genes are found in a rapidly evolving R-gene cluster containing numerous NB-LRR genes. The NB-LRR paralogue representing Rpg1b has been previously identified and a strong candidate for Rpg1r is now being analyzed further. Curiously, preliminary analyses indicate that the evolutionary history of Rpg1b includes a sequence exchange with the putative Rpg1r gene.

Molecular and metabolic events during induction of local and systemic acquired resistance

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Systemic acquired resistance (SAR) develops in response to a locally restricted pathogen inoculation and represents an enhanced state of broad-spectrum resistance at the whole plant level. The long-distance signals involved in SAR are still enigmatic. Although methyl salicylate (MeSA) has been reported as a crucial phloem-mobile SAR signal in tobacco, we show that MeSA is dispensable for SAR in Arabidopsis. In incompatible *Pseudomonas syringae*-Arabidopsis interactions, jasmonate biosynthesis is essential for MeSA formation, whereas in compatible interactions, the bacterial virulence factor coronatine is the main driving force for salicylate (SA) to MeSA conversion. This suggests that *P. syringae* uses coronatine-mediated volatilization of MeSA from leaves as a means to attenuate the SA-based defense pathway. In contrast to MeSA, flavin-dependent mono-oxygenase FMO1 functions as a central SAR component. Functional FMO1 is also required for beta-amino butyric acid-(BABA)-induced resistance. Our data suggest that FMO1 acts in concert with reactive oxygen species, SA and other SAR players within signal transduction in distant leaves to amplify defense responses at the systemic level. When analyzing changes in leaf lipid composition in response to *P. syringae*-attack, we found that desaturation of membrane sterols, i.e. sitosterol to stigmasterol conversion, is a significant metabolic process occurring after bacterial infection. After identifying Arabidopsis insertion mutants unable to express the respective sterol desaturase, we found that induced stigmasterol formation actually favors bacterial multiplication. Signaling events and subcellular aspects associated with stigmasterol accumulation, as well as consequences of sterol C22-desaturation for different membrane properties will be presented and discussed.

EHD2 inhibits endocytosis and signaling of Leucine-Rich receptor like proteins

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Pattern recognition receptors can trigger plant defense responses in response to specific ligands / patterns. EIX (ethylene-inducing xylanase) triggers a defense response via the LeEix2 receptor, while bacterial flagellin triggers plant innate immunity via the FLS2 receptor. Endocytosis has been suggested to be crucial for the process in both cases. Our work demonstrates that the EIX elicitor triggers internalization of its receptor LeEix2. Treatment with endocytosis, actin or microtubule inhibitors greatly reduced the internalization of LeEix2. Additionally, we demonstrate that plant EHD2 binds LeEix2 and is an important factor in the internalization and regulation of the induction of defense responses such as the hypersensitive response, ethylene biosynthesis and induction of pathogenesis-related protein expression, in the case of EIX/LeEix2 and the Cf family receptors (LRR receptors lacking a kinase domain), but does not appear to be involved in the FLS2 system (LRR receptor possessing a kinase domain). Our results suggest that different endocytosis pathways are involved in the induction of plant defense responses.

Biochemical activity and expression of ChsA, a protein involved in chemotaxis in *Azospirillum brasilense* Sp7

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We have previously identified a gene, *chsA*, involved in chemotactic response in *A. brasilense* Sp7, encoding for a protein containing a PAS sensory domain and an EAL domain (Carreño *et al.* 2009). Proteins containing an EAL domain have phosphodiesterase activity (PDE-A) for the hydrolysis of c-di-GMP [cyclic-bis (3'-5') dimeric GMP], a compound known to function as a second messenger in a broad spectrum of cellular processes including motility, biofilm formation and cellular differentiation. To determine if ChsA was involved in hydrolyzing c-di-GMP the *chsA* gene was cloned in an expression vector. ChsA protein was then expressed and purified by affinity chromatography. The activity detected in the presence of *bis-p*-nitro phenyl-phosphate (bis-*p*NPP) as a substrate was of 0.59-μM min^{-1} mg^{-1} protein, demonstrating that ChsA displayed a phosphodiesterase activity. As ChsA displays characteristic signaling protein architecture, we propose that the redox state of the cell is sensed through the PAS domain and directly coupled to the transmitter EAL module, showing PDE-A activity. A transcriptional fusion between the promoter region of the *chsA* gene and the *gusA-gfp* reporter genes was constructed in the broad host range plasmid pRU1156 (Karunakaran *et al*. 2005). Using this plasmid we observed that *chsA* was expressed both at the free-living state and during colonization of wheat roots, suggesting that ChsA may play a role in biofilm formation on the root surface. Colonization with the chsA mutant is in progress to determine the role of ChsA in biofilm formation on the root system. This work was supported by a CONACyT grant Ref. CB-2005-01-49227, and EETL was recipient of a CONACyT scholarship.

Type VI pili are involved in pathogenicity and biofilm formation of *Acidovorax avenae* subsp. *citrulli*
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Bacterial fruit blotch (BFB) of cucurbits is caused by the Gram-negative bacterium *Acidovorax avenae* subsp. *citrulli* (*Aac*). BFB gained importance in the late 1980s, after devastating outbreaks in watermelon fields in several USA states. Since then, BFB has spread worldwide, and has been reported in other cucurbits such as melon, pumpkin, squash and cucumber. *Aac* is a seedborne pathogen of highly destructive potential. Under favorable conditions, *Aac* spreads rapidly throughout transplant nurseries and in the field, leading to seedling blight or at a later stage, fruit rot. Strategies for managing the disease are limited and there are no reliable sources of BFB resistance. Despite the economic importance of BFB, there is little knowledge on basic aspects of the pathogen's biology and on the molecular basis of its interaction with its host plants. To identify *Aac* genes associated with pathogenicity, we generated a transposon mutant library on the background of *Aac* strain M6, and screened it for reduced virulence on seed transmission assays with melon. Here we report the identification of a *pilM* mutant with significantly reduced virulence. *pilM* encodes a protein involved in assembly of type IV pili (TFP). Further characterization of this mutant revealed that *Aac* requiresTFP for twitching motility and wild type levels of biofilm formation. Significant reductions in virulence and biofilm formation as well as abolishment of twitching motility were also observed in insertional mutants affected in other TFP genes. We also show for the first time, evidence supporting that *Aac* possess the ability to colonize and move thorough the host xylem vessels. Our results also support that polar flagella also play an important role in biofilm formation and virulence of this plant pathogenic bacterium.

***Thymus transcaspicus* essentil oil inhibits malaria vector *Anopheles stephensi* Liston**
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Resistance development in mosquitoes toward synthetic larvicidal and mosquitocidal agents has resulted in failure in their management and control. Exploitation of plant volatile oils with aroma therapeutic values is considered to be a valuable tool to combat this vector and, consequently the malaria outbreak. Larvicidal activity of *Thymus transcaspicus* essential oil (EO) was tested against larvae of *Anopheles stephensi*. Different concentrations of the EO were used against the parasite and the results were compared with that of synthetic insecticide, malathion. The results indicated a strong larvicidal activity of the EO (LC50 and LC90 values of 125 and 250 ppb respectively). The larvicidal activity was dose dependant. Considering the potential larvicidal activity of *Thymus transcaspicus*, the results of this study can be extended for the control of mosquitoes especially at breeding sites to control field populations of *Anopheles stephensi*.

Downy mildew *ATR5* belongs to non-RXLR family of effectors and triggers *RPP5*-dependant immune responses in *Arabidopsis*
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Understanding the mechanisms of microbial pathogenesis and plant-microbe interactions has motivated plant pathologists for a long while. Microbe-associated molecular pattern molecules (MAMPs) and effector molecules have been found to perform inter- and intracellular tasks as adaptation factors and manipulators of the defence network. *Arabidopsis-Hyaloperonospora* pathosystem has been playing a significant role in uncovering major complementary *AVR-R* genes. Cloning of *ATR1* and *ATR13* from *H. arabidopsidis* (formerly *H. parasitica*) and others including *Avr1b-1* and *Avr3a* from *Phytophthora* species has enabled the identification of common conserved regions including the N-terminal RXLR and dEER motifs. Arabidopsis La-*er* accession carries *RPP5*, which recognizes *ATR5* from Noks1/Noco2 and Emoy2 isolates. We have been carrying out map-based cloning of *ATR5* using F_2 mapping populations derived from different crosses between isolates of *H. arabidopsidis*. A genetic interval for *ATR5* has been established and a physical map of *ATR5* was constructed using the publicly available genomic and BAC-end sequences, as well as the BAC contig data. Further delineation of the *ATR5* locus was carried out and the gene has been placed on a single BAC clone. Fine mapping has put the gene to a 25kb interval. Bioinformatic studies supported by expression analysis revealed the presence of five genes, three of which have the characteristics of effector molecules. Interestingly, none of these candidates have an RXLR motif. Transient expression studies using bombardment assays have identified *ATR5* that gives an *RPP5* dependent defence response. Recent work on the function, evolution and further analysis will be presented.

The role of the LRR-receptor kinase, APR219 in plant immunity
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Many LRR-RKs have an important function for immune activation. Apr219, a novel LRR-RK seems in some unknown way to affect the defense response against the phytopathogen Pseudomonas syringae pv. tomato DC3000. In an effort to identify Apr219 interacting partners we have screened a tomato cDNA yeast two-hybrid library. By this, we were able to identify a Map kinase kinase (LeMKK4), a protein phosphatase (LePP2C), a Casein kinase I (LeCKL3/4), and a protein kinase (LePk) as positive interactors of Apr219. The specificity of this screen was verified as we tested several other MKKs, and protein phosphatases without any identifying any interaction. We therefore feel confident that the proteins identified are true interactors of Apr219. We have in addition established that Apr219 activities most likely are restricted to one or a few plant species.

Developing electrophysiological techniques to detect changes in apoplastic redox status during plant/microbe interactions
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The apoplast matrix that surrounds the plant cell is a dynamic microenvironment that mediates communication between the cell and its surroundings and is often the first line of defense against invading microbes. Redox reactions, which involve the transfer of electrons and provide most of the energy for life, are considered to play critical roles in host-microbe signaling. We have adapted different techniques to detect changes in the apoplastic redox potential that occur immediately or several hours after plant-microbe interactions. By using cell suspensions inoculated with various bacteria we have been able to use platinum-based electrodes for continuous monitoring of the apoplast/extracellular redox status, which directly appears to correlate with changes in extracellular phenolics and other redox sensitive metabolites, as well as other physiological responses. By using different bacterial partners or mutants the presence, loss or change in timing of these responses can be observed and possible connections made between redox status and other biochemical/physiological events. We are attempting to monitor similar events using palladium electrodes (Phytogram, Agricultural Electronics Corporation, Tucson, AZ) in greenhouse grown tomato plants as viroids spread through the plant. By improving sampling precision these techniques should aid further studies aimed at identifying specific metabolic pathways that are either activated or not activated during the first few hours of the tomato/microbe interaction.

Wheat leaf rust, *Puccinia triticina*, genome resource building and generation of a protein profile from isolated haustoria
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Genomic resources for the obligate pathogen, the wheat leaf rust fungus *P. triticina* (Pt) are being generated. We constructed a database with 45000 ESTs (cDNA from resting and germinated urediniospores, appressoria, haustoria and teliospores, and sexual cycle stages, i.e., pycnio- and aeciospores developing during alternate host infection on *Thalictrum speciosissimum*; see poster by R. Linning). A NSF-USDA grant was received to sequence the genomes of four isolates including parents of a mapping population, and to aid annotation by deep-sequencing cDNA pools from various structures (as above). Comparative analysis of Pt ESTs to the related stem rust, *P. graminis*

tritici (Pgt) genome, has been performed. Haustoria, fungal feeding structures which develop inside host cells upon infection, are thought to secrete 'effectors' which perturb host cells to divert nutrients to the haustoria for uptake and to suppress hostile host responses; some 'effectors' have also been shown to possess avirulence functions, eliciting host resistance responses. The haustorial proteome and 'secretome' are therefore of great interest. Haustoria were enriched from infected leaves by sucrose density gradient centrifugation and analyzed using several proteomics techniques: two-dimensional gel electrophoresis (IEF x SDS-PAGE and SDS-PAGE x SDS-PAGE), and by multidimensional protein separation with analysis by liquid chromatography-mass spectrometry (GeLC-MS). Over 130 proteins were tentatively identified using a Mascot search engine querying the Pt EST unigene set, Pgt genomic resources, and the NCBI non-redundant database. Some proteins were identified in both Pt and Pgt while others were Pt-specific. For validation, RT-qPCR analysis was performed on a subset of the protein-coding genes.

Towards the identification of gene networks induced by *Puccinia graminis* f. sp. tritici Ug99

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Stem rust, caused by the obligate fungal biotroph *Puccinia graminis* f. sp. tritici (Pgt), has been a serious problem wherever wheat and barley are grown. The East African stem rust race, Ug99, is a serious current threat because it is virulent against the majority of wheat varieties currently in production. We have recently conducted a genetical genomics experiment in order to address the ongoing threat of hyper-virulent stem rust outbreaks. This approach combines the complementary strengths of quantitative genetics and transcriptomics to connect loci that confer resistance with gene expression networks induced during infection. Analysis of transcriptomic and infection phenotype data from Ug99- and mock-inoculated lines belonging to the segregating Q21861 x SM89010 doubled haploid population not only confirmed the essential role played by a known resistance locus (*rpg4*/*Rpg5*), but also identified two novel eQTL hotspots. These two eQTL alter the expression of many disease responsive genes, and are positionally coincident with adult plant resistance QTL identified in this study. We have constructed a disease defense network nucleated by these loci and are currently testing the roles of predicted nodes via Barley stripe mosaic virus-induced gene silencing. This network contains not only barley orthologues of the well-known defense genes: *CeBIP, EDR1, WRKY71* and *WRKY45*, but also new unknown regulators. Because the eQTL appear to tie together *R*-gene mediated and basal defense networks, we consider the functional identification of their precise roles in integrating these systems a key step toward understanding how to achieve durable resistance against rusts.

BAK1 is involved in attenuation of EIX-induced defense responses

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Plant-microbe interactions involve numerous regulatory systems essential for plant defense against pathogens. An ethylene-inducing xylanase (EIX) of *Trichoderma viride* is a potent elicitor of plant defense responses in specific cultivars of tobacco (*Nicotiana tabacum*) and tomato (*Solanum lycopersicum*). The EIX receptors (*LeEix1* and *LeEix2*) were isolated in our lab. Structural analysis of these receptors suggests that they belong to a class of leucine-rich repeat cell-surface glycoproteins with a signal for receptor-mediated endocytosis. Both receptors are able to bind EIX while only LeEix2 mediates defense responses. The main purpose of the present work is to investigate the molecular mechanisms which allow plants to specifically activate defense responses after EIX elicitation. The brassinosteroid co-receptor, BAK1 (SERK3) was found to be involved in defense signaling through the flagellin receptor, as a BAK1 knock-out mutant was impaired in flagellin responses. Our results demonstrate that BAK1 binds LeEix1 but not LeEix2 in yeast two hybrid and bimolecular fluorescence complementation assays. We have recently found that LeEix1 attenuates EIX induced internalization and signaling. Interestingly, in BAK1 silenced plants, LeEix1 was no longer able to attenuate plant responses to EIX. We suggest that LeEix1 functions as a decoy receptor for LeEix2, possibly though its interaction with BAK1. Maya Bar and Miya Sharfman contributed equally to this work.

DELLAs control plant immune responses by modulating the balance of JA/SA-signalling and reactive oxygen species levels in plants

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GA promotes plant growth by stimulating degradation of negative regulators of growth called DELLA proteins. We show that DELLAs promote susceptibility to virulent biotrophs and resistance to necrotrophs, partly by altering the relative strength of salicylic acid (SA) and jasmonate (JA) signalling, and modulation of reactive oxygen species (ROS) levels in plants. The Arabidopsis *tetra* and *penta* DELLA mutants (that lack four and five DELLA genes, respectively) are very susceptible to the fungal necrotrophic pathogen *Alternaria brassicicola*, but more resistant to the bacterium *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000. The *tetra* mutant is partially insensitive to gene induction by Methyl-Jasmonate (MeJA), whereas the constitutively active dominant DELLA mutant *gai* is sensitized for JA-responsive gene induction, implicating DELLAs in JA-signaling and/or perception. To examine the impact of *penta* DELLA knockout on the flg22 response and *Pst* DC3000 challenge, we have carried out expression profiling with microarrays. Microarray data analysis revealed that several SA-responsive genes are induced and JA-responsive genes are repressed in *penta* compared to wild type plants. In addition, several genes involved in the detoxification of ROS are repressed in *penta* compared to wild type plants. In addition, we found increased accumulation of flg22-induced ROS in *penta* compared to wild type plants. In contrast, several genes involved in ROS detoxification are up-regulated in *gai* compared to wild type plants. This suggests that DELLA proteins control plant immune responses by modulating the balance of JA/SA-signaling and ROS levels in plants. The analysis of defence responses of other GA signalling mutants to different pathogens as well as data from various hormone and metabolite measurements will also be presented.

Exopolysaccharide production in the symbiotic soil bacterium, *Sinorhizobium meliloti*: What is the role of *syrA*?

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S. meliloti engages in a nitrogen-fixing symbiosis with roots of leguminous plants. In this intimate and complex interaction, the bacteria induce root nodule formation, invade the plant root, and are released into nodule cells, where they differentiate into nitrogen-fixing "bacteroids." Bacterial and plant determinants important for each stage have been characterized. To invade roots successfully, *S. meliloti* cell surface components such as exopolysaccharide (EPS-I & EPS-II) and cyclic beta-glucan are required; therefore, characterizing the regulatory circuits involved in production of these components is essential to our understanding of the symbiosis. We study two regulatory circuits that influence EPS-I abundance: *exoS-chvI-exoR* and *syrM-syrA*. The ExoS-ChvI two component system activates expression of EPS-I biosynthesis genes; ExoR is a periplasmic regulator that negatively affects ExoS-ChvI activity. In free-living bacteria, SyrM, a LysR family regulator, activates expression of *syrA*; overexpression of syrA leads to dramatically increased EPS-I. Expression of *syrA* is strongly induced in nodules, independent of *syrM*. Rather, *syrA* is expressed from a symbiosis-specific promoter. *syrA* is enigmatic--it is dispensable for an effective symbiosis and the mechanism by which it influences EPS-I production is unknown. Analyses using our dual-genome Affymetrix Symbiosis Chip show a significant overlap of expression changes between an *exoR* mutant and a *syrA* overexpressing strain. Currently, we are investigating whether *syrA* plays a role in the *exoS-chvI-exoR* regulatory circuit.

The *Xanthomonas campestris* pv. *vesicatoria* type III effector protein XopJ inhibits protein secretion: Evidence for interference with cell wall – associated defense responses

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The phytopathogenic bacterium *Xanthomonas campestris* pv. *vesicatoria* (Xcv) uses the type III secretion system (T3S) to inject effector proteins into cells of its host plants. The molecular mechanisms by which type III effectors suppress host defense responses are far from being understood. Based on sequence similarity, *Xanthomonas* outer protein J (XopJ) is a member of the YopJ/AvrRxv family of SUMO peptidases/acetyltranferases, although its biochemical activity has not yet been demonstrated. Confocal laser scanning microscopy revealed that green fluorescent protein (GFP) fusions of XopJ are targeted to the plasma membrane when expressed in plant cells which most

likely involves N-myristoylation. In contrast to a XopJ (C235A) mutant disrupted in the catalytic triad sequence, the wild type effector GFP fusion protein was also localized in vesicle-like structures co-localizing together with a Golgi marker protein, suggesting an effect of XopJ on vesicle trafficking. Using cell biological and biochemical approaches we were able to show that XopJ blocks protein secretion possibly at the stage of vesicle docking at the plasma membrane. Transgenic Arabidopsis plants with inducible expression of XopJ showed reduced papillae-associated callose deposition when challenged with an avirulent *Pseudomona syringae* strain. Taken together our data indicate that the virulence function of XopJ is to inhibit cell wall-associated defense reponses.

Elucidating the molecular mechanism of priming for stronger defense in *Arabidopsis thaliana*

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Upon infection by a pathogen plants can acquire immunity to a broad spectrum of pathogens. The acquired immunity is frequently associated with the accumulation of antimicrobial PR proteins and with the so-called "priming" of cells. Priming is the phenomenon that enables cells to respond to much lower levels of a stimulus in a more rapid and robust manner than non-primed cells. It has been hypothesized that priming involves accumulation of dormant signaling components that are not used until challenge exposure to pathogens. However, the identity of such signaling components has remained elusive. We show that during development of acquired immunity in *Arabidopsis*, priming is associated with accumulation of mRNA and inactive proteins of mitogen-activated protein kinases (MPK) 3 and MPK6. Upon challenge infection with *Pseudomonas syringae* pv. *maculicola*, these two enzymes were more strongly activated in primed plants than in non-primed plants. This elevated activation was linked to enhanced defense gene expression and development of acquired immunity. In addition, priming of defense gene expression and acquired immunity were lost or reduced in *mpk3* or *mpk6* mutants. Our findings argue that pre-stress deposition of the signaling components MPK3 and MPK6 is a critical step in priming plants for full induction of defense responses during acquired immunity.

Secretory processes for small molecules in Arabidopsis non-host immunity

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Induced defence responses in plants usually involve cell polarization, comprising rearrangement of actin cytoskeleton, directed movement of particular organelles and targeted secretion at the site of pathogen contact. This may also include trafficking and secretion of antimicrobial compounds. Our recent study revealed a novel pathogen triggered metabolism pathway for glucosinolates, amino acid-derived thio-glucosides characteristic for crucifer plants, that so far were mainly known as insect deterrents. This pathway requires at least two enzymatic components: CYP81F2 P450 monooxygenase and PEN2-myrosinase. CYP81F2 is essential for the pathogen-induced accumulation of 4-methoxyindol-3-ylmethyl glucosinolate, which in turn is activated by PEN2 for antifungal defense. PEN2-dependent indole glucosinolate hydrolysis is an active process involving directed movement and concentration of the respective enzyme(s) to the cell periphery at fungal entry sites, which likely generates high local concentrations of metabolism product(s) that are critical for plant resistance. Of note, genetic evidence suggests that PEN2-myrosinase is involved in the same biochemical pathway as PEN3/PDR8 ABC-transporter, which was also found to focally accumulate beneath sites of attempted fungal entry of leaf epidermal cells. These enzymes may constitute together a secretory immune response pathway for small molecules with broad-spectrum antimicrobial activity.

Effect of heavy metal toxicity on *Frankia* sp. and its symbiosis with black alder (*Alnus glutinosa* (L.) Gaertn)

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Frankiae are ubiquitous nitrogen fixing and root nodule-forming actino-bacteria. They are major players of actinorhizal symbiosis which is estimated to be responsible for 15 to 25% of nitrogen fixation on Earth. Among the 200 plant species frankiae colonize are alders. Alders are pioneers trees and shrubs well known for their robustness, their initiation of plant succession and their use in revegetation strategies. As soil bacteria, frankiae are exposed to anthropogenic stresses such as heavy metal contamination, yet survive as saprophytes and enter symbiosis. This suggests that *Frankia* spp. could have developed resistance mechanisms to allow its proliferation as soil saprophyte. Using a 96-well plate assay, 5 *Frankia* sp. strains (ACN10a, ACN12a, ACN14a, AvcI1 and CcI3) were exposed to 11 concentrations of 14 metals (Al, As, Cd, Co, Cu, Cr, Mg, Mn, Mo, Ni, Pb, Se, V and Zn). The strains' survival was quantitated by a tetrazolium salt reduction method, coupled to spectrophotometry. Results showed that the strains can tolerate heavy metal concentrations ranging 10 µM to 20 mM, depending on the metal. In addition to these results, we have addressed the fundamental concern of how heavy metals affect the *Frankia*-alder symbiosis establishment. We developed an axenic hydroponic plant culture method in which black alder (*Alnus glutinosa*) and *Frankia* sp. strain ACN14a were exposed to one concentration of each of 14 metals. These plant development and nodulation trials reveal the negative impact metals have on *Frankia* sp. infectivity. Effectivity of the symbiosis was verified by acetylene reduction assay done with nodules. The results of this study will pave the way for the elaboration of revegetation strategies tailored to site conditions using frankiae and alders.

Random mutagenesis to improve the activity of the polygalacturonase inhibiting protein (PGIP)

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PGIPs are plant cell wall proteins that specifically inhibit the activity of endopolygalacturonases (PGs) produced by fungi during infection. The interaction with PGIPs limits the destructive potential of PGs and may trigger plant defence responses. With the aim of generating PGIPs with improved and novel recognition capabilities, we have subjected to random mutagenesis by error prone PCR the gene *pgip2Q224K* encoding a mutated form of PGIP2 from *Phaseolus vulgaris* with no function against PG of *Fusarium phyllophyllum* (FpPG). The mutated proteins were expressed in *Pichia pastoris* and tested against several PGs. After screening 2400 trasformants we have isolated two proteins with a gain of function against FpPG, suggesting that our method is useful for obtaining inhibitors with novel specificities. Moreover we have isolated an inhibitor that shows an improved inhibition capability against PGs of *Fusarium graminearum* and *Colletotrichum lupini.* We are isolating other PGIPs and analyzing them against several PGs of important phytopathogenic fungi.

Genetic dissection of the role of *NFP* in Nod Factor perception in *Medicago truncatula*

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nfp mutants of *Medicago truncatula* are impaired in all responses to purified Nod Factors (NF) and unable to develop any symbiotic interaction with *Sinorhizobium meliloti*. *NFP* encodes a LysM-RLK with three extracellular LysM domains. LysM domains were first described in bacterial peptidoglycan-binding proteins, and are involved in chitin binding in rice. As NFs are lipochitooligosaccharides, LysM domains could be involved in NF binding in Legumes, but no binding data is available so far. We're using a genetic approach to test the role of *NFP* in Nod factor recognition and host specificity. The symbiotic interaction between *M. truncatula* and *S. meliloti* requires sulphated NFs whereas non sulphated NFs are involved in Pea/*R. leguminosarum* recognition. In our approach, we're using chimeric constructs to replace the extracellular part of NFP by the one from its Pea ortholog, SYM10, and test their ability to restore nodulation in *nfp* mutant plants, especially to rhizobia producing non sulphated NF. We're also testing different LysM domain swaps to better understand the function of individual domains during the interaction with *S. meliloti*. As the expression pattern of *NFP* is regulated upon inoculation with *S. meliloti,* first restricted to an epidermal area and then strongly induced in the cortex, we're also developing an approach involving tissue specific expression of *NFP* to better understand

the role of this gene during infection. This work was funded by the French Agence Nationale de la Recherche (contract ANR-05-BLAN-0243-01 "NodBindsLysM") and in part by the European Community Sixth Framework Program (contract MRTN-CT-2006-035546 "NodPerception")

Transcriptome-assisted elucidation of white pine blister rust life cycle
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White pine blister rust, caused by the heteroecious macrocyclic rust fungus *Cronartium ribicola* J. C. Fisch, has devastated North American populations of five-needles pines since its introduction. Characterizing the genomic determinants of the various spore stages could improve our understanding of their roles and help designing better control strategies. A normalized cDNA library constructed from a bulk of aecidiospores germinated for 16 h on leaves of *Ribes nigrum* was assembled and yielded 3035 singletons and 335 contigs, for a total of 3370 unisequences. Following a reciprocal homology search on plant and fungal sequence databases, fungal sequence representation was estimated at 19% (636 unisequences). A total of 213 sequences showed significant homology (< e-04) with pathogenicity, virulence or effector genes from fungal, Oomycete and bacterial pathogens of the PHI-base (Version 3.1; 966 genes). Eighty-four putative single nucleotide polymorphisms (SNPs) were detected in 43 different contigs. These resources will be invaluable for the annotation of the *C. ribicola* genome sequence as well as for population studies. Comparing the cDNA libraries of germinating aecidiospores, urediniospores, and basidiospores should provide insight into the molecular mechanisms underlying the host-specific infectivity of the different spore types produced by *C. ribicola*.

Virulence of *Magnaporthe oryzae* is regulated through specific transcription factors
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Magnaporthe oryzae's highly destructive effect on rice and other cereals has been well documented. To manage and potentially control this highly evolved pathogen, we must first understand the complexities of its pathogenecity. Infection depends upon the ability to produce a specialized, highly melanized appressorium. Previous analyses have shown numerous genes to be differentially expressed early during infection process. Two of these genes are; a bromodomain containing protein discovered to interact with the N-terminal CPKA. The cyclic AMP pathway is central in appressoria formation and, bromodomains have been shown to be involved in transcriptional activation. The second is a nuclear localized bHLH transcription factor with possible alternative transcripts expressed in appressoria. Targeted gene deletions demonstrated that both transcription factors are required for normal appressorium formation, and mutants exhibited significantly reduced lesion size and number when inoculated on host leaves. Deleting the bHLH containing protein in particular, resulted in the nearly complete loss of appressorium formation and ability to cause disease. These genes are currently being characterized in further detail. Our studies will help to elucidate and potentially aid in the control of the severely damaging effects of *M. oryzae*.

Deciphering the role of TIR domain in Flax rust resistance proteins
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The majority of known *R* genes belong to the Nucleotide Binding-Leucine rich repeat (NB-LRR) family, and a major subclass of this family contains an N-terminal domain related to the *Drosophila* Toll and human Interleukin-like Receptor (TIR). In animals, Toll-Like Receptors TIR domains play the role of cytoplasmic signalling domains but the precise role of this domain in plant NB-LRR resistance proteins is poorly understood. In flax, the polymorphic L locus encodes TIR-NB-LRR proteins with specific recognition of different Avr proteins of the flax rust fungus (*Melampsora lini*), and previous studies demonstrated that direct R/Avr protein interaction correlates with gene-for-gene specificity. We have shown that mutations in the L6 TIR domain do not affect the interaction between L6 and its corresponding Avr partner AvrL567, but disrupt HR induction *in planta.* Furthermore, overexpression of the TIR domain + 39 residues from the NB domain of a couple of L allele induces spontaneous cell death *in planta*. Taken together, these results support a signalling role for L TIR domain. We also showed that the TIR domain is not required for Avr interaction in yeast, but TIR polymorphism do affect this recognition, indicating that the TIR domain is also involved in the balance between R intramolecular interactions and R/Avr interaction.

The mutualistic interaction of *Arabidopsis thaliana* and *Piriformospora indica* - A metabolomics approach
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The mutualistic root endophyte, *Piriformospora indica*, was co-cultivated in hydroponic culture with *Arabidopsis thaliana* seedlings. The known growth-promoting effect of this fungus could be verified under these conditions. Interaction of the fungus with the plant roots significantly stimulated seedling growth. Upon co-cultivation for two weeks, methanolic extracts were prepared from roots and leaves of the plantlets, as well as of the fungal mycelia. In addition, the culture medium was reduced and pre-fractionated via solid-phase extraction. All extracts were analyzed by non-targeted metabolite profiling using UPLC-ESIqQTOF-based mass spectrometry, which has the potential to detect a broad spectrum of mostly secondary metabolites. Differential metabolite patterns were detected in culture media, roots and mycelia of co-cultivated *versus* individually grown plants and fungus. In contrast, metabolite pattern in leaves showed no significant alteration upon co-cultivation. Most recent results on the metabolite-based analysis of the mutualistic interaction will be presented.

WRKY III transcription factor family in plant stress signalling
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Perception of stresses by the plant leads to reprogramming of the plant transcriptome and subsequent activation of physiological and metabolic changes resulting in adaptation of the plant. Distinct types of stresses might trigger different adaptive responses that are mediated by a complex signal transduction network including transcription factors (TF). The WRKY transcription factor family is specific to plants and appears to be involved in the regulation of various physiological processes including pathogen defence, senescence, wounding and drought responses. WRKY70, which belongs to the WRKY group III, was a node of convergence in plant defence signalling. WRKY70 is a central component mediating cross-talk between SA and JA pathways. In this context, our project is to elucidate the contribution of WRKY III transcription factors family in the regulation of plant stress signalling in *Arabidopsis*. To this aim, a general study of the 13 *Arabidopsis* WRKY III TF was initiated. Expression patterns of WRKY III TFs show a common induction after SA treatment that confirms putative function in plant defence for the whole family. In addition, differential inductions pattern between WRKY III TFs were observed in response to various biotic and abiotic stresses that indicate function in distinct signalling pathways for each WRKY III TF. To investigate their function in plant defence signalling, a reverse genetic approach is used. Plants down regulated for each WRKY (T-DNA mutant or RNAi line) were subjected to Pseudomonas/Botrytis infection and cold/ozone treatment to evaluate these lines for stress tolerance. Finally, to identify crosstalk and regulation between WRKY signalling pathways, WRKY III TF interaction network was characterized by yeast 2-hybrid and co-immunoprecipitation.

Rhizosphere mycoflora of some species of Myristicaceae of the Western Ghats of Karnataka, India
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Rhizosphere mycoflora of *Gymnacranthera farquhariana, Knema attenuata, Myristica dactyloides, M. fatua* var. *magnifica* and *M. malabarica* was studied by soil plating and soil dilution methods. The soil samples were collected from the rhizospheres of each tree species in three seasons namely, summer, monsoon and post-monsoon over a period of two years from the forests of Gersoppa, Uttara Kannada district of Karnataka. The physico-chemical parameters such as soil moisture, temperature, pH, EC, OM, elements like N, P, K, Ca, Cu, Fe, Mn, Mg, Na and Zn were analysed using standard procedures. A total of 99 species of fungi were isolated from the rhizosphere of five tree species and 49 species from the non-rhizosphere region. Only six out of 49 species isolated from non-rhizopshere soils were not observed in the rhizosphere soils. However, fifty out of 99 rhizosphere fungal species were not observed in the non-rhizopshere soils. The Sorensen's index showed the dissimilarity between the mycoflora of rhizosphere and non-rhizosphere soil

to the extent of 35-38%. Among the five tree species, a maximum of 73 fungal species were isolated from the rhizosphere of *M. fatua* var. *magnifica* followed by 67 species in *G. farquhariana,* 65 species in *K. attenuata,* 64 species *M. malabarica* and 63 species in *M. dactyloides.* Whereas 37 species were common to the rhizosphere of all the five tree species and 17 species were observed in the rhizosphere of only one of the five tree species. The dissimilarity was 12–28% within five tree species. Season-wise maximum number of fungal species were recorded during monsoon in all plant species and minimum during post-monsoon as well as summer. The physico-chemical parameter of the rhizosphere soil varied in different plant species in different seasons.

Functional characterization of SRFR1, a suppressor of effector-triggered immunity in *Arabidopsis*

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Mutations in *SUPPRESSOR OF rps4-RLD 1* (*SRFR1*) encoding a tetratricopeptide repeat-containing protein enhance effector-triggered immunity to bacterial pathogens expressing the effector protein AvrRPS4. The mutant *srfr1* alleles are recessive, suggesting that genetically SRFR1 functions as a negative regulator of AvrRps4-triggered immunity. This is supported by our observation that several defense-related genes are upregulated in the *srfr1* mutants. This upregulation however does not trigger constitutive defense responses in the mutant plants. Similar to an increasing number of resistance and resistance-associated proteins, SRFR1 displays nucleo-cytoplasmic localization and may function as a multimer. In preliminary transient expression assays SRFR1 interacts with components of the plants defense network. Elucidating SRFR1 function will allow us to understand how plants tightly regulate resistance responses.

The *WRKY72* transcription factor is involved in a basal defense pathway conserved between tomato and Arabidopsis

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WRKY transcription factors are known to play critical roles in plant defenses to various pathogen and pests. By microarray analyses we identified a WRKY transcription factor to be transcriptionally upregulated in tomato in response to root knot nematodes (*Meloidogyne* spp; RKN). This WRKY is an ortholog of *Arabidopsis thaliana* (Arabidopsis), *AtWRKY72*, a member of subgroup IIb, and therefore named as *SlWRKY72*. IIb-type WRKYs have so far not been genetically proven to play roles in defense. Functional characterization by virus-induced gene silencing implicated a role for *SlWRKY72* in both basal defense to aphids as well as gene-for-gene resistance to RKN and aphids mediated by the *R* gene *Mi-1*. According to the publicly available Arabidopsis microarray data, *AtWRKY72* is upregulated by a number of pathogens and elicitors. Functional characterization of *AtWRKY72* T-DNA insertion lines showed that this gene is also important for basal defense to *Hyaloperonospora parasitica* but not to *R* gene-mediated defenses in Arabidopsis. These results suggest that the function of WRKY72 in basal defense against different pathogens is conserved between tomato and Arabidopsis. Downstream targets of WRKY72 in Arabidopsis will be identified by microarray analysis of the T-DNA insertion lines.

Paranodulation and nitrogen fixation of wheat roots in non-sterile soil

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Symbiotic nitrogen fixation is the most efficient, environmentally friendly, and inexpensive means of providing plants with biological nitrogen. For the farm experiment, we selected bacterial cultures that exhibited the highest nitrogen fixation rates in model experiments which were isolated from the rhizosphere of wheat. Wheat plants were grown in pains containing two Kg with sandy-clay-loam soil. The experiment was carried out in 6 variants with 10 replications as following. 1) Wheat without any treatment (control) 2) Wheat + Abiological nodulation agent (ANA) (2-4-D) 3) Wheat + Biological nodulation agent (BNA) *(Bacillus polymyxa Strain 43)* 4) Wheat + mixed cultures of diazotrophic bacteria (*Arthrobacter* sp + *Xanthomonas* sp). 5) Treatment 4 + ANA 6) Treatment 4 + BNA. The activity of nitrogen fixation in wheat roots in was measured by the acetylene method. We observed the formation of nodule-like structures (*P*- nodule) on wheat roots both after seedlings were treated with the abiogenic nodulation agent or biogenic nodulation agent and after the plants were inoculated with the mixed nitrogen-fixing culture. The formation of *P*- nodules on wheat roots was observed in the 14th day of plant growth. The study of the nitrogenase activity on the wheat plants showed that nitrogen fixation peaked on the 31st days of plant growth, and, was maximum in variant 4 (plant + mixed nitrogen-fixing culture).

Role of phosphorylation on the silencing suppressor activity of the movement protein TGBp1 of the Potato X virus and the relationship with its ATPase activity

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Potato virus X (PVX) is a member of the potexvirus whose RNA genome codes for the viral replicase, three movement proteins (MPs: TGBp1, TGBp2 and TGBp3) and the viral capsid protein. Mounting evidence suggests that phosphorylation events can regulate MP functions. We have previously demonstrated that PVX TGBp1 is phosphorylated by a *Nicotiana tabacum* CK2-like kinase (Módena et al., 2008). The aim of this project is to evaluate the role of phosphorylation on the silencing suppressor and ATPase activities of the PVX TGBp1. Based on in silico analysis of sequences from different potexvirus TGBp1s, we identified two threonine residues possibly subjected to phosphorylation. We have developed different TGBp1 mutants where the identified threonines (T193 and T215) have been replaced by alanine (A) or aspartate (D) residues. Silencing experiments show that the TGBp1-T215A mutant is partially defective on its silencing suppressor activity whereas TGBp1-T215D loses all silencing suppressor activity. On the other hand, we observed that TGBp1-T193A has less suppressor activity due to the fact that it is less stable than wild-type TGBp1. We also show that although protein levels of TGBp1-T193D mutant are normal, it was not able to restore wild-type suppressor activity. Finally, ATP hydrolysis assays show that the ATPase activities of TGBp1-T193A and TGBp1-T215A were similar to wild-type TGBp1, whereas the revertant versions were significantly reduced. These results suggest that phosphorylation of PVX TGBp1 plays an important role in regulating the ATPase activity which might be necessary for silencing suppression.

The analysis of the *Blumeria graminis* in vivo proteome indicates a "stressed-out" fungal partner in a compatible interaction

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We have carried out a survey of the powdery mildew *Blumeria graminis* f. sp. *hordei* proteome in conidia, sporulating epiphytic hyphae and haustoria in infected epidermal cells. A total of 827 proteins were identified by a variety of LC/mass spectrometric techniques in combination with the recently sequenced genome of powdery mildew (www.Blugen.org). We use Gene Ontology/Panther to classify the proteins and map the relevant ones to metabolic pathways (MapMan). This gives an indication of the metabolic status of the fungus in the three stages. A comparison of the proteins found in the tissues reveals striking differences in the classes of genes that are fully and functionally expressed. The surprising finding is that there is a high prevalence of stress related proteins in the haustoria. This suggest that in spite of the fully "compatible" nature of this interaction, the intracellular structures of the pathogen are coping with "stress" imposed by the host, possibly in the form of ineffectual defence responses. For example there are a high proportion of proteins identified in haustoria that are involved in coping with reactive oxygen species, for example hydrogen peroxide, which is itself produced by the barley in response to infection. Moreover, many of the proteins only detected in the haustoria are predicted to be secreted, which may play a role as effectors required for the establishment of the disease. This research provides a unique insight in the development of powdery mildew disease such as the discovery of novel secreted fungal effectors which may influence the plant susceptibility and enhance pathogen virulence during the disease.

Characterisation of the synthesis and secretion of proteins from zoospore peripheral vesicles in *Phytophthora nicotianae*

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During the initial interaction between plants and motile *Phytophthora* zoospores, proteins undergo regulated secretion from three types of vesicles found in the zoospore cortex. All three vesicle types are formed in hyphae

after the induction of asexual sporulation. As part of the characterisation of the function of the secreted proteins, we are investigating spatial and temporal aspects of the synthesis and packaging of proteins into the secretory vesicles. Immunofluorescence microscopy using antibodies raised against proteins found in the so-called large peripheral vesicles and small ventral vesicles was used to determine the timing of vesicle formation and packaging of secreted and non-secreted proteins into these two vesicle types. The immunocytochemistry was complemented with quantitative real-time PCR to determine the timing of vesicle protein gene expression relative to the timing of vesicle formation during asexual sporulation. These results showed differential patterns of gene expression, vesicle formation and protein secretion during asexual sporulation.

The microbial antagonist, *Lysobacter enzymogenes,* infects the unicellular green alga, *Chlamydomonas reinhardtii,* intracellularly

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The Gram negative bacterium, *Lysobacter enzymogenes,* is a known antagonist of lower eukaryotes and other bacteria. Recent studies indicate that *L. enzymogenes* establishes pathogenic interactions with a number of these hosts, including nematodes, fungi and the bryophyte, *Physcomitrella patens,* by infecting them intracellularly. While early research suggested that members of the genus *Lysobacter* lyse blue-green and green algae, internalization of algae by *Lysobacter* has never been reported. In this study, we demonstrate that a more intimate interaction is possible between *L. enzymogenes* and the green alga, *Chlamydomonas reinhardtii.* Co-inoculation of *L. enzymogenes* and *C. reinharditii* results in lysis of algal cells, but the majority of algal cells remains resistant to lysis. This initially resistant population subsequently undergoes four distinct stages of infection: attachment of bacteria to the host cell wall, internalization of *Lysobacter* within *Chlamydomonas* cells, bacterial replication within algal cells and finally, degradation of host cells with concomitant release of bacterial cells. Bacterial secretion systems play a major role in their pathogenesis of higher organisms and two of the most notorious—the type III and type IV secretion systems—as well as numerous virulence traits whose expression is controlled by the Crp-like protein (Clp) global regulator, appear to be implicated in the *L. enzymogenes* infection process of *C. reinhardtii*. Based on these findings, we propose the use of the *C. reinhardtii-L. enzymogenes* pathosystem as a platform for investigating the molecular basis of host-pathogen interactions.

The *Pseudomonas syringae* type III effector HopG1 targets mitochondria, alters plant development and suppresses effector- and PAMP-triggered innate immunity

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The bacterial plant pathogen *Pseudomonas syringae* uses a type III protein secretion system (T3SS) to inject type III effectors into plant cells. A primary target of plant pathogenic type III effectors is the plant innate immune system, which consists of both effector-triggered immunity (ETI) and pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI). The type III effector HopG1 is broadly conserved in T3SS-containing bacterial plant pathogens. We show that HopG1 from *Pseudomonas syringae* pv. tomato DC3000 is injected into plant cells and suppresses both ETI and PTI. Furthermore, HopG1 is targeted to plant mitochondria and its N-terminal two-thirds are sufficient for this localization. This targeting was confirmed by immunofluorescence colocalization with a mitochondrially targeted red fluorescent protein and by subcellular fractionation experiments. Constitutive expression of *hopG1* in *Nicotiana tabacum* (tobacco), *Lycopersicon esculentum* (tomato), and *Arabidopsis thaliana* dramatically alters plant development and mitochondrial function. The alterations cause dwarfism, increased branching and reduced fertility. These findings suggest that plant innate immunity requires mitochondrial functions and that phytopathogenic bacteria target this organelle to promote disease.

Molecular characterization of three AvrBs3-like effectors from the Arabidopsis pathogen *Xanthomonas campestris* pv. *armoraciae*

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Xanthomonas campestris pv. *armoraciae* (*Xca*) is a *Brassicaceae* pathogen and several strains can infect the model plant *Arabidopsis thaliana*. *Xca* strain 5 carries three genes (*hax2*, *hax3*, and *hax4*) encoding members of the large AvrBs3-family of effectors. Effectors of this family employ distinct structural features that are essential for their molecular activity within the plant cell. They contain nuclear localization sequences and a transcriptional activation domain that both are essential and enable these effectors to act as transcriptional activators of plant genes. The central domain of effectors from this family consists of a tandem array of typically 34 amino acids. The repeats are nearly identical but vary at certain positions. The repeat region mediates interaction of the effector with promoters of target plant genes. Hax3 and Hax4 contain 11.5 and 14.5 repeats of 34 amino acids each, respectively, whereas Hax2 contains 21.5 repeats of 35 amino acids each. Together, the three *hax* genes are required for full pathogenicity of *Xca* strain 5 on radish plants. We will present recent progress on identification of target genes for Hax2, Hax3, and Hax4 from Arabidopsis.

The effect of plant defense on the uncultured bacterial community

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The plant leaf harbors a diverse assemblage of bacteria, ranging from pathogenic to symbiotic. Little is known about how plant defenses affect the uncultured bacterial community in nature. The goals of this project are to: 1) survey the uncultured bacterial community associated with *Arabidopsis thaliana* in the wild, 2) determine how the uncultured microbial community structure is influenced by host plant defenses. First we characterized the uncultured microbial communities associated with *A. thaliana* and compared endophytic and epiphytic communities, as well as communities associated with roots and rosettes, different field sites and different time points. We used 454 pyrosequencing to sequence the V6 hypervariable region from the 16S rRNA gene. Analysis of 24 samples yielded 201,222 partial bacterial 16S rRNA sequences. The epiphytic community was found to be more species rich than the endophytic community. The most abundant genera identified in the community are *Pseudomonas* and Sphingomonas, and members of the Oxalobacteraceae, Rhizobiaceae, and Microbacteriaceae families. A field experiment was performed with *Arabidopsis* defense mutants: coi1-1 (JA-insensitive mutant), npr1-1 and sid2-1 (two mutants impaired in SA signaling), and pad3-1 (lacking the antimicrobial compound camalexin). The bacterial community associated with these mutants is currently being analyzed. This experiment will provide information about how variations in plant defense influence the plant-associated uncultured bacterial community.

Evolutionarily distinct RXLR effectors from distantly related oomycetes target the plant exocyst

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The endocytic cycle plays a crucial role in plant defence. For example, the recycling of membrane receptors has been shown to be essential for their signalling activities. It is logical therefore that plant pathogens will attempt to manipulate the endocytic process. Using yeast-2-hybrid analysis we have discovered that the *Phytophthora infestans* effector AVR3a interacts with the potato homologue of Sec5. Sec5 is a component of the multi-subunit exocyst vesicle tethering complex which is specifically involved in trafficking between Golgi and the plasma membrane. We have also found that the Arabidopsis Sec5 homologue is targeted by diverse RXLR effectors from *Hyaloperonospora arabidopsidis,* indicating that it is a pivotal target for oomycete pathogenicity. Moreover, we show that evolutionarily distinct RXLR effectors from either *P. infestans* or *H. arabidopsidis* interact strongly with Sec5 homologues from either potato or Arabidopsis, indicating that these effectors are functionally related and capable of similar interactions in diverse plant hosts. Using fluorescent protein tags, we localise Sec5 and Sec5-effector interactions during infection. We show, using virus-induced gene silencing, that *Sec5* does indeed contribute to host innate immunity.

The barley-*Pyrenophora teres* interaction: Mapping defence response genes in the host and analysing the population genetic structure of the pathogen

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Barley net- and spot-form of net blotch diseases are caused by two *formae* of the fungus *Pyrenophora teres* (*P. t.* f. *teres* [*Ptt*] and *P. t.* f. *maculata* [*Ptm*]).

In the first part of this study, the genetic diversity among 3 *Ptt* and 2 *Ptm* populations sampled from within separate barley fields located in South Australia was investigated using microsatellite markers. The screening of markers across 20 loci revealed 90% of them to be polymorphic. The *Ptt* and *Ptm* populations showed a comparable level of genetic similarity, as indicated by similar Nei's gene diversity measures (0.38 and 0.39) and same Nei's G_{ST} (0.03). Low pairwise comparisons of θ values (< 0.008 and 0.018) indicated there was no significant differentiation between populations within each group. The level of genetic diversity increased when the *Ptt* and *Ptm* populations were analysed as one group, with pairwise comparisons of θ values showing there was significant genetic differentiation between them (θ >0.571). In the second part of this study, 21 defence response [*DR*] genes associated with the barley-*P. teres* incompatible interaction were mapped in barley using doubled-haploid mapping populations. The 21 *DR* genes were distributed across all seven barley chromosomes, with at least one gene mapping to within 15 cM of another on chromosomes 1H, 2H, 5H and 7H. Additionally, some *DR* genes appeared to co-localise with regions harbouring known resistance genes or quantitative trait loci for net blotch resistance on chromosomes 6H and 7H, as well as loci associated with resistance to other barley leaf diseases. The results of both studies combined give an insight into the genetic structure of the host crop and pathogen population and will aid the implementation of biocontrol strategies for barley net blotch disease.

Analysis of *Cercospora beticola* mating type gene structure in north central USA

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Cercospora beticola Sacc. is a necrotrophic fungus that causes Cercospora leaf spot of sugarbeet (*Beta vulgaris*). The fungus is considered to be the most destructive foliar pathogen of sugarbeet worldwide, causing significant economic losses primarily due to reduction in sucrose concentration and root yield. Field isolates of *C. beticola* are well-known for high levels of variability, particularly with regard to morphological characteristics and fungicide resistance. Nonetheless, despite the diversity found in *C. beticola* populations, no teleomorph has been described for *C. beticola*. The mating type genes *MAT1-1-1* and *MAT1-2-1* genes of *C. beticola* were recently cloned and used to characterize populations in Western Europe, Iran, and New Zealand, but no such work has been carried out on North America isolates of *C. beticola* to our knowledge. We therefore characterized the mating type gene structure of *C. beticola* isolates from sugarbeet production area of north central United States. In addition, isolates were tested for sensitivity to several classes of fungicide to determine if mating type correlated with variability in fungicide sensitivity. A detailed analysis of mating type gene structure and the relationship to fungicide sensitivity will be presented.

Characterization of the plant molecular mechanisms governing the Nod-independent symbiosis between *Aeschynomene* and photosynthetic *Bradyrhizobium*

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The most studied plant / bacteria symbiosis is the legume-rhizobia interaction which results in the formation of nodules, a specialized organ in which bacteria fix nitrogen for the plant benefit. The establishment of this symbiosis involves a complex molecular dialogue between the 2 partners for their mutual recognition. Nod factors (derived from lipochitooligosaccharide) synthesized by rhizobia and encoded by nod genes, are recognized by specific plant kinases. This first step of recognition triggers a succession of events leading to the formation of nodule. This molecular recognition process has been described in all rhizobia / legume couples characterized so far, but the universality of this paradigm has been recently overturned by our study of the *Bradyrhizobium* photosynthetic-*Aeschynomene* symbiosis. Indeed, an analysis of the genomic sequences of two photosynthetic *Bradyrhizobium* strains failed to detect the presence of canonical nod genes from these bacteria; this demonstrates that Nod factors are not required to induce nodular organogenesis in *Aeschynomene* (Giraud et al. Science, 2007). This result raises the question of the degree of conservation of the symbiotic signalling pathway in this original Nod-independent interaction. Our purpose is thus to study the plant molecular mechanisms governing this new interaction and in particular to determine whether the signalling pathway triggering nodulation in *Aeschynomene* is common to the one described in the model legumes *Medicago truncatula* and *Lotus japonicus*. We are currently developing molecular tools in *Aeschynomene* with the aim of study the function of candidate plant genes implied in the early phases of the interaction with photosynthetic *Bradyrhizobia*. First results and considered approaches will be presented.

The constitutive expression of a glutaredoxin from C. chinense leads to a reduced accumulation of the tobamovirus pepper mild mottle virus strain I (PMMoV-I) in N. benthamiana plants

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By mRNA differential display of compatible and incompatible PMMoV-I and –S-C. chinense interactions, a cDNA band was isolated whose expression was induced in both interactions. The nucleotide sequence analysis and subcellular localization by confocal microscopy revealed that it corresponded to a mono-thiol glutaredoxine (grx) from the chloroplast. Transgenic N. benthamiana plants expressing either the full-length grx or a truncated form that accumulates to the nuclei has been obtained. A reduced accumulation of PMMoV-I is observed at latest stages of infection that results in a major recovery of the infected plants as well as in a increased seed production in these plants.

Genomic studies of *Albugo candida – Arabidopsis thaliana* interactions

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Genomic studies of *Albugo candida – Arabidopsis thaliana* interactions White rust, which is caused by the obligate oomycete *Albugo candida* (Pers. ex. Fr.) O. Kuntze, affects Brassicaceae species including cultivated Brassica crops and the model plant *A. thaliana*. The response of *A. thaliana* cultivars has been used to define host defense mechanisms against different *A. candida* isolates. Two resistance genes, *RAC1* (resistance against isolate Acem1) and *WRR4* (a broad spectrum white rust resistance gene), have been characterized. We have taken a genomic approach to identify *A. candida* effector molecules that are involved in the interaction with its host plant. A database of approximately 42,000 ESTs from *A. candida* isolate Ac2v generated from infected tissue or from spores was developed. cDNAs encoding proteins with similarity to known elicitors from other oomycetes, as well as several 'RXLR' type effectors were identified. Comparison of the cDNA frequency from libraries generated from spores with those from infected tissue revealed that genes encoding elicitors as well as several unknown proteins were up-regulated during the infection. We also initiated a project to sequence the *A. candida* Ac2V genome using the 454 sequencing platform. To date, close to 550 Mbps of genomic sequence have been generated which has been assembled into 32 Mb of contigs with an average length of 7.5 kb and N50 of 27.6 kb. The largest contig in the assembly was 165.9 kb and nearly all (99%) the ESTs generated from Ac2V infected tissues or spores were identified on the assembled contigs.

Towards understanding the role of aphid salivary gland proteins in plant infestation

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Aphids are amongst the most devastating hemipteran sap-feeding insects of plants. During epidemics, aphids reach high population densities and consequently, induce extensive feeding damage. In addition, they vector the majority of described plant viruses worldwide. Aphids feature well developed salivary glands that consist of several specialized cells that are likely to produce proteins required for establishing successful interactions with host plants. *Myzus persicae* (green peach aphid) is considered a generalist, with host plants in over 40 plant families, and is an important pest worldwide. We hypothesize that during plant infestation *M. persicae* secretes sets of salivary gland proteins that enhance the aphid survival and fitness as well as affect host range specificity by modulating host cell processes. Our aim is to identify and characterize these proteins and to elucidate the molecular mechanisms underlying their functions. Genomics resources recently became available offering unprecedented opportunities for investigating aphids and the perturbations they cause in plants. We applied a data mining strategy combined with functional assays to identify and functionally characterize secreted salivary gland proteins from *M. persicae*. We identified 115 proteins

from a salivary gland EST database (3233 ESTs) that are predicted to be secreted. Currently, we are screening this set of proteins for effects on aphid survival, fitness and host range specificity using in planta over-expression followed by aphid challenge as well as RNAi in aphids. In addition, we are using transient over-expression assays in *Nicotiana benthamiana* to investigate whether these proteins affect plant cell processes, especially those involved in defense. Progress on our efforts will be presented.

Xanthomonas campestris pv. campestris exploits _N_-acetylglucosamine during infection

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Xanthomonas campestris pv. *campestris* (*Xcc*), the causal agent of black rot disease of Brassicaceae, possesses specific CUT systems (Carbohydrate Utilization systems containing TonB-dependent transporters), devoted to the scavenging of plant carbohydrates. We identified a new *Xcc* CUT system involved in the exploitation of *N*-acetylglucosamine (GlcNAc) from plant origin. This GlcNAc CUT system, which is under the control of NagR and NagQ regulatory genes, is divided into an upper pathway involved in the external generation and uptake through the outer membrane of GlcNAc-containing carbohydrates and a lower pathway corresponding to the uptake through the inner membrane and catabolism of GlcNAc. We show that this system is used during the infection of host plants. Indeed, a *nagA* mutant, affected in GlcNAc catabolism and sensitive to this compound, is unable to grow *in planta* and to induce disease symptoms. This phenotype is reversed when mutations in the upper pathway are introduced in *nagA* mutants, thus underlying the importance of the upper pathway in the generation of GlcNAc *in planta*. Based on glycoside hydrolase activities belonging to the upper pathway, we propose that glycans from glycoproteins might be the source of GlcNAc for *Xcc* during infection. Our comparative study shows that the *Xcc* CUT system is specifically conserved in phytopathogenic *Xanthomonadaceae* suggesting that the degradation of glycans of plant origin is a common feature of this bacterial family. Although GlcNAc is an important nutrient source for chitinolytic bacteria, the exploitation of this molecule by phytopathogenic bacteria was never suspected. Therefore, this work broadens the source of GlcNAc metabolized by bacteria and sheds new light on metabolic capabilities of plant pathogenic bacteria.

A medium-throughput strategy for expression/purification of _Phytophthora infestans_ RxLR effectors for structure/function studies

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Recent investigations have identified the effector repertoire of the potato late-blight pathogen *Phytophthora infestans*. One set of effectors, classified according to the presence of an RxLR sequence motif which mediates translocation into plant cells, have received particular attention. Despite harboring a readily identifiable host-targeting sequence, the biological functions of these effectors remain poorly understood; largely because they share little sequence homology with proteins of known function. Determining the three-dimensional structure of *P. infestans* RxLR effectors is an exciting alternative for understanding protein function and directing further study. The validity of this approach has been demonstrated numerous times for effectors translocated by plant and mammalian pathogens via a type III secretion system. Here, we present current progress towards this aim, focusing on medium-throughput expression/purification studies of a sub-set of *P. infestans* RxLR effectors for which expression is known to be induced during infection of potato. Purified RxLR effectors will be used for both biochemical and structural studies.

Gain of disease susceptibility in Arabidopsis by inactivating a lectin receptor kinase or by overexpressing a _Phytophthora infestans_ RXLR effector

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Phytophthora infestans secretes numerous RXLR effectors that could play a role in colonizing host plants. One of these is IPI-O, which - like other RXLR proteins - contains at its N-terminus a signal peptide for secretion and a RXLR-dEER motif for host cell internalization whereas the C-terminal domain is required for effector functions. The RXLR domain of IPI-O partly overlaps with a RGD cell adhesion motif, which has been shown to bind to a lectin receptor kinase in *Arabidopsis* that may function as an effector target. This lectin receptor kinase (LecRK79) participates in protein-protein interactions to mediate cell wall-plasmamembrane adhesions and it has been observed that IPI-O can disrupt these adhesions. Transgenic *Arabidopsis* lines expressing *ipiO* and *Arabidopsis LecRK79* knock-out lines (*lecrk79*) were analyzed in their response to pathogen infection, in particular to *Phytophthora*. Both, *lecrk79* and the *ipiO* expressing lines show a change in susceptibility to infection with oomycete pathogens and are behaving as phenocopy lines. Disrupted cell wall-plasmamembrane adhesions in both lines are likely to reduce defence responses. Accordingly, elicitor triggered cell death can be suppressed in *ipiO* expressing lines and *lecrk79*. In incompatible interactions with *Phytophthora brassicae* and *P. infestans* the expression of *LecRK79* increases, suggesting a role in hypersensitive responses. Furthermore, when overexpressing *LecRK79* in Arabidopsis, disease resistance is promoted. Our observations strongly suggest that LecRK79 plays a crucial role in disease resistance and point toward involvement of the RXLR effector IPI-O in the infection process.

New structures of O-specific polysaccharides of _Azospirillum_ spp.

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Lipopolysaccharides (LPSs) were isolated from the outer membranes of the bacteria *Azospirillum brasilense* SR55 and *A. lipoferum* SR65 grown in a liquid malate medium to the end of the exponential growth phase. These microorganisms had been isolated from the rhizospheres of wheat grown on poor saline soils in a Saratov region arid zone with a sharply continental climate. The bacteria are of interest as promising inoculants for bread cereals grown in problem agricultural zones. By using mild degradation, we isolated O-specific polysaccharides (OPSs) from the LPSs. Chemical and spectroscopical investigations of the OPS of SR55 established that its repeating unit was an octasaccharide whose main chain contained two α-D-galactoses, three nonsubstituted L-rhamnoses, and two substituted L-rhamnoses, with one being methylated at position 3 and the other being substituted by α-D-glucuronic acid at position 2. This is the first time that a heterooligosaccharide has been found as part of an OPS repeating unit among *A. brasilense* strains; also, this is the first time that uronic acid, characteristic of *Azospirillum* capsular polysaccharides, has been found as part OPS. We showed that the OPS of SR65 was a neutral PS that contained a tetrasaccharide whose main chain was built up from three L-rhamnoses associated by α-1-3 glycosidic linkages, with one rhamnose being substituted by β-D-glucose at position 3. Interestingly, the OPS of *A. lipoferum* SR65 and the previously studied OPSs of *A. brasilense* S17 and *A. lipoferum* SpBr17 have polysaccharides with the same sugar composition, but their repeating units have different structures. Thus, the established structures have supplemented the list of *Azospirillum* OPSs in which D- or L-rhamnose is the predominant sugar.

Identification of oomycte effector targets using _in planta_ co-immunoprecipitation

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Oomycete pathogens deliver a variety of effector proteins into plant host cells to suppress defense responses and enable successful colonization. The mechanisms involved in transport of oomycete effectors and host defense manipulation remain unclear. In this study, we aimed to find target proteins of the 52 validated oomycete effectors including several with avirulence activity (RXLR family) to elucidate their biological function and derive hypotheses about the effector mode of action. We used an in vivo co-immunoprecipitation (co-IP) assay to identify the targets of our effectors. We made expression constructs by replacing the secretion signals with the Flag tag and cloning into pJL-TRBO, a binary plasmid derived from a modified *Tobacco mosaic virus*. We delivered effector constructs into the leaves of *Nicotiana benthamiana* and transiently overexpressed them by agroinfiltration. We then harvested the leaves 2-3 days after infiltration and extracted total proteins. Effector proteins and their interactors from the plant were co-IPed with anti-FLAG resins under non-denaturing conditions followed by elution using 3X FLAG peptides. Eluted proteins were then run on SDS-PAGE, gel slices were excised, digested with trypsin, and identified by LC-MS/MS. Accepted proteins were

required to have Mascot scores of more than 50 and two or more unique peptides identified. We are using reverse co-IP, split YFP assay and yeast two-hybrid system to confirm interactions between effectors and the putative targets. We will report and discuss identified effector target proteins and any alterations in plant immunity resulting from overexpression or virus-induced gene silencing of these targets.

Interactions of human enteric pathogens with *Dickeya dadantii* in soft rot lesions on lettuce and cilantro leaves

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Multiple outbreaks of salmonellosis and *E. coli* O157:H7 infections have been linked to leafy vegetables in the US and Europe, revealing that human pathogens can exploit the plant habitat and thereby affect public health. We have investigated the fitness of *Salmonella enterica* and E. coli O157:H7 in the lettuce and cilantro phyllosphere, and their interaction with soft rot plant pathogens such as Dickeya dadantii (*Erwinia chrysanthemi)* and *Pseudomonas viridiflava*. Both enteric pathogens reached large population sizes and formed complex biofilms on leaves infected with D. dadantii. Microarray analysis of the gene expression profile in S. enterica cells colonizing lettuce and cilantro soft rot lesions caused by *D. dadantii* revealed that the pathogens deploy a large part of their transcriptional machinery for the utilization of propanediol and ethanolamine, substrates that result from degradation of plant cells by the soft-rot pathogen. Population sizes of propanediol-defective mutants of *S. enterica* in macerated cilantro leaves were reduced 10-fold compared to the parental strain. Also, *S. enterica* genes involved in the transport of, or regulated by, quorum sensing autoinducers were upregulated in *D. dadantii* soft rot lesions, indicating the possibility of cross-talk between the plant and human pathogens. Additionally, S. enterica cells recovered from soft rot lesions were more invasive of eukaryotic cells than when grown in culture. Our observations suggest that plant pathogens contribute to creating a hospitable niche for enteric pathogens on plants, which may also affect their infectious dose in humans.

The *Ustilago maydis* pathogenicity island 19A facilitates biotrophic development and tumor formation in maize

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The biotrophic fungus *Ustilago maydis* causes smut disease in maize. During biotrophic growth this fungus penetrates the cuticle, establishes a mycelial network within the plant apoplast and finally induces tumors on all aerial parts of its host. The fungal effectors facilitating these biotrophic interactions have remained elusive. We have identified the *U. maydis* cluster 19A, a 43kb genomic locus comprising 24 plant-induced genes for novel secreted proteins. This gene cluster is required for tumor formation and anthocyanin induction. Microscopic analysis has shown that cluster 19A mutants are able to penetrate and proliferate within the leaf epidermis while hyphal proliferation in the mesophyll layer remains restricted to vascular tissue. This is associated with the induction of necrotic lesions, most likely due to elicitation of a late plant defense response. Genetic dissection of this locus has revealed that at least 8 effector genes additively contribute to the described phenotypes. Respective, biologically active GFP fusion proteins have been generated to localize the major effectors during *U. maydis* infections and upon transient expression in maize. The isolation of yeast-two-hybrid interactors suggests that some of the secreted effectors might interfere with defense responses and development of the plant host. To elucidate the impact of 19A effectors on host responses we are currently performing microarray analyses comparing the host transcriptomes upon infection with wild type and different 19A mutant strains. Results of these studies will be presented.

Genotype-dependent response of wheat to the *Fusarium* mycotoxin deoxynivalenol and associated gene network components

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Fusarium fungi can attack the heads of small-grain cereals resulting in Fusarium head blight disease (FHB). These fungi have the ability to produce trichothecene mycotoxins such as deoxynivalenol (DON) in infected grain. Wheat cultivars differ in their resistance to both FHB disease and their tolerance of DON. The mycotoxin DON acts as a virulence factor for *Fusarium* fungi, facilitating fungal spread within wheat tissue and the development of FHB disease. Like FHB disease, DON induces premature bleaching of wheat spikelets. The ability of wheat spikelets to resist DON-induced bleaching is genotype-dependent. In wheat cultivar (cv.) CM82036 DON resistance is associated with a quantitative trait locus, Fhb1, located on the short arm of chromosome 3B. Gene expression profiling (micro-array and real-time RT-PCR analyses) of DON-treated spikelets of progeny derived from a cross between cv. CM82036 and the DON-susceptible cv. Remus discriminated ten toxin-responsive transcripts associated with the inheritance of DON resistance and Fhb1. These genes do not exclusively map to Fhb1. Based on the putative function of the ten Fhb1-associated transcripts, we discuss how cascades involving classical metabolite biotransformation and sequestration processes, alleviation of oxidative stress and promotion of cell survival might contribute to the host response and defence against DON.

Poly(ADP-ribosyl)ation and host-pathogen interactions

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Poly(ADP-ribosyl)ation is a post-translational modification in which ADP-ribose polymers are attached to a protein. Activation of poly(ADP-ribosyl)ation has been linked in animals to the DNA damage response and programmed cell death. We have discovered that poly(ADP-ribosyl)ation processes are activated during, and have an influence on, plant responses to pathogen attack. We initially hypothesized a role for poly(ADP-ribosyl)ation in plant defense responses when we detected up-regulated expression of the poly(ADP-ribose) glycohydrolase gene *PARG2* after infection with *Pseudomonas syringae* pv. *tomato* or *Botrytis cinerea*, or upon treatment with flg22, a bacterial flagellin-derived elicitor of innate immune responses. We also find that levels of ADP-ribose polymer increase moderately upon infection and that poly(ADP-ribosyl)ation of discrete proteins occurs. Paradoxically, while *PARG1* gene expression is not significantly altered during plant defense responses, *parg1* but not *parg2* mutants display exaggerated responses to MAMPs such as flg22 or elf18. However, loss of either *PARG1* or *PARG2* results in an accelerated onset of *Botrytis*-induced disease symptoms. In addition to genetic approaches, experiments with pharmacological inhibitors of poly(ADP-ribose) polymerase (PARP) have revealed disruption of some but not all flg22-elicited responses. Callose deposition, lignin deposition and seedling growth inhibition are significantly altered, but the oxidative burst and expression of defense-associated genes remain normal after MAMP elicitation of plants treated with PARP inhibitor. Our more recent progress in defining the contributions of poly(ADP-ribosyl)ation to plant-pathogen interactions will be discussed.

Quantitative traits control the timely onset of post-invasive defense responses to non-adapted powdery mildews in Arabidopsis

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Immune responses to non-adapted powdery mildews in Arabidopsis require two parallel secretory pathways restricting fungal entry into leaf epidermal cells (pre-invasion resistance). One pathway involves PEN1, SNAP33, and VAMP721/722 SNARE proteins for vesicle-mediated exocytosis (Kwon et al., 2008). The second pathway engages peroxisome-associated PEN2 and the plasma membrane-resident ABC transporter PEN3 to deliver indole glucosinolate metabolites into the apoplast (Lipka et al., 2005; Stein et al., 2006, Bednarek et al., 2009). A common feature of *pen1* and *pen2* mutants is cessation of invasive fungal growth coincident with a host cell death response. Genetic analysis demonstrated that post-invasive fungal growth restriction requires the signalling proteins EDS1, PAD4, and SAG101 (Lipka et al., 2005). To identify other components of post-invasive resistance, the *pen2* mutant line was EMS-mutagenized and the obtained M_2 population was screened for altered infection phenotypes following inoculation with non-adapted *Blumeria graminis* f. sp. *hordei* and *Erysiphe pisi* conidiospores. One line (named #157, Col 0 *pen2*-1) showed both enhanced *B. graminis* and *E. pisi* epiphytic hyphal growth without differences in entry rates compared to

pen2 mutant plants. This suggests a delay in the onset of post-invasive defense responses in mutant #157. We have undertaken a map-based cloning approach to isolate the locus in mutant #157 that controls the timely onset of post-invasive defense responses. Unexpectedly, we found an ecotype-dependent variation of the *pen2* infection phenotype and a quantitative variation of the #157 phenotype in Col and Ler backgrounds. To overcome this complication, we have adopted a gene isolation strategy utilizing QTL analysis combined with SOLEXA whole genome re-sequencing of line #157.

Signaling pathways involved in *Brassica napus* defense response against pathogens contrasting in their infection strategies

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Plant defense responses are controlled by mechanisms dependent on hormone signaling. It has been generally suggested that resistance to bitrophic pathogens is conferred by salicylic acid (SA)- dependent defense mechanisms, while resistance to necrotrophs is modulated by jasmonic acid (JA)- signaling. More complicated situation occurs after attack by pathogen utilizing the both strategies, called hemi-biotroph. In order to identify signaling pathways involved in host interaction with hemi-biotroph we focused on the fungal pathogen *Leptosphaeria maculans*, a causal agent of blackleg disease of oilseed rape. In our experiments we investigated the activation of SA-dependent and SA-independent pathways recording the expression of marker genes by means of RT-qPCR. The primers were designed on ESTs with high homology to *Arabidopsis* genes. For comparison, both biotrophic pathogen, *Turnip yellow mosaic virus* (TYMV), causing only mosaic and blotching symptoms in Brassica plants without any necrotic symptoms, and a necrotrophic one, *Sclerotinia sclerotiorum*, inducing tissue necroses, have been taken under the study. Expression of marker genes *PR1* (SA-dependent), *AOS* (JA-dependent), *HEL* (ethylene-responsive), and *SYP121* (ABA-responsive) in *B. napus* plants infected by pathogens differing in their lifestyle indicates considerable overlap in defense signaling.

Comparative analysis of type III secretion in bacteria that cause disease in plants and mushrooms

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Many pathogenic bacteria, including the phytopathogen *Pseudomonas syringae*, utilize a molecular syringe known as a type III secretion system (T3SS) to inject 'effector' proteins into host cells, where they promote virulence and suppress host defence responses. Despite these well characterized functions, T3SSs are increasingly being identified in bacteria that are not commonly associated with plant or and animal diseases, such as *Pseudomonas fluorescens*. Through multi-locus sequence typing and degenerate PCR we have determined the distribution of T3SSs in the *P. fluorescens* species complex, notably in strains pathogenic to cultivated mushrooms. We have used Illumina/Solexa sequencing technology to generate genomic data for several mushroom pathogenic strains for the purpose of comparative genome analysis and we are currently using bioinformatic methods to identify and assess the distribution of T3SS effectors in mushroom pathogenic *Pseudomonas*. Potential target organisms for these effectors include fungi, plants or other organisms that have a significant impact on the productivity of agricultural and mycocultivation systems. We are currently performing functional studies using heterologously expressed effectors, gene knockouts and reporter fusions to understand the potential roles of T3SSs in these strains.

Changes in *Capsicum chinense* transcript population induced by root infection with *Phytophthora capsici*

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Plants have evolved a number of defense strategies to protect themselves from pathogen invasion. Pathogen challenge can trigger an integrated set of signal transduction pathways, in distal and local tissue. Phytophthora blight, caused by the oomycete pathogen *Phytophthora capsici*, is a devastating disease on peppers. We studied the interaction of *P. capsici* with *Capsicum chinense* (habanero pepper) which is an important crop in Yucatán, México. We specifically examined the transcriptional changes that occur in systemic tissues after root inoculation with a compatible isolate of *P. capsici*, using the differential display technique. Among the findings, we detected transcripts whose deduced aminoacid sequences belong to diverse metabolic processes, including alternative nitric oxide synthesis, nitrogen metabolism and trafficking of intracellular vesicles. These results suggest an influence of the infection over defense responses, as well as the basal metabolism. The expression of these genes was also studied in response to various treatments, like external application of plant hormones such as salicylic (SA) and jasmonic (JA) acids. MGC has a CONACYT PhD fellowship 204985; this project is supported by CONACYT P54831.

The coffee CaNDR1 protein restores bacterial resistance in the *Arabidopsis thaliana ndr1-1* mutant and is localized in the plasma membrane

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Coffee leaf rust, caused by the fungus *Hemileia vastatrix*, is a major disease of coffee (*Coffea arabica*). The manipulation of genes that regulate disease resistance pathways in *C. arabica* is a promising strategy for achieving broad spectrum resistance in this species. The *CaNDR1* gene, displaying sequence homology to the *Arabidopsis thaliana NDR1* gene, is up-regulated during the hypersensitive reaction (HR) of *C. arabica* to *H. vastatrix*. The role of *CaNDR1* in disease resistance was examined by genetic complementation of the *A. thaliana ndr1-1* mutant using a 35S::*CaNDR1* cDNA construct. Wild-type (Col-0), *ndr1-1* mutant and three *35S*::*CaNDR1* T3 *ndr1-1* lines of *A. thaliana* were inoculated by leaf infiltration with *Pseudomonas syringae* pv *tomato* (Pst), and bacterial growth in inoculated leaves was monitored for up to 96 h. Over-expression of CaNDR1 restored resistance to Pst strain DC3000 carrying avirulence gene *avrRPT2* in the three *35S*::*CaNDR1* lines of *A. thaliana ndr1-1*. HR-like symptoms were visualised in leaves inoculated with Pst DC3000 (*avrRPT2*). Analysis of transcript accumulation of a set of defence-marker genes showed that *CaNDR1* over-expression in the *ndr1-1* mutant restored the defence gene expression patterns observed in the wild-type plants. *Agrobacterium tumefasciens*-mediated transient expression assays were conducted in tobacco (*Nicotiana benthamiana*) for assessing the cellular sublocalization of the CaNDR1 protein. Western-blot analysis using anti-HA antibodies identified a single HA-tagged CaNDR1 band in membrane protein extracts and imaging in confocal microscopy detected the GFP-fused CaNDR1 in the plasma membrane. These data show that CaNDR1 is a true homolog of NDR1 and may be a suitable regulator of disease resistance pathways to improve coffee for enhanced and broad spectrum resistance to pathogens.

TILLING and ecoTILLING in grapevine

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The availability of two annotated grapevine genome sequences provides a solid foundation for functional genomic tools. To characterize gene function in relation to powdery mildew pathogenesis, we have pursued two reverse genetic approaches centered on TILLING technology. First, in collaboration with the Seattle TILLING Project, we adapted established protocols for application in grapevine. Using an M2 population of EMS-induced mutants, we found that even in a highly heterozygous organism like grapevine, TILLING can be used to detect induced point mutations in candidate genes. Second, we are screening wild and cultivated *Vitis* germplasm for naturally-occurring functional variants of candidate genes, through application of ecoTILLING. For gene diversity projects such as ecoTILLING, abundant diversity is available through the USDA-ARS National Plant Germplasm System (NPGS), which maintains and distributes over 4200 accessions of *Vitis* spp. This molecular characterization of pathogenesis-related gene diversity at NPGS will interface with disease resistance screens we recently completed in the same collection. In addition to enhancing our understanding of gene function in pathogenesis, these complementary approaches based on TILLING technology could provide new germplasm for use in grapevine breeding programs.

Nitrogen transports can act as natural switches for plant resistance signaling

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Nitrogen levels have been associated to enhanced susceptibility of plants to different pathogens. However, mutation in a high affinity nitrate transporter (HATS) codified by *NRT2.1* enhances resistance and is not associated to different nitrate levels in normally fertilized plants. *nrt2.1* shows lower susceptibility to *Pseudomonas syringae* with reduced disease rates and lower bacterial growth inside the plant tissue. This reduced susceptibility is linked to an enhanced SA production only once the infection is established. In addition, ABA levels do not change in *nrt2.1* during the infection while it is increased in wild type plants. Coronatine less Pst DC3118 produce reduced symptoms on Ws background while it grows as Pst DC3000 in *nrt2.1*. Therefore it seems that *nrt2.1* has some disruption in ABA signaling which probably results in a deficient ABA control by the pathogen. This establishes a possible link between nitrate transporters and plant responses to biotic stresses.

Localization of DIR1 during Systemic Acquired Resistance in Arabidopsis

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Long distance signaling between plant tissues is important in disease resistance pathways such as Systemic Acquired Resistance (SAR). SAR is induced by an initial priming infection in one leaf resulting in resistance throughout the plant to normally virulent pathogens. A key feature of SAR is the movement of mobile signals from the induced leaf to distant leaves where these signals are perceived. Research in our lab using dir1-1, a SAR-defective Arabidopsis mutant, indicates that DIR1 is involved in the long distance signaling stage of SAR. Western analysis demonstrated that DIR1 protein is present in phloem sap of SAR-induced wild type, but not mock-induced or dir1-1 plants suggesting that DIR1, which encodes a putative lipid transfer protein, may be involved in transporting a lipid signal to distant tissues to establish SAR. Expression of DIR1 in one leaf of dir1-1, using Agrobacterium-mediated transient transformation, followed by SAR induction, rescued the dir1-1 SAR defect suggesting that expression of functional DIR1 in the induced leaf is sufficient for a successful SAR response. If DIR1 is a SAR long distance signal, it should move from the induced leaf to distant leaves during SAR. Preliminary Agrobacterium-mediated transient transformation experiments suggest that DIR1 does move to distant leaves during SAR. We are also developing a cucumber-Arabidopsis SAR model combining the excellent genetics of Arabidopsis and the robust SAR response of cucumber. Preliminary experiments indicate that phloem exudates from SAR-induced cucumbers contain a DIR1-like protein that can rescue the Arabidopsis dir1-1 SAR defect suggesting that both species use similar long distance signaling mechanisms during SAR.

A pseudo-ratiometric analysis reveals actin polymerization sites in legume living root hair cells

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Actin dynamics plays a pivotal role during growth, cell expansion, intracellular transport of pathogens (e.g. *Listeria*), polar growth, cytoplasmic streaming, and growth oscillations. It can also modulate ion channel activity in animal cells and controls the opening of stomata in plant cells. Previously, we have reported that the actin cytoskeleton of the root hair cells responds with a rapid fragmentation of the actin bundles after Nod factors exposure. This data supports the idea that actin microfilaments have an important function during the rhizobial interaction and during mycorrhizal association. In this work, the capability of fluorescently labeled cytochalasin (Fl-Cyt), which binds to the plus end of microfilaments where polymerization occurs, was analyzed. A pseudo-ratiometric analysis using sub-lethal drug concentrations and a cytoplasmic reference dye was developed. These experiments demonstrate that Fl-Cyt is able to label the plus ends of the actin microfilaments and that these polymerization sites are localized at the tip of the root hair. Time-lapse imaging unravels the dynamics of the polymerization sites showing continuously reorganization. To our knowledge this is the first time that actin polymerization sites are visualized in a plant tip growing cell using this approach. Furthermore, fast changes induced by Nod factors and reorganization of the polymerization regions were observed once root hairs reorient growth in the presence of Nod factors. These results support the notion that actin polymerization plays a key role during the Nod factors response and more importantly during polar growth.

Characterization of prevailing geminiviruses in Mexico using a rolling circle amplification protocol

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The family *Geminiviridae* includes plant viruses which circular ssDNA genome is encapsidated in a characteristic geminate particle. These pathogens had caused dramatic losses in crops around the world, especially in tropical and subtropical countries. In Mexico, about a dozen of geminiviruses species had been reported since the 70s and the list keeps growing up. Detection and identification protocols are indispensable to counteract its dissemination. In this work, a partial identification system based on rolling circle amplification followed by digestion with several enzymes (RCA-dig) was standardized. Our results show that two geminiviruses, *Pepper golden mosaic virus* (PepGMV) and *Pepper huasteco yellow vein virus* (PHYVV) prevail in *Solanaceae* crops such as pepper and tomato. These viruses were first detected in Mexico and cloned in the 80s. When they infect the same plant, induce synergistic symptoms. The RCA-dig system allowed us to corroborate that PepGMV and PHYVV are still present in Mexican fields. Several isolates of PepGMV and PHYVV from various states (samples took between 2005 and 2007) were cloned and characterized. Using this technique with new samples (collected between 2008 and 2009), we were able to distinguish PepGMV or/and PHYVV (single or mixed) infections. The RCA-dig allowed us to establish a reference guide (protocol) to carry out a rapid analysis of samples from several regions of Mexico. The protocol is expected to allow the detection of emerging viruses without the need of cloning and sequencing and to help in viral dispersion studies.

The role of *IAP1* in Age-Related Resistance in *Arabidopsis thaliana*

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Age-Related Resistance (ARR) has been observed in numerous plant species, resulting in increased disease resistance as the plant matures. Evidence from our lab suggests that ARR in Arabidopsis to *Pseudomonas syringae* pv *tomato* involves the accumulation of salicylic acid (SA) in the intercellular space where it may act as an anti-microbial agent. EDS1 and PAD4, key regulators of SA accumulation during basal resistance in young plants are also required for the ARR response. Additionally, *IAP1* (important in the ARR pathway) was identified in an ARR mutant screen and shown to be required for ARR as well. Both intercellular and intracellular SA accumulation was reduced in *iap1-1* compared to wild type, suggesting that *IAP1* lies upstream of SA accumulation during ARR. The *iap1-1* mutation does not affect basal resistance in young plants, implying that it is specific to the ARR response, although R-gene mediated resistance to *Pst (AvrRps4)* and *Pst (AvrRpt2)* is partially compromised suggesting that the two pathways share common elements. Mapping of *IAP1* is ongoing. Identification and characterization of *IAP1* will provide important insights into the ARR defense pathway.

***Orobanche* seed-stimulating compounds (strigolactones) in root exudates of the soybean wildtype Bragg and its supernodulating mutant *nts1007* are altered by P/N levels and by mycorrhization**

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Strigolactones, apart from being seed-germination-stimulating compounds for parasitic weeds e.g. *Orobanche*, recently have been identified to be important signals for arbuscular mycorrhiza (AM). Once mycorrhizal the levels of seed-germination-stimulating compounds (strigolactones) in root exudates are reduced and, thus, their involvement in the AM autoregulation has been suggested. Higher P- and N-levels in the soil are known to reduce the attachment of parasitic weeds to plants and also affect AM root colonization. At high P-levels, alone and in combination with high N-levels, mycorrhization

is reduced. There are some data available that these alterations of the susceptibility of plants to parasitic weeds and to AM fungi are linked with alterations in the exudation of seed-germination-stimulating compounds. In the present work we tested this hypothesis with the soybean Bragg and its supernodulating mutant *nts1007*, which is deficient in AM autoregulation. In Bragg and the mutant P- and P/N-application reduced AM colonization. Changes of the percentage of seed-germination, indicating alterations of seed-germinating compounds, were determined in a bioassay with *O. foetida*. Exudates of mycorrhizal and nonmycorrhizal plants at different P and N levels were tested. P- and N-application to nonmycorrhizal plants resulted in nearly similar levels of seed-germination as in control plants. Without P/N application mycorrhization reduced the seed-germination activity of the root exudates of Bragg to half and of the mutant to a third, whereas in mycorrhizal plants with P or N a germination activity was nearly absent. The implication of these changes of the seed-germination activity of root exudates, as an indicator for the presence of strigolactones, on AM root colonization is discussed.

Characterization of effector proteins in *Xanthomonas axonopodis* pv. *manihotis*

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Many plant pathogenic bacteria inject effector proteins into the host cytoplasm during infection through the type three secretion system (TTSS). The Type III secretome has been shown to play an important role in bacterial virulence or aggressiveness in susceptible hosts. Several effector proteins have been identified in the phytopatogenic species of the genus *Xanthomonas.* We obtained a partial genome sequence of *Xanthomonas axonopodis* pv *manihotis* (*Xam*)*,* the causal agent of the Cassava Bacterial Blight (CBB), using Solexa technology. We identified effector proteins in *Xam* based on their homology with sequences present in other species of *Xanthomonas, Pseudomonas* and *Ralstonia*. Using site-directed mutagenesis and HR suppression assays, we characterized several effectors. Two of them showed interesting phenotypes. The first one, *pth*B, is a member of the AvrBs3 protein family that encodes 13.5 tandem repeats of 34 amino acids each, as well as two nuclear localization signals and an acidic activation domain. We obtained a *pth*B mutant in the CFPB1851 strain and carried out structure-function studies using versions of the gene which lack different domains in the protein. The second effector is HpaF, which contains an LRR domain and has been associated with suppression of host defenses in other xanthomonads. Changes in the pattern of pathogenicity and virulence of the mutants were assessed by inoculations in greenhouse grown cassava plants. This is the first approach to elucidate the mechanisms that *Xam* uses to cause disease in cassava.

***Verticillium* resistance in tomato is a concerted effort of early and late defence response genes**

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Race-specific resistance is usually elicited because of the presence of resistance genes in host plants. In tomato (*Solanum lycopersicum*), the two resistance genes *Ve1* and *Ve2* fulfill this requirement against the fungal wilt pathogen *Verticillium dahliae* race 1 (Vd1). However, the molecular factors and mechanisms leading to this resistance response are still unknown. In this study, we identified early and late defence response genes specifically upregulated in resistant plants compared to their susceptible counterparts. After Craigella resistant (CR) and susceptible (CS) tomato plants were infected with Vd1, the successful establishment of *Verticillium* infection was verified by fungal biomass and symptom indexing assays. RT-PCR analysis reveals that the genes coding for ethylene response factor-2, beta-1,3-glucanase, cyclin-dependent kinase inhibitor and IMP dehydrogenase/GMP reductase exhibit early upregulation in resistant plants (starting at 4 days post-inoculation [dpi]) compared to susceptible ones. Meanwhile, the genes for a 14-3-3 protein and pre-mRNA processing factor 8 are only upregulated in the resistant plants at later stages (starting at 8 dpi). Additionally, the higher expression of IMP dehydrogenase/GMP reductase were actually caused by both suppression in the compatible interaction and induction in the incompatible one. It was observed that the early response genes mimic the pattern of *Ve* gene expression while late response gene upregulation correspond to the initiation of symptom recovery and fungal elimination in resistant plants. The identification of these genes provides insights into the overall mechanisms resulting in resistance against *Verticillium* in tomato plants.

Comparative analysis of expression profiles in shoots and roots of tomato systemically infected by *Tomato spotted wilt virus* reveals organ-specific transcriptional responses

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Tomato (*Solanum lycopersicum*), a model species for the Solanaceae, is severely affected by *Tomato spotted wilt virus* (TSWV) worldwide. Although the remarkable environmental and agronomic importance of this interaction, the molecular mechanisms underlying virus infection and symptoms development are far to be understood. To elucidate the transcriptional response of plants to TSWV infection, microarray analyses were performed on shoots and roots of systemically infected tomato. Several differentially regulated genes were identified, with remarkable differences between shoots and roots. Results were validated by qRT-PCR on selected genes. Compared to control plants, virus infection induced differential expression of 2,385 genes in shoots and 1,166 genes in roots. Although virus concentration was similar in the two organs, gene expression comparisons detected both co-regulated and differently regulated genes. A total of 465 genes, mainly involved in defence and stress response, signal transduction and primary metabolism, showed conserved up- or down-regulation in both organs. Many of the genes that were not co-regulated were involved in categories as photosynthesis and amino acid metabolism. A detailed analysis highlighted, in addition, differences in abscissic acid (ABA) metabolism, response to hormones, synthesis of and response to reactive oxygen species, metabolism of carotenoids and polyamines. In particular, ABA production seems to increase in roots while several ABA-responsive genes are up-regulated in shoots, suggesting an involvement of this hormone in the development of plant symptoms during systemic infection.

Histochemical and cytochemical investigations of phenols in leaves of banana infected by the *Mycosphaerella fijiensis*

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Several types of biotic and abiotic stimulations are known as potential activators of phenolic compounds production on plants with defense mechanisms. Different studies showed that the increase of the synthesis of phenolic compounds on plants after pathogens attack is frequent (Matern et al., 1995; De Ascensao et al., 2003). For resistant plants, defense responses based on phenolic compounds production are characterized by the early and rapid phenols accumulation on infection site, resulting in effective pathogen insulation. Resistant "Calcutta 4", partially resistant "Pisang Madu" and susceptible "Grande Naine" banana tree cultivars to the black Sigatoka were infected *in vitro* by *Mycosphaerella fijiensis* and studied in the presence of polyphenols on the processes of plant-pathogen interaction, during the kinetics of 5, 7,10, 15, 21, 25 days after inoculation. Cuts with 60 µm of thickness with cryomicrotome assistance were colored with: DMACA, Vannilin-HCl and Neu's reagent and then observed with optical and epifluorescence microscopy. After 15 days, it was observed for the "Calcutta 4" and "Pisang Madu" cultivars the presence of condensate tannins and catechins characterized by the reddish color with Vannilin-HCl of some mesophyll cells when compared with the control plant. It was not observed any presence of condensate tannins and catechins for the susceptible "Grande Naine"cultivar. It was concluded that the presence of condensate tannins and catechins is directly related with the plant defense mechanisms against pathogens for the *M. fijiensis*-*Musa acuminata* interaction. This is the first report of the presence of condensate tannins and catechins for this pathosystem.

ERF transcription factors regulating early nodulin gene expression in response to symbiotic rhizobial Nod factors

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Rhizobium lipo-chitooligosaccharides known as Nod factors (NF) play a key role in the establishment of the symbiotic interaction that leads to nodule development in legumes. NF signal transduction involves multiple receptor-

like kinases and oscillatory calcium responses that are necessary for the subsequent transcription of ENOD genes. In the model legume Medicago truncatula, ERN1, 2 and 3 are ERF transcription factors that are directly involved in the transcriptional regulation of ENOD genes in epidermal root hair cells in response to NFs. All three ERNs bind to so-called NF-box cis-regulatory sequences found within NF-responsive ENOD gene promoters, and gene expression is regulated by antagonistic activities: ERN1 and 2 act as transcriptional activators, whereas ERN3 appears to act as a repressor. Significantly, full ERN1 and 2 transcriptional activities is dependent upon a highly conserved C-terminal domain, which is absent in ERN3. In addition, the closely-related ERN1 and 2 genes are co-regulated in root hairs in response to NFs, which raises the question as to whether they operate synergistically during early NF signalling. Despite the similar expression patterns in root hairs, quantitative RT-PCR analysis reveals that ERN1 and ERN2 are differentially expressed during later stages of nodulation. This suggests that both common and independent pathways are regulated by these transcription factors. The spatio-temporal expression profiles of individual ERN genes are currently being analyzed by means of ERN promoter-GUS and GFP-ERN protein fusions expressed in transgenic M. truncatula roots. This should reveal to what extent ERN genes are co-regulated and the respective localisation of these ERN factors throughout symbiosis.

Identification and characterisation of the *RPP13/ATR13* recognition complex between downy mildew and Arabidopsis

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Elucidation of complex mechanisms driving host-pathogen co-evolution has been of great interest and presents a challenge. The studies involving the *Arabidopsis-Hyaloperonospora arabidopsidis* pathosystem have contributed extensively to our understanding of the genetics of host-pathogen co-evolution. We have been studying a co-evolutionary model based on the *Arabidopsis RPP13* resistance gene with the highest reported level of sequence diversity among known R-genes, and the highly variable cognate effector gene *ATR13* from *H. arabidopsidis*. Previously, sequence analysis of the *RPP13* gene resulted in numerous distinct clades, and the recognition of *ATR13* by *RPP13-Nd* has been attributed to the alleles in a single clade. In addition, ATR13 independent recognition of various *H. arabidopsidis* isolates by an *RPP13* (Rld-2) allele in a different clade suggested the presence of novel ATR molecules. Furthermore, recognition of various alleles of *ATR13* by resistance genes other than the *RPP13* has been shown. Overall, this has revealed the presence of a complex co-evolution between *Arabidopsis* and *H. arabidopsidis*. In order to provide an in-depth view of such a complex system, we are currently using map-based cloning approaches both in *Arabidopsis* and *H. arabidopsidis* to identify and characterize the genetic components of the *RPP13/ATR13* complex. Recent progress towards the cloning of these genes will be presented.

Molecular characterisation of integrated sequences of Banana streak virus in the banana plant genome

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The genome of banana (Musa sp.) harbours multiple integrations of several species of Banana streak virus (BSV), certainly resulting from illegitimate recombination between host and viral DNA. Surprisingly, this pararetrovirus does not require integration for its replication. Some integrations, only existing in the Musa balbisiana genome (denoted B), are infectious by reconstituting a functional viral genome. To date, four widespread species of BSV (Goldfinger -BSGfV, Imové - BSImV, Mysore - BSMysV and Obino l'Ewai - BSOLV) have been reported as integrated into the B genome and as infectious, under stress conditions, resulting in viral infection of the banana plant. In order to study BSV expression from such viral integrants, a characterisation of infectious integrants (eBSV) was undergone by studying both a Musa BAC library obtained from the wild diploid M. balbisiana cv. Pisang Klutuk Wulung (PKW) containing the four BSV species described above and one interspecific genetic cross using carrier PKW. The organization of eBSGfV was fully characterized recently in our lab (Gayral et al., 2008). eBSGfV results from a single event of integration corresponding to an allelic insertion extensively rearranged, containing at least one full-length viral genome. Although the four BSV species present important differences with each other, the organisation of the three other eBSVs looks like eBSGFV. Indeed, preliminary data indicate that each of them is extensively rearranged in PKW and present as two insertions at the same locus. This suggests an allelic insertion resulting from a single even of integration. Experimental evidences to demonstrate BSV expression and to validate the infectious nature of every eBSV are on the way.

Role of disease resistant gene in shaping genetic incompatibility in Arabidopsis thaliana

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Previous work suggested a Dobzhansky-Muller type, two-gene interaction is the basis of poor performance in necrotic F1 hybrids of wild strains of Arabidopsis thaliana. I will discuss a case of hybrids in which two independent systems cause incompatibility. One system is due to an epistatic interaction between two linked loci on chromosome 3; one of these maps to the *Recognition of Peronospora parasitica 1* (*RPP1*) cluster. This complex locus, where TIR-NBS-LRR type of disease resistance genes highly diverge both in sequence and number in different strains, is emerging as a cause for multiple cases of hybrid incompatibilities. The role of this class of R genes, which can trigger autoimmune responses in hybrids, will be discussed in the context of epistatic interaction with the closely linked locus. The second incompatibility system appears to map to a single locus, *Accelerated Cell Death* 6 (*ACD6*), which suggests that accumulation of divergent alleles even at one gene can cause genetic incompatibility. This heterozygous disadvantage case seems to underlie several other necrotic hybrids as well. Implication of divergent alleles of disease resistant gene in adaptation and speciation will be discussed.

Characterization of the *Bax inhibitor-1* promoter in *Arabidopsis thaliana*

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Programmed cell death (PCD) is a physiological process found in all organisms that is important for normal development, maintenance of homeostasis and in response to biotic and abiotic stresses. The Bax-inhibitor-1 (BI-1) is a cell death regulator that has protective function under different stress conditions that can trigger PCD. For example, overexpression of barley BI-1 in barley interferes with cell death and compromises non-host resistance, and its overexpression in carrots provides resistance to *Botrytis cinerea*. Expression of plant *BI-1* is enhanced during senescence, in response to wounding and to various biotic and abiotic stresses. Since expression of plant *BI-1* correlates with its protective role in response to various stress, we have been studying how expression of *BI-1* is regulated in the plant model *Arabidopsis thaliana*. We have found that expression of *AtBI-1* is stimulated by UV-C irradiation, which also triggers PCD in *A. thaliana* seedlings. Using promoter deletions and GUS reporter gene analysis, we have identified a promoter region that is required to maintain low levels of *AtBI-1* expression in absence of UV-C. Deletion of this sequence increases expression of GUS reporter gene to a level close to that obtained after UV-C irradiation. How this sequence could regulate AtBI-1 expression in response to UV-C will be discussed.

Genes associated with pathogenicity distinguish Australian pathogenic isolates of *Fusarium oxysporum* f. sp. *vasinfectum* from non-pathogenic *Fusarium oxysporum*

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Fusarium oxysporum f. sp. *vasinfectum* (*Fov*) is the causal organism of a cotton wilt disease. Australian isolates of this pathogen fall into two vegetative compatibility groups, VCG 11 and VCG 12, that are distinct from overseas Fov isolates and are thought to have evolved in Australia. It is likely that *Fov*, like the tomato wilt pathogen *F. oxysporum* f. sp. *lycopersici* (*Fol*), secretes effector proteins into xylem of infected cotton plants. Bioinformatic analysis of genes expressed in *Fov* infected cotton identified several Fusarium cDNAs encoding secreted proteins, some with homology to *Fol* genes and some with homology to no known sequences. A few of these putative cysteine rich effector proteins are specific to the VCG11 and 12 pathogenic *Fov* isolates and absent from genetically related non-pathogenic *F. oxysporum*

isolates collected from Australian cotton fields as well as several other formae speciales of *F. oxysporum* tested. These *Fo* isolates were also screened for twelve effector genes from *Fol* and only one was found in the VCG11 and 12 isolates of *Fov*. VCG11 and 12 isolates also contained an expanded copy number and a conserved RFLP pattern for a fungal transcription factor gene (*FTF*-1) from *F. oxysporum* f. sp. *phaseoli* and another gene from *Fov* with homology to short-chain dehyrogenase both of which have been associated with pathogenicity. The presence of the same effector proteins and the same additional copies of two more genes associated with pathogenicity in two distinct VCGs of *Fov* along with their absence in genetically related non-pathogenic *Fo* isolates strongly suggests presence of a pathogenicity chromosome or island in *Fov* and efforts are continuing to identify any such chromosome(s) or regions(s).

Functional role of the cytosolic tail of the Cf-9 resistance protein from tomato

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The Cf-9 protein of tomato confers resistance to races of the leaf-mould pathogen *Cladosporium fulvum* that expresses the cognate Avr9 protein. Cf-9 is a trans-membrane protein with a large extracellular N-terminal leucine rich repeat (LRR) domain and a small cytoplasmic tail at the C-terminus. While regions within the LRR domain are involved in determining specificity towards Avr9, little is known about the role of the cytsolic C-terminal domain in Cf-9 function. We have taken a targeted mutagenesis approach to study the role of individual amino acid residues within the CTD of Cf-9 protein. Amino acids were selected for analysis based on their conservation amongst Cf-9 homologues and/or potential involvement in phosphorylation (S and T). The YPAWF and II motifs with similarity to canonical endocytosis motifs YxxΦ and LL were also targeted for mutagenesis. Mutations were introduced to change CTD residues to A and in the cases of S and T, also to D to mimic effects of phosphorylation. The Cf-9 mutants were assayed for necrosis induction by co-agroinfiltration with Avr9 in leaves of *Nicotiana benthamiana* and *N. tabacum*. Mutations of affecting the interval between S834 and S842 resulted in various degrees of loss of activity, which was most pronounced in the case of the P838A mutation. The T835D mutation was also severely impaired whereas the S834D mutation remained fully functional. These experiments have defined a region of eight amino acids within the CTD of Cf-9 that play a role in Cf-9 function and also identified two amino acids within this region, T835 and P838 that are vital for its function. The possibility that this region is involved in binding the Cf-9 interacting proteins CITRX or VAP27 is being investigated.

Methods to study PAMP-triggered immunity using model *Solanaceae* plant-pathogen interaction systems

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Plants have evolved multi-layered defense mechanisms that allow them to suppress the growth of pathogenic microbes that attempt to colonize their tissues. In the first line of defense, plants use pattern recognition receptors (PRRs) that recognize structurally conserved microbial features called pathogen-associated molecular patterns (PAMPs). This recognition event leads to PAMP-triggered immunity (PTI). Examples of well-characterized PAMPs include bacterial flagellin and translation elongation factor Tu (EF-Tu). The PRRs for these PAMPs are two of the best-studied receptors, FLS2 and EFR, which recognize the conserved amino acid peptide of flagellin (flg22) and EF-Tu (elf18). Much of the research on PTI has been performed with *Arabidopsis*, and most of the PTI assays have been standardized for this species. There is a need then for robust PTI assays in other systems, including model systems involving *Solanaceae*-microbe interactions. We have optimized molecular, cellular, and whole-plant methods to measure PTI responses in *Solanaceae* species, particularly tomato (*Solanum lycopersicum*) and *Nicotiana benthamiana*. These methods allow high-throughput screens and detailed characterizations of potential PTI-related genes, which will facilitate the study of plant innate immunity in economically important *Solanaceae*-pathogen interactions.

ERF proteins diversity and defense response

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Several ERF transcription factors are believed to play a crucial role in the activation of plant defense responses, but little is known about relationships between diversity of this family and the functions of groups or individual ERF in this process. In the present study 200 ERF genes from unigene cotton database were identified. Conserved amino acid residues and phylogeny reconstruction using the AP2-conserved domain suggested that the classification into 10 major groups used for Arabidopsis and rice is applicable to the cotton ERF family. Based on in silico studies we predict that group IX ERF genes in cotton are involved in jasmonate (JA), ethylene (ET) and pathogen responses. To test this hypothesis, we analyzed the transcript profilies of the group IXa subfamily in the regulation of specific resistance to Xanthomonas campestris pathovar malvacearum (Xcm). The expression of four members of the group IXa was induced upon challenge with Xcm. Furthermore, the expression of several ERF genes of the group IXa was induced synergistically by JA in combination with ET suggesting that the encoded ERF proteins may play key roles in the integration of both signals to activate JA- and ET-dependent responses.

Rapid cloning of *R2* homologues in wild *Solanum* using functional allele mining with RxLR effectors of *P. infestans* and *R2* cluster information

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Molecular insight or R-Avr interactions in *Solanum* can lead to significant progress in acquiring genetic resistance against *Phytophthora infestans*, the major pathogen on cultivated potato. Recently a functional affector profiling system for detecting R-Avr pairs was developed (Vleeshouwers et al. 2008). Using this system we identified numerous new candidate R-Avr interactions by an initial screening of 24 wild *Solanum* species for response to 55 candidate effectors of *P. infestans*. In this study, we focused on a detected interaction of an RxLR effector (Kamoun 2006) in *S. schenckii*. A functional allele-mining with this RxLR (PexRD) effector in 144 wild *Solanum* genotypes resulted in additional responding plants of *S. edinense* and *S. hjertingii*. Subsequent genetic studies in *S. edinense* led us to potato linkage group IV (Park et al. 2005), which contains the functional *R* genes, *R2*, *R2-like*, *Rpi-blb3* and *Rpi-abpt* (Lokossou et al., in press). By using specific primers for these *R*-genes we amplified and sequenced 21 candidates of the *R2* family from the three wild *Solanum* species. Some of the amplified homologues are identical or highly similar to the recently cloned *R2-like* and *Rpi-blb3*, respectively, but others show much more variation, and the *R2* family appears highly expended and diverse in *Solanum*. Since *R2-like* and *Rpi-blb3* respond to *P. infestans* efefctor *PiAvr2*, candidate R genes will be cloned and tested for responses to the PexRD effector and *PiAvr2*. We hypothesize that evolutionary dynamics between *PiAvr2*/PexRD effector and the *R2* cluster may have created the observed diversity on plant and pathogen side. These experiments may bring us new insights in the co-evolution of an *R*-gene cluster and its interacting effectors.

Corresponding metabolic reactions in host and pathogen modulate opposing functions of defense and virulence

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The outcome of pathogenesis is usually governed by the potency of complementary mechanisms of host defense and pathogen virulence. We show that analogous metabolic reactions catalyzed by glycerol-3-phosphate (G3P) dehydrogenase (dh) in a plant and its fungal pathogen modulate defense and virulence in the respective organisms. Inoculation of Arabidopsis with Colletotrichum higginsianum was associated with an increase in G3P levels and a concomitant decrease in glycerol levels, in the host. Plants impaired in plastidal G3P catabolism (act1), accumulated elevated levels of pathogen-

induced G3P and displayed enhanced resistance. The act1 mutation also improved resistance in hypersusceptible camalexin deficient plants. Overexpression of the host G3P generating G3Pdh (GLY1) also enhanced resistance to C. higginsianum. Correspondingly, a mutation in gly1 enhanced susceptibility to C. higginsianum. In vitro studies showed that exogenous application of G3P suppressed the transcription of fungal G3Pdh but not the host GLY1 gene. Knock-out mutations in the fungal G3Pdh dramatically reduced the virulence of C. higginsianum on wild-type plants, however G3Pdh-defective fungi remained virulent on mutant hosts defective in G3P generation. Together, these results suggest a novel and specific link between G3P metabolism in the host and pathogen during pathogenesis.

Host components mediating the sustained growth and reproduction of an obligate biotrophic fungus

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The sustained growth and reproduction of the obligate biotrophic fungus *Golovinomyces orontii* on *Arabidopsis thaliana* requires continued alteration of host primary and specialized metabolism to supply the fungus with its requisite nutrients and water, while suppressing/delaying cell death and senescence, and limiting defense responses. To elucidate host processes and components required at this stage of the powdery mildew interaction, laser microdissection was used to isolate cells at the site of infection at 5 days post infection (5 dpi) and to perform global Arabidopsis expression profiling. At 5dpi, powdery mildew colonies remain spatially isolated and quantitative indicators of fungal growth and reproduction including hyphal length, conidiophores per colony and conidia per conidiophore may be quantified. Seventy-seven transcriptional regulators exhibited altered expression at the site of infection. By coupling this information with identified site-specific functional processes and cis-acting regulatory element analyses for genes associated with these processes, we predicted site-specific process networks associated with photosynthesis, respiration, cold/dehydration responses, cell death suppression, salicylic-acid mediated defense responses, and cell cycle progression. Further analysis of T-DNA insertion mutants in a subset of these regulators and subsequent experimentation has resulted in two important findings: (1) cell cycle modulation is important at this phase of infection and (2) specialized metabolites are required for conidiophore formation. Funded by NSF2010 MCB-0420267 to MW.

***CaLEA73,* a LEA protein, is involved in plant tolerance to water and salt stress**

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Currently, one of the major problems that face the world agriculture productivity is the increasing of regions with extreme environment. The mechanism of plant to salt and water stress tolerance is a topic of intense research in plant biology. Special attention has been to the LEA proteins because it has been found that these proteins are associated with the tolerance to abiotic stress. These proteins accumulate during the last stages of seed maturation and have been reported that are increased in some tissues when the plants are under water stress, salt stress or low temperatures. *CaLEA73*, which expresses a LEA protein, was obtained from *Capsicum annuum cv*. Caballero seeds which were osmoprimed. *CaLEA73* was induced in *Capsicum annuum cv*. Caballero plants when they were under water stress. In order to analyze whether *CaLEA73* is able to increase the responses in plants to either salt and/or water stress we transform *Arabidopsis thaliana* plants with a construct containing the cDNA *CaLEA73* downstream of the constitutive promoter *CaMV35S*. The presence and expression of the *CaLEA73* transgene was confirmed by PCR and RT-PCR respectively. During the meeting we will show the stimulating results of the transgenic lines *35S:CaLEA73* under different stress conditions.

Hop/Sti1 is required for PAMP receptor biogenesis, transport to the plasma membrane and function in rice innate immunity

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Plants and animals have evolved similar innate immunity systems to prevent pathogen infections. Receptor-like kinases (RLKs) are receptors of microbe/pathogen-associated molecular patterns (M/PAMPs) in plants. However, biogenesis and maturation of these receptors are largely unknown. Here, we characterize a novel OsRac1-interacting co-chaperone, Hop/Sti1, as a key regulator of innate immunity in rice. Hop/Sti1a forms a core complex with a chitin receptor, RLK9, in endoplasmic reticulum together with Hsp90 and subsequently transport to plasma membrane via vesicle trafficking system. Hop/Sti1a has a role in rice blast resistance and is important for biogenesis and transport of RLK9 thereby functions in chitin-triggered immune response. Our results suggest that the biogenesis and transport of RLK9 is regulated by Hop/Sti1a. Based on the results, we propose a "defensome" model in which Hop/Sti1 may function as adaptor protein, linking M/PAMP receptors and molecular switch Rac GTPase together during innate immune response.

Identification of domains required for AvrPtoB$_{B728a}$ interactions with Pto family members from *Solanum lycopersicon* and its function to trigger defense responses in tomato VFNT cherry

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AvrPtoB$_{B728a}$ is a type III effector protein from *Pseudomonas syringae* pv. *syringae* (*Psy*) B728a that physically interacts with the Pto kinase in a yeast two-hybrid assay and elicits a *Pto*-dependent defense responses in tomato. According to previous studies, AvrPtoB$_{B728a}$ can trigger defense responses not only in a tomato cultivar containing Pto, but also in the tomato cultivars without the Pto kinase, such as VFNT cherry and Moneymaker. Silencing of *Pto* family members in tomato cultivar VFNT cherry abolishes gene-for-gene resistance, indicating that at least one of the Pto family members are required for this resistance. *Pto* locus in VFNT cherry has been sequenced and it contains *Prf*, *SlPtoA*. *SlPtoB*, *SlPtoC*, *SlPtoD* and *SlPtoF*. By means of yeast two-hybrid analysis, certain *Pto* family members can interact with AvrPtoB$_{B728a}$, and they express in tomato without any pathogen challenge. In this study, we use deletion approach to identify domains required for interactions with Pto family members in a yeast two-hybrid analysis. The biological functions of these deletion mutants are under investigation by pathogenicity assay with delivery of AvrPtoB$_{B728a}$ mutants from *Psy* B728aΔ*avrPtoB*$_{B728a}$ into leaves of tomato cv. VFNT cherry.

Identification of genomic region responsible from Soybean mosiac virus-London resistance breaking (SMV-LRB) against Rsv4 resistance gene in V94-5152 soybean host

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Soybean mosaic virus (SMV), a member of potyviruses, is the most common viral pathogen of legumes with soybean as the primary host (Hajimorad *et al*., 2003). Two SMV isolates, i.e., SMV-L and SMV-LRB were two viruses identified in soybean cultivated fields at London, Ontario, Canada. They were sequenced and found 99.6% sequence identical on their full-length genome. They belonged to the G2 pathotype of SMV strain groups in the US. These two viruses differed in overcoming the Rsv4 resistance gene present in V94-5152 soybean germplasm (Gagarinova *et al*., 2008). Both SMV-L and SMV-LRB viruses infected William 82 (susceptible host for both viruses). SMV-L could not infect or cause any symptoms in V94-5152 but SMV-LRB infected V94-5152 and induced severe symptoms. With the above background, infectious viral clones SMV-L and SMV-LRB were developed and their genomes were exchanged to develop chimeric virus clones. The SMV genome was divided into three parts namely, 1K, KA and AA respectively. 1K contained from start of the genome, P1 full gene, missing 87 bases at C terminal of HC-Pro. KA contained 87 bases at C terminal of HC-Pro, P3 full, 6K1 full, partial CI (missing 894 bases). AA has partial CI protein, full genes of 6K2, NIa-VPg, NIa-Pro, RdRp and CP. They were initially bombarded on William 82 soybean 12-15 days old seedlings as it is a susceptible host for both SMVs. The treatments were observed for symptoms and tested by ELISA. The positive samples for all the treatments were subsequently rubbed on new soybean 12–15 days old seedlings William 82 (control) and

V94-5152. They were observed for symptoms and tested by ELISA after 15 days of inoculation. Further details of results would be presented and discussed.

Molecular characterization and functional analysis of ubiquitin extension genes from the potato cyst nematode *Globodera rostochiensis*

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Ubiquitin is a highly conserved 76-amino acid protein found in every eukaryotic cell. It has been proposed that ubiquitin has many cellular functions including DNA repair, transcription regulation, regulation of cell cycle and apoptosis. We identified two ubiquitin extension genes (*Gr-Ubi1* and *Gr-Ubi2*) from the potato cyst nematode *Globodera rostochiensis* that encode proteins containing a monoubiquitin domain and a signal peptide enabling secretion. Southern blot analysis showed that multiple copies of the *Gr-Ubi* gene exist in the nematode genome and RT-PCR analysis revealed strong expression of the gene throughout the nematode life cycle. *In situ* mRNA hybridization localized the Gr-Ubi gene expression exclusively within the dorsal esophageal gland cell of both preparasitic and parasitic stages of the nematode, suggesting a possible secretion of these ubiquitin extension proteins during nematode parasitism. C-terminal GFP translational fusions were used to determine the subcellular localization of the ubiquitin extension protein in planta. Our preliminary results indicated that the protein is localized on the cell membrane and in the nucleus of infiltrated tobacco leaf cells. Potato transgenic RNAi and overexpressing lines for the *Gr-Ubi* gene are currently being developed to further investigate the role of these nematode-produced proteins in plant parasitism.

Elucidation of OsWRKY53-depending signaling pathway in rice defense responses

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Plants recognize pathogens by detecting elicitors *via* specific receptors that activate defense genes through intracellular signaling cascades. Transcriptional regulation of defense-gene expression is central to induced disease resistance in higher plants. WRKY proteins form a large family of plant-specific transcription factors that appear to play a regulatory role in a variety of stress responses. Using microarray analysis, we previously identified *OsWRKY53* as a chitin elicitor-induced gene from suspension-cultured rice cells. *OsWRKY53* was also induced in rice plants by infection with the blast fungus *Magnaporthe grisea*. A transient assay using the particle bombardment method showed that OsWRKY53 is a transcriptional activator, and overexpression of *OsWRKY53* resulted in enhanced resistance to *M. grisea* in rice plants. These results strongly suggest that OsWRKY53 is a transcription factor that plays important roles in elicitor-induced defense signaling pathways in rice. To reveal the regulatory mechanisms of *OsWRKY53*, transient gene expression assays using the firefly reporter gene were performed. As the results of transient gene assay, three tandem W-box elements existed in the upstream region of *OsWRKY53* function as an elicitor-responsive *cis*-element. These results suggest that some WRKY proteins are involved in the regulation of *OsWRKY53* expression. Moreover, to identify the OsWRKY53-dependent regulons comprehensively, we generated transgenic rice cells which constitutively express OsWRKY53-3xHA fusion protein, and established the experimental system for chromatin immunoprecipitation (ChIP). Now, we are trying to perform ChIP-on-chip analysis using the custom promoter array.

A subgroup of *Pseudomonas syringae* are common plant colonizers able to propagate on a wide variety of plant hosts despite lacking a *hrp/hrc* cluster and effector gene orthologues

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Pseudomonas syringae, as a species, is a pathogen of many different plant species, but any given strain of *P. syringae* is a pathogen of only a few plants. The host range of any particular strain of *P. syringae* is thought to be delimited by the host defenses elicited by Type III Secretion System (T3SS) – associated effectors (T3Es) and microbial-associated molecular patterns balanced against other T3Es that can suppress these defenses. Here, we characterize a subgroup of *P. syringae* which lack the *hrp/hrc* gene cluster encoding the typical *P. syringae* T3SS and any known *P. syringae* effector orthologues. *P. syringae* lacking the *hrp/hrc* cluster were isolated much more frequently from healthy plants than *hrp/hrc+* strains and do not cause a hypersensitive response on tobacco indicating a nonpathogenic lifestyle. Phylogenetic comparison shows that all *hrp/hrc-* isolates are more closely related to each other than to their nearest known pathogenic relative, the bean pathogen *Psy*B728a. *In planta* growth assays indicate that *hrp/hrc-* strains do exhibit a degree of host preference, despite lacking T3Es. The degree of this preference, and the ability to survive endophytically, varies among strains pointing to T3SS-independent factors contributing to host range. Genome sequencing of the *hrp/hrc-* strain *Psy*642 indicates that both toxins and an atypical T3SS may aid survival *in planta* and define host range. Ectopic expression of a typical *P. syringae* T3SS reduced growth of *Psy*642 on all plants tested. All *hrp/hrc-* isolates contain ice nucleation genes. Taken together, these findings may explain why bacteria in this group apparently are more common leaf colonizers than *hrp/hrc+ P. syringae* and suggest yet another successful survival strategy of this pathogen.

Glucosinolate metabolites required for MAMP-activated callose deposition response

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The perception of pathogen or microbe-associated molecular pattern molecules (MAMPs) by plants triggers a basal defense response analogous to animal innate immunity and is defined partly by the deposition of the glucan polymer callose at the cell wall at the site of pathogen contact. Transcriptional and metabolic profiling in *Arabidopsis* mutants, coupled with the monitoring of pathogen-triggered callose deposition, have identified major roles in pathogen response for the plant hormone ethylene and the secondary metabolite 4-methoxy-indol-3-ylmethylglucosinolate. Two genes, *PEN2* and *PEN3*, are also necessary for resistance to pathogens and are required for both callose deposition and glucosinolate activation, suggesting that the pathogen-triggered callose response is required for resistance to microbial pathogens. Our study shows that well-studied plant metabolites, previously identified as important in avoiding damage by herbivores, are also required as a component of the plant defense response against microbial pathogens.

A high-affinity cytochrome *c* oxidase in *Ralstonia solanacearum* contributes to growth in microaerobic environments and to full virulence on tomato

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Ralstonia solanacearum race 3 biovar 2 (R3bv2) is an economically important soilborne plant pathogen, causing bacterial wilt disease by infecting host plant roots in the soil. This pathogen chemotaxes towards host roots and root exudates, yet little is known about R3bv2 behavior in the host rhizosphere and early in bacterial wilt pathogenesis. To explore this part of the disease cycle, we used a novel taxis-based, promoter-trapping strategy to identify pathogen genes induced in the plant rhizosphere. This screen identified several genes whose promoters were upregulated in the presence of tomato root exudates. These included the known plant-responsive virulence regulators *hrpG*, *pehR,* and *vsrA* as well as the operon encoding type IV pili, a known virulence factor. Another identified gene encodes an assembly protein for a high affinity *cbb3*-type cytochrome *c* oxidase (*cbb3-cco*), which is important for respiration in low oxygen conditions in other bacteria. In R3bv2, *cbb3-cco* gene expression increased under low oxygen conditions and the *cbb3-cco* mutant strain grew more slowly in a microaerobic environment (0.5% O_2) that simulated potential oxygen status in the rhizosphere and host xylem vessels. Although the *cco* mutant could still wilt tomato plants, symptom onset was significantly delayed relative to the wild-type parent strain. Further, the *cco*

mutant did not colonize host stems as effectively as wild-type. These results suggest that R3bv2 encounters low oxygen environments during its interactions with host plants and that the pathogen uses this oxidase to help it succeed under these conditions.

Genetic and molecular analysis of *Xanthomonas oryzae* pv. *oryzae*, Northeast Thailand isolates

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IS-MPMI 2009 XIV Congress 52

Bacterial blight (BB), caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo), is one of the most serious diseases of rice in all major rice-growing area of Thailand. The most effective approach to control BB is the use of resistant varieties which is economically feasible, an environmental-friendly control method and easy for farmers to handle. However, the selection pressure from the usage of resistant cultivars may result in mutation of the pathogen population to overcome the resistance genes. Therefore, the use of diverse genetic resources for BB-resistance and a good understanding of pathogen diversity are the major key for a successful rice breeding program for bacterial leaf blight resistance. In this study, 9 Xoo isolates (from 7 locations in Northeast, Thailand, 2004) were differentiated into two pathotypes/races based on pathogenicity reaction of 10 rice near-isogenic lines (NILs) and 4 rice lines/cultivars carrying different resistance genes. Eighty isolates of Xoo from 12 provinces in northeast, Thailand were molecular analysis using PCR-based techniques, i.e. RAPD-PCR, rep-PCR and IS-PCR, with survey and selected 15 primers. Cluster analysis of DNA band patterns could divided Xoo isolates into 13 groups in which isolates form 10 provinces were clustered into a large group. Our results suggest that the pathogen isolates from northeast, Thailand had genetic diversity and were distributed to all parts of the region. Thus, this information is importance for further understanding of the population structure of Xoo in Northeast, Thailand.

Downstream signaling mechanisms of the Rx NB-LRR disease resistance protein

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Dominant disease resistance genes play an important role in plant immunity to pathogens. They are widespread throughout plant genomes and have been useful tools in agricultural improvement. The proteins they encode (R proteins) are most commonly of the NB-LRR class, containing a central nucleotide binding (NB) domain and carboxy-terminal leucine-rich repeats (LRR). These proteins function through recognition of distinct pathogen-encoded proteins and the subsequent induction of defense responses. While a picture of R protein structure and function is developing, relatively little is known about how pathogen recognition is translated into a defense signal. We have found that the highly conserved NB domain of the potato NB-LRR protein Rx is sufficient to initiate defense responses. By screening for proteins that physically interact with the Rx NB domain, we have identified several candidate downstream signal adaptors. We are using virus-induced gene silencing to evaluate the role of these signal adaptor candidates in Rx-mediated resistance responses, as well as responses mediated by other R proteins. We have also applied various functional assays to the characterization of these signal adaptor candidates, the results of which will be presented.

Hormonal interference and promotion of rice growth by the PGPR *Azospirillum lipoferum* 4B

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Azospirillum plant growth-promoting rhizobacteria (PGPR) can grow in intimate relation with roots of monocots, including wheat, corn, and rice, and were shown to facilitate the proliferation of plant roots and/or increase crop yields. *Azospirillum* PGPR benefit the plant via a variety of mechanisms, including associative nitrogen fixation and modulation of the plant hormonal balance. The latter could involve phytohormone biosynthesis noticeably indole-3-acetic acid (IAA), nitric oxide production and/or deamination of the direct plant ethylene precursor, i.e. 1-aminocyclopropane-1-carboxylic acid (ACC). However, most information was obtained from only a few *A. brasilense* strains. In silico genome analysis of the rice isolate *A. lipoferum* 4B showed the absence of any known IAA biosynthesis genes. However, we evidenced by HPLC that strain 4B is actually able to produce new auxin-like compounds, some of them active on rice. In addition, it is one of the few *Azospirillum* strains displaying the ACC deaminase encoding *acdS* gene and AcdS activity. In order to better characterize the plant beneficial traits of this atypical *Azospirillum* phytostimulatory strain, an *acdS-egfp* biosensor and a AcdS$^-$ mutant were constructed to determine whether the bacterial *acdS* gene is expressed on rice roots and contributes to its plant beneficial effect.

Relative importance of different high affinity iron uptake systems for virulence of *Cochliobolus heterostrophus*

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Iron is an essential micronutrient for nearly every organism on earth, including plant and animal pathogens. Host iron is bound to ferritin or other chelators, so successful pathogens must possess a method for high affinity iron acquisition to liberate it. Fungi have two known mechanisms for this. Many produce small Fe^{3+} chelating peptides called siderophores, which can be involved in iron acquisition or homeostasis. Fungi can also reduce Fe^{3+} at the cell membrane and transport it with a high affinity iron permease (FTR), a process called reductive iron assimilation (RIA). The maize pathogen *Cochliobolus heterostrophus* possesses both siderophore (produced by the nonribosomal peptide synthetase NPS6) and RIA mediated iron acquisition and both are iron regulated, suggesting involvement in iron homeostasis. *nps6* mutants display reduced virulence and increased sensitivity to low iron and oxidative stress. To test whether loss of RIA also affects virulence and sensitivity to low iron and oxidative stress, an *ftr*>-deletion strain was generated. Unlike *nps6*-deletion strains, *ftr* strains are like WT with respect to these characteristics. *nps6*;*ftr* double mutants, generated by crossing *nps6* to *ftr* mutants, display growth defects on complete medium compared to *nps6*, as well as a reduction in conidiation. Testing these mutants for sensitivity to low iron, oxidative stress, and virulence will indicate the relative importance of RIA *vs* siderophores for *C. heterostrophus*. If the double mutant can still grow *in planta*, it will indicate that the fungus has a novel method for iron acquisition.

Bacterial oligomers and polymers play opposite roles: MAMPs interact with each other and with host cell walls during induction of calcium signalling, which is suppressed by bacterial EPS

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Bacterial MAMPs are released *in planta* as complex mixtures along with endogenous oligogalacturonan elicitor (OGA). We studied the early responses in *Arabidopsis* of calcium influx and oxidative burst elicited by non-saturating concentrations of bacterial MAMPs, used alone and in combination: flagellin peptide (flg22), elongation factor peptide (elf18), peptidoglycan (PGN) and resulting muropeptides, lipo-oligosaccharide (LOS) and core oligosaccharides. This revealed that some MAMPs have additive (e.g. flg22 with elf18) and even synergistic (flg22 and LOS) effects, while some mutually interfere (flg22 with OGA). OGA suppression of flg22-induced defences was not due to interference with binding of flg22 to its receptor FLS2 and is not exerted at the level of BAK1 as shown by comparing responses in *bak1*- plants. The peptide elicitors are potent at sub-nanomolar levels, whereas in plant cells PGN and LOS only at high micromolar levels induce low and late host responses. This contrast seems to result from restricted access through the plant wall matrix of these macro- or supra-molecular MAMPs. Flg22 is restricted by ionic effects, yet rapidly permeates a cell wall matrix, whereas LOS, which forms micelles, is severely constrained, presumably by molecular sieving. MAMPs induce characteristic calcium influx signatures, but these are concentration-dependent and unlikely to explain differential induction of defence genes *PR1*, *PDF1.2* and *PAL1* by flg22, elf18 and OGA. Calcium influx is a prerequisite for defence responses and is prevented or reduced by diverse pathogens producing Ca^{2+}-chelating EPS in the apoplast.

Genetic and functional characterization of the RTM-mediated resistance

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The RTM-mediated resistance was identified to restrict long distance movement of the potyviruses, *Tobacco etch virus* (TEV), *Lettuce mosaic virus* (LMV) and *Plum pox virus* (PPV) in *Arabidopsis thaliana* (Whitham et al., 1999, Decroocq et al., 2006). At least three dominant genes named *RTM1*, *RTM2* and *RTM3* are involved in this resistance process. Remarkably, a mutation in anyone of these three genes is sufficient to abolish the restriction of the potyvirus long distance movement, suggesting that these genes act in an interdependent way to block the generalized invasion of the plant. *RTM1* encodes a lectin-like protein (Chisholm et al., 2000) and *RTM2* encodes a protein with similarities to small heat shock proteins (Whitham et al., 2000). Both of these genes are expressed in phloem-associated tissues (Chisholm et al., 2001). In order to elucidate the RTM-mediated resistance mechanism, we have undertaken several kinds of experiments: (1) Using a F2 population produced between the susceptible mutant *rtm3* and the RTM-mediated resistant accession Ws-2, *RTM3* was mapped and identified in a genomic interval including 22 genes in chromosome 3 which contains a large number of genes encoding MATH domain-containing protein; (2) Using PPV recombinants produced between a strain able to overcome the RTM resistance and another one unable to overcome this resistance, and after sequencing of LMV variants able to overcome the RTM resistance, we could show that a region of the viral coat protein contains the genetic determinant responsible for overcoming the RTM resistance. Finally, to better understand the mechanism of this original resistance, protein-protein interaction experiments have been performed between the different RTM proteins and the CP of potyviruses.

Identification of the genes involved in the assimilation of the oligosaccharides raffinose, melibiose, sucrose and lactose in *Erwinia chrysanthemi* 3937

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Erwinia chrysanthemi (syn. *Dickeya dadantii*) is a plant pathogenic bacterium which causes soft-rot disease by degrading the cell walls. The most abundant soluble carbohydrates in plant tissues are the two oligosaccharides, sucrose and raffinose. The model strain *E. chrysanthemi* 3937 use these oligosaccharides as carbon source for growth. In contrast, lactose is assimilated only by mutants with a de-repressed oligosaccharide transporter (Lac+ mutants). We analysed the 3937 genome for the presence of potential genes involved in the catabolism of galactosides or glucosides. We identified a group of five genes similar to the cluster *scrKYABR* involved in sucrose catabolism in various enterobacteria and a locus of three genes *rafRBA* homologous to some genes involved in raffinose catabolism in a few *E. coli* strains. The absence of additional *raf* genes present in other enterobacteria suggests that the corresponding functions are complemented by other *E. chrysanthemi* genes. The 3937 genome does not contain homologues of the genes *melA* and *melB* which are involved in melibiose catabolism in *E. coli.* Phenotypic analysis of *scr* and *raf* mutants confirmed the functionality of these genes and revealed cross-links between the assimilation pathways for melibiose, raffinose and sucrose in *E. chrysanthemi.* The names of these genes does not reflect their double function, with *raf* genes involved in melibiose catabolism and *scr* genes contributing to raffinose catabolism. We also demonstrated that the Lac+ mutants arise by de-repression of the transporter RafB. We analysed the role of CRP, RafR and ScrR in the regulation of these pathways. Finally, the importance of such catabolic pathways for the bacterial multiplication in the plant tissues was assessed during the infection process.

Involvement of horizontal gene transfer in the emergence of new pathotypes of *Ralstonia solanacearum*

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Ralstonia solanacearum is a widely distributed phytopathogenic bacterium that is known to invade more than 200 host species, mainly in tropical areas. *R. solanacearum* has been described as a highly flexible organism capable of counteracting plant resistance, leading to the emergence of a new pathological variant. We propose to investigate the fundamental role of horizontal gene transfer (HGT) in its genome evolution. In the *R. solanacearum* species complex, the natural ability to transform has been described as an ubiquitous physiological trait. Eighty percent of strains distributed in all phylotypes - likely species - have the ability to acquire free plasmids and/or genomic DNA (Coupat *et al*., 2008, FEMS Microbiol Ecol 66:14-24). Extent and number of transfer events were also evaluated by using selective antibiotics markers as well as by comparative genomic hybridization with DNA microarrays. Results showed that replacements might involve DNA fragments up to 90 kb and multiple integration events into the *R. solanacearum* genome. In addition, inter-phylotype HGT studies showed that transformants were able to acquire large DNA blocks (30 kb) containing pathogenicity determinants from the DNA donor (Guidot *et al*., 2009, ISME J. doi :1038/ismej.2009.14). The natural transformation mechanism thus appears as one of the main forces for the generation of emergent pathogens. We confirmed that a recombinant strain, which was found to be more virulent on tomato than its wild type parental strain, has acquired a type III effector which was not present in the wild type parental genome.

Characterization of the HrpK1 protein of *Pseudomonas syringae* – A putative translocator

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Pseudomonas syringae pv. *tomato* DC3000 requires a type III protein secretion system (T3SS) for plant pathogenesis. T3SSs allow bacteria to inject type III effector proteins (T3Es) across the eukaryotic plasma membrane into host cells. Translocation of T3Es depends upon the formation of a structure that is an extension off the tip of the Hrp pilus, known as a translocon, which can open the plasma membrane of eukaryotic cells. The translocon from animal pathogens is relatively well understood. However, because the plant cell wall acts as an additional barrier, the translocon in plant pathogens is likely very different from those of animal pathogens. The *P. syringae* HrpK1 protein is a type III-secreted protein and a putative translocator. In addition, *hrpK1* mutants have a reduction in growth and disease symptoms in host plants and a reduced induction of the hypersensitive response (HR) in tobacco. HrpK1 has been purified and used in pore-forming assays. Translocation assays suggest specificity of HrpK1 in promoting the translocation of different T3Es. Members of another group of type III-secreted proteins called harpins are also important for translocation. Experimental data using poly-mutants indicate that *hrpK1* plays a more significant role than harpins in terms of translocation of T3Es. A non-pathogenic *Pseudomonas fluorescens* strain expressing a functional T3SS and the T3E HopA1 causes an HR in tobacco, which is greatly reduced when *hrpK1* is mutated. Interestingly, in this system the *hrpK1* mutant phenotype is complemented by the other harpin proteins. These experiments along with other biological assays that further characterize the *P. syringae* translocon will be presented.

Do botcinic acid and botrydial phytotoxins have redundant functions in the fungal pathogen *Botrytis cinerea*?

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The fungus *Botrytis cinerea*, the causal agent of gray mould disease, affects more than 200 agriculturally important plant species. As a necrotrophic pathogen, this fungus secretes at least two nonspecific phytotoxins i.e. botrydial (a sesquiterpene) and botcinic acid (a polyketide). The botrydial biosynthetic gene cluster was previously characterized by reverse genetics. Botrydial mutants were constructed in several strains which allowed to characterize the toxin as a strain-dependent virulence factor (Siewers et al., 2005; Pinedo et al., 2008). One hypothesis to explain this strain-dependent effect is that, in some strains, the absence of botrydial is compensated by botcinic acid. In order to test this hypothesis, the botcinic acid biosynthesis genes were identified by transcriptomic approaches. The 21 polyketide synthase (BcPKS)-encoding genes predicted in *B. cinerea* genome were spotted on Nylon filters and their expression was studied in different

physiological stages. Two *BcPKS* genes were shown to be up-regulated during tomato leaves infection. Interestingly, one of the two candidate genes is part of a secondary metabolism gene cluster whose expression is regulated by the calcineurin signal transduction pathway as the botrydial gene cluster (Schumacher et al., 2008). Inactivation of this *BcPKS* gene abolished botcinic acid biosynthesis providing the evidence that it encodes a key enzyme for botcinic acid synthesis. In addition to single botrydial- or botcinic acid-non-producing mutants, double mutants were constructed. The phenotypic characterization of the whole set of mutants, together with in planta expression data of both botrydial and botcinic acid gene clusters, provides new insights about the role of these two toxins in *B. cinerea* infectious process.

Functional characterization of *Beet soil-borne mosaic virus* RNA-4-encoded protein

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Beet soil-borne mosaic virus (BSBMV) is a member of the Benyvirus genus together with *Beet necrotic yellow vein virus* (BNYVV), with similar genomic organization and both vectored by *Polymyxa betae*. The ability of BNYVV helper strain to replicate BSBMV RNA-3 suggests a common and conserved viral RNA selection mechanism for both viruses. We recently described a 1,733 nts long BSBMV RNA-4 (GenBank: FJ424610), which has been molecularly and functionally characterized. As for BSBMV RNA-3, full-length BSBMV RNA-4 cDNA clone permitted the obtention of infectious transcripts that BNYVV viral machinery is able to replicate and to encapsidate in planta. Moreover, such BSBMV RNA-4 can substitute BNYVV RNA-4 for an efficient transmission through the vector P. betae in Beta vulgaris plants. Two putative ORFs have been identified and could encode for peptides of 32 kDa (383-1,231 nts) and 13 kDa (885-1,241 nts) respectively. Using BNYVV helper strain, BSBMV RNA4's protein initiation codons have been studied by mutagenesis. We associated the local necrotic lesions phenotype to the protein expression onto mechanically inoculated *Chenopodium quinoa* plants. Flag or GFP-tagged sequences have been expressed in viral context. Western blot analyses of local lesions contents, using FLAG-specific antibody, revealed a high molecular weight protein, which suggest either a strong interaction of BSBMV RNA4's protein with host protein(s) or post translational modifications that need to be better investigated. GFP-fusion sequences permitted the sub-cellular localization of BSBMV RNA4's proteins. Yeast two hybrid system has been employed for search for p32 and p13 partners.

Molecular mechanisms of BABA-induced resistance to pathogens in Arabidopsis: the case of the protein kinase IBS1

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In order to resist pathogen attacks, plants have evolved a network of defense strategies. It has been shown that single molecules can enhance and accelerate the basal defense responses triggered by microbial pathogens if applied to plants ahead of the infection. This phenomenon is called potentialization or priming. We use the non-protein amino acid b-Aminobutyric Acid (BABA) to prime Arabidopsis defenses against a wide variety of pathogens (BABA-induced resistance or BABA-IR). As high doses of BABA lead to sterility in Arabidopsis, we screened a collection of T-DNA tagged Arabidopsis mutants (Versailles collection) and isolated mutants impaired in BABA-induced sterility (*ibs* mutants). Among these mutants, the *ibs1* mutant is compromised in BABA-IR against the bacteria *Pseudomonas syringae* pv. *tomato* and the oomycete *Hyaloperonospora arabidopsis*. Independent mutants with different T-DNA insertions in the *IBS1* gene (SALK mutants) confirmed the role of IBS1 in BABA-IR dependent on the Salicylic Acid pathway. The main priming molecular model suggests that the priming agent induces the production of proteins in their inactive forms, which are switched on to their active forms upon pathogen attack. Transcription factors and protein kinases are among our most likely candidates. Fitting in with this model, *IBS1* encodes a cyclin-dependent kinase-like protein which phosphorylation activity could lead to the activation of its target proteins that act in the defense pathways. Recent updates and highlights of three projects will be presented and discussed.

Role of type III secretion system and adhesins in the fitness of *Xanthomonas fuscans* subsp. *fuscans* in bean phyllosphere and in transmission to seeds

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Deciphering the mechanisms enabling plant pathogenic bacteria to disperse, colonize and survive on their hosts provides the necessary basis to set up new control methods. We evaluated the role of the type III secretion system (T3SS) and adhesins in two steps of the asymptomatic host colonization process: phyllospheric colonization and transmission to seeds. *Xanthomonas fuscans* subsp. *fuscans* is responsible for the common bacterial blight of bean, a seedborne disease. Unlike the wild-type *X. fuscans* subsp. *fuscans*, strains with mutations in T3SS regulatory genes were impaired in their phyllospheric growth as was *Escherichia coli* on bean. Strains with mutations in the *hrp* structural genes maintained the same constant epiphytic population densities as did *X. campestris* pv. *campestris* on bean in a non-host interaction. Among the five adhesins identified in *X. fuscans* subsp. *fuscans*, only the non-polar adhesin YapH was required for adhesion on leaves. Transmission to seeds by the vascular pathway was abolished for mutants in T3SS regulatory and structural genes, and remained possible but altered, for mutants in adhesin genes, except for mutant in *yapH* which behaved as the wild-type strain. Transmission to seeds by floral structures did not require any of the known adhesins and remained possible but with a low efficiency for *hrp* mutants and was repeatedly recorded for a non-host pathogen (*X. campestris* pv. *campestris*). *E. coli* did no transmit to bean seed. In conclusion, we showed that T3SS and bacterial adhesins are implicated in the various processes leading to host phyllosphere colonization and systemic transmission to seeds in the absence of symptoms in compatible interactions.

Molecular events leading to virulence at the *AvrLm4-7* locus in *Leptosphaeria maculans* field populations highlight a complex interaction between *AvrLm3* and *AvrLm7* avirulence functions

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The stem canker fungus, *Leptosphaeria maculans*, is the main pathogen of oilseed rape (*Brassica napus*) world-wide. Gene-for-gene interactions involving avirulence (AVR) genes of the pathogen and major resistance (R) genes in the plant are the rule in this pathosystem and condition the outcome of the interaction, i.e., resistance or disease. In France, the *Rlm7* resistance is currently deployed in commercial cultivars and provides us the opportunity to investigate initial molecular mechanisms conditioning the evolution of an AVR gene, *AvrLm4-7*, under selection pressure. *AvrLm4-7* specifies a double interaction specificity, because it is recognised by both the *Rlm4* and *Rlm7* R genes (Parlange et al 2009). Population surveys suggest that loss of the *AvrLm7* specificity necessitates complete loss of function or drastic down regulation of the gene. The analysis of 400 naturally occurring *avrLm7* isolates showed a large number of molecular mechanisms leading to virulence. Intriguingly, 99% of the *avrLm7* isolates gained the *AvrLm3* specificity, virtually absent in European populations. Genetic analysis showed that *AvrLm3* and *AvrLm4-7* are two distinct genes and complementation of an *AvrLm3* isolate with *AvrLm4-7* resulted into loss of the *AvrLm3* specificity thus indicating a functional interaction between the two genes. Detailed analysis on molecular events leading to *avrLm7* virulence and progresses toward map based cloning of *AvrLm3* will be presented.

Evolution of a nomadic subtelomeric disease resistance gene cluster in common bean

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The B4 resistance (*R*)-gene cluster, located in subtelomeric region of chromosome 4, is one of the largest clusters known in common bean (*Phaseolus vulgaris*, *Pv*). We sequenced 650 kb spanning this locus and annotated 97 genes, 26 of which correspond to Coiled-coil-Nucleotide-Binding-Site-Leucine-Rich-Repeat (CNL). Conserved microsynteny was observed between the *Pv* B4 locus and corresponding regions of *Medicago truncatula* and *Lotus japonicus*, in chromosomes *Mt*6 and *Lj*2, respectively. The notable exception was the CNL sequences, which were completely absent in these regions. The origin of the *Pv* B4-CNL sequences was investigated

through phylogenetic analysis, which reveals that, in the *Pv* genome, paralogous CNL genes are shared among nonhomologous chromosomes (4 and 11). Together, our results suggest that *Pv* B4-CNL derived from CNL sequences from another cluster, the Co-2 cluster, through an ectopic recombination event. Integration of the soybean genome data enables us to date more precisely this event and also to infer that a single CNL moved from the Co-2 to the B4 cluster. Moreover, we identified a new 528-bp satellite DNA, referred to as *khipu*, in the *Pv* B4 locus. It was shown to be specific to the *Pv* genus and present on most chromosomal termini, indicating the existence of frequent ectopic recombination events in *Pv* subtelomeric regions. Our results highlight the importance of ectopic recombination in *R*-genes evolution.

Regulation of *Pseudomonas* sp. DF41 biocontrol activity

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Sclerotinia sclerotiorum is an economically important plant pathogen that infects over 400 species, including canola (*Brassica napus* L.). A novel *Pseudomonas* species, DF41, isolated from the canola rhizosphere consistently demonstrates inhibition of *S. sclerotiorum in vitro* and in greenhouse assays. Through transposon mutagenesis, two mutants exhibiting a loss of antifungal (AF) activity have been identified. The first mutant, DF41-1278, carries an insertion in a biosynthetic gene required for the production of a cyclic lipopeptide (CLP) antibiotic. Thus, it appears that CLP production is essential for DF41 biocontrol. The second mutant, DF41-469, has an insertion in gacS, which forms part of the GacS/GacA two-component global regulatory system. Several other DF41 regulatory elements have been identified including a quorum-sensing (QS) system, the stationary phase sigma factor RpoS and a small regulatory RNA called RsmZ. We are currently elucidating the hierarchy of gene regulation in DF41. A DF41 strain harboring an autoinducer (AI) degradation gene (aiiA) that is no longer capable of QS, showed increased expression of rpoS'-'lacZ and rsmZ' –'lacZ transcriptional fusions; however, AI'-'lacZ expression was decreased. When grown in the presence of exogenous AI, rpoS and rsmZ are repressed, while AI gene expression is increased. Collectively, these findings indicate that in DF41 i) QS represses transcription of rpoS and rsmZ and ii) AI gene expression is positively autoregulated. Interestingly, DF41 carrying the aiiA gene exhibits increased AF activity in vitro. Analysis of a clp::lux fusion revealed that CLP gene expression is not increased in this background; therefore, another AF compound must account for the enhanced biocontrol.

A tomato calcineurin B-like protein and its target, a CBL- interacting protein kinase, define a novel Ca^{2+}-mediated signaling pathway in plant innate immunity

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A transient increase in intracellular Ca^{2+} is an early event in plants after pathogen perception and is necessary for full resistance activation. How this specific Ca^{2+} signature is decoded by calcium sensors and transformed into downstream responses leading to plant immunity is not well understood. To identify cellular components of the *Pto*-mediated hypersensitive response (HR) signal transduction pathway, we conducted a virus-induced-gene-silencing (VIGS) screen in *Nicotiana benthamiana*. A calcium sensor (calcineurin B-like; CBL) and a CBL-interacting protein kinase (CIPK) were identified in our random gene silencing screening. We tested their interaction by means of a yeast two hybrid assay and pull-down experiments and characterized the *Solanum lycopersicum* ortholog SlCIPK kinase activity *in vitro*. We found that the CBL and CIPK proteins participate in bacterial, fungal and nematode-elicited HR, thus being convergent elements in immunity signaling. Finally, we characterized the possible role of these proteins in the immune and susceptibility responses in tomato towards *Pseudomonas syringae* pv *tomato*. This is the first time that a CBL/CIPK signaling module has been implicated in biotic stress signaling. Since their participation in abiotic stress signaling is well characterized, our results establish CBLs and CIPKs as global mediators of plant stress responses.

Myzus persicae* (green peach aphid) salivary components induce defense responses in *Arabidopsis thaliana

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Myzus persicae (green peach aphid) feeding on *Arabidopsis thaliana* induces a defense response that can be quantified as reduced aphid progeny production. These increased plant defenses are measurable in the infested leaves but not in other parts of the plant. Similarly, infiltration of aphid saliva into Arabidopsis leaves causes only a local increase in aphid resistance. Further characterization of the defense-eliciting salivary components indicates that Arabidopsis recognizes a proteinaceous elicitor with a size between 3 to 10 kD. Genetic analysis using well-characterized Arabidopsis mutants shows that saliva-induced resistance against *M. persicae* is independent of the known defense signaling pathways involving salicylic acid, jasmonate, and ethylene. Among 78 Arabidopsis genes that were induced by aphid saliva infiltration ($P < 0.05$, >1.5-fold increase), 52 had been identified previously as aphid-induced. Conversely, among 47 down-regulated genes, 19 are also down-regulated by aphid feeding. Most genes that are significantly induced by aphid saliva treatment are known to be stress-responsive, but few are responsive to the well-known plant defense signaling molecules salicylic acid and jasmonate. Quantitative PCR analyses confirm expression of saliva-induced genes. In particular, expression of a set of *O*-methyltransferases, which may be involved in the synthesis of aphid-repellent glucosinolates, was significantly up-regulated by both *M. persicae* feeding and treatment with aphid saliva. However, this did not correlate with increased production of 4-methoxyindol-3-ylmethylglucosinolate, suggesting that aphid salivary components trigger an Arabidopsis defense response that is independent of this aphid-deterrent glucosinolate.

Identification of cellular mechanisms targeted by *E. amylovora* type III effector DspA/E

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Fire blight is a devastating bacterial disease of *Maloideae*, a tribe of *Rosaceous* plants including apple, pear and ornamentals. The causal agent is the enterobacteria *Erwinia amylovora* whose pathogenicity relies on a type III secretion system (TTSS). The TTSS allows bacteria to secrete in the plant apoplasm and/or inject into plant cells effector proteins called type III effectors (TTEs). TTEs are known to modify plant metabolism and/or to suppress plant defense mechanisms thus allowing bacteria to colonize plant tissue. It was shown that *E. amylovora* secretes and/or injects in plant cells at least 12 TTEs. Only two of those, DspA/E and HrpN, have been shown to be involved in the pathogenicity of *E. amylovora*. The TTE DspA/E is injected in plant cells and is absolutely required for pathogenicity as *dspA/E* mutants are non pathogenic. Furthermore, transient expression in plant cells indicates that DspA/E is sufficient to cause cell death in eucaryotic cells. To understand the function of DspA/E inside the plant cell, we constructed Arabidopsis transgenic plants expressing *dspA/E*. We analyzed the phenotype of transgenic plants, identified Arabidopsis genes whose expression was modulated by DspA/E and observed the localization of DspA/E in plant cells. Alltogether, these data allowed us to identify cellular mechanisms that are targeted by DspA/E.

Regulation of a new utilization pathway of xylan degradation products by the LacI repressor XyxR in *Xanthomonas campestris* pv. campestris

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The microbial degradation of plant cell wall is not only an important biological process but is of increasing scientific interest for biotechnological applications. *Xanthomonas campestris* pv. campestris (*Xcc*), the causal agent of black rot disease of Brassicaceae, is known for its ability to exploit plant compounds. This characteristic seems to be linked to the over-representation of TonB-dependent transporters (TBDTs) and the presence of numerous CUT systems (Carbohydrate Utilization systems containing TBDTs) required for the scavenging of plant carbohydrates. In this study, we describe a new *Xcc* CUT system involved in xylan utilization which is controlled by the LacI-type repressor XyxR (encoded by *XCC4101*). We show that xylo-oligosaccharides are better inducers than the monomer, xylose. This repressor regulates the expression of genes present in three loci. These loci encompass two TBDTs, two inner membrane transporters, two xylanase, a xylosidase and other degradative enzymes involved in the utilization of xylan and its degradation product. Based on indirect transport assays, we show that the two TBDTs of the xylan CUT system, XCC4120 and XCC2828, are required for the uptake of xylo-oligosaccharides, confirming their role in carbohydrate scavenging. Moreover, xylo-oligosaccharides and xylose are transported exclusively

across the two inner membrane transporters of the xylan CUT system. Xylan is the major component of hemicellulose in plant cell walls. Xylo-oligosaccharides uptake through TBDTs represents a new aspect in the exploitation of these molecules by bacteria. It suggests the existence of active and specific mechanisms for nutrient uptake through the outer membrane.

Characterization of protection markers in *Vitis vinifera* L. cv Chardonnay against *Botrytis cinerea* by proteomic analysis

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Grey mold is one of the main diseases affecting vineyard. Unfortunately, the use of chemicals treatments, known to damage environnement, remain nowadays the only solution to cope with *Botrytis cinerea*, the agent of grey mould. An alternative strategy is the use of elicitors to stimulate plant defense mechanisms and thus prevent disease. Nevertheless no elicitors has shown, to date, a real protective effect against grey mold in the vineyards, although most of them induce the expression of defense gene in controlled experiments. In this context, it appears crucial to characterize biomarkers which would be able to discriminate between grapevine defense stimulation and effective protection against *Botrytis cinerea*. The aim of this study is to unravel protection biomarkers by two dimentionnal electrophoresis proteomic analysis comparing between "protective elicitor" treated plant and plant stimulated by "a non protective elicitor". Our first results have identified three different elicitors with protective or non protective activity against *Botryitis cinerea*. Previous data shown clear diffences between this three elicitors. Further experiments should allow to identify specific protection biomarkers in grapevine against *Botrytis cinerea*. The identification of protection biomarkers would improve comprehension of plant defense mechanisms against *Botrytis cinerea* and allow to developp large scale screening tools for analysis of new elicitors confering grapevine protection against grey mold and other pathogens. This work was supported by the *Région Champagne Ardenne* and by the *Comité Interprofessionnel des Vins de Champagne.*

WAKomics: Large-scale functional analysis of the WAK genes involved in the rice/*Magnaporthe grisea*/ interaction

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Wall-associated protein kinases (WAKs) are a new group of receptor-like kinases (RLKs) recently identified in *Arabidopsis* and in rice. A gene responsible for the quantitative resistance to *Fusarium oxysporum* in *Arabidopsis*, RFO1, encodes a WAK-like protein (WAKL22). This finding suggests an important role for some WAKs in disease resistance. The first WAK in rice was recently identified as OsWAK1. The induction and overexpression of this gene in rice led to enhanced resistance to rice blast. Furthermore, WAKs also seem to interact with a wide range of proteins involved in cell-wall composition, phosphorylation or transcription processes. This is functional evidence that WAKs are novel RLKs playing an important role in plant disease resistance. Microarray data reveal differential expression of some WAKs in rice leaves infected with *Magnaporthe grisea*. This set of WAKs was analysed by real time PCR in a time course experiment and confirmed the microarray results. This analysis also revealed differential expression for some WAKs very early after infection (4, 6 or 8 hours post inoculation), suggesting an early role in pathogen recognition. To confirm a role of these WAKs in rice blast resistance, Knock-Out and transgenic rice lines with constitutive expression of a subset of WAKs were produced. Kinase assays, localisation and molecular interactions using a reporter system are also planned. Preliminary data supporting the role of this gene family in disease resistance in rice will be presented.

***Arabidopsis WAT1*, a homolog to *Medicago truncatula Nodulin21*, is essential for auxin homeostasis, secondary cell wall formation, and plant-pathogen interactions**

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We have identified an *Arabidopsis* mutant, *wat1* (*wall and tryptophan1*), with increased tolerance to *Ralstonia solanacearum*, a soil-borne vascular pathogen that causes bacterial wilt in numerous plant species. Our study of *WAT1,* an *Arabidopsis* homolog of *Medicago truncatula Nodulin21*, provides the first functional characterization of a member of the Plant-Drug/Metabolite Exporter family. From a developmental standpoint, *WAT1* encodes a novel tonoplast-localized transporter that is required for auxin homeostasis and secondary wall formation. To characterize *wat1* tolerance to *Ralstonia*, several approaches are being employed. We are performing cell wall phenotyping of *wat1* vs. wild-type roots using cytology and immunocytochemistry. Experiments with pectin antibodies revealed that *wat1* roots contain constitutively higher amounts of pectin, mainly localized in the stele, than in the wild-type. We have also determined the effect of the *wat1* mutation on pathogen multiplication. Bacterial density in the aerial portion of the plant is significantly reduced in *wat1*. To test if *wat1* tolerance is specific to *Ralstonia*, we tested a battery of vascular (*X. campestris* pv. *campestris, P. cucumerina, V. dahliae*) and non-vascular (*P. syringae* pv. *tomato, C. higginsianum*) pathogens. The systematic tolerance to vascular pathogens and the sensitivity to non-vascular pathogens suggest that mechanisms involved in *wat1* tolerance might be localized within the vascular system. To determine if ethylene, jasmonic acid, and/or salicylic acid signaling pathways are involved in *wat1* tolerance, double mutants were generated by crossing *wat1* with *ein2, coi1, jar1-1, sid2,* and NahG transgene. Finally, comparative transcriptomic analyses of *wat1* vs. wild-type have been performed to identify novel constitutive mechanisms associated with *wat1* tolerance.

Tobacco rattle virus* RNA1 as a vector to induce gene silencing on *Nicotiana benthamiana

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Virus-induced gene silencing (VIGS) is used for characterising the functions of plant genes. *Tobacco rattle virus* (TRV) that belongs to the (+)ssRNA viruses of the genus *Tobravirus* and has a bipartite genome can infect a large number of plant species. Previously used TRV VIGS vectors contain the silencing-inducing heterologous sequence inserted into RNA2 but have difficulties to induce gene silencing in our model plant *Gerbera hybrida* due to the spreading obstacle of TRV RNA2. In order to achieve efficient gene silencing on *Gerbera hybrida*, two new TRV VIGS vectors M1 and M2 were developed, in which the *16K* gene on RNA1 was partly deleted so to remove the recently identified 16K silencing suppressor protein and to vacate space for the silencing-inducing plant gene insert. A mutant (16Kstop) was also constructed to cause an early terminator of the 16K translation. Infectivity and gene silencing efficiency of the constructs were assessed. The infectivity of M1 and M2 was lower than that of the non-modified TRV RNA1. M1 and M2 carrying a fragment of the phytoene desaturase gene (PDS) gene at the position of 16K gene induced silencing of PDS on *Nicotiana benthamiana*, but with a limited efficiency as compared with the TRV VIGS vectors carrying the *PDS* gene fragment on RNA2. The 16Kstop mutant exhibited slower systemic virus movement but caused more severe necrosis symptoms in the systemically infected leaves of *N. benthamiana* than TRV carrying the wild-type RNA1. These results showed that the 16K protein and RNA sequence regulate virulence of TRV. Although they are dispensable for systemic virus infection and 16K acts as a suppressor of silencing, removal of the 16K protein or disruption of the 16K sequence with foreign gene inserts does not result in enhanced VIGS.

Priming of defense responses in rice cells by bacterial lipopolysaccharides

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"Primed state" is the physiological condition of plants in which they can more rapidly mount defense responses to biotic or abiotic stress. Manipulation of this ability of plants to establish a novel disease resistant crop seems to be

advantageous compared to the strategy that includes the constitutive activation of defense machinery. However, the molecular mechanism that leads to the primed state, which is the prerequisite for such applications, is largely unknown. We recently reported that bacterial LPSs act as a potent elicitor to induce defense responses associated with PCD in suspension-cultured rice cells. We also found that the pretreatment of the rice cells with a low concentration of LPS clearly induced primed state for following chitin elicitation. To confine the structure required for these biological activities of LPS, we examined the activity of LOS, a smaller molecule consisting of a defined oligosaccharide unit and Lipid A. LOS induced defense responses and showed priming activity in the rice cells as similar to the whole LPS molecules, indicating the importance of this structure for these biological activities. Chemical modification of LOS and the analysis of the activity of the products are underway to further clarify the critical structure for these activities. As some types of priming, such as SAR and ISR, have been known to involve phyotohormones for signaling, we are presently analyzing the dynamics of phytohormones during the establishment of LPS-mediated primed state, aiming to clarify the role of these phytohormones in priming/defense responses.

Today is a good day to die: Defining the molecular mechanisms of RPS5-mediated defense response

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Although plant NBS-LRR proteins were shown to mediate pathogen recognition over 15 years ago, the molecular mechanisms of their activation are still poorly understood. We are studying NBS-LRR protein activation using the Arabidopsis protein RPS5. RPS5 is an NBS-LRR resistance protein of the non-TIR class and is required for recognition of the *Pseudomonas syringae* effector AvrPphB, a cysteine protease that cleaves the host protein kinase, PBS1. RPS5 recognizes AvrPphB indirectly by monitoring the status of PBS1. Upon PBS1 cleavage, RPS5 is activated and triggers plant defense responses. Systematic truncation and mutation of PBS1 has shown that the kinase domain, but not kinase activity, of PBS1 is required for activation of RPS5. After cleavage by AvrPphB, a portion of the PBS1 cleavage products remain bound to RPS5. Microscopic analysis indicates that RPS5 and PBS1 are localized to the plasma membrane prior to AvrPphB. We are currently assessing whether RPS5 and/or PBS1 relocalize after activation. To identify potential downstream interactors of RPS5, we have purified RPS5-containing protein complexes and begun to characterize co-purifying proteins. We have also begun to examine the evolutionary relevance of the AvrPphB-PBS1-RPS5 system by assessing the ability of AvrPphB-related proteins to cleave PBS1 and activate RPS5 and the ability of AvrPphB to cleave distantly related PBS1 orthologs. Together, these experiments afford a better understanding of the mechanics of pathogen detection.

Molecular and biochemical characterization of Nodulin 22 of *Phaseolus vulgaris*: A protein implicated in adaptation to oxidative stress and "defense" mechanisms in root nodules

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We isolated a clone from a common bean (*Phaseolus vulgaris* L.) cDNA library derived from *Rhizobium*-infected roots. The deduced protein, called Nodulin 22 (Nod22) due to its molecular weight, contains a highly hydrophobic N-terminus, with signal peptide characteristics, and a C-terminal extension with high identity to the alpha-crystallin domains found in alpha-crystallin lens chaperones and other small heat-shock proteins (sHSPs). sHSPs family of molecular chaperones is unusually abundant and diverse in higher plants as opposed to other eukaryotes. In addition, their selective induction upon a variety of environmental stresses suggests that these proteins play an important role in stress tolerance. Indeed, Nod22 transcripts are accumulated from early to late stages in root nodule development and its expression is mimicked following paraquat (1,1'dimethyl-4,4'-bipyridinium ion) treatment in young roots. Remarkably, when this protein is over-expressed in *Escherichia coli* it confers protection against oxidative stress. Preliminary phenotypic characterization of transgenic 35S:Nod22 nodules as well as Nod22 RNAi nodules suggest that this protein is profoundly involved in the nodulation process.

Seed-transmission of *Peronospora* sp. on sweet basil – Development of a rapid and specific pathogen detection method

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Downy mildew (*Peronospora* sp.) of sweet basil (*Ocimum basilicum*) has become a major problem in Europe since 2001. The rapid spread of the disease in many commercial farms in different European countries led to the assumption that *Peronospora* sp. is transmitted through infected seeds. Glasshouse and growth chambers experiments with different seed samples obtained from commercial production confirmed that seeds are a potential source of pathogen inoculum. Methods used recently for identification of downy mildew on seeds are time consuming and non specific. Hence, the development of a rapid and specific method for detection of *Peronospora* sp. especially in basil seed samples was urgently necessary. Based on sequence information of the ITS-region a primer pair was used for a specific identification of the pathogen in basil seeds and plant samples. The use of our PCR-method allows an early detection of the downy mildew pathogen before symptom expression in plant samples and seeds. The detection of *Peronospora* sp. in seed samples with the new PCR- method supported the hypothesis that seeds are an inoculum source and responsible for the spread of the pathogen from the seed production area to the commercial basil production.

Vcp1 and Vcp2, two proteins that tame virulence of the biotrophic fungus *Ustilago maydis*

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Ustilago maydis is a biotrophic fungus and causes smut disease in its host maize. Recently, its genome was fully sequenced and genome wide expression analysis enabled the identification of gene clusters coding for novel secreted proteins which play an important role during the infection process of the host plant maize. Whereas many of the identified cluster-deletions lead to impaired pathogenicity one cluster was identified where the deletion causes a hyper-virulent phenotype. By subdeletions, the main effectors for the hypervirulence phenotype have been identified as the products of two related genes *vcp1* and *vcp2* (virulence controlling protein). We will present results from our current functional analysis of these two secreted proteins with respect to in vivo localisation, expression and putative Interaction partners. Furthermore the core-region of the proteins responsible for the tamed virulence was identified by mutagenesis and a working-model to explain the observed phenotype will be presented. The long term goal is to obtain a molecular understanding of the mechanism that restricts virulence in this biotrophic fungus.

Pep1, a novel effector protein of *Ustilago maydis* is required for the establishment of biotrophy

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The basidiomycete *Ustilago maydis* causes smut disease in maize. The biotrophic interaction between U. maydis and its host plant is initiated by direct penetration of cuticle and cell wall of maize epidermis cells. The invading hyphae are surrounded by the plant plasma membrane and proliferate within the plant tissue. We identified a novel secreted protein (Pep1: Protein essential during penetration) which is specifically expressed during biotrophic growth. Disruption mutants of pep1 are not affected in saprophytic growth and develop normal infection structures. However, Δpep1 mutants arrest immediately upon penetration of the epidermal cell and elicit strong plant defense responses that resemble a non-host response. By in-vivo immunolocalization, live-cell imaging and plasmolysis approaches we detected Pep1 in the biotrophic interface as well as its accumulation at sites of cell to cell passages. Site-directed mutagenesis identified the four cysteine residues in Pep1 as essential for function suggesting that the formation of disulfide bridges is crucial for proper protein folding. An ortholog of pep1 encoded by the barley covered smut fungus Ustilago hordei is also needed for penetration of barley. Moreover, uh-pep1 is able to complement the U. maydis Δpep1 mutant. From this we conclude that Pep1 has a conserved function in smut fungi. To understand the mechanism how Pep1 suppresses host defense responses we are currently identifying Pep1-interacting proteins by co-immunoprecipitation. The results from these studies will be presented.

Molecular characterization and tolerance to heavy metals of methylotrophic endophytic bacteria associated with mangrove ecosystems

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Mangrove ecosystem due to the high biologic productivity which demands a high nutrient availability at the beginning of the trophyc chain, microorganism activities are essential in the organic matter decomposition process and nutrient fixation. Bacteria species adapted to the mangrove conditions present a potential source of biotechnologies resources such as discovery of new bacteria species that produces enzymes. The genus *Methylobacterium* is constituted by the PPFMs (pink-pigmented facultative methylotrophic) bacteria. They can fix nitrogen, nodulate the host plant, produce cytokinin (plant hormone) and enzymes like pectinase and cellulose. These characteristics make *Methylobacterium* spp. important species on the plant growth promotion. In this context, it was selected only *Methylobacterium* sp. from samples in Bertioga, SP, Brazil, of places with and without oil spilling, of *Rhizophora mangle*, *Avicenia nitida* and *Laguncularia racemosa* plant species. It was assessed the isolates tolerance to different heavy metals: Cadmium, Lead and Arsenic in different concentrations (0,1mM, 0,5mM, 1mM, 2mM, 4mM and 8mM), and also the genetic diversity of *Methylobacterium* spp. by the sequence analysis of the gene 16S rRNA (ribosomal gene). The isolates from the place with oil spilling were grouped, suggesting that oil can select microorganism that tolerate oil compounds and change the methylotrophic bacteria community. Cadmiun is the most toxic heavy metal assessed in this work followed by Arsenic and Lead. It was found two isolates that are tolerant to cadmium, lead and arsenic that present a potential to be used to biorremediate this environment, immobilizating this metals that are present in the oil, from the mangrove with oil spilling.

Both lipid A and the O-antigen from *Burkholderia cepacia* lipopolysaccharides contribute to induction of defense genes

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Lipopolysaccharides (LPS) are ubiquitous, indispensable components of the cell surface of Gram-negative bacteria that have diverse roles in bacterial pathogenesis of plants. LPS are tripartite amphipathic molecules, consisting of a Lipid A moiety that is embedded in the outer leaflet of the phospholipid/protein bilayer, a core oligosaccharide, and a polysaccharide consisting of repeating units, the O-Antigen. The LPS from the endophyte, *Burkholderia cepacia*, has been used as a PAMP in several studies. Thus far the eliciting parts of the LPS lipoglycan have not yet been identified, but the Lipid A, the core and O-Antigen are potential elicitors of plant defense responses. The current study investigated the contribution of two subcomponents to the biological activity of LPS by monitoring the expression profiles of the genes induced in *Arabidopsis thaliana* seedlings in response to elicitation by either Lipid A or O-Antigen-core compared to the genes induced by intact LPS molecules. ACP-DDRT-PCR, in conjunction with pyro-sequencing, was found to offer an effective approach to study differential gene expression. Lipid A and O-Antigen-core were able to trigger gene expression associated with defense and thus to contribute to the plant responses launched following perception of LPS. However, more research still has to be carried out in order to distinguish between unique and convergent downstream responses.

Dihydrochalcones could play a dual role in resistance of *Malus* towards *Erwinia amylovora* through antioxidant and antibacterial activities

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Dihydrochalcones (DHC) represent a family of phenolic compounds found in *Malus* where they are present in large amounts in leaves (100 to 150 mg/g DW) and shoots. The involvement of these secondary metabolites and their oxidation products in resistance towards the necrogenic bacteria *Erwinia amylovora* (causative agent of fire blight), was investigated in two apple genotypes with contrasting susceptibilities. The constitutive phenolic pattern was analyzed by means of RP-HPLC-ESI-MS. Profiles revealed that phloridzin (phloretin-2-O-glucoside) is the major DHC present in leaves of the susceptible genotype (100 mg/g DW) whereas two additional compounds were detected in large amounts in the resistant cultivar (50 and 10 mg/g DW). These compounds were identified by ESI-MS and RMN 1H and 13C as sieboldin (3-hydroxyphloretin-4-O-glucoside) and trilobatin (phloretin-4-O-glucoside), respectively. Neither of the compounds exhibited significant antibacterial activity, however sieboldin showed a high antioxidant activity as evidenced by a strong DPPH* reducing activity and ex vivo myorelaxant rat mesenteric artery activity. During bacterial infection or *in vitro* oxidation, constitutive DHC are transformed into oxidation products showing both antibacterial and high antioxidant activities in the resistant genotype only. DHC transformation involves polyphenol oxidases (PPO) which are highly induced in leaves challenged with the bacteria in both genotypes. However, cultivar specific PPO profiles lead to the production of a differential set of transformation products. Thus, these results suggest a dual role for DHC and their oxidation products in resistance by creating i) a high constitutive or induced antioxidant status and/or ii) a direct antibacterial activity towards *E. amylovora*.

Dissection of the *Medicago truncatula/Aphanomyces euteiches* pathosystem revealed new molecular components involved in the interaction between plant and oomycetes

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The use of *Medicago truncatula* as a legume genetic model system has greatly increased our knowledge of molecular mechanisms of root symbiosis. In contrast, the genomic resources of this plant have not been exploited to characterize legume-pathogen interactions. Among the pathogens which are responsible for economic losses on legumes, we have focused our work on the interaction between M. truncatula and the oomycete *Aphanomyces euteiches*, a major root pathogen of pea and alfalfa. For both partners of the interaction, genomic approaches were developed. Using the nearly sequenced A17 line as the resistant parent and the F83.005 line as the susceptible one, a major QTL of resistance was mapped on the top of chromosome 3. Resistance is associated with a protection of the root stele characterized by additional pericycle division and increased lignification. Fine mapping allowed the identification of a 135 kb region rich in proteasome-related genes, suggesting a novel mechanism involved in oomycete resistance. To identify new pathogen effectors more than 20,000 ESTs were generated, clustered into 8,000 unigenes and deposited in a public database (AphanoDB). In silico analysis of the sequences revealed new biosynthetic pathways which were never described in oomycetes such as sterol biosynthesis, and the presence of putative virulence factors distinct from *Phytophthora*. Taken together, these approaches will lead to the large scale identification of plant and microbe genes playing a key role for the outcome of the *M. truncatula* - *A. euteiches* interaction.

A decade of proteomics for studying arbuscularmycorrhizal symbiosis: From axenic system to soilborne interactions

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Proteomics has emerged as a powerful tool to point out protein modifications in roots interacting with arbuscular mycorrhizal (AM) fungi and nitrogen-fixing rhizobia. Regarding the AM symbiosis, most of the proteomic studies were performed with *Medicago truncatula* inoculated with controlled AM inocula. Depending on developmental AM stage and amount of material available, untargeted and/or sub-cellular proteomics were applied to identify proteins whose accumulation was modified during the AM process. For the early stage, the protein patterns of non-inoculated and roots synchronized for appressorium formation in wild-type [A17], penetration-defective [*dmi3*] and autoregulation-defective [*sunn*]genotypes were compared, allowing identification of proteins potentially early involved in recognition, signalling and defence reactions. In mature *M. truncatula* mycorrhiza, sub-cellular proteomics targeted symbiosis-related membrane proteins eligible as involved in nutrient transport and signalling upon arbuscule functioning. The overlaps between the 2 common AM isolates (*G. mosseae*, *G. intraradices*) were studied through high throughput comparative proteomics allowing identifying, in addition to plant proteins, several AM proteins. A step forward metaproteomics of root responses to soil endogenous microbial communities was recently undertaken by combining the use of plant genotypes with contrasted behaviour towards the main symbionts occurring in natural communities (AM fungi and rhizobia) with comparative 2-DE proteome

profiling. All genotypes were grown in a silt-clay loam soil containing its own endogenous microbial communities and we expect to reveal the global root proteome orientation in response to one or the other natural micro-symbiont community.

Deletion of a fungal endophyte stress-activated MAP kinase disrupts host development

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The mutualistic association between the fungal endophyte *Epichloë festucae* and perennial ryegrass provides an excellent model for studying the signalling involved in regulating plant-fungal interactions. In this association, fungal growth is highly regulated and co-ordinated with host growth. Production of reactive oxygen species by the fungal NADPH oxidase complex is essential for this association, however, the exact nature of the signalling involved is unclear. The aim of this project was to test if the stress-activated MAP kinase, *sakA*, plays a role in this signalling. Seedlings were inoculated with wild-type E. festucae (WT) and a Δ*sakA* mutant. This mutant had reduced ability to colonise perennial ryegrass seedlings, however, where colonization was established both host and symbiont showed drastically altered phenotypes. Fungal biomass *in planta* was greatly increased and hyphae appeared hyper-branched, irregular in size and shape and were surrounded by an electron dense extracellular matrix, indicative of a host defense response. Hydrogen peroxide levels surrounding the hyphae were also much higher than in WT associations. The host displayed stunted growth with poor root system development and premature senescence. However, the most striking change was that the base of tillers infected with the mutant appeared bulbous and lacked anthocyanin pigmentation. Microscopic examination revealed that the host cells below the shoot apical meristem were disorganized and no longer formed linear cell files, causing the base to bulge. This suggests appropriate signalling by the stress-activated MAP kinase is essential for maintaining the mutually beneficial association with perennial ryegrass, and when this signalling is disrupted dramatic changes in host development result.

The involvement of *BAX INHIBITOR-1*-like genes in the interaction of plants with biotrophic powdery mildew fungi

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Host cell death or survival is crucial in the intimate organismic interplay of plants and parasites. In barley, over-expression of the conserved cell death inhibitor *BAX INHIBITOR-1* (*BI-1*) supports establishment of haustoria of the biotrophic powdery mildew fungus (*Blumeria graminis* f. sp. *hordei*) but limits success of pathogens that rely on host cell death. In contrast, knock-down of *BI-1* reduces penetration frequency of *B. graminis*. In *Arabidopsis thaliana*, we observed *AtBI-1* promoter activity at the site of infection by the powdery mildew fungus *Golovinomyces orontii*. *AtBI-1* over-expression supports development of powdery mildew fungi on *Arabidopsis* and barley. However, the knock-out of *AtBI-1* does not dramatically alter the powdery mildew infection phenotype. This might be due to functional redundancy of *BI-1*-like genes, of which several are present in the *Arabidopsis* genome. The investigation of *Arabidopsis* T-DNA insertion mutants revealed one *bi-1*-like mutant, which displayed strongly suppressed conidiophore production by *G. orontii* along with enhanced cellular defense responses. Data suggest a general function of BI-1 family proteins as regulators of plant-microbe interactions.

Investigation of *Verticillium dahliae*'s counter-defenses against potato

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Early dying syndrome causes major yield losses in potato worldwide. *Vertcillium dahliae* is the main component of the microbial complex causing this disease. Its management, in the absence of resistant cultivars, relies on alternative strategies such as cultural practices and crop rotations. In an earlier study, we pre-screened a set of bacteria and potential manure crops for their efficacy in reducing the impact of the disease under controlled and field conditions. The most effective treatments seemed to operate through the enhancement of the host's defense mechanisms. In the present study, we investigated the variations in potato secondary metabolites accumulation under controlled conditions, in response to these treatments and/or *V. dahliae*. The effective control treatments resulted into accumulation of the flavonol-glucoside rutin to levels that exceeded the threshold of toxicity to *V. dahliae* isolates (160 µM). Treatments with less control effectiveness induced the same compounds, especially rutin, to levels below the toxicity threshold. Through additional investigations, we demonstrated the ability of *V. dahliae* to cleave and use the sugar moiety of rutin as a carbon source and to further metabolize the remaining flavonol (quercetin) to by-products such as phloroglucinol and protocatechoylphloroglucinol carboxylic acid derivatives. This capability plays a dual role in *V. dahliae* pathogenicity by allowing it to overcome some of the plant defenses and to turn toxic compounds into by-products that serve its notoriety in the rhizosphere.

Functional genomics analysis of *Verticillium dahliae* towards identification of its pathogenicity-related genes

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Potato *Verticillium* wilt is a soil-borne plant disease caused by *V. dahliae*. The loss of potato yield due to *Verticillium* wilt may reach 30–50%. Furthermore, the quality of the infected potato tubers can be unacceptable by the consumers and processors. *Verticillium* wilt has been a challenge to the potato crops in many parts of Canada. Successful management of any plant disease goes through a good understanding of the interaction between the host and the pathogen. Our objective is to identify *V. dahliae* pathogenesis-related genes and further study their functions. We established a *V. dahiae*-potato differential model pathosystem. Integrated differential amplification of subtractive hybridization was carried out to identify pathogenesis-related genes in *V. dahliae*. One hundred eighty five differentially expressed transcripts have been sequenced and blasted. Numerous fragments showed an expected role as pathogenicity genes (i.e. Fragment 10-32 similar to exo-polygalacturonase, *Neurospora crassa*). Genetic analysis of selected differentially expressed genes will be carried out in our lab using RNA interference.

The plant innate immune system: Role of phospholipases and oxylipins

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Plant recognition of pathogenic effectors induces the complex suite of cellular and molecular events comprising plants inducible defense responses. We are studying the signal transduction pathway and defence mechanisms after the recognition of pathogenic effectors by plant resistance proteins. Recognition of either the effector AvrRpm1 or AvrRpt2 from *Pseudomonas syringae* by the respective resistance proteins RPM1 or RPS2 was found to cause the sequential activation of the two phosphatidic acid (PA) generating pathways, phospholipases C in combination with diacylglycerolkinase and phospholipase D. Inhibition of either of the pathways inhibited defence responses. Recognition of the pathogenic effectors also caused accumulation of the jasmonates 12-oxo-phytodienoic acid and dinor-oxo-phytodienoic acid. Interestingly the majority of these were found to be esterified to novel galactolipids, arabidopsides. These accumulate to surprisingly high levels, 7-8% of total lipid content and the accumulation of arabidopsides is downstream of the phospholipases. We have shown that arabidopsides posses anti-pathogenic activity. Their accumulation is transient and they are likely a source for delayed release of free jasmonates. In summary, our data supports that PA as well as oxylipins are integral and necessary components of the plant innate immune system.

Identification and characterization of bacterial effectors that interact with the plant protein folding catalyst cyclophilin

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Effector proteins are essential virulence determinants for many Gram-negative bacterial plant pathogens. These proteins are delivered into the host cell cytoplasm via the Type III Secretion System during infection and collectively contribute to pathogen fitness on host plants. Due to size constraints of the Type III Secretion System, effectors are delivered as either partially or completely unfolded proteins and it is hypothesized that many exploit plant folding catalysts for activation. One such folding catalyst, cyclophilin, has been identified. Cyclophilins are molecular chaperones that catalyze the *cis/trans* isomerization of peptidyl-prolyl bonds, a rate-limiting step

during protein folding. We have designed and implemented a targeted, high-throughput protein interaction screen to identify effectors that interact with the *Arabidopsis* cyclophilin ROC1. To date, eight effectors from different species of phytopathogenic bacteria have been identified in this screen. Functional analysis of effectors that require cyclophilin for activation is underway. Identification of the host activators of bacterial effectors will enable the production of enzymatically active effector proteins *in vitro* and facilitate investigations into their functions during the infection process.

Manipulation of the defense response to *Xanthomonas* by RNAi in citrus

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Citrus is one of the most economically-important fruit crops in Argentina. The province of Tucumán is the primary lemon-producing region, with an annual output of approximately 1.3 million metric tons. Nevertheless, citrus commercialization in the region has been severely affected by quarantine diseases. One of the most detrimental diseases is citrus canker, caused by bacterial phytopathogen *Xanthomonas axonopodis* pv. *citri* (*Xac*). The majority of commercial citrus cultivars are moderately to highly susceptible to *Xac*. Our laboratory is developing a variety of biotechnology-based strategies for cultivar protection against *Xanthomonas* species based on interference with the biological and molecular processes of *Xanthomonas* pathogenesis. Post-transcriptional gene silencing in members of the *Rutaceas* family, achieved through interference RNA (RNAi), is one such strategy. Double-stranded RNA expression vectors, encoding hairpin RNAs for citrus phytoene desaturase and callose synthase genes, respectively, were delivered to lemon leaves by transient infiltration with transformed *Agrobacterium*. Phenotypic, histo-anatomic and molecular analysis showed that both vectors were functional not only in lemon plants, but also in other species of the *Rutaceas* family. This system has enabled us to manipulate the basal immune response in citrus plants, as well as the genetic expression in select signalling pathways and metabolic routes.

***Arabidopsis thaliana* PEN1 (*At*SYP121) mediates triggering of innate immunity by bacterial lipo-oligosaccharides (LOS)**

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In eukaryotes, proteins of the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) family are believed to be required for docking and fusion of intracellular transport vesicles with acceptor/target membranes. The Arabidopsis syntaxin PEN1 (*At*SYP121) is a SNARE protein that has been shown to play a role in pathogen resistance in Arabidopsis towards fungi. We present data to show that Arabidopsis PEN1 is also involved in signal transduction leading to the induction of the innate immune responses by particular microbe-associated molecular patterns (MAMPs) of bacterial origin. Specifically we show that PEN1 is required for induction of *PR1* gene induction, callose deposition and generation of reactive oxygen species (ROS) by LOS but not by flagellin. These findings, which suggest multiple roles for PEN1 in determining plant resistance to pathogens, is discussed in the light of previously published work that shows internalisation of LOS on application to suspension cultured cells and the very recent work implicating PEN1 in resistance to fungal pathogens.

Transcriptomic analysis of gene expression elicited by the biocontrol agent *Trichoderma harzianum* T34 in *Arabidopsis thaliana*

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Trichoderma harzianum is a well-known biocontrol agent against a broad spectrum of plant pathogens and although the fungal responses after plant root colonization are well characterized, the transcriptomic information about plant responses elicited by this beneficial fungus is being explored. The aim of this work was to analyze changes in gene expression in Arabidopsis plants inoculated with *Trichoderma harzianum* T34 by hybridizing RNA samples to the Affymetrix GeneChip Arabidopsis ATH1 Genome Array that contains more than 22.500 probe sets representing approximately 24.000 genes. Although we were mainly interested in changes in the expression of genes related with plant defence, differentially expressed genes involved in plant responses to stress, regulation of transcription, signal transduction or plant metabolism, were identified. *T. harzianum* T34 modifies the expression of genes involved in different processes of plant growth and development, including systemic changes in Arabidopsis plants modulated by JA and SA.

Genetic diversity of *Xanthomonas* species pathogenic to citrus based on *avr* and *leucine rich protein* genes

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The genus *Xanthomonas* includes a group of phytopathogenic bacteria that have great economic importance around the world. Citrus canker, caused by *Xanthomonas citri* subsp. *citri*, is one of the main threats to citrus crops worldwide. Different forms of citrus canker have been described based on pathogen specificity and geographical origin, and are caused by other two *Xanthomonas* species: *X. alfalfae* subsp. *citrumelonis* and *X. fuscans* subsp. *aurantifolii*. In the present work we developed DNA primers and probes based on pathogenicity genes in order to study the genetic diversity of these three *Xanthomonas* species, specially a Brazilian population of *citri* strains. The presence of *avrXacE1*, *avrXacE2*, *avrXacE3* and *leucine rich protein* (*lrp*) genes were observed in almost all *citri* and *aurantifolii* strains, but *avrXacE2* an *avrXacE3* genes were absent in *citrumelonis* strains. The profiles generated by PCR-RFLP of the avr genes did not display any polymorphism after agarose gel electrophoresis. However the use of *avr* genes as Southern blot probes proved to be effective to differentiate *Xanthomonas* species pathogenic on citrus and to identify polymorphisms between strains belonging to the same species from Brazil. With the exception of the probe corresponding to *lrp* gene, the number of haplotypes identified (22) varied according to the gene/endonuclease used on Southern blot. The strains of subspecies *citri* possess a higher number of copies of the *avr* genes than subspecies *aurantifolii* and *citrumelonis*. Relatively high genetic diversity was observed in *citri* and *aurantifolii* Brazilian populations, independently of the citrus canker control method applied in the area of origin of the strains.

Lipoperoxidative events influence ochratoxin a biosynthesis in *A. ochraceus* during the interaction with *triticum durum* seeds

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In *Aspergillus nidulans*, *A. flavus* and *A. parasiticus*, lipoperoxidative events stimulate mycotoxin biosynthesis, conidiogenesis and sclerotia formation. Conversely, in *A. ochraceus* resveratrol, a well-known inhibitor of lipoxygenase and cicloxygenase activity, reduces the formation of oxidized lipids and hampers OTA biosynthesis. Which enzyme is the main target of resveratrol in this mycotoxigenic fungus? Is it present a lipoxygenase(*lox*) - like gene in *A. ochraceus*? A *lox*-like gene sequence (*AoloxA*-like; DQ087531) has been found in the genome of *A. ochraceus*. This gene fragment presents an high homology (47 identities, 78 positives %, score 341) with the *lox* genes of *A. fumigatus* (XP_746844) and of some plants (*Solanum tuberosum* – CAA64769; *Hordeum vulgare* – AAB70865). For better studying the effect of lipoperoxides on morphogenesis, OTA biosynthesis and the interaction between wheat seeds and *A. ochraceus* during fungal colonization an AoloxA null mutant (Δ*AoloxA*) has been generated. Δ*AoloxA* displays a different colony morphology with a remarkable delay in conidia formation and an induction of sclerotia development. The reduced lipoxygenase activity and lipoperoxides formation in Δ*AoloxA* seem to induce a strong reduction of OTA biosynthesis in comparison with the wild type. Further, the seeds of *T. durum* cv ciccio contaminated with Δ*AoloxA* did not accumulate 9-hydroperoxyoctadecadienoic acid and did not express *PR1* mRNA whereas WT stimulated both these events. The results obtained show that lipoperoxidation, driven by AoloxA-like, modulates morphogenesis, OTA biosynthesis and the interaction with the wheat seed in the mycotoxigenic *A. ochraceus*.

Exploiting a non-host Arabidopsis system to identify powdery mildew resistance genes in grapevine

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Powdery mildew is an obligate biotrophic pathogen which invades plant cells to gain access to nutrients. Most powdery mildews exhibit host specificity. For example, *Arabidopsis* is a host for *Golovinomyces cichoracearum* but displays penetration resistance to other non-adapted powdery mildew species. However, the Arabidopsis mutant, *pen1-1* allows penetration and feeding structure (haustoria) formation when infected by non-adapted powdery mildews (Collins et al., 2003). The European wine grape *Vitis vinifera* is susceptible to the powdery mildew species *Erysiphe necator* which is the most economically important fungal pathogen of viticulture worldwide. Genetic resistance to *E. necator* exists in the North American grape species *Muscadinia rotundifolia*, conferred by a single dominant locus, *Run1*. *Run1* resistance is mediated by the programmed cell death (PCD) of epidermal cells penetrated by *E. necator*. *Run1* has been introgressed into a *V. vinifera* background and fine-scale genetic mapping has localised *Run1* resistance to a region containing seven full-length TIR-NBS-LRR type resistance (*R*) gene candidates. As stable transformation of grapevine is a lengthy process we introduced each of these grape *R*-gene candidates into the Arabidopsis *pen1-1* background. The compromised penetration resistance of *pen1-1* mutants allows the non-host, *E. necator* to penetrate Arabidopsis epidermal cells and form haustoria. Using this non-host system we have identified a grape *R*-gene candidate which induces a strong PCD response to *E. necator* in the *pen1-1* mutant. This *R*-gene candidate has now been stably transformed into grapevine for characterisation and may ultimately control powdery mildew infection in vineyards.

Identifying legume autoregulation of nodulation signals

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Legumes can enter into symbiotic relationships with rhizobia resulting inthe formation of nitrogen fixing root nodules. Nodule numbers are internally regulated by the plant via phytohormones, Nod Factor (NF) perception and a long-distance signaling network termed Autoregulation Of Nodulation (AON). AON commences during the early stages of nodule development with the production of a root-derived signal called Q. Q is transported to the shoot where it, or a product of its action, is perceived by a LRR receptor kinase called NARK. This results in the altered expression of genes required for JA biosynthesis and also leads to the production of a Shoot-Derived Inhibitor (SDI). SDI is subsequently transported to the root where it acts to inhibit continued nodule development. Findings will be presented regarding our progress in characterizing and identifying soybean Q and SDI. A bioassay based on the expression of marker genes has been developed to identify Q in soybean xylem sap. Another bioassay based on nodule numbers has been developed to identify SDI in leaf extracts. Using this bioassay, we have shown SDI to be NARK- and Nod factor-dependent, heat stable, small, and likely not a peptide or RNA molecule. These bioassays are currently being used to confirm the presence of Q and SDI in samples following purification and fractionation procedures. In addition, we will report on our use of Virus-Induced Gene Silencing (VIGS) to confirm roles for signals identified by array and site-directed mutagenesis studies as components acting downstream of NARK.

Functional analysis of defense-related genes involved in the Casuarina glauca-Frankia symbiosis

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The ability of some plants to establish symbiotic relationships with certain soil bacteria and harness the ability of the bacteria to fix atmospheric nitrogen into ammonia has a tremendous impact on natural and agricultural ecosystems. In two groups of N2-fixing symbiotic interactions, the prokaryotic partners are soil bacteria - rhizobia in legume symbioses, and Frankia in actinorhizal symbioses - and N2 fixation takes place in a newly formed plant organ, the root nodule. Root nodule formation is an intricate and complex process that must be tightly regulated. In legumes, the picture emerging from recent research indicates that similar mechanisms are used by the plant to recognize and accommodate pathogens and symbiotic microbes. At the plant level, the induction of defense mechanisms has been associated with microsymbiont infection control and defense against external pathogens. Far less is known about the similarities between actinorhizal symbioses and pathogenesis, but recently the involvement of putative defense genes during nodulation has been suggested. The aim of our work is to understand the molecular mechanisms used by actinorhizal plants to control the nodulation process, particularly in what concerns the parallelism between nodulation and pathogenesis in the model system C. glauca-Frankia symbiosis. Five genes, encoding two chitinases (CgChi1 and CgChi3), a peroxidase (CgPox), a glutathione S-transferase (CgGST) and a hairpin-inducible protein (CgHin1), were found to be expressed preferentially in nodules as compared to roots. To shed light on the functions of theses genes, plant transformation and production and characterization of the encoded proteins are in progress and the results will be presented.

Role of cell cycle control in bacteroid differentiation of *Sinorhizobium meliloti*

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Rhizobia are Gram-negative soil bacteria that are capable of inducing the formation of nitrogen-fixing nodules on the roots of their leguminous host plants. In indeterminate nodules, induced by *Sinorhizobium meliloti* on its hosts, bacteria differentiate into bacteroids that become enlarged and polyploid through several cycles of endoreduplication of the genome uncoupled from cell division. Regulation of the cell cycle is probably affected during this differentiation process. The bacterial cell cycle is a highly organized process, precisely coordinated in space and time. We investigated dynamic sub-cellular localization patterns of proteins involved in the control of the *Sinorhizobium meliloti* cell cycle at single cell level in a microfluidics system. Moreover, the role of major players in cell cycle control, in cell cycle progression and bacteroid differentiation was also studied. *ctrA* depletion and overexpression strains were constructed and analyzed, and the *ctrA* regulon was defined by a combined bioinformatics and transcriptome approach. Symbiotic phenotypes of a mutant in the cell cycle kinase gene *divJ* and a *ctrA* overexpression strain suggested a role of these proteins in bacteroid differentiation.

Trichoderma harzianum* strains T22 and T39 induce in maize systemic resistance against *Fusarium verticillioides

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In Italy *Fusarium verticillioides* is probably the most common fungal species isolated from maize where causing heavy fumonisin contamination. Some species of genus *Trichoderma* induce systemic resistance and are considered effective biocontrol agents against some plant-pathogen fungi. In our study the strains T22 and T39 of *Trichoderma harzianum* were evaluated for their ability in reducing both *F. verticillioides* infection and lesion severity in commercial maize hybrid. After 15 days from the two strains soil treatment, maize roots were strongly colonized by *Trichoderma*. Stems of 30-day-old maize plant challenged with *F. verticillioides* showed a lesion reduction of 65% and 59% for T22 and T39 respectively. These reductions in infection area were associated with a considerable decreasing of necrotic tissues and a full recovery of plant health. Effect of *Trichoderma* strains in reduction of endophytic inoculum of *F. verticillioides* was also surveyed by quantitative Real Rime PCR. Analysis of enzymatic marker for ISR and SAR in roots and shoots revealed a differential kinetics response, in presence of *Trichoderma* colonization. Moreover, local transcriptomic response (*Pal*, *Lox13* and *Hpl*) in *F. verticillioides* infection was monitored for 96 hour post-inoculation by relative RT PCR. Data collected show the possibility to employ some *T. harzianum* strains as biocontrol agent in maize, also confirmed by the ISR molecular marker.

Transient expression of *avrGf1* in citrus

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Xanthomonas citri subsp. *citri* secretes multiple effector proteins into plant host cell via type three secretion system (T3SS). Recently, *avrGf1* was identified in *Xanthomonas citri* subsp. *citri* strain A^W (Xcc-A^W), which incites disease in Key lime and a hypersensitive reaction (HR) in grapefruit (Rybak *et al*., 2009). Strain Xcc-A^WD*avrGf1* showed similar symptoms to those incited by the wild type strain in grapefruit but lost the ability to trigger HR. Four deletion derivatves of *avrGf1* were created with mutations in both the N – and C – terminal coding regions of the 449 codon open reading frame to identify the regions responsible for the HR. A transient expression protocol using *Agrobacterium tumefaciens* strain GV31 was employed to test the ability of the constructs to elicit an HR in grapefruit leaves. HR elicitation was abolished in two mutants carrying deletions of 83 amino acids (aa) at the C – terminus and 116 aa at the N-terminus when compared with the full length gene. However, mutations performed in the N- and C-terminus, deleting 13 and 7 aa, respectively, did not affect the HR induction. Additionally, the *avrGf1* translocation was confirmed using a $AvrGf1_{1-106}$::$AvrBs2_{62-574}$ fused protein in Bs2 pepper plants. The results showed that the first 116 amino acids is important to translocation and HR elicitation and the last 83 aa also are involved in the ability to trigger cell death in grapefruit tissue. Thus, transient expression developed here for gene expression in citrus leaves offers a rapid and efficient tool for identifying genes involved in the citrus resistance or virulence.

An unknown type three secretion independent HR-elicitor from *Xanthomonas citri* subsp. *citri*

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Xanthomonas citri subsp. *citri* (Xcc) and *X. fuscans* pv. *aurantifolli* (Xfa) both incite bacterial citrus canker and trigger hypersensitive reactions (HR) in the non-host tomato. Xcc and Xfa rely on the type three secretion system (T3SS) to deliver effector proteins (T3-effectors) into the host cells for pathogenicity. Some T3-effectors are known to suppress the host defense mechanisms, while some are recognized by the plant surveillance systems, activating the defense responses that often include a HR. Xcc and XfaC are unusual in that strains with a defective T3SS trigger a HR on non-host tomato plants. *Xanthomonas perforans* (Xp), a pathogen of tomato, and a T3SS defective mutant of Xp also trigger an HR in tomato when harboring the pL450 clone from Xfa, indicating that the genes responsible for the T3SS-independent HR elicitor are present on the clone. Subcloning of the pL450 clone revealed a 3.0-kb fragment with T3SS-indepenpent HR elicitor activity. Sequence analysis revealed three open reading frames with no sequence similarity to known T3SS effectors, neither, flagellin related protein. Studies are undergoing to determine whether one or more of the candidate genes is involved in the T3SS-independent phenotype.

Quantification of PR proteins expressed in the rice - blast interaction during the resistance induction by abiotic and biotic inducers

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The strategy of induced resistance in plants to pathogens has shown promising results in relation to the resistance mechanisms with special reference to elucidation of processes involved in the expression of resistance. The objective of this investigation was to quantify related proteins "PRP's" expressed in the rice blast interaction during the resistance induction process by ASM Molecule (abiotic inducer) and by an avirulent isolate of *Magnaporthe oryzae* (biotic inducer). The model for analysis was based on pre-treatment of cultivars Cica-8 and Meica-1 with inducers (biotic and abiotic) followed by inoculation with isolates of challenging fungus *M. oryzae*. Also the activity of peroxides and beta-1,3-glucanase was tested. The experiment was conducted under controlled greenhouse and laboratory conditions. The induction of resistance was manifested in the reduction of leaf area affected and lesion type. The quantification of enzymatic activity of PRP's was highly successful corresponding to the results relating to the leaf blast severity quantification measured in greenhouse experiments.

The endo-β-1,4-glucanases influence plant susceptibility

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Endo-β-1,4-glucanases (EGases) depolymerise polysaccharides containing 1,4-β-D-glucan linkages and take part in cell wall editing processes such as elongation, fruit ripening and floral abscission. EGases are localized both in the membrane and secreted in the wall depending on their specific metabolic or catabolic role. Plant cell wall modifications are critical components in the response to different stress and several cell wall modifying enzymes have been recently involved as important factors of resistance or susceptibility. The EGases hydrolytic activity in the wall could modify parameters such as the mechanical barrier texture, the availability of nutrients for pathogens and probably the cell wall integrity sensors state. We found a linkage between the lack of EGase activity and pathogen response in tomato. Transgenic plants lacking Cel1 and Cel2 resulted more resistant to *Botrytis cinerea*, supporting the role of EGases as susceptibility factors (Flors et al, 2007). In Arabidopsis, according to the analysis of Affymetrix microarray data, some EGase genes are differentially regulated upon biotic stresses. Both the redundancy of the EGases Arabidopsis family and the complexity of the cell wall matrix degradation where many enzymes are involved, prevent severe phenotypes of single EGases T-DNA insertion mutants. Nevertheless, here we show that the absence of one EGase in individual Arabidopsis T-DNA mutants, influence the response to *B. cinerea* and *Pseudomonas syringae* infection. Parameters of the defence response and the susceptibility against these two pathogens were analyzed. Both *in vivo* and microarray data analysis point out the involvement of the EGases into the stress response network.

Global and cell-type gene expression profiles in tomato plants colonized by arbuscular mycorrhizal fungi

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In nature one of the most widespread mutualistic association is the arbuscular mycorrhizal (AM) symbiosis formed between soil fungi belonging to Glomeromycota and most land plants. The symbiosis develops in the plant roots where the colonization involves epidermal and cortical cells. In the root cortex the fungus develops intercellular hyphae and extensively branched intracellular hyphae called arbuscules which are considered crucial components of the interaction. The AM symbiosis has a multifunctional character: AM fungi improve plant nutrient acquisition and provide protection from biotic and abiotic stresses. To obtain an overview of transcriptional changes triggered in roots and shoots of tomato (*Solanum lycopersicum* L.) as a result of the colonization by the AM fungus *Glomus mosseae* the TOM2 microarray platform (Cornell University) was used. Expression profiles of 17 selected genes was confirmed for qRT-PCR. This analysis revealed 362 up-regulated and 293 down-regulated genes in mycorrhizal roots. Significant gene modulation was also observed in shoots: 85 genes showed increased transcript levels while 337 genes were down-regulated. Most responsive genes are ascribed to the following functional categories: primary metabolism, defence and response to stimuli, cell organization, protein modification and transcriptional regulation. In addition, to identify possible plant determinants of arbuscule formation, the cell-type expression profiles of a subset of genes induced in mycorrhizal roots were monitored taking advantage of the laser microdissection technology. Six genes specifically expressed in arbusculated cells have been identified: they are involved in auxin and abscissic acid metabolism, cell wall biogenesis and cytoskeletal dynamics.

Potential offensive role of the Type I secretion system in *Xylella fastidiosa*

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Xyllela fastidosa (Xf) is a xylem limited, insect vectored, plant parasitic bacterial species with multiple pathovars, some of which cause severe plant diseases, including Pierce's Disease (PD) of grapevine. Although Xf appears

to lack a Type III secretion system, the six available Xf genomes predict the presence of Type I secretion systems, including components involved in (defensive) antimicrobial chemical efflux and also in (offensive) repeats in toxin (RTX) effector secretion. Mutation of TolC, an outer membrane Type I system component common to both offensive and defensive Type I secretion, was previously shown in PD strain Temecula to result in complete loss of pathogenicity, *in planta* survival, and increased sensitivity to phytoalexins. Mutagenesis of Type I system components associated with RTX secretion, including homologs of *cvaA, cvaB* and *hlyBD*, involved in the secretion of colicins and hemolysins, respectively, resulted in mutants with reduced virulence on grapevines. The Temecula genome has 8 predicted hemolysins and 3 colicin V precursors. Six of the Temecula hemolysins were well conserved among the other 5 sequenced Xf strains and were also present in PD biocontrol strain EB92-1 (representing 5 host range variant groups). All 3 of the Temecula colicin V precursors were well conserved among the other 5 sequenced Xf strains and EB92-1 with at least 86% amino acid identity. Deletion of all three colicin V precursors in Temecula resulted in reduced virulence on grapevines, suggesting an important role for these Type I effectors in host colonization.

Gomesin, a β-hairpin antimicrobial peptide, induces *Xylella fastidiosa* biofilm formation at a sublethal concentration

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Xylella fastidiosa is the etiologic agent of a wide range of plant diseases, including citrus variegated chlorosis and Piece Disease, and is transmitted by xylem sap-feeding insects. In this work, we report the effects of sub lethal concentrations of gomesin (a β-hairpin antimicrobial peptide (AMP) isolated from spider) on gene expression profile of *X. fastidiosa*. AMPs are cytotoxic molecules produced by most organisms, including bacteria and arthropods. Therefore, we reasoned that *X. fastidiosa* might be exposed to AMPs produced by the microbial community that colonizes its plant or insect hosts. The minimal bactericidal concentration of gomesin to *X. fastidiosa* is 200-400 μM and the sublethal concentration used on our experiments was 50 μM. The gene expression profile of *X. fastidiosa* upon 60 min treatment with 50 μM gomesin was screened by whole genome DNA microarray hybridizations. The data revealed that treatment with sublethal concentration of gomesin modulates expression of 159 *X. fastidiosa* genes belonging to different functional categories, of which 143 were up-regulated and only 16 were down-regulated. Interestingly, the up-regulated set of genes includes genes for enzymes involved in exopolysaccharide synthesis, an important step in biofilm formation. Accordingly, we found that sublethal concentration of gomesin enhances production of biofilm by *X. fastidiosa in vitro* culture. On the other hand, pre-treatment of *X. fastidiosa* with sublethal concentration of gomesin prior to experimental infection of tobacco plants appears to delay the onset of disease symptoms suggesting that biofilm formation interferes with colonization of the plant xylem vessels. Supported by CNPq and FAPESP.

Defence spending slashed in the hyperaccumulator economy?

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Thlaspi caerulescens is a 'metal hyperaccumulator' – a plant that actively takes up and stores metals such as zinc, nickel and cadmium in its aerial tissues. It has been proposed that, while the plant is able to tolerate high concentrations of these metals, its potential pests and pathogens cannot. This is termed the 'elemental defence hypothesis'. We have shown that growth of the pathogen *Pseudomonas syringae* pv. maculicola M4 (*Psm*) is limited in plants exposed to metals, and that metal concentrations in the apoplastic fluid of these plants are sufficient to account for the observed reduction in bacterial growth. Furthermore, *Psm* mutants with increased or decreased metal tolerance show a corresponding increase or decrease, respectively, in their ability to colonise metal-treated plants. Since the plant appears to be defended against disease by metals, it is possible that investment of resources in other defences may be reduced in the hyperaccumulator (termed the 'trade-off hypothesis'). We have shown that inoculation of *T. caerulescens* with *Psm* does not trigger accumulation of salicylic acid or reactive oxygen species (ROS), as observed in the non-accumulating relative *Arabidopsis thaliana*. We have also failed to detect any callose deposition in *T. caerulescens* in response to either wildtype *Psm* or non-pathogenic bacteria. It therefore appears that elements of the basal defence response and of defence signalling are attenuated or lacking in the hyperaccumulator plant. We hypothesise that this is due to a trade-off between these conventional defences and metal-based defences, which renders them unnecessary.

Peroxidase/catalase expression in the phytopathogen *Rhodococcus fascians*: Comparison between a wild-type strain and a non-pathogenic, plasmid-free strain

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The phytopathogen bacterium *Rhodococcus fascians* induces the development of leafy galls on infected plants. Leafy galls are local amplifications of multiple buds inhibited for further outgrowth. The capacity of *R. fascians* to induce the symptoms depends on the presence of a linear plasmid named pFiD188 on which important virulence loci are located. A 2D-DIGE analysis was performed to compare the proteome of the pathogenic wild-type strain D188 and the non-pathogenic, plasmid-free strain D188-5. This analysis identified 77 spots differentially expressed. Among them, 31 spots showing more than 2 fold difference in expression were further analyzed by mass spectrometry. Homologies were found for 13 polypeptides, among which homologues of the peroxidase/catalase-MI85 protein from *Mycobacterium tuberculosis* and the catalase-peroxidase KatB from *Stappia aggregata* IAM 12614 were 3.2 and 2.2 times more present in strain D188, respectively. On the contrary, a homologue of a catalase from *Rhodococcus jostii* RHA1 was 2.8 times more present in strain D188-5. Catalases are known to play an important role in virulence by protecting the pathogen from the oxidative burst in its host. It has been suggested that photorespiration increases during the *R. fascians*-plant interaction, potentially causing the production of H_2O_2. RT-PCR analysis of the expression of the putative catalase genes in *R. fascians* was performed and the relevance of these catalases during the *R. fascians*-plant interaction is discussed.

Transcript profiling of Fusarium Head Blight-infected spikes reveals different mechanisms of resistance in wheat

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Fusarium Head Blight (FHB) is a devastating disease of wheat caused by trichothecene-producing *Fusarium graminearum* and related species. Trichothecenes are known factors of disease aggressiveness with a role in disease spread. In order to reduce the impact of this disease, we need to improve our understanding of the molecular mechanisms associated with FHB-resistance. The current study explores the differential transcriptome of three wheat genotypes (Superb, DH1 and DH2) in their response to trichothecene-producing (Tri5+) or trichothecene non-producing (Tri5-) *F. graminearum*, and to the trichothecene, deoxynivalenol. 'Superb' is a susceptible Canadian wheat cultivar that shares 75% genetic identity with each of the double haploid lines, DH1 (CIMMYT11-derived resistance to initial infection) and DH2 (Sumai3-derived resistance to disease spread). Uninoculated spikelets of point-inoculated heads were harvested in order to identify the changes in transcript accumulation associated with resistance. Surprisingly, among the differences in transcript accumulation observed in DH2, only a handful were associated with the plant-defense response, suggesting that resistance to disease spread is not an induced systemic resistance. Substantial differences in transcript accumulation were observed between treatments in DH1, and these included genes associated with cell wall lignification. Cell wall lignification of the spikelet glumes could potentially improve resistance to initial infection by blocking fungal penetration. In contrast, we speculate that upregulation of salicylic acid production in 'Superb' may divert phenylpropanoid substrate away from lignin production, thus contributing to susceptibility in this cultivar.

Genetic dissection of Verticillium wilt resistance mediated by tomato Ve1
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Vascular wilt diseases caused by soil-borne pathogens are among the most devastating plant diseases worldwide. The *Verticillium* genus includes vascular wilt pathogens with a wide host range. Although *V. longisporum* infects various hosts belonging to the Cruciferaceae, *V. dahliae* and *V. albo-atrum* cause vascular wilt diseases in over 200 dicotyledonous species including economically important crops. A locus responsible for resistance against race 1 strains of *V. dahliae* and *V. albo-atrum* has been cloned from tomato (*Solanum lycopersicum*) only. This locus, known as *Ve*, comprises two closely linked inversely oriented genes, *Ve1* and *Ve2*, that encode cell surface receptor proteins of the extracellular leucine-rich repeat (eLRR) receptor-like protein (RLP) class of disease resistance proteins. We show that *Ve1*, but not *Ve2*, provides resistance in tomato against race 1 strains of *V. dahliae* and *V. albo-atrum*, and not against race 2 strains. As expected, a Ve1-GFP fusion localizes to the plasma membrane in *Nicotiana benthamiana*. Using virus-induced gene silencing in tomato, the signaling cascade downstream of Ve1 is shown to require both EDS1 and NDR1. In addition, also NRC1, ACIF, MEK2, and SERK3/BAK1 are required for Ve1-signaling in tomato. In conclusion, we demonstrate that Ve1-mediated resistance signaling only partially overlaps with signaling mediated by Cf proteins, type members of the RLP-class of resistance proteins.

Analysis of LRR-RLKs involved in pathogen-associated immunity
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For a successful propagation of any organism a key capability is in the defense of pathogens, which requires the capacity to distinguish foreign from own structures. In mammalians immune response is a complex system with several layers of defined reactions allowing for a highly specific immune response. The first reaction, the so-called innate response is used for a fast initial response to a variety of pathogens thereby providing time to develop a more specific immune reaction via the adaptive immune response. The key components of the mammalian innate immunity the Toll-like receptors (TLRs) are well studied and some structure data of TLRs and their complexes with their cognate ligands became available recently. With respect to their possible counterparts in plants e.g. the flagellin receptor FLS2 molecular characterization started just a few years ago. We are interested in the structural and functional elucidation of the receptor-like kinases FLS2 and BAK1, both of which are involved in the immune response of plants to the bacterial elicitor flagellin. These receptor-like kinases comprise of an extracellular leucine-rich repeat domain (LRR) similar to those of TLRs, a single transmembrane domain and an intracellular serine/threonine kinase domain. By co-immunoprecipitation it has been shown that FLS2 and BAK1 form a receptor complex upon flagellin treatment. However, it is so far not clear, which domains are involved in the interaction betweenFLS2 and BAK1 and whether flagellin binds to the LRR domains of both receptor-like kinases. We would like to investigate the interaction parameters by using techniques like bimolecular fluorescence complementation (BiFC) and fluorescence resonance energy transfer (FRET).

Identifying the components of resistance signaling pathways in soybean
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Soybean is the world's largest legume crop and a major source of edible plant oils and proteins. Despite the economic importance of the soybean crop and the devastating losses incurred by its diseases very little is known about the molecular mechanisms underlying host defense against pathogens infecting soybean. Although a large number of resources and genomic tools are available for soybean, studies related to gene functions have been lacking. This is largely due to the fact that rapid functional genomics tools have been unavailable for application in the soybean plant. We have used a novel Bean Pod Mottle Virus-derived system to silence soybean genes and examine their roles in defense against microbial diseases. Using this strategy we have identified soybean genes participating in basal, resistance gene (R)-mediated and systemic immunity. Our work has shown that reducing the levels of the fatty acid oleic acid induces a novel defense-signaling pathway that provides resistance against multiple pathogens. In addition, we have shown that GmRAR1 and GmSGT1-2 are essential signaling components required for extreme resistance against Soybean Mosaic Virus mediated by the R gene Rsv1. The identification of GmRAR1 and GmSGT1-2 could aid in the cloning of Rsv1 as well as the study of the mechanisms of extreme resistance to viruses. We find that although soybean defense signaling pathways utilize many conserved components the specific requirements for these vary significantly.

The atypical myrosinase PEN2 is associated with the periphery of a subcellular endomembrane compartment with unclear identity
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The atypical myrosinase PEN2 is a molecular component of nonhost resistance in *Arabidopsis* against nonadapted powdery mildew species (Lipka et al., 2005; Bednarek et al., 2009). Transient fluorescence labelling experiments suggested an association of the PEN2 protein with the periphery of peroxisomes (Lipka et al., 2005). PEN2 exhibits a unique C-terminal extension which is required for protein function in nonhost resistance (Lipka et al., 2005). Interestingly, we could recently show that GFP-fusions with the PEN2 C-terminal extension alone exhibit the same subcellular localisation pattern as functional full-length PEN2-reporter fusions, demonstrating a functional role of the unique C-terminus for subcellular localisation. In addition, we generated stable transgenic lines expressing functional PEN2-GFP and peroxisomal matrix localised dsRed. Confocal microscopy revealed that PEN2 co-localises with a subpopulation of mature epidermal peroxisomes. At sites of incipient fungal penetration, PEN2-tagged non-motile subcellular compartments accumulate which are smaller than mature peroxisomes and seem to lack the dsRed-specific peroxisomal marker. Oxidative stress, wounding and pathogen attack are known to induce peroxisome biogenesis gene expression (Lopez-Huertas et al., 2000), which suggests a pathogen-responsive peroxisome proliferation or *de novo* biogenesis. We currently favour a model in which PEN2 localises to pathogen-induced and functionally specialised peroxisomes that are formed at sites of attempted fungal invasion. Possibly, these compartments are identical with the ER/peroxisome intermediate compartment (ERPIC) recently proposed by Mullen and Trelease (2006). To test this working hypothesis we conduct systematic time course analyses with a set of suitable fluorescent subcellular marker lines.

Serotonin is a novel inducer of immune responses in rice
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Plants recognize pathogen infection and induce a series of immune responses including hypersensitive cell death. *Sekiguchi lesion* (*sl*), one of rice lesion mimic mutants, induces cell death in the absence of pathogens. We have identified the *SL* gene using map-based approach, which encodes a CYP71 subfamilly of cytochrome P450 monooxygenase. The SL protein has the tryptamine 5-hydroxylase activity that catalyzes conversion of tryptamine to serotonin. The *SL* gene was up-regulated by elicitor and pathogen infection. Moreover, the exogenous application of serotonin induced cell death and expression of defense-related genes in rice suspension cell cultures. These results suggest that serotonin may be a novel inducer of plant immune response. In addition, the serotonin-dependent expression of defense-related genes were highly reduced by suppressing express of the small GTPase OsRac1, a component of OsRac1 immune complex and mutant of the heterotrimeric G protein α subunit. These results indicate that the OsRac1 complex and Gα are required for serotonin-mediated immune responses.

Host-derived signals activate plant innate immunity
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We have shown that oligogalacturonides (OGs) protect Arabidopsis against fungal infection and that this protection is independent of ethylene, jasmonate and salicylic acid. Although OGs have been studied for years as elicitors of defence responses little is known about the elements involved in the signal transduction pathways triggered by these elicitors. We are using a reverse genetic approach to dissect the OG-dependent perception and transduction pathways. Genes strongly and significantly induced upon treatment with OGs

were selected from microarray data obtained from Arabidopsis plants treated with the elicitors, and knockout lines in these genes were obtained. Mutants are currently under characterisation and results on their possible involvement in OG-mediated signalling will be presented. Transcript profiling experiments show that responses triggered by OGs and the bacterial PAMP flg22 largely overlap in Arabidopsis. However, the two elicitors are not perceived through the same receptor and, in contrast to flg22, OG-mediated signalling does not require the co-receptor BAK1/SERK3. Our results suggest a possible role played by other members of the SERK family. Similarly to flg22, OG-triggered responses are blocked by the bacterial effector AvrPto, suggesting that this protein may physically interact with either the OG-receptor(s) or co-receptor(s).

Translocation of the biotrophic fungal effector AvrM into host plant cells

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Biotrophic pathogens secrete avirulence proteins (effectors) that are detected by host resistance proteins within plant cells. In the absence of host R genes, it is proposed that the principal role of these effectors is to manipulate plant cells, altering metabolism and allowing the pathogen to evade the host basal defence responses. Whereas effectors from pathogenic bacteria enter host cells via the Type III secretion system, the mechanism responsible for delivery of effectors from biotrophic fungal pathogens remains one of the key unanswered questions in the field of plant-pathogen interactions. A good model for studying the interaction between biotrophic fungi and plants is the infection of flax (*Linum usitatissimum*) by the flax rust fungus (*Melampsora lini)*. In this project, antibodies against the flax rust effector AvrM localized AvrM in infected flax leaves by immunofluorescence and transmission electron microscopy. These experiments provided direct evidence that AvrM is secreted by flax rust and taken up by host cells in contact with the fungal haustorium. A time course of infection is being analysed to further characterize patterns of AvrM synthesis and translocation into the host cell. Experiments are also underway to elucidate the mechanism behind AvrM uptake.

Molecular mechanisms of grapevine resistance to powdery mildew

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The commonly cultivated variety of grapevine, *Vitis vinifera* Cabernet Sauvignon, is highly susceptible to powdery mildew (PM), which is caused by the obligate biotrophic fungus *Erysiphe necator*. We have used a PM-resistant North American grapevine variety, *V. aestivalis* Norton, to compare gene expression profiles with Cabernet Sauvignon after PM inoculation. Differences in transcript levels of known plant defense-related genes such as *EDS1*, *MAPKK*, *WRKY*, *PR1* and *PR10* suggest that basal defenses are induced in Cabernet Sauvignon and constitutively activated in Norton (Fung *et al.,* 2008). Six putative *EDS1-like* (*EDL*) genes were found in the *V. vinifera* Pinot Noir genome. Based on gene chip data, *EDL* transcripts increased significantly in PM-infected Cabernet Sauvignon. *EDLs* also were constitutively expressed at higher transcriptional levels in resistant Norton than in susceptible Cabernet Sauvignon. However, the Pinot Noir genome sequence revealed that the gene chip probes for *EDLs* were not entirely paralog-specific. Here we focus on EDL6, which shows highest amino acid similarity with AtEDS1. Sequencing of Norton and Cabernet Sauvignon *EDL6* indicated that the predicted amino acid sequences are almost identical, but the promoter regions of *EDL6* are diverged, suggesting that the transcription of *EDL6* is regulated distinctly in the two varieties. We are testing the functional complementation of the Arabidopsis *eds1-1* mutant by Norton and Cabernet Sauvignon *EDL6* in assays using *Pseudomonas syringae* pv. tomato strain DC3000 expressing the avirulence gene *avrRPS4*. This study will provide a better understanding of the molecular mechanisms of grapevine disease resistance.

Regulation of cell death and innate immunity by two receptor-like kinases in Arabidopsis

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Arabidopsis BRI1-associated receptor kinase 1 (BAK1) has multiple functions in plant development and pathogen defense. Using co-immunoprecipitation and quantitative Mass Spectrometry analysis, BAK1-interacting Receptor Kinase (BIR1) was found to associate with BAK1 *in vivo*. Like the double mutant of *bak1* and *bak1-like 1,* the *bir1-1* mutant displays constitutive disease resistance and a seedling-lethality phenotype. Activation of MPK4 by the flg22 peptide is compromised in the *bir1-1* mutant, suggesting that BIR1 may function upstream of MPK4 to negatively regulate cell death and defense responses. Epistasis analysis showed that the *bir1-1* mutant phenotypes were partially suppressed by mutations in *PAD4* and *EDS1* and to a less extent by mutations in *EDS5*, *SID2* and *NDR1*. To further unravel defense pathways activated in *bir1-1*, we carried out a suppressor screen of *bir1-1*. One of the mutants, *sobir1-1* (*suppressor of bir1-1, 1*), strongly suppresses the cell death of *bir1-1*. Combining the *sobir1* and *pad4-1* mutations leads to complete suppression of all mutant phenotypes of *bir1-1*, indicating that *SOBIR1* functions in parallel with *PAD4*. *SOBIR1* encodes another receptor-like kinase and overexpression of *SOBIR1* activates cell death and defense responses, suggesting that SOBIR1 is a key component of a signal transduction pathway promoting cell death and disease resistance. Our data provide evidence that plants produce both positive and negative signals that are perceived by different RLKs to fine tune innate immune responses.

Transcription factor profiling has identified putative regulators of aphid resistance in *Medicago truncatula*

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Aphids are probably the largest group of sap-sucking insects causing serious damage to agriculture and natural ecosystems. Resistance to aphids has been identified in a number of plant species, yet the molecular mechanisms underlying aphid resistance remain largely unknown. The model legume *Medicago truncatula* widely used for plant biological studies has also been proved to be a valuable model plant for studying plant-aphid interactions. A single dominant gene conferring resistance to bluegreen aphid (BGA; *Acyrthosiphon kondoi* Shinji), named *AKR*, has been identified and mapped in the *M. truncatula* cv Jester. Using high-throughput quantitative real-time PCR technology, the transcription profiles of 760 putative *M. truncatula* transcription factor genes have been analyzed in a pair of susceptible and resistant near-isogenic lines of *M. truncatula* following BGA infestation for 6 and 12 hours. Eighty TF genes belonging to 30 TF families were responsive to BGA infestation. Among these, a significantly higher number of genes were responsive upon infestation in the resistant interaction than the susceptible interaction, which suggests a rapid transcriptional reprogramming in the resistant plant during the early stages of aphid infestation. Interestingly, members of AP2/EREBP, bHLH, C2H2(Zn) and WRKY gene families had a high representation and were specifically induced in the resistant plants following BGA infestation. A subset of these genes were further analyzed and shown to be associated with BGA resistance in other *M. truncatula* genetic backgrounds as well as with resistance to pea aphid. The results suggest that these TFs may play important roles in aphid resistance.

Implication of callose deposition in the OCP3-mediated disease resistance to necrotrophic pathogens

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The phytohormones abscisic acid (ABA) and methyl jasmonate (MeJA) control the signaling pathways responsive of the plant adaptive responses to drought and pathogenic fungi infections, respectively. OCP3 is a transcriptional regulator of the Homeobox family and that has been previously demonstrated to be required for mediating specific aspects of plant responses as mediated by both ABA and MeJA. The ocp3 loss-of-function mutant shows an JA-dependent enhanced resistance towards Botrytis cinerea and Plectosphaerella cucumerina that is accompanied by an enhanced drought tolerance and increased sensitivity to ABA. This phytohormone has been proposed to be involved in the callose deposition surrounding the infection

sites to restrain pathogen entry. In this work we show that the enhanced resistance of ocp3 plants towards B. cinerea and P. cucumerina goes along with an early and drastic increase of callose accumulation. These results suggest that OCP3 is implicated in the regulation of the rapid callose deposition involved in plant-pathogen interaction. To further study this observation we have generated a battery of mutants which allowed us to dissect the genetic requirements of the OCP3-mediated resistance towards necrotrophic fungi and the role played by ABA and JA in mediating the observed deposition of callose.

One for all and all for one: Three redundant transcription factors orchestrate multiple defense responses

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Three redundant members of the TGA family of bZIP transcription factors play a crucial role in mediating the salicylic acid-dependent defense response "systemic acquired resistance". Here we present evidence that they are also involved in signaling networks regulating the responses to pathogen associated molecular pattern (PAMPs) and to jasmonic acid in combination with ethylene. Due to their crucial role in multiple pathways, TGA factors can act as switch points that help to prioritize the most suitable pathway depending on the stage of infection and the pathogen.

Characterization and functional analysis of *Fusarium oxysporum* effectors

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Fusarium oxysporum f. sp. lycopersici (Fol) is the causal agent of tomato wilt disease. This soil-born fungus infects roots and invades the xylem vessels eventually resulting in wilting or even plant death. Plant pathogens secrete effectors to manipulate the host and facilitate infection. When an effector triggers immune responses on a resistant host it is called an avirulence protein (Avr). In tomato resistance to Fol is mediated by the *I*, *I-2* and *I-3* resistance genes that mediate perception of Avr1, Avr2 and Avr3, respectively. Upon host colonization Fol secretes many small proteins in the xylem (Six) of which 11 have been identified with mass spectrometric analysis. Some of them might represent effectors affecting resistance gene function. It was shown before that Avr1 (Six4) suppresses immune response induced by I-2 and I-3 to confer susceptibility. We have used Agrobacterium transient transformation assays (ATTAs) to co-express eight Six proteins, whose function is unknown, with different *R/AVR* gene pairs and the elicitor Inf1. We identified four Six proteins that suppress or enhance HR. These four will be used as baits to screen for their plant targets to understand how they function.

Plant genes involved in *Agrobacterium*-mediated genetic transformation

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Agrobacterium-mediated plant transformation is a complex process involving transfer of DNA and virulence (Vir) effector proteins from the bacterium to the plant. We have previously shown that numerous plant proteins are involved in transformation, either directly by associating with transferred Vir proteins, or indirectly by affecting plant cell biology processes important for transformation. We have identified more than 100 *Arabidopsis* mutants that are either resistant (rat) or hyper-susceptible (hat) to *Agrobacterium* transformation. Collectively, these mutants represent each of the transformation steps that occur within the plant cell, including bacterial attachment and biofilm formation, T-DNA and Vir protein transfer, T-complex nuclear targeting, T-DNA integration, and transgene expression. Over-expression of many of these "transformation" genes, such as those encoding particular core histones, results in increased transformation frequencies, both of *Arabidopsis* and of maize and other crop species. We have recently identified plant defense pathways as major factors influencing transformation. Over-expression of a UDP glucosyltransferase increases transformation, most likely by debilitating plant defense responses. We have also identified a myb transcription factor that may be a global negative regulator of transformation. Inhibiting expression of this myb transcription factor increases transformation 10- to 20-fold. One of the downstream targets of this myb transcription factor is a WRKY transcription factor gene. Manipulation of plant genes mediating transformation is a novel approach to enhancing transformation frequencies of recalcitrant plant species.

MOS11 in snc1-mediated resistance

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Plant uses highly specific R protein as a line of defense against specific effectors found in pathogens. Upon specific recognition of its cognate binding partners (or its effect), the R protein triggers a robust defense response to thwart pathogen invasion and growth. Our lab uses the Arabidopsis autoimmune model snc1 to dissect signaling events downstream of R proteins activation. The misssense *snc1* dominant mutant plants display dwarf morphology, constitutive PR gene expression, elevated salicylic acid level, and are resistant to most virulent bacterial and oomycete pathogens. We used T-DNA insertional mutagenesis to search for genes that when mutated would modify *snc1*-mediated resistance. One of such suppressors we found is *mos11* (*modifier of snc1, 11*). Loss of *mos11* partially suppresses dwarf morphology of *snc1*, it decreases endogenous SA level, and partly abolishes *snc1* enhanced resistance. MOS11 has no previous known function in Arabidopsis. In human, MOS11 homolog is part of a small protein complex involved in RNA splicing and export. Using a reverse genetic approach, Arabidopsis T-DNA lines corresponding to putative homologs of the human MOS11 complex were analyzed. We found that one mutant could also partly suppress *snc1*. The triple mutant *mos11*/ *new mutant*/*snc1* reverted to wild-type morphology. More detailed analysis of the *mos11* complex will be presented at the meeting.

Identification of virulence effector genes in *Sporisorium reilianum*

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Smut fungi are biotrophic plant pathogens that infect economically important hosts. *Ustilago maydis* and *Sporisorium reilianum* are two smut fungi that both parasitize maize but cause species-specific symptoms. While *U. maydis* induces spore-filled tumors on leaves, stems or flowers, *S. reilianum* triggers phyllody and spore formation exclusively in the inflorescence. We hypothesized that species-specific symptom development should involve secreted proteins that are either species-specific or are only weakly conserved between the two related fungi. Therefore, we sequenced and compared the genome of *S. reilianum* to that of *U. maydis*. The largest region of low sequence conservation exactly matched the gene cluster 19A, a previously identified virulence locus of *U. maydis*. Cluster 19A of *S. reilianum* comprises 29 genes, 21 of which code for secreted proteins. Deletion of the largest part of cluster 19A (25 genes) in *S. reilianum* resulted in increased fungal proliferation in maize leaf epidermal cells and slightly reduced virulence. In contrast, deletion of the second part (four genes) of cluster 19A led to dramatically reduced virulence, suggesting that this region contains important virulence factors for *S. reilianum*. To assess the contribution of each of the four genes to *S. reilianum* development, we are currently testing virulence of respective single gene deletion strains. Interestingly, in *U. maydis* important virulence determinants are encoded in the first half of cluster 19, whereas the second half contributes only weakly to virulence. This suggests that the closely related fungi *U. maydis* and *S. reilianum* utilize different effectors to facilitate colonization of their common host plant maize.

Assessing global levels of transcriptional activity involved in cold stress in arctic *Mesorhizobium* using microarray technology

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Arctic *Mesorhizobium* sp N33 is a cold adapted symbiotic bacterium and isolated from the nodules of indigenous plants (*Oxytropis arctobia*) in Canadian high arctic. This bacterium is able to establish better N2-fixing

nodules on the temperate cultivated legume sainfoin (*Onobrychis vicifolia*) and provides higher plant yield at 10°C than commercial temperate rhizobia. The molecular mechanism of cold adaptation in this psychrotrophic bacterium is poorly understood. To decipher the transcriptomes activities on the genome-wide scale of this bacterium during various stages of cold stress, a DNA microarray technology was established. One way ANOVA analysis for microarray data showed that 424 transcripts (ESTs) were significantly different with a P Value < 0.005 and produced six hierarchical clusters. With a P Value < 0.001 only 206 transcripts showed significantly differential expression and produced three hierarchical clusters. All 424 ESTs were sequenced and compared with GenBank and their biological pathways were ascertained using EXPASy and KEGG databases. Furthermore, to validate the results of microarray analysis, seventy seven primer pairs were designed for differentially expressed genes over the set of experimental conditions and a quantitative-real time-reverse transcription PCR (Q_RT_PCR) strategy is under investigation.

Using a *GSTF8* promoter system to analyse plant responses to auxin-like herbicides and the fungal pathogen *Rhizoctonia solani*

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Plant glutathione S-transferases (GSTs) are detoxifying enzymes that play a key role in defending plants against xenobiotic compounds. One well-studied GST is *GSTF8,* whose expression in increased in response to a range of stresses, such as salicylic acid (SA), auxin, and hydrogen peroxide. *GSTF8* expression is also induced by the auxin-type herbicide dicamba, suggesting that *GSTF8* has a role in detoxifying this compound. Auxin-like herbicides are widely used to control broadleaf weeds, but there is very little information about the molecular effects of dicamba, a benzoic acid. Therefore, we have performed microarrays and analysed the sensitivity of auxin-resistant mutants to various concentrations of dicamba and show that although there is some overlap with the signalling pathways induced by the synthetic auxin 2,4-dichlorophenoxy acetic acid, there are also significant differences in the longer-term molecular effects. In addition to dicamba, our lab has also discovered that the *GSTF8* promoter is also induced by the fungal root pathogen *Rhizoctonia solani. R. solani* AG8 is the major pathogenic strain in wheat; however, Arabidopsis is resistant to this strain. A forward genetic screen of Arabidopsis seeds carrying the *GSTF8* promoter fused to a luciferase reporter gene (*GSTF8*::LUC) produced a mutant called LOF1 (loss-of-function mutant 1). In LOF1 the *GSTF8* promoter fails to respond to *R. solani* infection. Through cloning of LOF1, we hope to gain insight on mechanisms by which the plant defends itself against *R. solani.*

***Ram1*: Molecular basis of signalling specificity in mycorrhizal symbiosis**

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Despite dramatic differences between the two processes common genes have been identified that are required in the early signalling events leading to both rhizobial and mycorrhizal endosymbioses (Parniske et al., 2008). For their ability to establish symbiotic interactions with both N-fixing bacteria and arbuscular mycorrhizal (AM) fungi legume plants provide the best biological system for the identification of novel signalling components responsible for signal specificity. With this aim a fast neutron mutagenized *Medicago truncatula* population was screened to identify mutants impaired in AM symbiosis (Myc-) but showing wild type responses to *Sinorhizobium meliloti* infection (Nod+). Here we show the identification and characterization of the *RAM1* gene (*Reduced Arbuscular Mycorrhiza 1*). In *ram1* plants symbiosis with multiple AM fungi is blocked at very early stages but the capability of inducing hyphal branching in germinated AM fungal spores (Akiyama K. et al. 2005) is retained. The absence of any obvious developmental phenotype together with normal behaviour in response to pathogens indicate the highly specific *RAM1* function in symbiosis.

Effect of the hypersensitive response on *Pseudomonas syringae* pv. *phaseolicola* colony formation *in planta*

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Pseudomonas syringae pv. *phaseolicola* (*Pph*) is the seed borne causative agent of halo blight in the common bean, *Phaseolus vulgaris*. Gene for gene interactions underpin varietal resistance and race structure in the *Pph*/bean interaction. *Pph* race 4 strain 1302A contains the avirulence factor *avrPphB*, which matches resistance gene *R3* and causes a rapid hypersensitive reaction (HR) in *P. vulgaris* cultivar Tendergreen. *avrPphB* resides on a 106kb genomic island designated PPHGI-1, which if lost from 1302A, causes change to a virulent phenotype that produces water-soaked lesions typical of *Pph* disease. Evolution of 1302A virulent strains (by loss of PPHGI-1) occurs by selective pressure due to exposure to *R3* based HR in *P. vulgaris* Tendergreen leaf tissue. In a separate study, we have shown PPHGI-1 can be horizontally transferred from *Pph* 1302A to *Pph* race 6 strain 1448A, creating 1448A::PPHGI-1 that also triggers the HR in Tendergreen. We have tagged these strains with fluorescent proteins (YFP and RFP introduced individually into each chromosome). We have used the tagged strains to examine colony development *in planta* and interactions between avirulent and virulent bacteria within the leaf apoplast. The HR controlled by the *R3* gene has striking effects on colony morphology. Bacterial multiplication is strongly influenced by the proportion of inoculum delivering the AvrPphB avirulence protein. The microenvironment within the leaf creates localized stress conditions that affect the retention of PPHGI-1.

Bacterial AvrPtoB exploits the endogenous ubiquitination machinery to degrade FLS2

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An important layer of active defense in plant immunity is the detection of pathogen-associated molecular patterns (PAMPs) mediated by cell surface receptors. The *Arabidopsis thaliana* pattern recognition receptor FLS2 recognizes bacterial flagellin (flg22) and triggers a suite of defense reactions to restrict bacteria from entry into plant tissues and limit bacterial proliferation, referred to as PAMP-triggered immunity. To overcome this level of defense, pathogenic bacteria deliver effector molecules into the plant cell that interfere with PAMP responses. AvrPtoB, an effector from *Pseudomonas syringae* pv. *tomato* (PtoDC3000), suppresses immune responses elicited by FLS2. We show that AvrPtoB, through its N-terminus, associates with FLS2 and this interaction is enhanced by flg22 stimulation. AvrPtoB is active as an E3 ligase to catalyze poly-ubiquitination of the FLS2 kinase domain *in vitro* and *in vivo* leading to the degradation of FLS2, which is required for full virulence of PtoDC3000. We propose a model, in which AvrPtoB initially binds to FLS2 independently of its E3 ligase function to prevent acute signaling, and subsequently exploits the endogenous degradation pathway by ubiquitinating FLS2 to permanently down-regulate PAMP signaling. Furthermore, we will discuss ubiquitination of FLS2 by mapping target residues and the type of ubiquitination, analyzing natural variation in the ubiquitination sites, and identifying the endogenous E3 ligases.

Levels of non-host resistance of Arabidopsis to Asian soybean rust (*Phakopsora pachyrhizi*)

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The causal agent of Asian soybean rust (*Phakopsora pachyrhizi*, ASR), has recently spread from its origin in tropical and subtropical areas of Asia and Australia to Africa, South-America (2001) and the United States (2004). All commercial soybeans are susceptible to the fungus and natural resistance based on *R*-genes has been quickly overcome in the field. In this context, the identification of additional sources of resistance might assist in combating the pathogen, which can cause up to 80% yield loss when not restricted by fungicides. A very stable type of resistance in plants is non-host resistance (NHR), relying on different resistance levels and molecular redundancy. The model plant Arabidopsis is a non-host to ASR, showing pre-haustorial resistance accompanied by deposition of hydrogen peroxide, superoxide and callose. Analysis of mutants in non-host resistance of Arabidopsis to barley powdery mildew (*Bgh*) suggested similar mechanisms in defeating these non-host pathogens, since the penetration mutants *pen2* and *pen3* were found to allow intercellular growth in the mesophyll, while on wild-type plants, the interaction is stopped on epidermis level. However, even if the triple mutant *pen2 pad4 sag101* allows sporulation of *Bgh*, additional level(s) of resistance seem to be involved in ASR-non-host resistance, since ASR is able to form haustoria but not to reproduce on this mutant. The establishment of haustoria is a crucial step in the arrangement of a biotrophic interaction. We are interested in identifying the crucial factors allowing haustoria establishment and therefore biotrophy. To this end, results of a whole-genome expression analysis will be presented.

Identification of host protein partners involved in the *Rx*-mediated resistance

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Rx is a potato resistance (R) gene of the NBS-LRR (nucleotide binding site-leucine rich repeat) family which confers a durable resistance against *Potato virus X* (*PVX*) (Bendahmane *et al*., 1995). Even if recently a direct interactor of *Rx* protein has been identified through different approaches (Tameling and Baulcombe 2007; Sacco *et al.,* 2007), lots of information are still missing about the host proteins involved in this resistance cascade. The goal of this project is to identify new genes required for the *Rx*-mediated resistance. For that purpose a collection of Rx-expressing EMS mutant tomato (cv Micro-Tom) has been generated and screened with a partial resistance-breaking strain, KH2. During the screening, five mutant lines which survive PVX KH2 inoculation and do not display the systemic necrosis infection phenotype observed in their unmutated parent, have been identified. However, the *Rx* resistance against a common PVX isolate, CP4, still appear to be effective in these mutants, suggesting that they are affected in the specificity and/or intensity of the *Rx*-mediated resistance. A genetic analysis has revealed that all the mutations are independant and that 3 of them are dominant. A molecular characterization has also been pursued, starting with the study of the *Rx* expression, which appears to be normal in all mutants. To make sure that the mutation in these lines does not affect genes that are already known to be involved in the *Rx*-mediated resistance as for example RAR1, SGT1, HSP90 or RanGap2 (Azavedo *et al*., 2002 and 2006; Moffett *et al.*, 2002), the sequences and expression of these genes have also been analysed. Moreover, inoculation of PVX isolates mutated at the two CP key amino acids (121 and 127) for resistance breaking is under the way to analyse if the recognition specificity is altered in these mutants.

***R. etli* CFN42 responds to nitrogen oxides through the reductases coding by *nirK* and *norC* genes**

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The biological denitrification process consists of the sequential reduction of nitrate or nitrite to nitrogen gas coupled with energy conservation when oxygen concentrations are limited. The Cu- containing Nir (encoded by *nirK)* catalyzes the reduction of NO_2- to NO. Because of its toxicity NO has to be immediately reduced to nitrous oxide by NorC and NorB. Therefore, to remove the NO, reactions for reduction of nitrite and nitric oxide most be regulated coordinately. Denitrification capability is a widespread process in procaryotes including nitrogen-fixing bacteria. Among rhizobia only *B. japonicum* is a true denitrificant. Other rhizobia such as *S. meliloti* or *R. sullae*, are only partial denitrifyers and *Rhizobium etli*, the common bean symbiont, is a non-denitrifying bacteria. The presence of *nirK*, *norCBQD* and *nnrR* genes in *R. etli,* suggests the possibility that NirK and NorCB have a N-oxides detoxifying role. To gain insight into how *R. etli* responds to reactive nitrogen species, we carried out a functional characterization of the reductases encoding by *nirK* and *norCB* genes. Our results shown that: (i) *nirK* gene is functional both in free-living conditions and in bacteroids; (ii) Nir activity increases in response to N-oxides; (iii) to have a full Nir activity and nitrite-uptake the action of FixKf, NnrR and pseudoazurin proteins is necessary; (iv) NorC is important to reduce NO; (v) *norC* expression is under the control of NnrR protein through FixKf. By expression analysis we demonstrated the participation of common regulatory proteins for N_2-fixation and N-oxides detoxification genes, indicating that these processes may perform complementary functions in *R. etli*. This work is partially supported by grants IN201406 and 202109 (DGAPA, UNAM).

Use of 454-pyrosequencing method as a quantitative gene expression tool in geminivirus-infected and recovered plants

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Geminiviruses are a group of plant viruses whose genomes are circular ssDNA. *Pepper golden mosaic virus* (PepGMV) is a geminivirus that infects major Mexican crops such as pepper (*Capsicum annum*). A host recovery system has been characterized in geminivirus-infected pepper plants (after 2-3 leaves with severe symptom expression, new leaves are "recovered", symptomless). To identify differentially expressed genes (mock *vs* symptomatic *vs* recovered tissues), one cDNA library from each stage was sequenced by 454-pyrosequencing method. A Reference Transcriptome (RT, pepper genome is not available) was first obtained by assembling 70,743 ESTs (Sanger sequencing, several organs) with the 454 "reads" to obtain 32,738 contigs. The quality of the hybrid assemblage was corroborated by a BLAST analysis to two different Solanacea ESTs database. To verify the fidelity and quantitative characteristics of pyrosequencing, several 454-runs from two libraries were performed. The reads obtained for each run were aligned against the RT. Using the Fisher's test we corroborated that there is not differential expression between different runs from the same library. The 454-reads from each condition were then aligned to RT and differentially expressed genes were identified by Fisher's test. Following this procedure we were able to identify over-expressed (o) and repressed (r) contigs from symptomatic (147-o; 107-r) and recovered (145-o; 119r) leaves. At least, 12% of o-contigs and 30% of r-contigs did not align to any reported sequence. Our results support the idea that the pyrosequencing method is reliable and it can be used as a quantitative strategy. The use of emerging technologies will provide new tools to study important biological phenomena such as recovery in geminivirus-infected plants.

Functional characterization of a 68kb deletion that specifically affects pathogenicity of *Ralstonia solanacearum* race 3 (potato) strains

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On the basis of taxonomy studies, *Ralstonia solanacearum* is considered as a complex species for its very large infra-specific diversity. Molecular methods and sequence comparisons were used to evaluate intraspecific variation among the strains, grouping these in four monophyletic groups which correlate with their geographical origin (Fegan and Prior 2005); however it is clearly established that the host range of the strains does not correlate with phylogeny. The strains that specifically attack potato (and to a lesser extent tomato) such as strains IPO1609 and UW551, called potato strains are considered as nearly 'clonal' (>90% identity at the genomic level). However, we noticed that these two strains showed a striking difference in pathogenicity: IPO1609 is scored as not pathogenic on tomato plants in contrast to what observed for strain UW551 (pathogenic on tomato). We therefore focused our research in understanding what makes the difference in pathogenicity between these 'nearly isogenic' strains. A comparison of the draft genome sequences identified a 68 kb deletion specifically on the megaplasmid replicon of IPO1609. This 68kb region does not contain any Type III or Type II Secretion System genes that could explain this phenotype. We generated an identical 68 kb deletion in the UW551 strain and found that the resulting mutant strain ΔUW551 presented the same defect of pathogenicity on tomato as the IPO1609 strain. The identification of the genes from this 68kb region that are essential for the pathogenicity of these strains will be presented.

Induced systemic resistance in *Agrostis stolonifera* and *Nicotiana benthamiana* by the volatile organic compound, (2R,3R)-butanediol is associated with priming of gene expression

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Induced systemic resistance (ISR) was activated by the bacterial volatile organic compound, (2R,3R)-butanediol against several plant diseases, but not by (2S,3S)-butanediol, which is reported to be an ISR-inactive compound. The active (2R,3R)-butanediol reduced by 77% the density of anthracnose lesions caused by *Colletotrichum orbiculare* on *Nicotiana benthamiana*. Soil application of (2R,3R)-butanediol primed expression of the pathogenesis-related (PR) genes, *NbPRb-1b*, basic *NbPR-2* and *NbPR-5dB*, but did not affect expression of genes involved in SAR in *N. benthamiana*, *NbPR-1a*, *NbPR-3Q* and acidic *NbPR-5*. For *Agrostis stolonifera*, soil application of (2R,3R)-butanediol reduced the area of diseased leaves by 90 to 95% for Microdochium patch caused by *Microdochium nivale*, brown patch caused by *Rhizoctonia solani* or dollar spot caused by *Sclerotinia homoeocarpa*. Expression of the jasmonate-related genes, *AsAOS1* and *AsOPR4*, and the PR gene, *AsGns5*, was primed by (2R,3R)-butanediol, but expression of SAR-associated genes was not affected. ISR was activated by (2R,3R)-butanediol in both monocots and dicots, resulting in resistance and priming of gene expression like that produced by application of ISR-inducing rhizobacteria.

Investigation of the cellular basis for resistance to ergot infection in wheat
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The aim of this study is to gain a cellular and molecular understanding of the interaction between the mycotoxin producing plant pathogen *Claviceps purpurea* and its host hexaploid wheat (*Triticum aestivum*). Although detailed molecular studies have been carried out on factors affecting pathogenicity of *C. purpurea* on rye, we set out in this work to make the first detailed description of the interaction between *C. purpurea* and wheat, focusing on elucidation of mechanisms by which wheat can resist infection. To this end we will present the results of microscopy studies covering early events in the infection of wheat varieties showing a range of degrees of susceptibility. In the fully susceptible interaction, *Claviceps* spores germinate on the stigma hairs and grow through tissues of the wheat flower enveloping the ovule within three days, faster than previously reported in rye. By six to seven days the whole wheat ovary is colonised and the fungus enters its sphacelial stage whereby conidiospores are exuded from the plant in a sticky phloem sap residue known as honeydew. The structures observed at each of these stages will be presented. *Claviceps* cannot be chemically controlled and this drives the need to identify and exploit natural resistance to this pathogen. Partial resistance was identified in a UK wheat variety in previous work, and having established the timing and nature of the fully susceptible interaction at the cellular level, our next objective was to determine which stages in the development of a *Claviceps* infection are delayed or constrained in partially resistant backgrounds. We have also commenced gene expression studies and will present preliminary data on changes in host gene expression in early stages of infection of a fully susceptible wheat variety.

Characterization of a novel *Arabidopsis* resistance gene and its cognate effector from *Hyaloperonospora arabidopsis*
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Phytopathogenic oomycetes cause a large number of devastating plant diseases. We are using the *Arabidopsis thaliana/Hyaloperonospora arabidopsis* pathosystem as a tool to better understand the mechanisms underlying oomycete pathogenesis and plant disease resistance. Using a bioinformatics approach we have identified a number of putative effector genes in the recently assembled genome of *H. arabidopsis* isolate Emoy2 based on the presence of a putative secretion signal and the RXLR motif, which is associated with effector delivery into the plant cell. We have cloned and expressed forty candidate effectors to assess putative functions. One effector, ATR39, was found to induce a hypersensitive response on the *A. thaliana* ecotype Weiningen (Wei-0) when delivered by *Pseudomonas fluorescens* containing the Type III secretion system. ATR39 is highly conserved among *H. arabidopsis* isolates and, like other cloned oomycete effectors, encodes a protein of unknown function. Recognition of the effector segregates as a single locus in a Wei-0/Col-0 F2 mapping population and maps to a cluster of putative NBS-LRR resistance genes. Interestingly, despite being able to recognize ATR39, Wei-0 remains susceptible to the Emoy2 isolate of *H. arabidopsis*, indicating a complex interplay between factors derived from pathogen and host. Here we present our progress on the cloning and characterization of this novel pair of avirulence effector and resistance gene.

Tolerance to trehalose, a possible key mechanism underlying partial resistance to clubroot in Arabidopsis?
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Partial resistance under polygenic control is more and more considered as an efficient tool for sustainable control of plant pathogens. However, underlying mechanisms are hardly understood. We work on molecular and physiological basis of quantitative partial resistance harbored by *Arabidopsis thaliana* to *Plasmodiophora brassicae*, a soil-borne pathogen causing the clubroot disease. *P. brassicae* synthesizes large amounts of trehalose which is accumulated in all plant parts during infection. This disaccharide, rarely accumulated in plants, has been described as a potential effector that would possibly enhance carbon availability for the pathogen. The aim of this study is to determine if the tolerance to trehalose is a possible key mechanism underlying partial resistance to clubroot. Trehalose tolerance was quantified by measuring root growth and anthocyans accumulation in plants subjected to exogenous treatments with trehalose. The accession Bur-0, partial resistant to clubroot, was found to be tolerant to trehalose. Dissection of the genetic architecture of trehalose tolerance was performed through QTL analyses on a RIL progeny from the cross Bur-0 x Col (susceptible to both clubroot and trehalose). The relationships between genetic factors implied in trehalose tolerance and clubroot quantitative partial resistance will be discussed.

Characterization of *Hyaloperonospora arabidopsidis* effectors using heterologous delivery, mixed infection, and next generation sequencing
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Successful colonization by pathogens depends on the delivery of effector molecules that suppress host defence responses and manipulate host metabolism in order to avoid recognition and obtain nutrients. It is estimated that the oomycete plant pathogen *Hyaloperonospora arabidopsidis* (*Hpa*) delivers more than a hundred effector proteins into host cells during infection, with a group of effectors characterized by the presence of a RXLR motif, which was recently shown to be required for translocation of *Phytophthora* effectors into the plant cytoplasm (Duo *et al.*, 2008; Whisson, S.C., *et al*. 2007). Because the outcome of the interaction between the pathogen and the plant is the result of the concerted action of multiple effectors delivered into the plant cell, we are developing protocols to assay the contribution of individual RXLR effector candidates from *Hpa* to the outcome of its interaction with *Arabidopsis* plants when delivered simultaneously using the pEDV system (Sohn *et al*. 2007) expressed in *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000) and Illumina sequencing technology. We infected plants with a mixture of *Pst* DC3000 strains carrying different plasmid-borne *Hpa* effector candidates and used the abundance of a given effector sequence three days after inoculation to infer its contribution to the pathogen success or failure during infection. Our results using a mixture of 38 *Hpa* effectors in accessions Col-0 and Ws-0 allowed us to identify one effector that enhances bacterial multiplication in Col-0 but not in Ws-0, one effector that enhances bacterial multiplication in Ws-0 but not in Col-0, and one effector that enhances bacterial multiplication in both accessions. This work is funded by the Gatsby Charitable Foundation; DG and JLB are funded by HFSP.

Bacterial and fungal antagonists of *Rhizoctonia solani* on lettuce: Biocontrol efficiency and rhizosphere microbial community response
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Based on the colonization ability of lettuce roots as prerequisite for effective plant antagonist interaction and disease suppression, two bacterial antagonists (*Pseudomonas jessenii*, *Serratia plymuthica*) were selected as promising control agents against the soil-borne pathogen *Rhizoctonia solani* on lettuce. Presently the effect of the soil type on colonization ability was investigated by *rfp* labelled bacterial antagonists. The fungal antagonist (*Trichoderma viride*) chosen showed high mycoparasitic activity against *R. solani*. The effect on lettuce growth and on disease suppression of bottom rot were tested in single and combined application of bacterial and fungal antagonists. No significant improvement of plant growth was observed by either one of the antagonists in single or combined treatment. However, an effective disease suppression effect was evaluated. Plate counts and DGGE fingerprints of *Pseudomonas*-specific *gacA* genes amplified from total rhizosphere community DNA confirmed that *P. jessenii* established as dominant *Pseudomonas* population in the lettuce rhizosphere. Furthermore, the DGGE fingerprint revealed that the *R. solani* heavily affected the bacterial and fungal community structure in the rhizosphere of lettuce.

***NENA* is required for root symbiosis and encodes a putative component of the nuclear pore**
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Genetic analysis of symbiosis defective mutants has identified a signaling network that unites early responses to symbiotic fungi and bacteria during

onset of arbuscular mycorrhiza (AM) and root nodule symbiosis. A central role within the network has been ascribed to symbiosis-induced calcium spiking. Moreover, two putative nucleoporins, Nup133 and Nup85, are involved in the signaling process. During a genetic screen for AM-defective *Lotus japonicus* individuals the *nena* mutant was isolated. AM development of *nena* is impaired during penetration of the outer root layers by *Glomus intraradices*, leading to abortion of colonization. Absence of calcium spiking after Nod factor treatment points to an early defect in the signaling pathway. Nodulation deficiency in *nena* is more severe at higher (24°C) than at lower (18°C) temperature. We identified the causative mutation by map-based cloning in a gene that is similar to *SEH1*, encoding a WD40 repeat protein. Complementation of *nena* mutants by *Agrobacterium rhizogenes*-mediated transformation and the phenotypes of additional *SEH1* alleles obtained by TILLING corroborate gene identification. SEH1, Nup133 and Nup85 belong to the same Nup84 subcomplex of the nucear pore in yeast. Although sequence conservation is low, interaction of NENA with LjNUP85, revealed by yeast two-hybrid and tobacco BiFC analyses, as well as peri-nuclear localization of translational NENA fusions to fluorescent proteins fit to the nucleoporin annotation. The lack of distinct pleiotrophic effects in *nena* mutants indicates a specific involvement of nuclear pore components in the regulation of root symbiosis.

Identification of signaling pathway controlled by *Enhanced Disease Resistance 1 (EDR1)* gene

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Loss-of-function mutations in the *Arabidopsis* gene *EDR1* (*ENHANCED DISEASE RESISTANCE 1*) confer enhanced resistance to the powdery mildew pathogen *Golovinomyces cichoracearum*, and enhanced ethylene-induced senescence. *EDR1* encodes a protein kinase similar to *CTR1* (a negative regulator of ethylene responses). To identify components involved in the *EDR1* signaling pathway(s), we screened for mutations that suppress *edr1*-mediated phenotypes. One suppressor mutation was mapped to the *KEEP ON GOING* (*KEG*) gene, which encodes a multi-domain protein that includes a RING E3 ligase domain, a kinase domain, ankyrin repeats, and HERC2-like repeats. The KEG protein appears to negatively regulate the protein level of the transcription factor ABI5 (ABCISIC ACID INSENSITIVE5). Together with the evidence that *edr1* displays enhanced sensitivity to both exogenous ABA and drought, we now believe that ABA signaling plays a central role in *edr1*-mediated phenotypes. Analysis of the defense marker gene *PR-1* indicates that the *keg-4* suppressor mutation can also inhibit beta amino butyric acid (BABA)- induced priming of defense responses. We are currently mapping and cloning additional *edr1* suppressor mutations using a bulked-segregant analysis strategy combined with whole genome Illumina sequencing. Transcriptome analysis of the *edr1* mutant has shown that many defense-related genes are upregulated in *edr1* plants relative to wild-type plants, both before and after pathogen infection. Notably, genes associated with reactive oxygen species and phosphorelays were induced earlier and to a higher level in *edr1* plants. Knock-out mutations in some *edr1*-elevated genes enhanced susceptibility to powdery mildew, which is a strong indication of genetic interaction with *EDR1*.

ABA and auxin signaling in plant defense to *Leptosphaeria maculans*

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Beside the two TIR-NB-LRR encoding genes in the RLM1 locus, the short TIR-NB encoding RLM3 gene and camalexin, the plant hormones ABA and auxin play important roles in the successful colonization of *L. maculans* in Arabidopsis. We have further found that ABA is induced by *L. maculans* recognition, a reaction which is enhanced by BABA pre-treatment. BABA also activates defense responses downstream of ABA and enhances ABA sensitivity, but BABA cannot complement absence of the RLM1 gene. That BABA acts both upstream and downstream of ABA indicates that it affects a feedback loop regulating ABA homeostasis. Both ABA and BABA responses are however blocked in *abi1* and *abi4*. Studies of the susceptible callose synthase mutant *pmr4* and additional mutants strongly suggest that an unknown key resistance factor is present downstream of ABI4. Our approach to identify the direct targets of ABI4 is to use an inducible system followed by array and candidate gene analysis, on which data will be presented. Furthermore, one *L. maculans* susceptible mutant *lms5* is impaired in its auxin response resulting in enhanced growth on auxin containing media. *lms5* has decreased endogenous IAA content compared to wild-type (*Ler*) and shows a "spotty" disease phenotype similar to *rar1*. The mutation is mapped to chromosome 1, and assessment of knock-out mutants in a target location, revealed two genes, a small heat shock protein HSP17 and a F-box protein to be candidates. Additional studies of mutants impaired in auxin signaling and the SCF-complex propose involvement of LMS5 in protein degradation events. Updated results on LMS5, which not seem to be involved in defense to *Pseudomonas*, *Hyaloperonospora* or other necrotrophic fungi will be presented. The work is funded by the Swedish Research Councils VR and Formas.

Microarray analysis reveals up-regulation of a cascade of defense response genes in transgenic rice lines containing single blast resistance gene *Pi-k^h*

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Rice is one of the most important staple foods of half of the world population. Among biotic stresses, rice blast caused by *Magnaporthe oryzae* is a serious threat to the cultivation of rice at global level. This disease can be effectively managed by using resistant varieties. For this, identification, mapping and cloning of blast resistance genes are very important. The R gene *Pi-k^h* conferring high degree of resistance to rice blast was fine mapped and cloned by our group from indica cultivar Tetep (Sharma *et al*., 2005). Transgenic rice line (Taipei 309) was highly resistant to rice blast. To understand the co-regulation of genes in incompatible interaction between blast resistance gene *Pi-k^h* and *M. oryzae,* microarray analysis was performed. For this study, three biological replicates of each of transgenic and non-transgenic were taken. *M. oryzae* strain PLP-1 was used for spray inoculation on transgenic and non-transgenic plants under controlled conditions. Leaf samples were harvested after 72 hours post inoculation. Total RNA was isolated and used for hybridization of Affymetrix microarray chip containing 51279 rice genes. The data were analysed by using ArrayAssist software. Lists of genes were categorized as per Gene Ontology (GO) score. A total 68 genes were up regulated in transgenic rice line. The *Pi-k^h* gene was found to co-activate the battery of transcriptional regulators like Peroxidase, Dof zinc finger, MADS box and CapD. The highest up regulated gene was peroxidase having FCA value 24. These results confirmed the direct involvement of *Pi-k^h* gene in the co-expression of defense response genes in incompatible interaction.

Diffusible signals from arbuscular mycorrhizal (AM) spores lead to transcriptional changes in host but not in non-host plants

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It has been demonstrated previously that germinating spores of AM fungi (AMF) release one or more small (< 3kDa), heat-stable, partially lipophilic and diffusible compounds that elicit a transient cytosolic calcium elevation in soybean cells. Because the molecule(s) is (are) of exclusively fungal origin, we wanted to investigate whether the presence of the plant could act as an enhancing or stimulating factor, and if, on the other hand, the diffusible signals released by the fungus could lead to transcriptional changes in root cells. For that, two different experimental systems were used. In the first one, the water in which spores of *Gigaspora margarita* were germinated ("myc-water") was applied to 3-week old, P-starved plantlets of *Lotus japonicus* or Arabidopsis (negative control). In the second one, a modified sandwich system was used, with an additional nitrocellulose membrane preventing physical contact between the AMF spores and the roots, but allowing the exchange of diffusible signals from both partners. Microarray results are currently being validated by RT-qPCR on Lotus and Arabidopsis. The emerging picture, though, already suggests that i) host plants respond to the "myc-water" also at the transcriptional level; ii) non-host plants do not; and iii) the diffusible signals produced by germinating AMF spores are influenced by the presence of the host plant.

Identification of pathogenic effectors in *Xanthomonas axonopodis* pv. *manihotis* using a genetic screen

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Cassava Bacterial Blight, caused by the gram negative bacterium *Xanthomonas axonopodis* pv. *manihotis* (*Xam*), is the most important bacterial disease of this crop. This bacterium possesses a type III secretion system (TTSS) that secretes and translocates effector proteins into the host plant cell, facilitating the infection process and interfering with signaling and defense. The set of these translocated proteins is considered an important pathogenicity factor. At present there is only one effector protein, PthB, reported in *Xam* (Verdier, 2004). In this work we developed an *in vivo* genetic screen system similar to that developed by Guttman et al (2002) for *Pseudomonas*, which is based on the modular structure of effectors. Several combinations of Avr genes and host plants were tested for the implementation of the system. The best combination was AvrRpt2 and Arabidopsis. A transposon fusion with an orphan AvrRpt2 was transformed into Xam and a screen for HR was performed. Additionally, we are developing a parallel system to validate the translocation of putative effector proteins identified in *Xam* using bioinformatic tools and homology with previously characterized effector proteins in other species of this genus. For this approach we created chimeric proteins between the secretion signal of putative effectors and the C-terminus of AvrBs2 from *Xanthomonas campestris* pv *vesicatoria* (Roden, 2004). The identification of new effector using both approaches will greatly contribute to our understanding of the molecular strategies used by this bacterium to cause disease in cassava.

New mechanistic insights into the virulence activity of the *Xanthomonas* type III effector AvrBs3

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Xanthomonas campestris pv. *vesicatoria* is the causal agent of bacterial spot disease on pepper and tomato. Pathogenicity depends on a type III secretion system which translocates effector proteins into the plant cell cytoplasm. One well-studied effector is AvrBs3 which induces hypertrophy symptoms in susceptible plants and is recognized by the *Bs3* resistance gene in resistant plants leading to the HR. The AvrBs3 activities depend on the central protein region of 17.5 nearly identical, tandem repeats of 34 amino acids, one of two nuclear localization signals and an acidic activation domain. The latter are typical motifs of eukaryotic transcription factors. We previously demonstrated that AvrBs3 acts as a transcription factor and induces the expression of host genes resulting in the induction of hypertrophy and the HR dependant on the plant genotype. DNA binding of AvrBs3 and activation of *UPA* (*UPA*, upregulated by AvrBs3) gene promoters was studied in detail. Here we present new insights into the underlying molecular mechanism of AvrBs3 activity.

The "repertoire for repertoire" hypothesis: Repertoires of type three effectors may explain host specificity in *Xanthomonas*

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Host range of many plant pathogenic bacteria is restricted to one or a few host plant species reflecting a tight adaptation of bacteria to specific hosts. Two hypotheses could explain host specificity: either it can be explained by the phylogenetic position of the strains, or, alternatively, by the association of virulence genes enabling a pathological convergence of phylogenetically distant strains. In this latter hypothesis, host specificity would result from the interaction between repertoires of bacterial virulence genes and repertoires of genes involved in host surveillance systems and defences (a "repertoire for repertoire" hypothesis). To challenge these two hypotheses, we selected 132 *Xanthomonas axonopodis* strains representative of 18 different pathovars which display different host range. First, the phylogenetic position of each strain was determined by sequencing the housekeeping gene *rpoD*. This study showed that many pathovars of *Xanthomonas axonopodis* are polyphyletic. We thus investigated the distribution of 37 T3E genes in these strains by both PCR and hybridization methods. Our study revealed that T3E repertoires are highly variable between these strains, both in terms of T3E presence and of size of repertoires. T3E repertoires comprise both core and flexible gene suites that likely have distinct roles in pathogenicity and different evolutionary histories. Our results showed a correspondence between composition of T3E repertoires and pathovars of *Xanthomonas axonopodis*. For polyphyletic pathovars, this strongly suggests that T3E genes explain a pathological convergence of phylogenetically distant strains. Finally, our results support the "repertoire for repertoire" hypothesis as the molecular basis of host specificity for plant pathogenic bacteria.

Understanding interactions between phytopathogenic *Phytophthora* effector IpiO and the host resistance protein RB

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Species of phytopathogenic *Phytophthora* are well known for their ability to cause disease on economically important crops, with almost 100 recognized species targeting close to 300 different hosts. The host resistance protein RB, isolated from wild potato, specifically recognizes the *P. infestans* IpiO effector to elicit resistance. Using *P. infestans* isolates collected worldwide, we have found that IpiO is universally present, explaining the broad-spectrum phenotype of RB. However, we have found that multiple *IpiO* variants exist within a given P. infestans isolate. Importantly, some *IpiO* variants are recognized by *RB* (*IpiO1* and *IpiO2*) and some are not (*IpiO4*). We have determined that *IpiO* alleles not recognized by *RB* can be recognized by *RB*-like genes from other potato species. Most importantly, we have found that IpiO alleles are present in other phylogenetically distinct *Phytophthora* species. Therefore, *RB* may not only confer resistance to late blight of potato and tomato, but is potentially a source of resistance to other *Phytophthora* species on related or unrelated hosts. Further elucidation of the molecular events involved in *IpiO* recognition will be presented.

Analysis of the OsRac1 complex (Defensome) involved in rice innate immunity

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We have previously shown that OsRac1, a small GTPase in rice, is involved in reactive oxygen species production, defense gene activation and initiation of cell death during defense response. Recently, we isolated OsRac1 interacting proteins by affinity chromatography and yeast two-hybrid screen. These results indicate *in vitro* interaction of these proteins with OsRac1, but their interaction *in vivo* is not clear. Therefore, we used methods that allowed isolation of a native protein complex to analyze the components of OsRac1 complex *in vivo*, and study how the composition of the OsRac1 complex is altered by PAMPs treatment in suspension cell cultures. Gel filtration was performed with proteins extracted from transgenic suspension cell cultures expressing constitutively active form of OsRac1 (CA-OsRac1) or dominant negative form of OsRac1 (DN-OsRac1). Proteins fractionated by gel filtration were probed for proteins involved in innate immunity by western blotting. The CA-OsRac1 complex was composed of a higher molecular weight complex than DN-OsRac1 complex. To investigate the response of OsRac1 complex to elicitors, elicitor-treated transgenic suspension cell cultures expressing wild type form of OsRac1 were analyzed. Results indicate that the OsRac1 complex was composed of a larger complex at 10 minutes after elicitor treatment, and the complex size was reduced 20 minutes after the treatment. Taken together, these results suggest that except for OsRac1 the proteins of the OsRac1 complex form a protein complex before the activation of OsRac1, and when defense signaling is triggered by elicitors, OsRac1 is rapidly activated and forms a larger complex from the preformed complex. After the elicitor-triggered signaling is terminated, OsRac1 is likely to be inactivated and dissociates from the preformed complex.

Functional evaluation of plant defence signalling against *Fusarium graminearum* and *F. culmorum* in Arabidopsis floral tissue

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Fusarium head blight (FHB) infections of cereal crops cause considerable losses to grain quality and contaminate grain with mycotoxins. The two main causative agents of this disease on UK wheat crops are the pathogens *F. culmorum* (*Fc*) and *F. graminearum* (*Fg*). The molecular basis of resistance to FHB in cereal species is poorly understood but it is QTL based and fusarium

species non-specific. We have previously demonstrated that *Fc* and *Fg* conidia can infect the floral tissues of Arabidopsis to cause disease symptoms on flowers, siliques and upper stem tissue (Urban et al., 2002). Deoxynivalenol (DON) mycotoxin production is detected in infected Arabidopsis flowers but unlike in wheat heads does not contribute to fusarium pathogenicity (Cuzick et al., 2008a). The Arabidopsis floral model provides a highly tractable experimental system. Through a reverse genetics approach we have discovered that both NPR1 and EDS11 are independently required for floral resistance against fusarium, whereas the salicylic and ethylene (ET) signalling pathways are either not required or have only a minimal effect on the interaction outcome (Cuzick et al., 2008b). Previously, EDS11 had only been reported to be required for basal defence against virulent bacteria (Volko et al., 1998). Intriguingly, the lack of SGT1b signaling protein leads to enhanced arabidopsis floral resistance to fusarium, whereas a lack of STG1a or RAR1 had no discernable effect (Cuzick et al., 2009). New data will be presented on, additional signalling mutants, interspecies differences, the identification of a novel induced phenolic compound associated with fusarium hyphal arrest as well as our current working hypothesis on the spatial and temporal signalling networks co-ordinating floral defence.

Growth in a changing environment: How microbe-associated molecular patterns (MAMPs) affect plant growth

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Plant growth is affected by many environmental cues. Besides obvious parameters such as light and temperature, plant growth is also affected by biotic stress. For example, highly conserved microbial molecules known as MAMPs (for Microbe-Associated Molecular Pattern) inhibit seedling growth in susceptible Arabidopsis plants. However, the molecular mechanisms underlying this effect are unknown. We are members of a Swiss consortium which wants to study plant growth in a changing environment using a systems biology approach. To obtain meaningful mathematical models of plant growth, large amounts of quantitative data are required. Therefore in situ techniques will be established to measure overall plant growth non-invasively, in realistic conditions and with high temporal resolution. Growth will be described on the basis of several morphological parameters concomitantly with the determination of global gene expression signatures. In our study, we focus on the effect of various MAMPs on the growth of Arabidopsis, and we present here our initial results. Common molecular patterns emerging from the studies of ourselves and our partners will enable us to identify the underlying gene regulatory network. This will allow us to construct quantitative models of growth control at the whole plant level. Funding by a grant of the Swiss Initiative in Systems Biology is gratefully acknowledged.

Elicitor-responsive 20-bp element of the *Tobacco mosaic virus* resistance gene *N*

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We cloned the upstream DNA fragments of the *Tobacco mosaic virus* resistance gene *N* from Nicotiana tabacum cv. Samsun NN and fused them to the GFP reporter gene for promoter assays. *Agrobacterium*-mediated transient gene expression in *N. tabacum* cv. Samsun nn lacking the *N* gene allowed us to evaluate promoter activity with or without the expression of the elicitor p50 (a helicase domain of the virus replicase) and/or N protein. Individual expression of p50 or N protein stimulated the *N* promoter, although the stimulation by N protein was less prominent than that of p50. The coexpression of p50 and N protein resulted in higher stimulation of the *N* promoter than the individual expression, which indicated that a cooperative mechanism may be involved in the promoter stimulation by the two effectors. Through a deletion analysis of the upstream sequence, we identified a minimum elicitor-responsive region that was critical for promoter stimulation by p50 and N protein. Furthermore, we found that a 20-bp sequence overlapping the minimum region conferred a similar responsiveness to a truncated *Cauliflower mosaic virus* 35S promoter. Thus, we conclude that the 20-bp sequence was essential and sufficient for the promoter stimulation by the elicitor p50 and N protein.

Beneficial microorganism *Trichoderma harzianum* induces tolerance to multiple environmental and physiological stresses during germination in seeds and seedlings

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Trichoderma spp. are fungi that are plant symbionts which induce changes in plant physiology. Some of these effects occur very early in the life of plants. A seed treatment with *T. harzianum* T22 overcomes physiological stresses in seeds induced by aging; these effects occur rapidly and are manifested both by increased germination and by more rapid emergence of radicles from aged, treated seeds versus untreated seeds. The same treatment also induces substantial tolerance to the effects of osmotic and salt stresses in germinating seeds, in either aged or fresh seeds. The effects on these physiological or abiotic stresses also are evident as larger seedlings when grown in the greenhouse. In addition, biotic stresses from the seed rotting pathogen Pythium ultimum are also controlled by the same treatment. The very early events, which are manifest as increases in radicle emergence rates from seeds, probably are initiated before T22 comes into contact with the living parts of the seeds. Seed treatments with T22 are with conidia which do not germinate until at least 16 hr after imbibition begins and then the seed coat must be traversed by the hyphae before contact with living seed parts. Thus, there appears to be a remote communication between T22 germinating on seeds and the seeds themselves that results in a reduction in physiological and biotic stresses. Certain volatile compounds produced by T22 may be involved. A common mechanism for overcoming either intrinsic or extrinsic physiological stresses may be via alleviation of free radical effects.

FaeD and FaeT, two feruloyl esterases of *Erwinia chrysanthemi*

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Ester cross-linkages involving phenolic acids have an important role in the structural integrity of the plant cell wall. Feruloyl esterases (4-hydroxy-3-methoxycinnamoyl-sugar hydrolase) responsible for cleaving the ester-link between ferulate and the xylan or pectin chains have been identified in plants and associated microorganisms. Feruloyl esterases act as accessory enzymes for the complete degradation of the plant cell wall. The importance of feruloyl esterases in the modification of the plant cell wall structure conducted us to search whether *Erwinia chrysanthemi* (syn. *Dickeya dadantii*) possesses such esterases. *E. chrysanthemi* strains are responsible for soft-rot diseases on many plant species. These phytopathogenic enterobacteria are able to secrete several enzymes which attack the constituents of the plant cell wall, including a large set of pectinases. By screening an *E. chrysanthemi* gene bank, we detected two genes encoding feruloyl esterases. These genes were characterized and their expression analyzed. While *faeT* is weakly transcribed in all the tested conditions, the *faeD* transcription is highly induced in the presence of phenolic acids and controlled by the product of the adjacent gene, *faeR*. While FaeT is an intracellular protein, FaeD is an extracellular enzyme secreted by the Out system which mediates the secretion of the extracellular pectinases. The enzymes FaeD and FaeT were overproduced in *E. coli* and their main biochemical properties were determined. Finally, the role of these enzymes in the *E. chrysanthemi* virulence was assessed by analysis of the *faeD* and *faeT* mutants.

Cloning of *Pb1*, the durable panicle blast resistance of rice

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Rice blast, caused by *Magnaporthe oryzae*, is one of the most wide spread and destructive diseases of rice. The panicle blast occurring after heading stage causes decreased yield and lowered quality of brown rice. *Pb1* is a panicle blast resistance gene, which was identified as a major quantitative gene in rice cultivars derived from an indica rice cultivar, Modan (Fujii et al., 2000). The blast resistance by *Pb1* is more effective during adult stages (adult resistance), in particular for panicle blast, than during young stages, and has not experienced breakdown for more than 20 years (durable resistance). We isolated *Pb1* by map-based cloning to investigate the mechanisms underlying its characteristic traits of resistance. High-resolution genetic mapping and sequencing of a BAC clone overlapping with neighboring markers narrowed down the *Pb1* region to 26kb genetic interval. In this *Pb1* region, five open reading frames were predicted, but of these gene expression was detected for only P15 and P18. Complementation test showed that P15, but not P18, conferred blast resistance to rice. Pb1 encoded a protein of CC–NBS–LRR

family of 1,296 amino acids. Expression analysis showed that the strength of blast resistance roughly paralleled with the expression levels of *Pb1* at different growth stages. *Pb1*', which is located about 60kb upstream of *Pb1*, is almost identical with *Pb1* with only one amino-acid difference, suggesting that these two genes have arisen by gene duplication. Expression levels of *Pb1* was about 150-300 times higher than those of *Pb1*' in recombinant lines having each of them. These results suggest that the acquisition of new expression pattern by the gene duplication event is the mechanism by which the blast resistance gene *Pb1* has generated.

Recognition and virulence function of the *Pseudomonas syringae* effector AvrRps4

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Understanding the molecular mechanisms by which bacterial effectors contribute to disease and are recognized by resistant host plants is crucial to gain insights into host-pathogen cross-talk. The *P. syringae* type III effector AvrRps4 is recognized by the *Arabidopsis* nucleo-cytoplasmic TIR-NB-LRR receptor RPS4, triggering localized programmed cell death and restriction of pathogen colonization. RPS4 activation is connected to downstream transcriptional reprogramming and resistance through the nucleo-cytoplasmic immune regulator, EDS1. We showed previously that nuclear localization of RPS4 is required for its function. It is still unclear how and where inside the cell AvrRps4 is perceived by its cognate R protein. Also, a virulence activity of AvrRps4 remains to be elucidated. To explore in which subcellular compartment AvrRps4 recognition takes place, several inducible and overexpression lines of AvrRps4 fused to a functional or mutated nuclear localization (NLS) or nuclear export signal (NES) were genarated in *Arabidopsis* and tested for AvrRps4 function. In a complementary approach, *P. syringae* strains expressing AvrRps4-NLS or -NES were tested for AvrRps4 recognition upon enforced localization. Co-Immunoprecipitation experiments suggest that AvrRps4 and RPS4 do not form a stable complex in resistant or susceptible plant cells. We hypothesize that AvrRps4 recognition is indirect and are screening for interaction partners using immunopurified AvrRps4 protein.

Exploiting the *Phytophthora infestans* genome to determine targets for sustainable potato protection

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A new strategy in the fight against potato late blight, caused by the Oomycete *Phytophthora infestans,* is to identify pathogen effector proteins likely to be secreted during infection and translocated into host cells to manipulate host metabolism and defence responses. Many of these effectors are targeted by host surveillance systems to trigger resistance that is effective and potentially durable. AVR3a*,* the first effector characterized from *P. infestans*, was found to contain N-terminal RxLR and dEER motifs required for transport across the host plasma membrane. Developing genomic resources have allowed large-scale computational screening for this conserved motif to reveal approximately 500 rapidly diverging *P. infestans* effectors. We are cloning candidate *P. infestans* RxLR-EER effectors that are induced during plant infection, some containing predicted PFAM domains. These effectors are being expressed transiently in collections of cultivated and wild *Solanum* species maintained within the Commonwealth Potato Collection (CPC) to identify sources of resistance (*R*) genes that recognise specific effectors. In this way, we have identified AVR2, recognised by the *R2* resistance gene which maps to the more durable resistance locus present in cv. Stirling and, furthermore, CPC accessions that recognise both the virulent and avirulent alleles of *Avr3a*. By silencing effectors in *P. infestans* we have identified >15 that are functionally essential and >10 that are redundant for virulence. In addition, studying allelic variation of avirulent effectors in isolates from around the world reveals the selection pressures imposed on these genes in pathogen populations.

Understanding the leucine-rich repeat containing proteins FLS2 and EFR through conservation mapping and mutagenesis

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Each plant genome codes for hundreds of proteins with leucine-rich repeats (LRRs), a domain responsible for protein-ligand interactions. Many of these proteins are involved in plant immunity, including Arabidopsis FLAGELLIN SENSING 2 (FLS2) and EF-TU RECEPTOR (EFR). Because of the ability of the LRR structure to bind to a diverse array of ligands, it is of interest to identify locations on the surface of the LRR that have either been conserved or diversified throughout evolutionary history. We have developed a method of conservation mapping that highlights spatial clusters of residues that are divergent or conserved, among a group of homologous proteins, on the surface of LRRs. Crystal structures of LRR domains with their ligand, as well as mutagenesis studies of LRR domains, suggest that our method correctly identifies functionally important regions of LRRs. A web-accessible implementation of the LRR conservation mapping tool is under development. To further apply this knowledge, we are undertaking site-directed randomized mutagenesis of AtFLS2 and AtEFR to further understand functionally significant regions within the LRRs of these proteins. Furthermore, selective amino acid swaps (along the beta-strand region of the LRR) between AtFLS2 and its tomato ortholog, LeFLS2, may generate receptors with hybrid specificity between the two receptors. Finally, randomizing mutagenesis is being pursued to evolve FLS2 receptors with novel specificity to previously unrecognized flagellin peptides. These studies further the understanding of LRR specificity, modification, and evolution.

Effects of *Phytophthora infestans* and glucan effectors on the accumulation of secondary metabolites and transcrips of defense related genes in potato

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Late blight, caused by *Phytophthora infestans* (Mont) de Bary is a major disease of potato. Potato susceptibility to *P. infestans* has been suggested as an outcome to potato defense suppression by *P. infestans* glucans released only by compatible races of the oomycete under in vitro conditions. Some glucans are able to suppress phytoalexin accumulation and induction of glucanases. The objective of this investigation was to analyze in a whole plant (in vivo), the effects of glucans from race C (mating type A2), the elicitor eicosapentanoic acid (EPA) and *P. infestans* isolates on the accumulation of secondary compounds and defense related transcripts in the susceptible and the partially resistant potato cultivars "Russet Burbank" and "Defender" respectively. For this purpose, we used HPLC and RT-PCR analysis. Potato cultivars inoculated with *P. infestans* accumulated different secondary metabolites. Glucans from race C, added after EPA dramatically suppressed the accumulation of secondary compounds in Russet Burbank and Defender, compared with the glucans alone. In addition, the glucans added after EPA enhanced the symptoms of late blight in Russet Burbank inoculated with US-8, with a dramatic reduction in the accumulation of secondary metabolites. However, in Defender inoculated with US-8 treated with the glucans added after EPA, the accumulation of secondary metabolites was also reduced, while late blight symptoms were not detected. The RT-PCR analysis showed the suppression of different genes in the phenylpropanoids pathway, the terpenoids pathway and signaling genes. Finally the analysis of some genes using RT-PCR showed high similarity with the HPLC analysis of putative final products and the Potato-*P. infestans* interaction.

***Bacillus subtilis* cyclic lipopeptides as elicitors: How are they perceived by plant cells?**

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The rhizobacterium *Bacillus subtilis* secretes a broad range of surface-active and antimicrobial cyclic lipopeptides from the surfactin, fengycin and iturin families. Surfactins and fengycins to a lower extend but not iturins, tightly interact with plant cells and stimulate the induced systemic resistance (ISR) at the micromolar level. They constitute a novel class of bacterial elicitors with a possibly specific mechanism of action that we wanted to further investigate using tobacco suspension cells. Based on the amplitude of the early defense responses induced by multiple structural variants and on their insertion rates in plasma membranes, our results highlight the importance of the length of the acyl chain and of the amphiphilic nature of surfactin (cyclized and charged peptide) for optimal interaction and defense-inducing activity. Surfactin still

readly interacts with plant cell membrane after heat or protease pre-treatments, suggesting that specific recognition by putative receptors is not obvious. Treatment with micromolar concentration of surfactin is not associated with any marked phytotoxicity or adverse effect on the integrity and growth potential of the treated tobacco cells. This suggests that these molecules could interact without irreversible pore formation but in a way sufficient to induce disturbance or transient channelling in the plasma membrane that can in turn activate a biochemical cascade of molecular events leading to defensive responses.

Plant-expressed RNAi constructs to induce knock-down of fungal genes

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Fungal diseases are a major cause of yield losses in crop species grown worldwide. We are pursuing a novel approach to fight fungal pathogens, which includes the generation of plants expressing RNAi-constructs that share sequence homology with fungus-specific genes. Our working hypothesis is that the expression of these constructs in infected plant cells may result in host-induced gene silencing (HIGS). To this end, house-keeping genes highly conserved amongst fungal pathogens are preferentially used. To confine transgene expression to host cell types that are potentially infected, the HIGS-constructs are driven by tissue-specific promoters such as the epidermis-specific GstA1 promoter of wheat. Besides constructs that contain a single inverted-repeat sequence, the study also includes the test of triple-sequence combinations targeting several fungal mRNAs at a time. Preliminary experiments on transient and stable expression of those constructs in barley showed that the intimate cellular interaction between host and pathogen provides an adequate situation allowing plant-produced, fungus-specific double-stranded RNA to impede fungal development. The main objective of this project is the generation of transgenic barley exhibiting high resistance against a broad range of fungal plant pathogens.

A cyst nematode secretory protein alters host polyamine signaling to mediate susceptibility

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Plant-parasitic nematodes secret proteinaceous effectors, which are critical for host invasion and initiation of feeding sites. The mechanisms by which these effectors mediate susceptibility remain elusive. One effector, which we term 10A6, was cloned from the sugar beet cyst nematode *Heterodera schachtii,* a compatible pathogen of Arabidopsis. 10A06 is an uncharacterized protein previously cloned from *H. glycines*. The *H. schachtii* 10A06 contained an open reading frame of 858 bp encoding a 285 amino acid protein with an N-terminal signal peptide for secretion. Sequence alignment of *H. glycines* and *H. schachtii* 10A06 proteins revealed a strong homology between both orthologues. Scanning the GenBank database, including the recently published root-knot nematode *Meloidogyne incognita* and *M. hapla* genomes, revealed the absence of homologous sequences, suggesting that 10A06 is a cyst nematode-specific gene. 10A06 is expressed exclusively in the dorsal esophageal glands during early parasitic stages. Transgenic Arabidopsis plants expressing 10A06 showed morphological irregularities and increased nematode susceptibility. Using a yeast two-hybrid screen we identified Arabidopsis spermidine synthase (SPDS) as a specific interactor. *SPDS* mRNA abundance was elevated in transgenic plants expressing 10A06. Furthermore, the expression profiles of SPDS and other polyamine biosynthetic genes were found to be differentially regulated in response to *H. schachtii* infection. Transgenic lines expressing a *SPDS* promoter::*GUS* construct showed an increase in GUS expression in the *H. schachtii*-induced feeding sites. Collectively, these data indicate that 10A06 acts as an effector modulating polyamine signaling to promote pathogenesis in Arabidopsis.

Involvement of the *hrpW* in virulence of *Pseudomonas cichorii* for virulence on eggplant but not on lettuce

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Pseudomonas cichorii causes necrotic lesions on eggplant and rot on lettuce. The nucleotide sequences of *P. cichorii*'s *hrp* genes and their genetic structure were homologous to those of *P. viridiflava* BS group strains, suggesting a common ancestor of *hrp* clusters between *P. viridiflava* BS group strains and *P. cichorii* strains (Araki et al. 2006; Hojo et al. 2008). Furthermore, Hojo et al. (2008) showed that pathogenicity of *P. cichorii* is *hrp*-dependent on eggplant but not on lettuce. An operon encoding a harpin, HrpW, and HrpW-specific chaperon is located in *hrp* genes. Both a *hrpW*-mutant (CWN1) and a HrpW-specific chaperon gene-deleted mutant (ΔWchap) of SPC9018 lost their virulence on eggplant but not on lettuce. Population of CWN1 drastically decreased in eggplant after inoculation. On the other hand, population of ΔWchap had retained equally after infiltration, similar as that of a *hrpL*-mutant. Virulence of those mutants and their populations on eggplant was restored by transformation with the *hrpW* operon, originating from SPC9018. Two hybrid analysis showed association of HrpW with HrpW-specific chaperon. These data suggest that HrpW may be essential for virulence of SPC9018 on eggplant but not on lettuce, assistant with HrpW-specific chaperon.

Transient suppression of *TERT* gene attenuates development of citrus canker caused by *Xanthomoans axonopodis* pv. *citri*

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TERT (telomerase reverse transcriptase) is one of the components of telomerase that mediates maintenance of telomere length and is closely associated with cellular proliferation. Previously we reported that telomerase activity increased in citrus leaves after infection with *Xanthomonas axonopodis* pv. *citri*, the causal agent of citrus canker. To investigate the role of telomerase in development of the symptom, we first defined a sequence of citrus *TERT* gene mRNA and generated a construct induces RNA interference which carries a transcriptional unit of expressing short-hairpin RNAs under the enhanced CaMV-35S promoter. The *Agrobacterium tumefaciens*-mediated expression of the construct in citrus plant brought the suppression of *TERT* gene expression. To observe the effect of *TERT* gene silencing on virulence, *X. axonopodis* pv. *citri* was challenge-inoculated into the pre-infiltrated area of the *A. tumefaciens* transformant. Interestingly, the canker formation was delayed and attenuated compared to that was observed in non-infiltrated area, while the severity of water-soak lesion that occurs before appearance of hyperplastic canker was not affected. These data suggest that telomerase activity may play an important role in eliciting canker symptom on citrus plants.

Development of a DNA macroarray assay for the detection of the post-harvest pome fruit pathogens *Penicillium*, *Botrytis* and *Mucor*

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The ability to detect and identify plant pathogens both rapidly and accurately is fundamental to disease diagnostics and thus disease management. The conventional approach to testing for fungal plant pathogens has been based primarily on microscopic and colony morphology. These procedures may take days or weeks to complete and are, in some cases, relatively insensitive and inaccurate. The purpose of this study was to develop and test a DNA-based assay for the rapid detection and identification of the post-harvest pome fruit pathogens *Penicillium*, *Botrytis* and *Mucor*. DNA macroarray technology is a reverse dot blot technique that uses labeled sample DNA as probes in an array with specific oligonucleotides anchored on a nylon solid support. This technology is advantageous in that it allows the simultaneous identification of numerous fungal pathogens in a single test. DNA array technology has been developed to identify pre-harvest fungal pathogens of apple. Our research expanded the DNA macrarray technology to focus on post-harvest pathogens of pome fruit, such as *P. expansum*, *B. cinerea* and *M. piriformis*. The specificity of the macroarray was confirmed by examining the hybridization patterns with a variety of post-harvest pathogens (no cross-reactivity) and the sensitivity of the probes allowed the detection of less than 1 ng of pathogen DNA per macroarray. Spore loads were monitored throughout the 2007 and 2008 growing seasons as a mechanism to predict post-harvest disease incidence for apples. DNA was extracted from aerial spore samples and plant tissues and hybridized against the macroarray. The spore loads determined are being compared with disease incidence observed on the fruit post-harvest to assess the ability of the DNA macroarray to aid in disease forecasting.

Exploiting transcript profiling data to explore compatible rice – *Magnaporthe oryzae* interactions

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Whereas the molecular events associated with disease resistance are largely studied, those occurring in susceptible plants have received less attention, especially in Monocots. We chose the rice/*Magnaporthe oryzae* system to explore the molecular modifications that are induced in susceptible plant tissues following pathogen attack. Among the genes that respond to colonization by *M. oryzae*, we aim to identify plant genes required for the infection process. To obtain a global picture of the host processes that are altered during pathogen attack, we performed Affymetrix microarray hybridization experiments using RNA from rice leaves infected with a virulent isolate of *M. oryzae*. Changes in rice transcript levels at 3 and 4 days post inoculation were measured. In addition to the up-regulation of a large number of defense-related genes, this analysis revealed new features indicating extensive reprogramming of host gene expression. Microarray expression data were confirmed using quantitative RT-PCR for over 95% of analyzed genes. Detailed expression patterns after infection with a virulent versus an avirulent isolate were obtained for a set of 50 genes. Candidate host compatibility genes specifically or preferentially modulated in response to the virulent strain were selected for functional analyses. Rice T-DNA and Tos17 insertion lines and overexpressor lines for these candidate genes are being characterized. The response to infection by different *M. oryzae* strains is being assessed for the insertion mutants. Through this reverse genetics approach, we can expect to pinpoint plant defences suppressed by the pathogen to promote disease as well as other host processes targeted by the fungus to facilitate pathogen multiplication and nutrition.

EDR1 positively regulates nonhost defense response independently of PEN2

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PEN2 is required for nonhost preinvasion resistance of *Arabidopsis thaliana* against powdery mildews, and it also functions in nonhost resistance against *Colletotrichum* species that are hemibiotrophic fungal pathogens. It has recently been shown that *PEN2* encodes a myrosinase critical for synthesis of antifungal metabolite. In this study, we report that EDR1 is involved in nonhost peinvasion resistance of *Arabidopsis* against *Colletotrichum*. It has been reported that the *edr1* mutant increased resistance against adapted powdery mildew via enhanced activation of defense responses mediated by salicylic acid (SA). We show that nonadapted *Colletotrichum* increases the efficiency of plant invasion and formed lesions in the *edr1* mutant. The *edr1 pad4* mutant is still defective in nonhost preinvasion resistance, suggesting that reduced resistance in the *edr1* mutant is not due to activation of SA signaling. The *edr1* mutant also exhibits enhanced susceptibility to a necrotrophic fungal pathogen *Alternaria brassicicola*. These data indicate the positive role of EDR1 in resistance against certain fungal pathogens unlike response to the powdery mildew. To assess the relation between PEN2 and EDR1, we generated the *edr1 pen2* mutant. Importantly, the double mutant exhibits more severe defects in resistance against nonadapted *Colletotrichum* in comparison with each single mutant, indicating that EDR1 regulates nonhost resistance independently of PEN2-related antifungal pathway. Together with further studies including the microarray analysis of the *edr1* mutant, we will discuss EDR1 function in nonhost preinvasion resistance.

An *Arabidopsis* signal transduction element that contributes to oomycete susceptibility

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Phytopathogenic oomycetes cause some of the most devastating diseases affecting agricultural crops and natural ecosystems. Fungicide treatments and resistance breeding approaches are basically inefficient against these microorganisms. Our aim is to identify plant functions that are manipulated by oomycetes during the infection process in order to promote the establishment of disease. An analysis of the *Arabidopsis thaliana* transcriptome and reverse genetics approaches revealed a subset of activated genes, which are involved in signal perception and transduction during the compatible interaction with the obligate biotrophic oomycete pathogen, *Hyaloperonospora arabidopsidis*. One of these genes codes for a putative leucine-rich repeat receptor-like kinase. Its transcription is locally activated in haustoria-harboring cells, and appears to positively affect oomycete development in plant tissues. The principal findings for the role of this receptor-like kinase, for normal plant development and for the compatible plant-oomycete interaction, will be presented.

Influence of two independents type II secretion systems in the pathogenic process of *Xanthomonas axonopodis* pv. *citri*

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Throughout the genome of the bacterium *Xanthomonas axonopodis* pv. *citri* (Xac), the etiological agent of citrus canker, were identified two operons (*xps* and *xcs*) that encompass proteins for the type II secretion system (TIISS). This system is characterized as essential for pathogenesis and virulence of some bacteria, however, in Xac its functionality as well as the implication with host interaction are still poorly understood. We have analyzed the function of the two operons that code for the TIISS in Xac and their activity during the interaction with sweet orange (*Citrus sinensis* L. Osb.). Mutations in different genes of the operons rendered phenotypic alterations in disease symptoms and affected the initial steps of biofilm formation. Quantitative gene expression analyses revealed that the bacterium use both TIISS, however in a different fashion during the contact with the leaf tissue, and that the *xps* operon is highly more active. Analysis of secreted enzymes showed that the *xps* operon is responsible for secretion of amylases, proteases and cellulases, while the *xcs* operon is not. Confocal microscopy investigations found that the bacterial biofilm organization is influenced by both TIISS. This is the first evidence of the independent influence of both TIISS in the pathogenic process of Xac.

Novel plant loci specific for *Rhizobium* accommodation and symbiosis

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Legume plants have the ability to form a specialized nitrogen-fixing organ, called the root nodule, when interacting with host-specific symbiotic bacteria, collectively called rhizobia. The colonization of roots by rhizobia requires host plant functions that mediate the initiation and progression of the infection thread into the root cortex, thus allowing for the subsequent formation of functional root nodule symbiosis. We characterized three *Lotus japonicus* mutants, LjS3-BA, LjB32-BB and LjS29-1, in which bacterial infection was severely inhibited. These mutants formed uncolonized white nodules and nodule primordia, on the surface of which rhizobia accumulated. No or very limited bacterial colonization of the host root was observed in lines LjS3-BA and LjB32-BB, while LjS29-1 mutant had large number of microcolonies but only 3 to 4 oversized pink nodules were formed. In addition to symbiotic phenotype, LjS3-BA and LjB32-BB showed distorted trichomes and collapsed seed pods, while these characteristics were wild-type in LjS29-1. Mycorrizal infection was normal in all three mutant lines when challenged with *Glomus intraradices*. By performing allelic complementation and genetic mapping, we defined these lines as carrying mutations in novel symbiotic loci in *Lotus japonicus*. Map based cloning of the underlying genes is in progress, which is expected to uncover new elements of the plant genetic mechanism for rhizobial accommodation.

Phenome analysis in barley for genes of PAMP-triggered immunity to powdery mildew

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Based on candidate gene approaches and transriptome analyses, genes have been selected for transient over- and under-expression in barley during

interaction with the barley powdery mildew fungus *Blumeria graminis* f. sp. *hordei*. This led to the identification of putative signal transduction components such as the small RAC/ROP GTPase RACB or the cell death regulator BAX INHIBITOR-1 as host factors involved in susceptibility to powdery mildew. Stable transgenic mis-expression of these genes revealed functions in interaction with *B. graminis* and other pathogenic fungi such as *Fusarium graminearum* or *Magnaporthe oryzae*. Transcriptome analysis of transgenic barley in interaction with *B. graminis* was carried out for identification of RACB or BAX INHIBITOR-1 mediated transcriptional reprogramming. This generated a second generation of genes for testing via transient induced gene silencing. In parallel a complementary approach has been started for high throughput phenomic analysis of entire candidate-gene families involved in perception of pathogen-associated molecular patterns, signal transduction and polarized defence responses.

The oomycete *Crinkler* (*Crn*) gene family encodes a novel class of host-translocated effectors that target the nucleus

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Oomycete-host associations feature dynamic interplay between host defence and specialized pathogen machineries that subvert immunity. The *crinkler* (*crn*) gene family encodes a large class of secreted proteins that share a conserved N-terminal LFLAK motif, which is followed by diverse C-terminal domains. Transient expression of various CRN C-termini in plants result in cell death in some but not all cases, reflective of diverse functional roles. We hypothesized that the crinkler genes encode a large family of host-translocated (cytoplasmic) effector proteins that require a conserved N-terminal (D1) domain for host-translocation. To substantiate this hypothesis, we conceived a translocation assay in the *Phytophthora capsici-Nicotiana benthamiana* interaction system. Expression of *Avr3a* in *P. capsici* conditions avirulence on transgenic *N. benthamiana* plants carrying R3a, a phenotypic outcome that requires a functional translocation motif. We developed and used the AVR3a translocation assay to test whether diverse CRN N-termini could functionally replace the AVR3a translocation domain. These experiments revealed that (1) CRN D1 domains mediate translocation and (2) that the conserved LFLAK domain is required for this function. Finally, localization studies of CRN effector domains in planta revealed accumulation of GFP-CRN fusion proteins in the nucleus, suggesting that this class of effectors target the host nucleus during infection. These results implicate the CRN protein family as a large, novel class of cytoplasmic effectors in oomycetes that collectively perturb host processes. These findings highlight the abundance of effector classes that oomycetes have evolved and raises new questions about the evolutionary forces that shape diverse effector repertoires.

Rice gene activation by transcription activator-like effectors of Xanthomonas oryzae pvs. oryzae and oryzicola

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Recent studies of the molecular mechanisms by which *Xanthomonas* species cause disease in plants have revealed transcriptional activation of host genes by bacterial Transcription Activator-Like Effectors (TALEs) to be an important factor in pathogen virulence and plant R gene mediated resistance. Several TALEs have been shown to upregulate the expression of one or more specific host genes, and one, AvrBs3, demonstrated to bind specifically to its target gene promoter to activate transcription. The basis for TALE target specificity and transcriptional activation, however, is unknown. Whole genome microarray analyses of rice (*Oryza sativa*) showed significant upregulation of remarkably different sets of genes after challenge with either *Xanthomonas oryzae* pv. oryzae (Xoo) or *Xanthomonas oryzae* pv. oryzicola (Xoc), the causal agents of bacterial blight and bacterial leaf streak in rice. Mutational analysis of Xoc has revealed a correlation between individual TALE activity, upregulation of specific rice genes, and virulence. To identify elements critical for specific transcriptional activation, we have used a transient transformation and expression system to compare the activation strength resulting from wild type TALE/rice promoter interactions with activation resulting from mutated TALE/rice promoter interactions. Results of this study are reported, focusing on a rice gene targeted by distinct TALEs from Xoo and Xoc. Knowledge of the basis for specificity in TALE/promoter interactions could enable significant advances in crop disease control and even gene therapy in humans and animals.

Evidence for a conserved regulon involved in development and pathogen defense in monocot and dicot plants

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At least two antagonistic components of plant resistance against the fungal powdery mildew disease are ancient and have been conserved since the time of the monocot-dicot split. These are the seven transmembrane domain containing MLO/MLO2 protein and the syntaxin ROR2/PEN1, identified in barley (*Hordeum vulgare*) and *Arabidopsis thaliana*, respectively. Additionally, syntaxin-interacting SNARE proteins (VAMP721/722 and SNAP34) as well as a myrosinase (PEN2) and an ABC transporter (PEN3) contribute to antifungal pre-invasion resistance in either the monocot barley or dicot Arabidopsis. Here we show that these genetically defined components of penetration defense share a similar set of co-expressed genes in the two plant species. Most of the co-expressed Arabidopsis genes possess a common cis-regulatory element that may dictate their co-ordinated expression. We exploited gene co-expression to uncover novel components in Arabidopsis involved in both antifungal defense and the timely onset of leaf senescence. Together, our data provide evidence for an evolutionarily conserved co-regulon that functions in both plant innate immunity and development.

Identification of the *Pm8* rye resistance gene and its suppressor gene in wheat to understand the mechanisms of resistance gene suppression

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The genetic improvement of tolerance to biotic and abiotic stresses is essential in wheat breeding. One successful strategy to improve yield and increase disease resistance has been based on the introgression of rye (*Secale cereale* L.) genes into wheat cultivars. The 1RS chromosome arm derived from the rye cultivar 'Petkus' carrying the race-specific resistance genes *Yr9*, *Lr26*, *Sr31* and *Pm8* is the most widely used rye-wheat translocation since it mediates resistance to yellow rust, leaf rust, stem rust and powdery mildew, respectively. So far more than thirty powdery mildew (*Pm*) resistance genes have been genetically characterized in wheat, but only an allelic series of the resistance gene *Pm3* has been cloned and molecularly analysed. We are aiming at the cloning and molecular characterisation of the rye powdery mildew resistance gene *Pm8* and its suppressor gene present in a subset of wheat lines. Identification of a resistance gene and its suppressor is of great interest considering the frequent occurrence of genetic suppression, especially in lines with introgressed alien chromatin. We have identified candidate genes for *Pm8* and its suppressor which are currently tested using a transient transformation assay. First results will be presented.

Phosphate transporter ChPho1 is required for pathogencitiy of *Colletotrichum higginsianum* on *Arabidopsis*

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In a forward genetic screen for pathogenicity genes in the crucifer anthracnose fungus, *Colletotrichum higginsianum*, the insertional mutant *path*-12 failed to produce necrotic lesions on *Arabidopsis thaliana* plants. The mutant formed normal melanised appressoria but these rarely penetrated host epidermal cells. The mutant carried a tandem T-DNA insertion in a gene homologous to fungal Major Facilitator Superfamily transporters, in particular plasma membrane-localised, high-affinity proton symporters involved in the uptake of inorganic phosphate. Complementation with a wild-type copy of this gene, designated *ChPho1*, fully restored pathogenicity and penetration ability to mutant *path*-12. Functional complementation of a *Saccharomyces cerevisiae* quintuple phosphate transporter mutant (lacking *Pho84*, *Pho87*, *Pho89*, *Pho90* and *Pho91*) confirmed that *ChPho1* mediates phosphate uptake. Expression analysis showed that *ChPho1* is phosphate repressible. *ChPho1* was expressed during pre-penetration development on the plant surface and biotrophic growth in the plant apoplast, but not during necrotrophic growth in dead tissues. These findings suggest that phosphate availability could be a limiting factor for *C. higginsianum* during penetration and early growth *in planta*.

Characterisation of the uptake kinetics of *ChPho1* expressed in a yeast phosphate transporter mutant will be presented.

Identification and characterization of host factors involved virus infection in pepper

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Viruses are completely dependent on the host factors to complete their life cycle. Although the roles of host genes in virus infection are easily postulated, identification and functional characterization of host genes in virus infection are still in the beginning stage. To find essential host factors for virus life cycle, we enlisted candidate host genes using pepper and tomato expressed sequence tags (EST) information. Seventeen candidate genes including the eukaryotic translation initiation factors, translation elongation factors, and release factors were selected. Virus-induced gene silencing (VIGS) approach offers a rapid and efficient mean to knock down expression of given genes in plants. Here, we applied *Tobacco rattle virus* (TRV)-based VIGS screening methods to identify new host factors involved in virus infection. To investigate roles of candidate genes in virus infection, we used a GFP tagged *Cucumber mosaic virus* (CMV-GFP), *Tobacco mosaic virus* (TMV-GFP), and *Potato virus X* (PVX-GFP), which allow to track virus replication, cell-to-cell movement, and systemic movement. Identified host genes for virus infection will be presented and their possible roles will be discussed.

***Os*WRKY mediates the defense signaling via transcriptional activation of *OsNPR1* and *OsICS1* in rice**

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WRKY proteins are key regulators of SA- and pathogen-mediated defense-signaling pathway. We identified a pathogen- and SA-inducible *WRKY* that was early induced and reached a maximum at 6hr after SA treatments. By transgenic approach, over-expression of the *OsWRKY* resulted in strong induction of pathogenesis-related (*PR*) genes and enhanced disease resistance to *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). RNA interference-mediated knock-down of the *OsWRKY* (*OsWRKY*-RI) caused to abolish induction of *PR* genes in response to SA and enhance the susceptibility to pathogens. *OsNPR1* was also constitutively expressed in *OsWRKY*-OX lines. Therefore we suggest this *Os*WRKY plays as a positive regulator in defense-signaling pathway and its downstream genes are NPR1 and PR genes. We further confirmed that NPR1 and PR genes are direct target genes of this *OsWRKY* by transient assays with their promoters. *OsICS1* was also constitutively expressed in *OsWRKY*-OX lines. In addition to that the level of SA in *OsWRKY*-OX lines was increased compared to non-transgenic lines. All together, this *OsWRKY* plays as a positive regulator a role in defense signaling pathway and transcriptional activation of *OsNPR1* and *OsICS1*.

Biological control of common bacterial blight of bean with rhizosphere antagonists

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Common bacterial blight caused by the varieties *fuscans* and no *fuscans* of *Xanthomonas campestris* pv. *phaseoli*, though recent AFLP characterization of a collection of the two varieties confirmed the recent proposal to reclassify the varieties as distinct species, is an important bean disease worldwide. The disease, though resistant/tolerant cultivars are available, may cause important crop loss and, due to the limited availability and efficacy of chemical measures, new and alternative control measures appear necessary to develop. The aim of this study was to assess the potential of bacteria isolated from bean rhizosphere to control the above disease. Sixty and 57 out 162 bacterial isolates obtained from bean rhizosphere inhibited the growth of the varieties of *X. c.* pv. *phaseoli* as well as strains of common bean bacterial and fungal pathogens such as *Pseudomonas syringae* pv. *phaseolicola*, *P. s.* pv. *syringae*, *Curtobacterium flacumfaciens* pv. *flacumfaciens*, *Rhizoctonia solani, Macrophomina phaseolina* and *Sclerotinia sclerotiorum,* respectively. Furthermore, the majority of the antagonist isolates were shown to produce lytic enzymes. The above 60 antagonistic bacteria were evaluated for their possible interference on the pathogenicity and/or virulence of a strain of *X. c.* pv. *phaseoli* var. *fuscans* in *in vitro* and in greenhouse cotyledon and trifoliate pathogenicity leaf assays, respectively. Seven out 60 isolates were demonstrated to protect plant tissues in either *in vitro* or in greenhouse pathogenicity assays with a positive correlation between the assays. In particular, in *in vitro* and in greenhouse assays lesion reduction ranging from 30 to 66% were observed, respectively. Some of the factors involved in the protection mechanism will be presented.

Functional and structural characterization of flagellin glycan of *Pseudomonas syringae*

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Flagellins, components of flagellar filament, are known to be potent elicitors in *Pseudomonas syringae*. Previously we found that flagellin from *P. syringae* is a glycoprotein. Furthermore flagellin glycosylation affects HR-inducing ability of flagellin and is required for virulence against the host plant. DNA sequence analysis of flagellum gene cluster revealed that two genes (*fgt1* and *fgt2*, previously called as *orf1* and *orf2*) located upstream of flagellin gene, *fliC*, encode putative glycosyltransferases for flagellin glycosylation. Deletion mutants for *fgt1* (Δ*fgt1*) and *fgt2* (Δ*fgt2*) in *P. syringae* pv. *tabaci* produced nonglycosylated flagellin and partially glycosylated flagellin, respectively. Both mutants reduced the abilities for swarming motility and adhesion, and virulence to cause disease symptoms. Glycosylated residues in flagellin protein are identified on six serines. One of the glycan structure attached to S201 was identified. Ser 201 glycan is composed of two rhamnoses and one modified 4-amino-4,6-dideoxyglucose (viosamine). In this study we generatedthree mutants strains in *P. syringae* pv. *tabaci* which are defective in the genes encoding dTDP-viosamineaminotransferase (VioA) and dTDP-viosamine acetyltransferase (VioB) for dTDP-viosaminebiosynthesis and a gene encoding putative viosamine transferase (vioT). Flagellin molecular masses of the resultant mutants were observed to be smaller than that of wild-type strain in MALDI-TOF MS analysis. Both swarming motility and virulence on host tobacco leaves were also reduced in all three mutants. These results suggest that three genes are essential for transferring of modified viosamine to the rhamnose in flagellin and for virulence.

Enhanced pathogenicity of viable reassortant between infectious clone of *Tomato yellow leaf curl virus* and satellite DNAβ molecules associated with Far East Asian begomoviruses

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Yellow leaf curl disease of tomato plants is a very acute problem for tomato cultivation in Japan. Its causative agent, *Tomato yellow leaf curl virus* (TYLCV), has been identified. DNAβ is not associated with TYLCV. Yellow dwarf disease has also been observed in tomato cultivation in Japan. *Tobacco leaf curl Japan virus* (TbLCJV) and *Honeysuckle yellow vein mosaic virus* (HYVMV) as its causative agent have been identified. Two DNAβs are associated with TbLCJV and HYVMV and depend on TbLCJV and HYVMV for replication. DNAβs with TbLCV and HYVMV induce typical leaf curling, yellowing and sever stunting symptoms in tomato. We examined whether TbLCJV DNAβ-Ibaraki, HYVMV DNAβ-Nara and *Tomato leaf curl Philippines virus* (ToLCPV) DNAβ-Laguna1 can replicate by TYLCV-[Tochigi] and induce more severe symptoms than those induced by TYLCV-[Tochigi]. These DNAβs were supported for replication by TYLCV-[Tochigi] and induced more severe symptoms than those induced by TYLCV-[Tochigi] alone in tomato and *Nicotiana benthamiana* plants. The iteron present in TYLCV-[Tochigi] was not found in DNAβ-Ibaraki, DNAβ-Nara and DNAβ-Laguna1, suggesting a more relaxed specificity for begomovirus Rep binding during their replication. The DNAβs appears to be capable of using Rep from a diverse range of begomoviruses.

AtMIN7 is required for salicylic acid-dependent basal defense in Arabidopsis

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The plant pathogenic bacterium *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000) uses a type three secretion system to translocate effector proteins into the plant cell to promote pathogenesis. The *Pst* DC3000 conserved effector locus (CEL) carries the functionally redundant effectors HopM1 and AvrE, and the ΔCEL mutant of *Pst* DC3000 displays substantially reduced growth on wild type Col-0 plants. HopM1 interacts with and promotes destabilization of the Arabidopsis ADP-ribosylation factor guanine nucleotide exchange factor (ARF GEF) MIN7 via the 26S

proteasome. Likewise, in *min7* mutant plants, HopM1 and AvrE are not completely necessary for bacterial multiplication. HopM1 has been shown to inhibit salicylic acid (SA)-dependent plant basal defense, and our ongoing experiments show an altered SA response in *min7* plants. Benzothiadiazole (BTH) is an SA analog that elicits SA-dependent defense. When plants are pre-treated with BTH, *Pst* DC3000 multiplication is reduced in Col-0, but *min7* plants are unable to fully restrict the growth of *Pst* DC3000 following BTH treatment. This indicates a deficiency in SA-dependent defense in *min7* plants. There are also changes in extracellular protein secretion in *min7* plants in response to BTH. *min7* plants lack a group of proteins found in the intercellular wash fluid of Col-0 following treatment with BTH. Additionally, to better understand the role MIN7 in pathogenesis we are investigating its cellular function. ARF GEFs promote the exchange of GTP for GDP on ARF GTPases, small GTPases that are critical regulators of vesicle trafficking. We are searching for potential MIN7 substrates that may function in host defense.

The critical role of epicuticular wax of *Arabidopsis* tissues for powdery mildew *Golovinomyces orontii* to recognize host surface and to establish the infection

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The powdery mildew Golovinomyces orontii is an obligate biotrophic fungus that infects *Arabidopsis thaliana*. For the successful infection, powdery mildew has to recognize the plant surface, invade into the host epidermal cells and establish the infection. Within one day, powdery mildew differentiates the haustorium in the host cell to absorb the host nutrition, and further grows mycelium and produces more spores. Increase in the plant hormone salicylic acid is characteristic plant response at the later stage of infection, when the secondary and tertiary infections occur. Molecular genetic studies have revealed a number of factors involved in the salicylic acid signaling pathway, and more recently, factors involved in the plant response at the fungal invasion stage. To further elucidate the mechanism underlying the establishment of powdery mildew infection on *Arabidopsis*, we have taken cell biological approach. Powdery mildew pathosystem is ideal for microscope observation for following reasons; (1) The fungal life cycle completes on the plant tissue surface; (2) Fugal spores are large enough to be detected under light microscopes, and infected and uninfected cells are clearly differentiated; (3)The early infection stages are well defined along the time course; (4) The early infection process is highly synchronized among spores. We have investigated *G. orontii* infection on various *Arabidopsis* tissues and found that fungi could hardly establish infection on stems and siliques, tissues that have heavy epicuticular wax deposition. Comprehensive studies using *Arabidopsis* waxless mutants and on environmental effects on wax deposition and powdery mildew infection are underway, and the role of epicuticular wax on powdery mildew infection will be discussed.

Avenacin saponin has anti-microbal activity to fungal pathogens for cereals

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Triterpenes are natural products that, like sterols, are synthesized from 2,3-oxidosqualene. Simple triterpenes have been identified in the leaf waxes ofvarious plant species. Some plants also produce elaborate triterpene glycosides (known as saponins). Triterpene glycosides are commonly anti-fungal (unlike simple triterpenes) and have been implicated in plant defense. Oat (*Avena*) is unusual amongst the cereals in that it produces anti-microbial triterpene glycosides. These root-derived compounds (avenacins) confer broad spectrum resistance to soil-borne diseases; *Gaeunmannomyces graminis* var. *avenae*, *Fusarium anenaceum* and so on (Mery et al, Phytochem. 25(9) 2069-2073, 1986). On the other hand, the importance of saponin detoxification in fungal pathogenesis has recently been demonstrated for some isolates of the fungus *G. graminis* var. *avenae*, which produces the enzyme avenacinase. Avenacinase detoxifies the triterpenoid oat root saponin avenacins, and is essential for pathogenicity of *G. graminis* var. *avenae* to oats. Here, to investigate the potentiality of avenacin for plant defense in other valuable cereals, we report fungicidal activity of avenacin to fungal pathogens; *Magnaporthe grisea*, *Bipolaris oryzae*, and *Blumeria graminis* f. sp. *Hordei*, for other cereals (rice and barley).

***SIX4*, an avirulence gene carried by race 1 of tomato wilt fungus, is truncated by putative transposable element-insertion in a newly emerged strain**

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Fusarium oxysporum f. sp. *lycopersici* (*FOL*) is the pathogen of soilborne wilt of tomato. In the pathogen, three races 1, 2, and 3 have been determined based on the specific pathogenicity to tomato cultivars. The compatible or incompatible relationships between races and cultivars can be explained by the interactions between avirulence gene(s) carried by *FOL* and resistance genes carried by tomato according to gene-for-gene theory (Flor 1956); for example, race 1 carrying *AVR1* is avirulent to tomato cultivars having a resistance gene *I*, and race 2 and 3 having no *AVR1* are virulent to the tomato cultivars having *I*. Houterman (2008) found the race 1-specific genome locus *SIX4*, which determines the avirulence to *I*; in other words, *SIX4* is the *AVR1* in *FOL*. In 2008, a strain of *FOL* which overcomes the races 1-resistance due to *I* emerged in Kochi, Japan. The strain was phylogenetically classified in to the Japanese race 1-clade (Kawabe 2005; Hirano 2006) based on rDNA-IGS region sequence. Real time-PCR using the specific primer-probe set (Yoshioka 2009) revealed that the strain carried *SIX4* genomic locus, indicating that the strain was race 1. However, we found that the amplicon from the strain was about 770 bp longer than that from race 1 using a *SIX4*-specific primer set. Sequencing of the amplicon revealed that in the strain *SIX4* was truncated by a putative transposable element belonging to the *hAT* family (Kempken 2001). All together, we assume that the new strain of *FOL* found in this study had overcome *I* by transposon insertion in the corresponding avirulence gene. As far as we know, this is the first report of an avirulence gene disrupted by a transposable element in nature which may give new insight in studying evolution of pathogenicity in plant pathogenic fungi.

Community proteogenomics of phyllosphere bacteria

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The plant phyllosphere, an ecologically and economically important environment, is colonized by numerous microorganisms, which affect plant health and growth. We applied a novel approach combining large-scale metaproteomics with metagenomics to get insights into the community composition and physiology of the bacterial inhabitants of the phyllosphere *in situ*. Additionally, our samples originating from agriculturally grown soybean and clover and from naturally grown *Arabidopsis thaliana* plants were characterized by 16S rRNA gene based analyses. Our data showed that all plants were predominantly colonized by *Alphaproteobacteria*. 260 Mbp of metagenome data were generated for one of the samples using 454 pyrosequencing technology. These data and all entries of the public Refseq database were used for the identification of proteins based on peptide analysis via RP-HPLC-ESI-MS/MS. Identification of more than 2,200 bacterial proteins in the different samples enabled a comparative analysis of the metaproteome of these samples, which revealed a surprisingly high consistency in the bacterial proteomes. We anticipate that the identity of abundant and ubiquitous commensal phyllosphere bacteria in combination with a better understanding of their physiology in this habitat is helping to exploit these bacteria with respect to potential plant beneficial effects in the future.

Characterization of defense-signaling mechanisms downstream of the rice panicle blast resistance gene *Pb1*

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Pb1 (*Panicle Blast 1*) confers the resistance to blast disease caused by a fungal pathogen *Magnaporthe grisea*. The blast resistance by *Pb1* is durable, of broad spectrum for fungal races, and is more effective during reproductive stages than vegetative stages. Recently, *Pb1* gene was isolated by map-based cloning and encoded a coiled-coil-nucleotide-binding-site-leucine-rich repeat (CC-NB-LRR) protein. The barley R protein *MLA,* the CC-NB-LRR-type R protein for *Bluminia graminis*, has been reported to translocate from cytosole to the nucleus and interacts with WRKY transcription factors (TF) after

pathogen recognition. By analogy, we tested if Pb1 interacts with WRKY TFs of rice by using yeast two-hybrid and GST pull-down procedures, revealing that Pb1 specifically interacts with WRKY45, which is a key TF of salicylic-acid signaling pathway and induced resistance by plant activators. RNAi-mediated downregulation of *WRKY45* expression in *Pb1*-containing rice line partially compromised the blast resistance. These results indicate that the Pb1 defense signaling is at least partially mediated by WRKY45. Of note, MLA-interacting WRKY TF is a transcriptional repressor whereas WRKY45 is a transcriptional activator, suggesting different molecular mechanisms involved in the two signaling pathways. Maize *ubi1*-promoter-driven overexpression of Pb1 enhanced blast resistance during vegetative growth stage. We are characterizing the transformants to further validate the Pb1-WRKY45 defense signaling.

MAPKs phosphorylate WRKY8 and induce defense-related genes in *Nicotiana benthamiana*

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Mitogen-activated protein kinase (MAPK) cascades play pivotal roles in plant innate immunity. We have purified StMPK1, which is activated in potatoes treated with hyphal wall elicitor of a potato pathogen *Phytophthora infestans*. The predicted amino acid sequence of StMPK1 showed strong similarity to stress-responsive MAPKs, such as tobacco SIPK and Arabidopsis AtMPK6. To investigate downstream signaling of StMPK1, we identified several PPSs (protein phosphorylated by StMPK1) using *in vitro* expression cloning method. To dissect the biological function of PPSs in plant defense, we employed virus-induced gene silencing (VIGS) in *Nicotiana benthamiana*. VIGS of *NbWRKY8* (*NbPPS8)* enhanced disease susceptibility to a virulent strain of *P. infestans* and *Colletotrichum orbiculare*. NbWRKY8 was phosphorylated by SIPK, Ntf4 and WIPK *in vitro*. MAPK-mediated phosphorylation of NbWRKY8 enhanced the binding activity to W-box sequence in vitro and the transcriptional activity *in vivo*. By using alanine scanning mutagenesis, we identified 5 serine residues in N-terminal region of NbWRKY8 as potential phosphorylation sites. To find candidate genes regulated by NbWRKY8, we isolated cDNAs corresponding to transcripts that were down-regulated in *NbWRKY8*-silenced plants by cDNA subtraction method. We identified five unique cDNA clones including two *NADP-MEs*. Expression of NbWRKY8^{DDDDD}, a phosphorylation-mimicking mutant of NbWRKY8, highly induced the expression of *NADP-MEs*, compared with wild-type NbWRKY8. These results suggest NbWRKY8 is phosphorylated and activated by MAPKs and induces expression of downstream genes involved in the defense responses.

Visualization ofternary complexes involved in innate immunity in living plant cells by using aBiFC-based FRET-FLIM

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A small GTPase, OsRac1, plays a key role in rice (*Oryza sativa*) innate immunity as part of a complex of regulatory proteins. Previously, using affinity column chromatography and yeast two-hybrid assays, we showed that OsRac1 forms a complex that includes RACK1A (Receptorfor Activated C-Kinase 1), RbohB (respiratory burst oxidase homolog), RAR1, SGT1and HSP90. Among these proteins, OsRac1 directly interacts with RACK1A and the N-terminal region of OsRbohB. However, the spatio-temporal dynamics of the components of this protein forming a complex remains unknown. We found that interaction between Rac GTPases and Rboh is ubiquitous and that a substantial part of the N-terminal region of Rboh, including the two EF-hand motifs, is required for the interaction. Furthermore, the direct Rac–Rboh interaction was supported by further studies using *in vitro* pulldown assay, a nuclear magnetic resonance titration experiment, and *in vivo* fluorescence resonance energy transfer (FRET) microscopy (Wong et al., 2007). RACK1A protein interacts with the GTP form of Rac1 that positively regulates RACK1A at both the transcriptional and posttranscriptional levels. RACK1A transcription was also induced by a fungal elicitor and by abscisic acid, jasmonate, and auxin. Analysis of transgenic rice plants and cell cultures indicates that RACK1A plays a role in the production of reactive oxygen species (ROS) and in resistance against rice blast infection. In this study, we developed new methods which can be applied to the study of protein-protein interactions and formation of ternary complex in plant living cell. Here we combined FRET (fluorescence resonance energy transfer)-FLIM (fluorescence lifetime imaging) with BiFC (bimolecular fluorescence complementation) analysis to investigate the dynamics of OsRbohB and RACK1A, forming a complex with OsRac1 *in vivo*.

Does *Glomus intraradices* affect gene expression of mycotoxic *Fusarium* strains in infected potato plants?

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Fusarium trichothecenes are a group of fungal toxins that cause problems to humans and animals that have consumed infected fruits and vegetables. In addition, these mycotoxins cause disease on plants infected with *Fusarium* trichothecene-producing strains. Here, we address the question of whether the production of trichothecenes by pathogens is affected on potato plants inoculated with *Glomus intraradices*. We have isolated 29 fungal isolates from infected potato plants. PCR and DNA sequencing of ITS1 regions were used for rapid identification. All isolates belong to *Rhizoctonia solani* and *Fusarium* spp. Three strains identified as *F. sambucinum*, *F. culmorum* and *F. oxysporum* f. sp*., lycopersici*, showed an aggressive infection of potato plantlets. We therefore hypothesized that these strains could produce thrichothecene. To test this hypothesis, we first used PCR primers which specifically amplify *tri1*, *tri4* and *tri5* genes involved in thrichothecene synthesis in *Fusarium* species. The three virulent strains showed PCR bands for these genes with the expected fragment size. Chemical analysis assays will be conducted to identify trichothecene chemtypes production in *in vitro* cultures. Currently, we have set up an experiment where plants are inoculated with *Glomus intraradices* and then infected with the thrichothecene-producing strains. As a control, non-mycorrhizal infected and non-infected plants are used. cDNA libraries will be constructed using RNA extracted from potato plants from different treatments. qPCR will be used to quantify gene expression of *tri1*, *tri4* and *tri5* genes. b-tubulin and EF 1a will be used as house-keeping genes.

A VAMP72 controlled secretion pathway has been recruited for symbiosome formation in *Medicago truncatula*

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Rhizobium bacteria are hosted inside root nodule cells as transitory organelles, symbiosomes (SB), where each bacterium is surrounded by a plant-derived membrane, the symbiosome membrabe (SM). We hypothesized that SB formation evolved from non-symbiotic endomembrane processes. We studied the role of a broad set of membrane identity markers in symbiosome formation in the model legume *Medicago truncatula* (Medicago). Here we will discuss the role of SNAREs (*N*-ethylmalemide sensitive factor attachment protein receptors) controlling vesicle fusion belonging to the VAMP7 family which contains two subfamilies: VAMP72 (controlling fusion to the plasma membrane) and VAMP71 (specific for vesicular transport towards vacuole). We cloned four VAMP72 genes from Medicago. Two of these are present in a "legume-specific" branch of the phylogenetic tree due to a legume specific duplication event. These genes are also expressed in the infection zone of the nodule and this is the site where rhizobia are released from infection threads and SBs are formed. Using GFP fusions as well as antibodies against these VAMPs we showed that they are located at SMs as soon as the rhizobia are released from the infection threads. Functional analysis of the VAMP72 genes using an RNAi approach showed that the two genes that are expressed in the infection zone have an essential role in establishing symbiosis. In the nodule they are essential for release of the rhizobia from the infection threads as well as for SB formation and maturation. Related Arabidopsis VAMP72s are required for focal secretion in a defense response upon attack by a fungal pathogen (Kwon et al., 2008). Therefore we currently study the role of the Medicago VAMP72s in the interactions with arbuscular mycorrhiza and biotrophic pathogenic fungi.

Molecular factors regulating Scots pine Antimicrobial peptides (*SP-AMP*) gene expression

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A novel family of antimicrobial proteins has been discovered, so-called Sp-AMP in *Pinus sylvestris* (Scots pine). Using a combination of Northern analysis and Real-time quantitative RT-PCR, we studied the expression of Scots pine *AMP* during challenge with either a pathogenic (*Heterobasidion annosum*), mutualistic (*Lactarius rufus)* or saprotrophic (*Stereum sanguinolentum)* fungi. The result showed increased Sp-*AMP* expression at a very early stage when challenged with the pathogenic, mutualistic or saprotrophic fungi. However, *Sp-AMP* expression is eventually attenuated at

prolonged incubation with mutualistic or saprotrophic fungi but remained high with the pathogenic fungi. In a separate study, we investigated if fungal cell wall chitin, chitosan or glucans has effect on the *AMP* expression. All three compounds provoked strong necrotic reaction on the roots. Interestingly, only chitosan (deacetylated chitin) induced the *Sp-AMP* expression. The study was repeated by inoculating Scots pine roots with fungal material devoid of cell wall (protoplast) or with yeast mutants devoid of chitin or glucan. Additionally, using Arabidopsis model with loss-of- function mutants in chitin responsive genes and in plant chitinase, we further investigated the regulation of defensin (the closest homologue to *Sp-AMP* in *Arabidopsis*). This was followed by a complimentary study on the possible mediatory role of salicylic acid and jasmonic acid as signaling pathways in *Sp-AMP* regulation. The results will be presented and discussed with reference to possible role of AMP in defence against phytopathogenic fungi of conifer trees.

Characterisation of the Horse Chestnut bleeding canker pathogen, *Pseudomonas syringae* pathovar *aesculi*

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In the United Kingdom there are approximately 470,000 Horse Chestnut (*Aesculus hippocastanum*) amenity trees. The last 6 years has seen the spread in the UK of bleeding canker disease of Horse Chestnut, which can lead to loss of limbs and tree death. The disease has now been attributed to the bacterium, *Pseudomonas syringae* pv. *aesculi (Pae)*. Very little is known about this pathogen, and most of the work done so far is limited to a macroscopic description of the symptoms. We are interested in characterising the origin of this pathogen, it's evolution and the factors that allow it to cause disease. Furthermore, we are interested to know how this pathogen is able to spread throughout the *A. hippocastanum* population in the UK so rapidly. We obtained a representative selection of *P. syringae* pv. *aesculi* isolates: 6617 (Glasgow), 6619 (Winchester), 6620 (Ewelme, Oxfordshire), 6623 (Alice Holt) and 6631 (a Belgian isolate). A number of experimental tests were carried out to identify the best strain to use for experimental investigations and as candidates for genome sequencing. These include virulence in *A. hippocastanum* and *Nicotiana benthamiana*; ability to exchange genetic material; phylogenetic analysis by MLST (Multilocus Sequence Tagging) analysis. We found that the strains all caused disease in A. *hippocastanum* and *N. benthamiana*. We could also detect molecular diversity in the MLST analysis. On the basis of the preliminary results that we have obtained, we chose the more aggressive strains 6617 and 6623 for further study of this dramatic emerging pathogen to understand exactly how it causes disease and is spreading throughout the tree population.

Characterization of ecologically distinct *Ralstonia solanacearum* strains through comparative *in planta* transcriptome analysis

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The widely distributed plant pathogen *Ralstonia solanacearum* causes bacterial wilt, one of the world's most destructive plant diseases. This soilborne pathogen attacks a wide range of crops in warm and tropical climates worldwide, infecting host plants through roots and then colonizing the xylem vessels. Genome sequences are available for a phylotype II Race 3 biovar 2 strain (R3bv2), UW551, and a phylotype I strain, GMI1000. R3bv2 is a major problem in the highland tropics and temperate zones, while strains like GMI1000 cause large losses at tropical temperatures. Although these strains maintain distinct ecological niches, at tropical temperatures both grow equally well in culture and cause comparable wilting on tomato. The limited comparative data about *R. solanacearum* strains at a global level indicates that about 10% of UW551 genes have no counterpart in the GMI1000 genome. Either the functions of these R3bv2-unique genes or differential expression of common genes could explain the significant epidemiological differences between strains. To test the latter hypothesis, we used custom-designed DNA microarrays for each strain to measure global transcriptional differences between UW551 and GMI1000 during early pathogenesis of susceptible tomato plants and mid-log phase growth in rich medium at the tropical temperature of 28°C. This comparative gene expression study will identify distinct and shared mechanisms that these strains use during wilt disease development and will strengthen our understanding of the intimate dynamics between *R. solanacearum* and its tomato host.

Colonization of barley roots by *Pseudomonas putida* is influenced by Fis

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Fis (factor for inversion stimulation) is a small homodimeric nucleoid-associated bacterial protein that is well known from studies of *Escherichia coli* as a trigger of fast growth of bacteria under conditions of abundance of nutrients. Fis regulates transciption of large numbers of genes and thereby can serve as a very important link between signals from the environment and bacterial physiological state. Very little is known about the functions of Fis in *Pseudomonas putida* a soil bacterium able to colonize plant roots. The aim of our studies was to investigate whether Fis could influence colonization of barley roots (*Hordeum vulgaris*) by *Pseudomonas putida* strain PaW85. We constructed *P. putida* strains either over-expressing Fis (strain F15) or reducing Fis expression by anti-sense RNA (strain FB) and studied ability of these strains to colonize barley roots in comparison with wild-type strain. 4 days pre-germinated barley seedlings were inoculated either with pure culture of cells of F15 or FB or in mixture with cells of wild type strain. We estimated persistence of each strain on roots alone or in competition experiments during 21 days. Cells of F15 and FB were tagged by gentamycin resistance gene and cells of wild-type strain PaW85 by streptomycin resistance gene. We found that cells of *P. putida* wild-type strain effectively out-competed F15 cells over-expressing Fis but not those of FB cells where the expression of Fis was artificially reduced. Taking together, the results obtained in this study indicate that Fis participates in regulatory network influencing ability of *P. putida* to colonize plant roots.

Understanding the regulation of *Pseudomonas syringae hrp* gene expression at the level of transcription

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The hypersensitive response and pathogenicity (*hrp*) regulon encodes a type three secretion system, and associated effector and regulatory proteins in *Pseudomonas syringae* pv. tomato DC3000. Probably all *hrp* genes are recognised via their "hrp-box" motif by HrpL, an alternative sigma factor. HrpL expression is σ^{54}-dependent; the activity of which is governed by two co-dependent AAA+ enhancer binding proteins, HrpR and HrpS. The activities of HrpR and HrpS are regulated by two additional proteins, HrpV and HrpG, which are encoded within the *hrp* regulon, to provide a natural feedback system. HrpV and HrpG are novel proteins with no known homologues. To further understand the control networks that govern HrpL expression, we have used an *in vivo* bacterial 2-hybrid assay to study the interactions between HrpV, HrpG, HrpR, HrpS and truncated mutants of HrpV and HrpG to determine functionally important domains. We have shown that HrpV inhibits HrpR/HrpS via binding to HrpS alone. Additionally we are studying the *in vitro* activities of HrpV, HrpG, HrpR and HrpS at the hrpL promoter in abortive initiation and UV-photocrosslinking assays, and via assessment of bacterial RNA polymerase (RNAP) holoenzyme ($E\sigma^{54}$) interactions. It has been demonstrated that HrpV reduces the ability of HrpR/HrpS to form a transcriptionally active $E\sigma^{54}$ open-complex. Phenotypes of DC3000 *hrpV* and *hrpG* knockout mutants have been analysed by monitoring symptom development and population growth (compared to wild-type) in the host *Arabadopsis thaliana*. DC3000 deleted for HrpV shows no phenotypic changes, however when deleted for HrpG, a significant decrease in both population growth *in planta* and symptom development is observed. A refined model for control of *hrp* gene expression will be presented.

Toward the identification of the *Hyaloperonospora arabidopsidis* RXLR-(EER) effectors that suppress PAMP-triggered immunity in *Arabidopsis thaliana*

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Oomycete pathogens include *Phytophthora infestans*, the causal agent of potato late blight, or *Hyaloperonospora arabidopsidis*, responsible for downy mildew of *Arabidopsis*. *In silico* analysis predicts that the oomycete genomes encode for a large number of so-called RXLR-(EER) effectors that are assumed to manipulate diverse host cellular activities as part of the infection strategy of the pathogen. In order to identify the repertoire of RXLR-(EER) effectors from *H. arabidopsidis* that suppress plant immunity triggered by the recognition of invariant microbial structures termed Pathogen-Associated Molecular Patterns (PAMPs), we use an *Arabidopsis* protoplast-based system according to the protocol developed in Jen Sheen's lab. For the read-out we

use methods that we set-up to measure 1) post-translational MAP kinase activation 2) PAMP-induced gene expression by qRT-PCR 3) pFRK1-Luc reporter gene assay 4) cell death. Our data show that some candidate RXLR-(EER) effectors are able to suppress both Flg22-mediated MAPK activation and the transcriptional activation of early Flg22-inducible genes. Subsequently, complementary biochemical and physiological approaches are currently performed to ascribe functions to RXLR-(EER) effectors as well as discovering their respective targets within the plant cell.

Characterization of RanGAP2 properties involved in the Rx-mediated resistance

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Plant NB-LRR proteins have been shown to confer resistance to very different types of pathogens. This innate immunity is based on the specific recognition of a pathogen-produced effector by an NB-LRR protein leading to the activation of defense responses, and often an hypersensitive response (HR) which suppresses the pathogen. The potato *Rx* gene encodes an NB-LRR protein which confers resistance to potato virus X (PVX) vie recognition of the viral coat protein (CP). It has been previously shown that an Rx-interacting protein, Ran GTPase activating protein 2 (RanGAP2) is required for the Rx-mediated responses. RanGAP proteins are ubiquitous in eukaryotes and known to be involved in several cellular processes such as the nucleocytoplasmic traffic during interphase. Moreover, two RanGAP proteins exist in potato, but only RanGAP2 is able to bind and mediate the resistance conferred by Rx. In order to characterize the functional properties of RanGAP2 related to the Rx-mediated resistance, we have constructed different mutants and swapped domains of RanGAPs. By co-immunoprecipitations and *in planta* transient expression assays, we have investigated if some known cellular functions of RanGAP2 are involved in the HR induction, as well as the specificity of RanGAP2 in the recognition of PVX CP by Rx.

Ulvan, a sulfated polysaccharide from green algae, activates plant immunity through the jasmonic acid signaling pathway

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The ability of plants to defend themselves against pathogens depends on the perception of signalling molecules, called elicitors, released during infection. Since elicitors induce plant defence, they might be considered as alternative tools for disease control in agronomic crops. Industrial use of elicitors needs the identification of abundant sources of these molecules and characterization of their biological activity. Here we report on the identification of a new polysaccharide purified from a crude extract of the green algae *Ulva* spp. and the characterization of it biological activity in plants. High and low molecular weight molecules contained in the extract were sized-fractionated. Analyses of the fractions revealed that biological activity was present only in the fraction of high molecular weight. Physical and chemical analyses of this fraction showed that it contained most exclusively a high molecular weight sulfated polysaccharide named ulvan, whose main constituent is a disaccharide unit, β-D-glucuronosyluronic acid (1→ 4) L-rhamnose 3 sulfate. Response to ulvan were compared to those induced upon methyl jasmonate (MeJA) and acibenzolar-S-methyl (a salicylic acid analog) treatments on the legume *M. truncatula* using oligo microarrays allowing the monitoring of more than 16,000 genes. Interestingly, ulvan gene expression signature showed significant similarity to MeJA and typical responses controlled by the JA pathway, such as induction of protease inhibitor activity. Expression of ulvan responsive genes was found to be dependant of JAR1 in *Arabidopsis* plants. Altogether, our results show that ulvan induced plant defense response through the jasmonate signalling pathway and highlight the use of functional genomics to develop new bioactive compounds for plant protection.

***Pseudomonas syringae* targets plant HSP70 for virulence**

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Pseudomonas syringae is a pathogen that needs the type III secretion system to inject effectors into plant cells for a successful infection. We previously characterized HopI1 as a chloroplast-localized effector that suppresses accumulation of the defense signal salicylic acid. HopI1 has two striking features: a variable repeat region rich in P/Q amino acids and a J domain. HopI1's J domain is essential for its virulence activity. J domains in many proteins interact with HSP70 and modulate its activity. Here we report that HopI1 acts as a typical co-chaperone of Hsp70 by increasing its ATP hydrolysis. HopI1interacts with a major HSP70 isoform (and possibly other isoforms) via its J domain; these proteins form a complex(es) in chloroplasts, as shown by co-IP and LC-MS/MS analysis. An intact J domain is critical for HopI1's binding to HSP70. This is consistent with the observation that J domain mutants of HopI1 lack virulence activity. Interestingly, the major HopI1-interacting HSP70 isoform is necessary for HopI1's virulence role; in a mutant reduced in this isoform, the difference in growth of wild-type *P. syringae* and Δ*hopI1* strains is smaller than in wild-type Arabidopsis. Deletion of the P/Q region does not influence binding to HSP70, although this region is important for HopI1's virulence activity. The P/Q region may be important for bringing client proteins to HSP70, which might facilitate their folding or affect their ability to assemble or stably reside in complexes.

The *Pseudomonas syringae* type III effector HopU1 ADP-ribosylates and inhibits the activity of a plant RNA-binding protein involved in stress responses

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The bacterial plant pathogen *Pseudomonas syringae* employs the type III protein secretion system (T3SS) to inject effector proteins into host cells leading to suppression of immune responses and promotion of bacterial growth and disease. Many *P. syringae* effectors have been shown to suppress plant innate immune responses; however, the majority of their enzymatic activities and their plant substrates remain unknown. Recently we reported that the *P. syringae* type III effector HopU1 is a mono-ADP-ribosyltransferase (ADP-RT) active on an artificial substrate and proteins in *Arabidopsis thaliana* and tobacco. Employing a proteomic approach with ADP-RT assays and mass spectrometry we identified the major HopU1 substrates in *A. thaliana* to be several RNA-binding proteins that possess RNA-recognition motifs (RRMs). One of the targets AtGRP7 was shown to be a component of plant innate immunity (Fu et al., 2007 Nature 447:284-288). Here we show further characterization of HopU1 and AtGRP7. In addition, we found using tandem mass spectrometry that an arginine residue in position 49 (R49) of AtGRP7 was ADP-ribosylated by HopU1. This modification resulted in significantly decreased binding affinity of AtGRP7 to RNA, measured by gel shift assays and kinetic analyses. Consistent with the reduced RNA binding, structural modeling of the RRM domain of AtGRP7 indicated that R49 would likely make a direct contact with RNA. Taken together, our data suggests that *P. syringae* has evolved to target an amino acid residue within an RNA-binding protein by disrupting its ability to bind RNA and in doing so favors plant pathogenesis.

Abscisic acid interacts antagonistically with salicylic acid-signaling pathway in rice-*Magnaporthe grisea* interaction

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Plant hormones play pivotal signaling roles in plant-pathogen interactions. Each hormone mediates a distinct defense pathway, but crosstalks between them are also important for regulating plant defense and pathogenesis. Here we report that antagonistic interaction of abscisic acid (ABA) signaling with salicylic acid (SA) defense signaling pathway in rice plays a pivotal role in rice–*M. grisea* interaction. We have previously shown that BTH, a functional analog of SA, enhances resistance of rice plants against blast fungus *M. grisea* by activating OsNPR1 and WRKY45, the key regulators of the SA pathway with each regulating one of two pathways branched downstream of SA. In this study, we found that exogenous application of ABA suppressed transcriptional upregulation of both *OsNPR1* and *WRKY45* induced by SA/BTH. This negative regulation accounts for previously reported enhanced *M. grisea* susceptibility by ABA application. Overexpression of *OsNPR1* or *WRKY45* mostly negated the enhanced susceptibility by ABA application, indicating that ABA acts upstream of both *OsNPR1* and *WRKY45* in the SA-signaling pathway. Determination of ABA contents revealed that high levels of ABA were accumulated in hyphae (mycelium) of *M. grisea* as compared to its conidia. Taken together, these results suggest a pathogenic strategy of *M. grisea* for infecting rice plants: *M. grisea* presumably uses ABA synthesized for itself to elevate local ABA level in invaded rice cells and consequently suppress the SA signaling pathway, which should facilitate infection of compatible *M. grisea* to rice.

GDSL lipase 1 in systemic resistance associated with ethylene signaling in Arabidopsis

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Arabidopsis GDSL LIPASE 1 (GLIP1) plays an important role in plant immunity, eliciting both local and systemic resistance in plants. GLIP1 functions independently of salicylic acid but requires ethylene signaling. Enhancement of *GLIP1* expression in plants increased resistance to pathogens including *Alternaria brassicicola*, *Erwinia carotovora* and *Pseudomonas syringae*, and limited their growth at the infection site. Furthermore, local treatment with GLIP1 proteins was sufficient for the activation of systemic resistance, inducing both resistance gene expression and pathogen resistance in systemic leaves. The *PDF1.2*-inducing activity accumulated in petiole exudates in a GLIP1-dependent manner and was fractionated in the size range of less than 10 kDa as determined by size exclusion HPLC. These results suggest that GLIP1 may mediate the production of a systemic signaling molecule(s). The fractions containing *PDF1.2*-inducing activity were further subjected to GS/MS analysis, which led to the identification of two lipid molecules (FA-Xs) as potential mobile signals. The accumulation of FA-Xs in systemic leaves was mediated by GLIP1 and ethylene-associated treatments, but not by SA. Local treatments with FA-Xs elicited systemic resistance in a NPR1-dependent manner. These results suggest that FA-Xs may function as systemic signals and that the biosynthesis of FA-Xs is specific to GLIP1 and ethylene signaling, but converge into SAR for the induction of systemic immunity.

Two Arabidopsis bHLH transcription factors involved in cyst nematode feeding site formation

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The soybean cyst nematode (SCN, *Heterodera glycines*) is one of the most damaging parasites of soybean causing an estimated one billion dollar annual loss in the United States. Successful SCN parasitism depends on the formation and maintenance of feeding sites (syncytia) in host roots, which is highly regulated by the interaction between the cyst nematode and the host. We are studying cyst nematode – plant interactions using the model system of Arabidopsis and the sugar beet cyst nematode (BCN, *Heterodera schachtii*), a close relative of SCN. By using an integrated research approach we have determined that two basic Helix-Loop-Helix (bHLH) transcription factors are involved in cyst nematode syncytium formation. Overexpressing one of these genes in Arabidopsis altered the morphology of the roots and the susceptibility to the sugar beet cyst nematode. As early as three days after inoculation, genes for both transcription factors were upregulated in the developing feeding sites. By using yeast- two-hybrid analyses and bimolecular fluorescent complementation assays, we discovered that these two bHLH transcription factors can dimerize. We hypothesize that this heterodimer specifically forms in the developing cyst nematode feeding site and is involved in the reprogramming of root cells into this new cell type. Further expression analyses are under way to determine target genes regulated by both transcription factors.

Characterization of the *Pseudomonas syringae* type III effectors HopO1-1 and HopO1-2, which are putative mono-ADP-ribosyltransferases

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Pseudomonas syringae uses a type III secretion system (T3SS) called the Hrp T3SS to inject type III effectors into plant cells and suppress plant immune responses. Translocated type III effectors carry out specific functions within the host cell and collectively are required for *P. syringae* to cause disease. *Pseudomonas syringae* pv. tomato DC3000 injects greater than 30 effector proteins into plant cells. Among these are the HopU1, which is a mono- ADP-ribosyltransferase (ADP-RT), and HopO1-1 and HopO1-2, which are predicted ADP-RTs. ADP-RTs are well known bacterial toxins in animal pathogens where they ADP-ribosylate and modify specific proteins. HopU1 was recently shown to ADP-ribosylate *Arabidopsis* RNA-binding proteins including the glycine-rich RNA-binding protein *At*GRP7 (Fu *et al.* 2007 Nature 447:284-288). The alignment of conserved regions with known ADP-RTs shows HopO1-1 and HopO1-2 also have three conserved regions of the ADP-RT active site. HopO1-1 and HopO1-2 GFP-fusions localize to plant plasma membrane of *N. benthamiana* leaves in *Agrobacterium*-mediated transient assays. This is consistent the putative myristoylation site contained at HopO1-1's amino terminus. Interestingly, HopO1-2 does not have an obvious myristoylation site and, therefore, it may localize to the plasma membrane via a different mechanism. Recombinant HopO1-1 and HopO1-2 are almost insoluble when expressed in *E. coli*, which has made them difficult to study. Increase of the cell culture volume allowed small amount of soluble proteins and they may possess weak ADP-RT activity. The study of HopO1-1 and HopO1-2 transgenic plants is expected to show whether they are capable of suppressing plant immunity and whether their activity is dependent on their ADP-RT active sites.

The secretome of *Melampsora larici-populina*

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The recent completion of the poplar leaf rust genome sequence, *Melampsora larici-populina*, together with the availability of the poplar genome, is providing an unprecedented opportunity to study the establishment of biotrophic relationships. The genome sequence of *M. larici-populina* has revealed that this fungus is equipped with an arsenal of approximately two thousand secreted proteins (more than 10% of the genome), only one third of which have identifiable homologs in the genome of *Puccinia graminis* f. sp. *tritici*, the wheat stem rust. Around 10% of the secretome encode highly divergent proteins belonging to different families with conserved cysteine residues, the largest family encompassing more than 100 members, mostly found in gene clusters. Similarities with effectors previously described in Pucciniales were also uncovered, such as multiple homologs of the *AvrM* gene found in *Melampsora lini* or of the Rust Transferred Protein (*RTP1*) gene from *Uromyces fabae*. In order to identify other effectors which could act as crucial determinants for biotrophic development, we elaborated a multifaceted approach using transcriptomics and evolutionary analyses. The global analysis of the transcriptome during the infection process was conducted using whole genome oligoarrays. EST libraries obtained from spores and infected leaves were also compared. The strategy for the evolutionary analysis included intra- and inter-specific comparative genomics, and tests for the presence of positive selection, by taking advantage of the available resources. Such information is currently being used for prioritizing candidate effector genes for functional studies.

A wheat *Mla* homolog confers powdery mildew resistance in wheat

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The obligate fungal pathogen *Blumeria graminis* causes the powdery mildew disease in wheat and barley. Fascinatingly, plant-invasion by formae speciales (*Bgt* and Bgh) exhibits strong host specificity, even though the pathogens are highly similar. The intracellular CC-NBARC-LRR resistance proteins PM3 and MLA contribute to race-specific resistance in wheat and barley, respectively. To shed light on the role of *Mla*-like sequences in wheat powdery mildew defense, the diploid wheat species *Triticum monococcum* was searched for *Mla* homologs. One sequence discovered encompassed an intact, full-length open reading frame (ORF) and was designated *TmMla1*. Comparison of the organisation of *Mla* loci in barley (Morex) and wheat revealed both striking similarities, but also a huge reshuffling of DNA sequences. Functionality of *TmMla1* was assessed by transient-overexpression in wheat and barley epidermal cells. So far we could not identify a *Bgh* isolate that is recognised by *TmMla1*. Surprisingly, we identified one *Bgt* isolate in wheat that was recognised by TmMLA1 and also a fully virulent isolate, demonstrating the race-specific action of this protein against wheat powdery mildew. Since it is known that the CC domain of MLA10 interacts with HvWRKYs1/2, we also checked interaction with TmMLA1. Indeed, we found a weak interaction between HvWRKY1 and the TmMLA1 CC domain in yeast two hybrid studies, suggesting a similar mode of action as for MLA1. To deepen our understanding of TmMLA1 function we intend to search for wheat WRKYs interacting with TmMLA1-CC. Most recent data will be presented.

Role of promoters in conferring resistance to apple scab

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Apple (*Malus x domestica*) is one of the important fruit crops of the world. Most of the present day apple varieties are susceptible to apple scab which is

caused by the fungus *Venturia inaequalis*. Genetic modification is an attractive option for introducing scab resistance into existing high quality apple cultivars. Then, the availability of appropriate resistance genes and knowledge about their expression levels are of prime importance. Apple scab resistance genes, *Vf1* and *Vf2* (*Venturia ineaqualis*; *Malus floribunda*) were isolated along with their native promoters, coding and terminator sequences. In order to study, the nature and the functional length of the *Vf1* and *Vf2* promoters, native gene promoters for the two *Vf* genes are being tested in two lengths (*Vf1* short promoter (SP)-312 bp, *Vf1* long promoter (LP)-1990 bp; *Vf2* short promoter (SP) - 288 bp, *Vf2* long promoter (LP) - 2007 bp). As a control, the apple rubisco promoter (1600 bp), which is a strong promoter, was included in testing expression of *Vf1* and *Vf2* genes. Putative plant transformants were obtained for all the above mentioned constructs. They were analyzed for the presence of the gene of interest through PCR. To assess the resistance at phenotypic level these transformants are being micrografted on root stock plant in the greenhouse. The transformants are then tested after manual inoculation with monospore isolates of the scab fungus. To assess the expression of both *Vf* genes quantitative RT-PCR will be performed. Preliminary results will be presented.

The second chitin elicitor binding protein (CEBiP2) in rice cell

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Chitin and its fragments as a typical fungal MAMP trigger various defense responses in a wide range of plant species. We recently isolated CEBiP, chitin elicitor binding protein, from rice cells and showed that CEBiP plays an important role as a cell surface receptor for chitin elicitor signaling. During the purification of CEBiP, we also obtained a minor protein by the GlcNAc8-Sepharose affinity chromatography. This protein, named CEBiP2, was identified as a membrane glycoprotein consisting of two LysM motifs in the extracellular domain and three amino acid residues in the intracellular region. CEBiP2 showed a high sequence homology to CEBiP, with 39% identity. Interestingly, the time courses of the induction of *CEBiP* and *CEBiP2* after chitin elicitor treatment were very different, peaking at 3h after the treatment for CEBiP while 15 min for CEBiP2. To study the function of CEBiP2, we established transgenic rice cells of over-expressing *CEBiP2 (CEBiP2*-EX) and also suppressing *CEBiP2* expression (*CEBiP2*-RNAi). Several lines of these *CEBiP2*-RNAi transformants significantly reduced the expression of *CEBiP2* but not of *CEBiP*. Many of them also showed the decreased level of chitin-induced ROS generation. From the affinity labeling experiment with biotin-GlcNAc8, we found CEBiP2 possesses specific binding activity for chitin elicitor. These results indicate that CEBiP2 may also play a role for chitin elicitor signaling in rice in addition to CEBiP. How these two chitin elicitor binding proteins share their function in rice cell is under investigation.

CRT1, a new player in *R* gene-mediated immunity in plants

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Resistance (*R*) gene-mediated immunity confers rapid and strong protection against pathogen infection in a wide range of plants. The Arabidopsis R protein HRT recognizes the coat protein (CP) of Turnip Crinkle Virus (TCV) and triggers resistance responses including the hypersensitive response (HR). CRT1 was identified in a genetic screening for mutants compromised in recognition of the TCV-CP. CRT1 is an ATPase carrying a 'GHKL' ATPase motif. Interestingly, CRT1 physically interacts with several R proteins besides HRT and modulates *R* gene-mediated resistance to bacteria and oomycete pathogens in addition to TCV, suggesting that CRT1 is a universal factor functioning in *R* gene-mediated resistance. Moreover, interaction of CRT1 with otherwise interacting R proteins appears to be disrupted upon activation of the R proteins by either recognition of the corresponding avirulence factor or by overexpression of the R protein; this result suggests that the CRT1-R protein interaction is dynamic. Silencing of the CRT1 homologues in *Nicotiana benthamiana* also compromised cell death triggered by tomato Pto, but not by a constitutively active MAPK kinase. These results raise the possibility that the CRT1 family functions i) in *R* gene-mediated immunity in a broad range of species and ii) upstream of the MAPK cascade. Further characterization of the CRT1-dependent transcriptome and additional CRT1-interacting proteins will be discussed.

The mitogen-activated protein kinases WIPK and SIPK regulate the levels of jasmonic and salicylic acids in wounded tobacco plants

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Jasmonic acid (JA) and salicylic acid (SA) are key signaling molecules which regulate plant wound and defense responses. JA mainly induces resistance to necrotrophic pathogens and insects, whereas SA induces resistance to biotrophic pathogens. Accumulation of JA and SA are induced by wounding and/or pathogen attack, however, the mechanisms underlying activation of JA and SA biosynthesis are poorly understood. In tobacco (*Nicotiana tabacum*), wounding induces rapid activation of two mitogen-activated protein kinases (MAPKs), wound-induced protein kinase (WIPK) and SA-induced protein kinase (SIPK). Our previous studies have suggested that activation of WIPK is required for the production of wound-induced JA. However, the exact role of WIPK remains unresolved. To define the roles of WIPK and SIPK in the wound response, we generated transgenic tobacco plants in which *WIPK* alone, *SIPK* alone or both *WIPK* and *SIPK* were silenced by RNA interference. In addition, transgenic plants were generated which over-express *NtMKP1*, a gene encoding tobacco MAPK phosphatase. In *NtMKP1*-overexpressing plants, wound-induced activation of both WIPK and SIPK are reduced. Here we show that wound-induced JA production was reduced compared with non-silenced plants in all of the *WIPK*-, *SIPK* and *WIPK*/*SIPK*-silenced plants. *NtMKP1*-overexpressiong plants also exhibited reduced JA production in response to wounding. Moreover, wounding resulted in an abnormal accumulation of both SA and transcripts for SA-responsive genes in *WIPK*/*SIPK*-silenced and *NtMKP1*-over-expressing plants, but not in *WIPK*-silenced and *SIPK*-silenced plants. These results suggest that WIPK and SIPK play an important role in JA production in response to wounding, and that they function cooperatively to control SA biosynthesis.

Intense defense reactions against *Phytophthora infestans* in transgenic potato plants expressing the avirulence protein Avr3a

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Induced expression of avirulence genes (Avr) under the control of pathogen-inducible promoters in plants containing the corresponding R-gene is expected to create plants with a high level of resistance against several pathogens. Upon interacting with the R protein of the intrinsic resistance gene the Avr protein will initiate a hypersensitive reaction (HR) at the site of infection, arresting the further invasion of the pathogen. However, beyond their pathogen-responsiveness, the promoters need to tightly regulate the expression of the Avr-gene in a spacial and timely manner, to avoid or reduce detrimental effects to non-infected tissues and the whole plant. We used the first published Avr-gene from *P. infestans*, $Avr3a^{KI}$, to implement the concept in transgenic potato plants. The expression of $Avr3a^{KI}$ was regulated by synthetic pathogen-inducible promoters of different strengths. Depending on the promoter, we observed high-intensity defense reactions against *Phytophthora infestans* in some transgenic potato lines, resulting in the strong regression of the pathogen. However, this reaction was always linked to the formation of spontaneous HR-like lesions which, eventually, could spread throughout most of the plant. More research is therefore necessary to put tighter control on the system.

The activation of OsRac1 by R protein plays a critical role in innate immune responses in rice

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Most resistance (R) genes encode nucleotide binding site-leucine-rich repeat (NBS-LRR) proteins, which act as intracellular receptors for pathogen signals. It is largely unknown how NBS-LRR resistance proteins transduce signals to their downstream effectors and thereby execute innate immunity in plants. We have previously found that the small GTPase OsRac1 plays an important role in R gene-mediated defense response in rice. Therefore, we searched for OsRac1-interacting molecules and found that OsRac1 directly interacted with *Pit* which is a NBS-LRR-type R protein for rice blast fungus. Pit formed the

complex with both the constitutively active and the dominant negative forms of OsRac1 in the plasma membrane in an activity-dependent manner. Transient expression of the active form of Pit and the constitutively active form of OsRac1 induced hypersensitive response and reactive oxygen species production in the leaf of *Nicotiana benthamiana*. The expression of the dominant negative form of OsRac1 suppressed Pit-induced hypersensitive response as well as reactive oxygen species production. The knock down of OsRac1 diminished Pit-mediated defense response against rice blast fungus. The active form of Pit activated the OsRac1 activity in rice protoplasts. These results suggest that OsRac1 forms a complex with R proteins and acts as a downstream effector of R proteins, thereby inducing immune responses in rice.

Identification of DH-PH type GDP-GTP exchange factor for Rac/Rop GTPase in plant immune response

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Plants recognize infection of pathogens through function of disease resistance genes, and induce a series of immune responses including production of reactive oxygen species (ROS) and anti-microbial compounds, and expression of PR genes. Previously, we have demonstrated that Rac/Rop, one of small G proteins, plays an important role in plant immune responses. Transgenic rice plants expressing constitutively active mutant of rice OsRac1 showed enhanced resistance of virulent races of *Magnaporthe grisea* and *Xanthomonas oryzae*, whereas hypersensitive response induced by avirulent *M. grisea* is suppressed by dominant-negative form of Rac1. These results indicate that Rac/Rop GTPase plays important roles in R gene-mediated resistance and basal resistance. Recently, the PRONE domain-containing proteins have been identified as GDP-GTP exchange factor (GEF) for Rac/Rop GTPase. In fact, the PRONE-type GEFs were able to activate OsRac1 in vivo and in vitro. Here, we have isolated a new type of Rac/Rop GEF containing a Dbl homology (DH)-like domain and a pleckstrin homology (PH) domain. Biochemical experiments indicated that this DH-like protein have the GEF activity for Rac/Rop GTPase *in vitro*. The role of the DH-like GEF in plant immune response will be discussed.

F-Box genes in plant defense signaling

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Induced defenses and stress responses play a major role in plant disease resistance and are regulated by a network of interconnected signal transduction pathways with the plant hormones ethylene (ET), jasmonic acid (JA) and salicylic acid (SA) as crucial mediators. These specific hormone-mediated signaling cascades trigger distinct sets of stress-related genes leading tolerance or resistance. F-box proteins are important components in these stress responses. They act as a member of a SCF protein complex, which target subsrate proteins for modification and degredation and thereby allow plants to respond rapidly to environmental stress factors. Well characterized F-box proteins include EBF1 and EBF2. The most prominent JA signaling mutant is defective in the COI1 gene, while SON1 is related to SA signaling. The central aim of our research is to analyze the contribution of a group of F-Box genes in plant defense responses and to identify novel components in plant defense signaling. As model system for the studies we utilize Arabidopsis, *Erwinia carotovora* and *Botrytis cinerea*. We are screening mutant F-box genes for altered resistance to these pathogens. For candidate genes we found, we started to perform yeast-two-hybrid screens for interactors responsible for a stress related phenotype.

Oomycete genomics to investigate pathogen virulence mechanisms and host specificity

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It was hypothesised (Jones and Dangl, 2006) that some pathogens fail on a non-host because their effectors have not been selected to evade recognition by the plant and thus trigger resistance (non-host resistance, NHR). In order to make use of this kind of resistance for plant protection it is important to know how pathogens evade recognition or how they suppress the defence reactions. *Albugo* sp. (white rusts, Oomycetes) provide a very interesting system to analyse defence suppression. After infection of *Arabidopsis thaliana* (At) by a compatible *A. candida* strain, the plant becomes susceptible to formerly incompatible downy mildew pathogens (Cooper et al., 2008) as well as powdery mildews. This indicates a suppression of non-host resistance. To find these important suppressors, genomes of two *Albugo* strains have been sequenced using the Illumina genome analyser. We used VELVET (Zerbino and Birney, 2008) to assemble the whole gene space for each strain and we estimate the genome size of both strains to be ~45Mb. The assemblies together with expression data were used to computationally analyse for effectors. More than 100 RXLR and 70 RXLQ effector candidates could be predicted for each of the strains. Although both Albugo strains grow and sporulate on At only 6 RXLRs and 3 RXLQs were conserved between them. This divergence might be due to the fast evolution of effector genes due to arms race between pathogen and host (Dodds et al., 2006). Since both strains show different levels of adaptation and host range this facilitates the screen for important effectors. To verify the computational analyses, potential effectors are currently tested for their functionality to repress non host resistance using the effector detector system (Sohn et al, 2007). Funding by: Gatsby, DFG and ERC advanced investigator grants.

Induction of S glycoprotein-like protein and its participation in defense responses in Nicotiana plants against Ralstonia solanacearum

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RsRGA4 (Ralstonia solanacearum-responsive gene A4) encodes a polypeptide containing a signal peptide, bulb lectin domain and PAN apple domain. RsRGA4 is similar to S-locus glycoprotein (SGP) from Brassica rapa, and SGP-like protein from Ipomea trifida and Medicago truncatula. We therefore designated RsRGA4 as NtSGLP (Nicotiana tabacum S locus glycoprotein like protein) and NbSGLP (its N. benthamiana orthlog). NbSGLP is expressed in root, leaf, petal, gynoecium and stamen. Expression of NbSGLP was strongly induced in tobacco leaves inoculated with an avirulent strain of R. solanacearum (Rs8107) and slightly enhanced by inoculation with virulent R. solanacearum strain RsOE1-1. Expression of NbSGLP was significantly induced by inoculation with an hrp-deficient mutant of Rs8107. Expression was also induced by aminocyclopropane carboxylic acid and salicylic acid. NbSGLP was secreted in a signal peptide-dependent manner. Agrobacterium-mediated expression of NbSGLP induced PR-1a and EREBP expression, but not PR-4 expression. Deletion of the signal peptide of NbSGLP reduced its defensive genes-inducing ability. In addition, deletion of the bulb lectin domain or PAN apple domain completely abolished the defensive genes-inducing activity. Virus-induced gene silencing of NbSGLP in N. banthamiana enhanced the growth of Rs8107. Expression of PR-1a and EREBP was compromised, but PR-4 expression was somewhat induced in NbSGLP-silenced plants. On the other hand, silencing of NbSGLP did not interfere with the appearance of the hypersensitive response (HR). Moreover, growth of RsOE1-1 and appearance of wilt symptoms were accelerated in silenced plants. Taken together, NtSGLP/NbSGLP might have a role in plant defense against R. solanacearum.

Mediator is a novel regulator of plant defense

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The Mediator multi-protein complex, first discovered in yeast by Nobel laureate Roger Kornberg, is conserved amongst all eukaryotes and plays an important role in regulating transcription. The function of the Mediator complex is to act as a "universal adaptor" between DNA-bound transcription factors and the RNA polymerase II complex, therefore integrating regulatory signals from both activators and repressors to modulate transcription at the promoter site. Our research has shown that manipulating genes encoding Mediator subunits offers yet unexplored control points to regulate innate immunity and flowering time in plants. We have found that the Mediator subunit, MED25 (previously known as the flowering regulator, *PHYTOCHROME AND FLOWERING TIME1* (*PFT1)*) is required for the uncompromised expression of both SA- and JA-associated defense genes and resistance to the leaf infecting necrotrophic pathogens *Alternaria brassicicola* and *Botrytis cinerea*. Unexpectedly however, we found that PFT1/MED25 renders Arabidopsis more susceptible to the root-infecting fungal pathogen *Fusarium oxysporum*. Additionally, we have identified a second Mediator subunit, MED8, which similarly to MED25, regulates both resistance to *F. oxysporum* as well as flowering time. Analysis of a *med25/med8* double mutant demonstrated additive increases in both *F. oxysporum* resistance as well as flowering time. This work reveals a new layer of defense regulation

previously undiscovered in plants and provides novel opportunities for agricultural improvement.

LAP (bHLH) is a transcription factor activated by OsRac1 in rice innate immunity

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A Rac/Rop GTPase, OsRac1, and its immune complex which plays an essential role in rice innate immunity have been characterized well so far. However, regulatory genes downstream of OsRac1 have not been identified yet. *LAP* gene encoding a putative basic helix-loop-helix transcription factor was obtained by yeast two hybrid assay with LAX. Microarray analysis using *LAP-GR* cells showed that *WRKY, PAL*, *Myb, CCR* genes were induced in the cells by dexamethazone, indicating that the genes may be downstream of *LAP* gene. RT-PCR analysis confirmed that *WRKY* and *PAL* genes are regulated by *LAP*. The downstream genes were also up-regulated by sphingolipid elicitor, supporting that *LAP* gene could be involved in defense response in rice. *LAP* gene was up-regulated in CA-OsRac1 cells compared to that of the wild type cells, suggesting that it acts downstream of OsRac1. *LAP*-RNA interference (RNAi) rice plants had impaired resistance to a rice blast fungus, indicating that *LAP* gene could be involved in resistance against blast fungus. Taken together, our results indicate that *LAP* gene is a transcription factor activated by OsRac1 and suggest that this gene plays as important regulator in innate immunity of rice.

A plasma membrane transcription factor NIN coordinates nodulation and abscisic acid signalling in *Medicago truncatula*

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Two diverse developmental programmes are necessary to generate a nitrogen fixing nodule: bacterial infection via infection threads that are initiated at the root epidermis and the cell cycle must be activated in the root cortex to form the nodule primordium. *Nodule Inception (NIN)* functions in the initiation of infection threads in the root epidermis as well as reinitiation of cell division in the root cortex. However, its mechanism of action remains unknown. We report that NIN is plasma membrane (PM)-localised and relocalises to the nucleus by proteolytic cleavage in response to Nod factor (NF). After cleavage, NIN acts as a bifunctional transcription factor which has both features of a repressor and an activator in regulating epidermal and cortical nodulation genes. The dual requirement of NIN in both epidermal and cortical programmes suggests that NIN is a key regulator coordinating these diverse processes. In addition, NIN has important roles in abscisic acid (ABA)-mediated signalling responses. ABA which is a negative regulator of nodulation induces relocalisation of NIN to the nucleus and NIN is required for the ABA-mediated inhibition of the early nodulation gene (*ENOD11*) and calcium spiking driven by NF. Therefore, this work provides evidence that NIN mediates crosstalk between nodulation and ABA signalling.

Using ChIP-chip to characterize Ca^{++}/calcineurin transcription factor binding sites in *Magnaporthe oryzae*

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Significant progress has been made to define the central signaling networks in many organisms, but collectively we know little about the downstream targets and the genes they regulate. To reconstruct the regulatory circuit of calcineurin signal transduction via *MoCRZ1*, a *Magnaporthe oryzae* C2H2 transcription factor activated by calcineurin dephosphorylation, we are identifying the DNA motifs to which this protein binds *in vivo* using chromatin immunoprecipitation and microarray (ChIP-chip). The *mocrz1* mutant showed impaired growth in the presence of Ca^{++} and has a pathogenicity defect mainly due to the inability to penetrate. We developed a non-coding region array of the *M. oryzae* genome in collaboration with NimbleGen technology. MoCRZ1 bound DNA fragments from eGFP tagged strain at the C terminus under its native promoter was immunoprecipitated with antiGFP antibody. More than 340 genes were identified as candidate targets with genes encoding calcium signaling components, and those involved in small molecule transport, ion homeostasis, and cell wall synthesis/maintenance being highly represented. To support ChIP-chip results and to obtain a global view of gene regulation by calcium signaling, a comprehensive microarray gene expression studies were also conducted. These data will provide support for remodeling calcium/calcineurin regulated signal transduction circuits controlling development and pathogenecity of this plant pathogenic fungus.

Nicotiana benthamiana* genes induced by *Pectobacterium carotovorum

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Pectobacterium species are enterobacterial plant pathogenic bacteria that cause soft rot disease on diverse plant species. Most *Pectobacterium* strains encode a type III secretion system (T3SS), but appear to encode few T3SS secreted proteins. The *P. carotovorum* WPP14 T3SS is required for HR-like cell death in *Nicotiana benthamiana* 24 hrs post-infiltration (hpi). Within 48 hpi, maceration symptoms become evident on the edges of the region that has undergone an HR, but not in leaves infiltrated with WPP14 T3SS mutants. To test the hypothesis that the *P. carotovorum* T3SS is responsible for suppression of plant innate immunity, callose formation in inoculated leaves was examined. Surprisingly, none of the *P. carotovorum* strains was able to impede callose deposition in *N. benthamiana* leaves. Despite no suppression of callose formation and a lack of HR or maceration symptoms, the T3SS mutant strains were able to grow as well as wild type in leaves. Within 24 hpi, there was strong callose deposition along leaf veins. Examination of leaves infiltrated with *P. carotovorum* encoding the green fluorescent protein gene showed that the pathogen cells were located along the veins, suggesting that the cells moved within the leaves to plant veins. To determine if the HR induced by *P. carotovorum* was similar to that induced by *Pseudomonas syringae*, gene expression profiles in inoculated *N. benthamiana* leaves were compared. *P. carotovorum* induced far fewer *N. benthamiana* genes than *Pseudomonas syringae*. Together these data support a model where *P. carotovorum* uses T3SS-induced plant cell death to promote maceration in leaves.

***Serine/threonine protein kinase 1* gene (*CaSTPK1*) is specifically induced during the *Tobacco mosaic virus*-mediated resistance response in hot peppers**

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Hot pepper (*Capsicum annuum* L. cv. Bugang) plants exhibit a hypersensitive response (HR) upon infection with *Tobacco mosaic virus* (TMV) pathotype P_0. In a previous study investigating the molecular mechanism that underlies this resistance, hot pepper cv. Bugang leaves were inoculated with TMV-P_0, and the genes specifically up-regulated during the HR were isolated by microarray analysis. One of the clones, the *Capsicum annuum serine/threonine protein kinase 1* (*CaSTPK1*) gene was increased specifically in the incompatible interaction with TMV-P_0. The expression of the *CaSTPK1* gene was triggered by NaCl and not salicylic acid (SA). These results suggest that *CaSTPK1* might be involved in an SA-independent viral defense signal transduction pathway and salt stress.

The Arabidopsis *RPS6* disease resistance gene encodes a member of the TIR-NBS-LRR class of proteins

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To identify molecular and genetic interactions between plants and pathogens, the model system *Pseudomonas syringae* - *Arabidopsis thaliana* is extensively studied. Comparative studies with the CC-NBS-LRR genes *RPS2, RPM1* and *RPS5* and isogenic *P. syringae* strains expressing the corresponding effector genes have been particularly fruitful in dissecting specific and common resistance signaling components. Arabidopsis possesses approximately 140 putative resistance genes encoding nucleotide binding site (NBS) and leucine-rich repeat (LRR) domain-containing proteins. Toll/Interleukin-1 receptor (TIR)-NBS-LRR *R* genes represent the largest *Arabidopsis R* gene family, but to date *RPS4* is the only characterized *Arabidopsis* TIR-NBS-LRR *R* gene for which a *P. syringae avr* gene is known. We previously identified *hopA1* from

P. syringae pv. syringae strain 61 as an avirulence gene that signals through *EDS1*, indicating that the corresponding resistance gene *RPS6* belongs to the TIR-NBS-LRR class. Here we report the identification of *RPS6* based on a loss of resistance screen and map-based cloning. Among resistance proteins of known function, the deduced amino acid sequence of *RPS6* shows highest similarity to the TIR-NBS-LRR resistance protein RAC1 that determines resistance to the fungal pathogen *Albugo candida*. Similar to *RPS4* and other TIR-NBS-LRR genes, *RPS6* generates alternatively spliced transcripts, although some of the alternative transcript structures are *RPS6*-specific. The cloning of *RPS6* will contribute to a closer dissection of common and specific *EDS1*-dependent TIR-NBS-LRR resistance gene pathways in Arabidopsis.

A novel transmembrane protein is required for oxidative stress homeostasis and virulence in plant and animal fungal pathogens

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Regulation of reactive oxygen species appears to be very important for pathogenic fungi during cell differentiation and pathogenesis. In this report we demonstrate that *Alternaria brassicicola* ATM1, a novel transmembrane protein, is necessary for tolerance to oxidative stress and plant pathogenesis. *ATM1* encodes a predicted hybrid membrane protein containing an AMP-binding, six putative transmembrane, and FAD and NAD(P)-binding domains, and shows high sequence similarity to proteins found only in filamentous fungi including an animal pathogen *Aspergillus fumigatus*. Localization and gene expression analyses indicated that *ATM1* is associated with fungal Woronin body, a specialized peroxisome, and strongly expressed during conidiation and initial invasive growth *in planta*. *A. brassicicola ATM1*-deficient mutants exhibited abnormal conidiogenesis, rapid loss of cell integrity of aged conidia, hypersensitivity to oxidative stress, and excessive oxidative burst of its own during conidiation and plant infection compared with wild-type strain. Virulence assays on green cabbage plant showed dramatically reduced virulence of the *Δatm1* mutants. Analysis of one of the *ATM1* homologs, *AtmA* in *A. fumigatus* revealed that the *ATM1* is functionally conserved in both plant and animal pathogenic fungi. Collectively, these results suggest that *ATM1* is likely to be involved in maintaining oxidative stress homeostasis during conidiation and pathogenesis.

Bacterial T3S effector AvrBsT alters phospholipid signaling in *Arabidopsis*

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Arabidopsis thaliana ecotype Pi-0 is resistant to *Pseudomonas syringae* pathovar *tomato* (Pst) strain DC3000 expressing the *Xanthomonas* T3S effector protein AvrBsT. Pi-0 plants are resistant due to a loss of function mutation in a conserved alpha/beta hydrolase, SOBER1 (Suppressor of AvrBsT Elicited Resistance1) (Cunnac et al., 2007). The nature of SOBER1 activity and substrate specificity was unknown; however, SOBER1-dependent suppression of hypersensitive response (HR) in Pi-0 suggested that this enzyme might hydrolyze a plant lipid or lipid precursor important for HR. Lipid profiling and lipase studies of infected Pi-0 leaves showed that AvrBsT triggers phospholipase D (PLD)-dependent production of phosphatidic acid (PA), a second messenger associated with defense signaling. Analysis of SOBER1 enzyme activity revealed that it cleaves phosphatidylcholine (PC), a PLD substrate that is hydrolyzed to PA. This suggests that SOBER1 is controlling phospholipid signaling and/or metabolism in response to AvrBsT perturbation. To determine how AvrBsT alters lipid signaling, a Common Interacting Protein 1(CIP1) that physically interacts with AvrBsT and SOBER1 *in planta* was isolated. Structural modeling of CIP1 suggests that it might encode an apolipoprotein. Interestingly, CIP1 binds phospholipids, associates with microtubules and lipid-raft-like structures near the cell cortex, and inhibits AvrBsT-triggered HR in Pi-0. Taken together, these data are consistent with the model that AvrBsT alters phospholipids signaling by targeting CIP1. In plants lacking SOBER1, this results in the activation of PLD-dependent PA signaling and disease resistance.

The function of NDR1 in plant resistance to *Pseudomonas syringae*

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Arabidopsis NDR1 (non-race specific disease resistance-1) a plasma membrane localized protein, plays an essential role in resistance mediated by the CC-NB-LRR class of resistance proteins, including RPS2, RPM1 and RPS5. Infection with *Pseudomonas syringae* pv. tomato DC3000 expressing bacterial effector proteins AvrRpt2, AvrB, AvrRpm1 and AvrPphB, activate resistance by the previously mentioned R proteins. Whereas the expression of NDR1 and its genetic requirement in plant disease resistance has been detailed, the specific biochemical mechanism(s) of NDR1 function remains largely unknown. We are utilizing EMS mutagenesis, as well as activation tagging mutagenesis screens to identify a role for NDR1 in disease resistance signaling. Also, through the use of homology modeling and structure threading NDR1 was predicted to have a high degree of structural homology to Arabidopsis LEA14, a protein implicated in abiotic stress responses. This homology with LEA14 suggests NDR1 may have a role in nutrient loss. This has led to the use of electrolyte leakage, and site directed mutagenesis of specific NDR1 motifs to elucidate a role for NDR1 in nutrient loss related to *Pseudomonas syringae* infection.

Genetically determined adaptation to hosts, a new facet in the *Bradyrhizobium japonicum*-legume symbiosis

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Bradyrhizobium japonicum is an alpha-proteobacterium which either grows free-living in soil or resides as a nitrogen-fixing endosymbiont (termed bacteroid) inside the root nodules of its legume hosts soybean (*Glycine max*), mung bean (*Vigna radiata*), cowpea (*Vigna unguiculata*), and siratro (*Macroptilium atropurpureum*). We are interested in studying the molecular and genetic determinants that enable *B. japonicum* to adapt to the different environments it comes across in each of the four host plants. To possibly identify the bacterial genes that are responsible for such physiological adaptations, we performed genome-wide transcriptome analyses using a custom-made *B. japonicum* GeneChip from Affymetrix. RNA was extracted from *B. japonicum* wild-type-infected root nodules of the four hosts, hybridized to the microarrays, and the differences in gene expression induced by the different hosts were assessed using an ANOVA analysis with subsequent Tukey post-hoc test. Our study showed that *B. japonicum* indeed adapts disparately to the hosts as evidenced by gene expression changes that were specific to each host. The numbers of specifically induced adaptation genes were: 96 for soybean, 46 for mungbean, 4 for cowpea, and 26 for siratro. Among the soybean-specific genes was one that encodes a response regulator of a two-component regulatory system. When plants were infected with a knock-out mutant of that gene, we observed a much more severe symbiotic nitrogen fixation defect on soybean as compared with the other three hosts. Therefore, apart from the well-known *nod* genes for host-specific nodulation, *B. japonicum* seems to posses genes that specifically enable bacteroids to function efficiently in a particular host, but which may be less important in other hosts.

The jasmonate signaling pathway in tomato regulates susceptibility to a toxin-dependent necrotrophic pathogen *Alternaria alternata*

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The plant hormone, jasmonic acid (JA), is known to have a critical role in both resistance and susceptibility against bacterial and fungal pathogen attack. However, little is known about the involvement of JA in the interactions between plants and toxigenic necrotrophic fungal pathogens. Using the tomato pathotype of *Alternaria alternata* (*Aa*) and its AAL-toxin/tomato interaction as a model system, we demonstrate a possible role for JA in susceptibility of plants against pathogens, which utilize host-specific toxins as virulence effectors. Disease development and in planta growth of the tomato pathotype of *Aa* were decreased in the *def1* mutant, defective in biosynthesis of JA, compared with the wild-type (WT) cultivar. Exogenous methyl jasmonate (MeJA) application restored pathogen disease symptoms to the *def1* mutant and led to increased disease in the WT. On the other hand, necrotic cell death was similarly induced by AAL-toxin both on *def1* and WT, and MeJA application to the tomatoes did not affect the degree of cell death by the toxin. These results indicate that the JA-dependent signaling pathway is not involved in host basal defense responses against the tomato pathotype of *Aa*, but rather might affect pathogen acceptability via a toxin-independent manner. Data further suggests that JA has a promotional effect on susceptibility of tomato to toxigenic and necrotrophic pathogens, such that pathogens might utilize the JA signaling pathway for successful infection.

Genome-wide screening of type III effectors from *Burkholderia glumae*

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Effectors are a cocktail of molecules that are known to be translocated into the host cells via the Type III protein secretion systems in gram negative bacteria. Type III effectors are reported to play important roles in bacterial pathogenicity and triggering innate immunity of eukaryotic host cells. Complete genome sequences of *Burkholderia glumae* BGR1, the causative agent of bacterial grain rot of rice, have been finished recently. With newly available genome information, sets of effector candidates of *B. glumae* were screened systematically in this study. As type III effector candidates of *B. glumae*, 31 PAI (Pathogenicity Island) genes (Kang et al., 2008) and over 200 genes possessing a conserved PIP Box (Plant Induced Promoter box; consensus, TTCG-n_{16}-TTCG), a *cis*-acting regulatory element, sequences were identified from the *B. glumae* genome database and screened *in vivo* using ELISA assay with a cya (adenyl cyclase) reporter gene. So far, eight effector candidates were identified followed by the yeast two hybrid screening to identify host proteins interacting with them. Further characterizations of the target proteins of effectors would elucidate the molecular mechanism of *B. glumae* pathogenicity in rice.

Transformation of *Frankia* sp. strain CcI3 by fusion marker genes using liquid culture selection

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Frankia is a nitrogen-fixing actinobacterium that establishes root nodule symbiosis with actinorhizal plants. The molecular basis of the symbiosis is largely unknown because genetic manipulation of *Frankia* has not been feasible. In this study we used novel technical attempts to transform *Frankia* sp. strain CcI3. We generated fusion marker genes consisting of a tetracycline resistance gene with a high codon usage similarity to *Frankia*'s and promoters of the strain's translation initiation factor 3 gene. We flanked the fusion genes with genomic sequences from strain CcI3 in the expectation that they would be integrated into the targeted site by homologous recombination. We introduced the transformation constructs into *Frankia* cells by electroporation and selected transformants in liquid media. The growth of antibiotic resistant cells was dependent on the presence of construct DNA. Genomic PCR and reverse transcriptase-PCR analysis confirmed that the marker genes were introduced in the cells. Integration of the marker genes into the chromosome by homologous recombination did occur, but at a low frequency. Most of the constructs were not integrated in chromosome and existed as degraded molecules in the cells. Marker genes were declined in the transformant population during maintenance, showing that transformation was transient.

DNA homologous recombinational repair genes are involved in growth and pathogenicity of *Magnaporthe oryzae*

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Magnaporthe oryzae is the causal agent of rice blast, the most importantdisease of rice. DNA recombination is regarded as an important factor participating in the variability, from the facts that *M. oryzae* shows the choromosomal length polymorphism, and transposable elements insertion / recombination. In order to clarify the role of DNA recombination in pathogenesis and variability, homologs of the genes involved in DNA recombinatinal repair have been analyzed in this study. *Rhm51, Rhm52* and *Rhm54*, homologs for *S. cerevisiae RAD51, RAD52* and *RAD54,* respectively were cloned from *M. oryzae*. Northern blot analysis revealed that these genes are highly induced by Methyl methane sulfonate (MMS) and to some extent by Methyl Viologen (MV) and the level of induction increases with dose of treatment. The genes are also constitutively expressed at low levels during the cell cycle. GFP reporter assay revealed that *Rhm54* is expressed in invasive hyphae *in planta*. Disruption mutants of *Rhm51* and *Rhm54* were constructed using pDESTR system. *Rhm52* could not disrupted by repetitive attempts of transformation. *rhm51* and *rhm54* mutants show reduced growth and are highly sensitive to MMS and H_2O_2. Although spores from both mutants and wild type do germinate and form appressoria, *rhm51* and *rhm54* mutants have a reduction in sporulation capacity compared to wild type. Inoculation assay revealed that the number of lesions formed on compatible rice cultivar was reduced in *rhm54* mutants. These results indicate that DNA homologous recombinational repair genes are involved in growth and pathogenicity of *M. oryzae*.

Analysis of the involvement of PEN2-F1GH subfamily members in the pathogen entry control in *Arabidopsis* nonhost resistance to fungal pathogens

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Resistance of an entire plant species to all genetic variants of a particular parasite is referred to as nonhost resistance, and is expressed by every plant towards the majority of potentially pathogenic microbes. The family 1 glycosyl hydrolase (F1GH) PEN2 has been identified as a key component of *Arabidopsis* nonhost pre-invasion resistance to non-adapted powdery mildew fungi, as *pen2* mutants allow enhanced fungal invasion rates into epidermal leaf tissue (Lipka et al., 2005). Recent analyses showed that PEN2 acts as an atypical myrosinase that is required for the local release of fungitoxic glucosinolates (Bednarek et al., 2009). The *Arabidopsis* genome harbours 48 predicted F1GH. Phylogenetic analyses suggest that PEN2 forms a monophyletic side branch together with 9 other *Arabidopsis*-F1GHs and represents an evolutionarily recent acquisition of the *Arabidopsis* genome. This is supported by Western blot analyses which detect PEN2-unique C-terminal sequences in closely related *Brassicaceae* species (*A. halleri*, *A. arenosa*, *A. lyrata*) but not in more distantly related species such as *Arabis alpina* or *Olimarabidopsis pumila*. To dissect the functional diversification within the *Arabidopsis* PEN2-F1GH subfamily we are currently conducting reverse genetics and complementation experiments with transcriptional and structural chimeras. Here we show that T-DNA insertion mutants for the closest homologue of PEN2 (encoded by *At3g60120*) permit increased invasion of the non-adapted powdery mildews *Blumeria graminis* f. sp. *hordei* (*Bgh*) and *Erysiphe pisi* (*Episi*). *At3g60120* x *pen2* double k.o. mutants show a slight additive *pen* phenotype upon inoculation with *Bgh*, suggesting a contribution of the At3g60120 gene product to nonhost pre-invasion resistance in *Arabidopsis* leaf tissue.

Jasmonate signaling during *Pseudomonas syringae* pathogenesis

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We are interested in the mechanisms by which plant pathogens modulate normal host cell processes to promote tissue colonization and disease development. Successful pathogen infection involves entry into host tissue, suppression of general antimicrobial host defenses, and growth to high levels in the plant. The phytotoxin coronatine (COR), a virulence factor of the bacterial plant pathogen *Pseudomonas syringae,* is required at multiple steps during *P. syringae* pathogenesis, including: 1) entry into host tissue, 2) suppression of salicylic acid-mediated defenses subsequent to entry, and 3) promotion of disease symptom development. COR is a functional analog of the endogenous plant hormone jasmonic acid (JA), most closely resembling the active JA amino acid conjugate JA-isoleucine. Molecular and genetic studies indicate that COR stimulates JA signaling during *P. syringae* infection, and that an intact JA signaling pathway is required for full disease susceptibility in both Arabidopsis and tomato. To further elucidate the roles of COR and JA-mediated processes in *P. syringae* pathogenesis, we are investigating the AtMyc2/JIN1-mediated branch of the JA signaling pathway required for susceptibility to *P. syringae*. We are using a combination of forward and reverse genetic approaches to identify and characterize novel JA signaling components and to investigate their roles during pathogenesis of Arabidopsis. We have isolated a mutant that defines a gene encoding a novel component of the JA signaling pathway that negatively regulates JA signaling and COR sensitivity. Our progress towards characterizing this gene and its roles in regulating JA-mediated responses and *P. syringae* disease development will be presented.

Studies on mechanism of disease resistance induced by high temperature treatment

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Systemic acquired resistance (SAR), one of plant defense systems, is induced by pathogen infection through salicylic acid (SA) accumulation. SAR has been well characterized and set of *pathogenesis-related* (*PR*) genes has been identified as SAR marker genes. The induction of disease resistance by high temperature treatment has been reported in cucumber, in which SA is likely to take part. To investigate the detailed mechanism of this resistance, effects of high temperature treatment in *Arabidopsis* were examined. The treatment induced the expression of *PR* genes, the accumulation of SA, and disease resistance to *Pseudomonas syringae* pv. *tomato* DC3000. The *MBF1c* gene, reported as a required gene for SA accumulation during heat stress in *Arabidopsis*, was induced in our experimental condition. The High temperature treatment induced the expression the isochorismate synthase 1 *(ICS1)* gene, encoding an SA biosynthetic enzyme. Analyses using the *sid2* (*SALICYLICACID INDUCTION DEFICIENT 2*) mutant defective in *ICS1* and NahG transgenic plants expressing SA degrading enzyme, indicated that high temperature-induced disease resistance required SA biosynthesis.

Combined bimolecular fluorescence complementation (BIFC) and förster resonance energy transfer-fluorescence lifetime imaging microscopy (FRET-FLIM) reveals ternary snare complex formation in plant cells

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The ternary soluble *N*-ethylmaleimide sensitive factor attachment receptor (SNARE) proteins *At*PEN1, *At*SNAP33 and *At*VAMP721/722 were recently shown to function together in disease resistance as well as plant development. Such ternary SNARE complexes are the minimal machinery required for vesicle fusion. We showed by combining BiFC with FRET measured by FLIM (FRET-FLIM) that at the single cell level translational fluorescent fusions of the orthologous barley and Arabidopsis SNAREs; *Hv*ROR2/*Hv*SNAP34/*Hv*VAMP722 and *At*PEN1/*At*SNAP33/*At*VAMP722 form authentic ternary SNARE complexes in leaf epidermal cells. In the transient expression system used the SNARE partners still responded normally to fungal challenge by focally accumulating (FA) below fungal attack sites. This suggests that the underlying cellular machinery driving the FA response is still operational. We noted that co-expression of the SNAREs resulted in the formation of vesicle-like compartments with a similar size and shape as FA sites. We therefore propose that the local assembly of SNAREs when exceeding a critical size drives focal accumulation and focal targeting of cellular defences.

Arabidopsis post-invasive oomycete/fungal resistance requires the beta-glucan receptor GSRK

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Like animals, plants evolved surface pattern recognition receptors (PRRs) to recognize highly conserved non-self molecules derived from microbes, called microbe-associated molecular patterns (MAMPs). In Arabidopsis, three PRRs have been described to date: FLS2 binding directly to bacterial flagellin, EFR detecting bacterial elongation factor Tu, and CERK1, a putative receptor for chitin, a major fungal cell wall component. Here we present a novel Arabidopsis PRR, *At*GSRK (glucan-sensing receptor kinase) that is required for post-invasive oomycete/fungal resistance. *At*GSRK directly and specifically interacts with b-glucan through its extracellular C-type lectin domain. Impairment of b-glucan signaling and enhanced susceptibility to the virulent oomycete *Hyaloperonospora parasitica* and the ascomycete powdery mildew fungus *Golovinomyces orontii* in *atgsrk* mutant plants indicate that *At*GSRK restricts the growth of oomycete/fungal pathogens. Using gene interaction experiments, we also found that *At*GSRK plays a distinct role in fungal resistance compared to *At*CERK1. The conservation of single copy *GSRK* homologs in Arabidopsis, rice, and poplar suggests its conserved role in glucan sensing across plant species. Different cytoplasmic signaling domains in plant GSRK and the mouse/human glucan receptor Dectin-1 imply a convergent evolution of glucan sensing in plant and animal innate immune systems.

A Rab GTPase RabG3b functions in autophagy and regulates innate immunity in plants

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A central component of the plant defense response to pathogens is the hypersensitive response (HR), a form of programmed cell death (PCD). Rapid and localized induction of HR-PCD ensures that pathogen invasion is prevented. Autophagy has been proposed as a mechanism to control HR-PCD, but little is known regarding the functional relationship between autophagy and HR-PCD and the regulation of these processes in plant innate immunity. Previously, we reported that transgenic plants overexpressing a constitutively active mutant (RabG3bCA) display unrestricted HR-PCD upon infection, whereas no major difference between transgenic plants overexpressing a dominant negative mutant (RabG3bDN) and WT plants is observed. For the further functional analysis of RabG3b gene, we also constructed to RabG3b RNAi line (RabG3bRNAi) and investigate phenotypic differences in these RabG3b transgenic plants during pathogen treatments. Here, we show that a small GTP binding protein RabG3b plays a role in autophagy and regulates HR-PCD. Microscopic analyses indicate that vesicle trafficking and autolysosome formation are aberrant in RabG3bCA transgenic plants. Our studies identify RabG3b as an essential component in vesicle trafficking and autophagy, regulating HR-PCD in the plant immune response.

***Botrytis cinerea* and the compatible interaction with *Arabidopsis thaliana*: Does the fungus manipulate plant defenses?**

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Botrytis cinerea is a ubiquitous pre- and postharvest necrotrophic pathogen with a broad host range that causes substantial crop losses. *B. cinerea* conidia penetrate through the cuticle and epidermal walls leading to the death of invaded cells and to tissue softening, rot or necrosis, depending on the invaded parts. We want to explore and understand how a virulent strain of *B. cinerea* causes disease on *Arabidopsis thaliana*. Jasmonic acid (JA) was previously shown to be induced after infection of the plant by *B. cinerea* and to be involved in the deployment of basal resistance against this fungus. We have compared the gene expression pattern of *A. thaliana* leaves treated with JA with that of leaves inoculated with *B. cinerea* using DNA microarrays. Not too surprisingly, a group of similar genes can be found among the genes induced by JA or by *B. cinerea* infection. Similarly, the expression of other genes was repressed both after JA or *B. cinerea* inoculation. More interestingly, 36 genes were also identified that were repressed by JA and induced by *B. cinerea* infection. We hypothesize that the expression of these genes might be modified by *B. cinerea* to allow colonization of its host. We tested this hypothesis using a reverse genetic approach. The resistance of candidate genes-corresponding T-DNA mutants have been analysed upon *B. cinerea* infection. We found that at least 3 Arabidopsis genes are essential for *B. cinerea* colonization. We will describe more in detail one candidate resulting from this study, which appears to act in the Botrytis-mediated JA/SA crosstalk.

Molecular characterization and biotechnological applications of endophytic bacteria associated with mangrove forests

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Mangrove ecosystems are rich in organic matter, where microbial activity is responsible for major nutrient transformations and the microorganisms play important role in the nutrient recycling of the mangrove forest. Endophytic

microorganisms are those that live inside of a plant at least in a period of its vital cycle, and are found in tissues such as leaves, branches and roots. Apparently, they do not cause any damage to the host, which distinguishes them from the phytopatogenic microorganisms. The aim of this work was to study the diversity of endophytic bacteria from typically plant species from mangrove, such as *Rhizophora mangle*, *Avicenia nitida* and *Laguncularia racemosa*, by culture-dependent and denaturing gradient-gel electrophoresis (DGGE). Also, the evaluation of enzyme production such as amylase, esterase, lipase, protease, pectinase and cellulase, by the bacteria isolated from mangrove was tested, aiming a biotechnological potential aspect. The predominant main genera of endophytic bacteria found were *Methylobacterium*, *Bradyrhizobium*, *Novosphingobium*, *Sphingomonas*, *Pseudomonas*, *Flavimonas*, *Microbacterium*, *Xanthomonas*, *Stenotrophomonas*, *Pantoea*, *Klebsiella*, *Salmonella*, *Escherichia* and *Enterobacter*. The analyses by DGGE showed similarity of bacteria communities from endophytic groups. The results suggest that DGGE is a practicable protocol to asses the complex endophytic bacteria community of mangroves. The isolates presented enzymatic activity, which was ranked as follows: proteolytic (69%), amilolytic (56%), lipolytic (9%), esterasic (47%), pectinolytic (75%). Cellulolytic activity was not detected.

Identification and analysis of Arabidopsis WRKY33-interacting proteins in plant defense

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Arabidopsis WRKY33 transcription factor plays an important role in plant resistance to necrotrophic fungal pathogens. It has been previously shown that WRKY33 interacts with MKS1, a MPK4 substrate that contains the conserved VQ motif. In the absence of pathogen infection, MPK4, MKS1 and WRKY33 are present as nuclear complexes. After infection with the bacterial pathogen *Pseudomonas syringae* or treatment with its flagellin, activated MPK4 phosphorylates MKS1 and releases the MKS1/WRKY33 complexes, allowing WRKY33 to target the promoter of *PAD3* and activates its expression. This MPK4-releasing-WRKY33 model is consistent with the opposite roles of MPK4 and WRKY33 in the regulation of *PAD3*. However, the *mpk4* and *wrky33* mutants share the common phenotype of hyper-susceptibility to necrotrophic pathogens, suggesting existence of additional mechanisms in WRKY33-mediated gene expression. Using yeast two hybrid screens, we have recently identified additional WRKY33-interacting proteins. Interestingly, like MKS1, the newly identified WRKY33-interacting proteins also contain the conserved VQ motif. Bimolecular fluorescence complementation assays confirm that WRKY33 interacts with the VQ motif-containing proteins in the nuclear compartment. Like *WRKY33*, genes encoding some of the interacting proteins are induced by necrotrophic pathogens. We have recently isolated T-DNA insertion mutants and constructed overexpression lines for some of the WRKY33-interacting proteins. Characterization of the mutant lines and overexpression lines is currently in progress and will be presented.

Study of a type VI secretion system (T6SS) in *Agrobacterium tumefaciens*

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The newly discovered T6SS is highly conserved in animal and plant-associated Proteobacteria with the accumulative data suggesting its importance in pathogenesis of many pathogens. Our previous secretome analysis has identified Hcp (Hemolysin-coregulated protein) that is secreted via a T6SS in plant pathogenic bacterium *Agrobacterium tumefaciens* (Wu *et al.*, 2008). By analyzing the in-frame deletion mutants in each of the genes encoded in this *t6ss* gene cluster, we identified a total of 16 genes (including *hcp*) that are essential for Hcp secretion. By biochemical fractionation and Western blotting in wild type and each of the mutants defective in Hcp secretion, we determined the cellular localizations of several essential T6SS components and the effects of each mutant in the protein accumulation of other T6SS components. We further characterize the IcmF- and IcmH-family proteins $ImpL_M$ and $ImpK_L$, the conserved T6SS components in several known functional T6SS. By topology and biochemical fractionation studies, both $ImpL_M$ and $ImpK_L$ appear as integral inner-membrane proteins with N-terminal domains facing the cytoplasm and C-terminal domains exposed to the periplasm. Protein–protein interaction and protein stability analyses indicated a physical interaction of $ImpL_M$ and $ImpK_L$. Mutants of $impL_M$ with substitution or deletion in the Walker A motif failed to complement the $impL_M$ deletion mutant in Hcp secretion, which provides evidence that $ImpL_M$ may bind and/or hydrolyze NTP to mediate Hcp secretion. In conclusion, the IcmF-family protein $ImpL_M$ interacts with $ImpK_L$ and may function as an NTPase for substrate translocation and/or assembly of T6SS machine, which is comprised of at least 16 T6SS protein components spanning both inner and outer membranes, in *A. tumefaciens*.

Perception and processing of bacteria-derived peptidoglycans in *Arabidopsis thaliana*

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The primary plant immune response can be triggered by recognition of several invariant structures of pathogens, so-called pathogen-associated molecular patterns (PAMPs). One example for such a PAMP is peptidoglycan (PGN), an essential component of bacterial cell walls. It could be shown that PGN induces typical plant defense reactions, such as medium alkalinization, transcription of defense-related genes and accumulation of callose deposits (Gust et al., 2007; Erbs et al., 2008). PGN is comprised of repeating disaccharide chains of N-acetylglucosamine and β-(1-4)-N-acetylmuramic acid that are cross-linked by short peptide bridges. Thus, PGN has a heteropolymeric structure and the resulting insolubility is utilized in its isolation and purification from bacteria. In order to gain new insights on the perception of peptidoglycan in *Arabidopsis*, we started to isolate it from the plant pathogen *Pseudomonas syringae* pv. *tomato* DC3000 (*Pto* DC3000). The *Pto* DC3000 PGN can be isolated in a highly pure manner and it activates defense responses in *Arabidopsis* similar to other tested peptidoglycans enabling us to search for the corresponding receptor. Furthermore, because of the complex structure of peptidoglycan it is tempting to speculate that processing of PGN and the release of smaller fragments by a plant enzyme takes place prior to perception. We have characterized a putative *Arabidopsis* PGN-hydrolase that shows high sequence similarity to a chitin and PGN hydrolyzing endochitinase from *Hevea brasiliensis*. The role of this putative PGN-hydrolase in the processing and perception of PGN in *Arabidopsis* will be discussed.

Comparative analysis of haustorial secretomes of rust fungi

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Obligate biotrophic fungi are characterized by a prolonged co-existence of host and parasite. Besides their devastating effects on many plants, a notable feature of these organisms is the formation of haustoria, specially differentiated hyphae, penetrating the host cells. It is suggested that haustoria are not only formed for translocation of nutrients but also for exchange of information. It is likely that so called effector proteins, secreted into the interface or even into the host cell itself, are involved in suppression of plant resistance. In order to identify potential effector proteins we set out to investigate the haustorial secretomes of different rust species. We were able to isolate haustoria of three different rust fungi, *Uromyces fabae*, *U. appendiculatus* and *Phakopsora pachyrhizi*, and established extraction of high quality RNA. Currently we are working on cDNA libraries of *U. appendiculatus* and *P. pachyrhizi* which will allow selection of cDNA fragments exhibiting a signal sequence. The selection will be done using the "yeast signal sequence trap", already established with *U. fabae*. This will enable us to compare different rust secretomes across species and might lead to the identification of novel effectors, essential for pathogenicity.

Role of glutaredoxin GRXS13, an early salicylic acid-responsive gene, on the basal and stress-induced cellular redox state in Arabidopsis plants

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Salicylic acid (SA) and reactive oxygen species (ROS) are key signals produced by plants subjected to biotic and abiotic stress conditions. These signals trigger the activation of sets of genes that orchestrate the stress defense response. Increasing evidence supports the idea that SA is crucial to control cellular redox homeostasis in stressed plants. Looking for clues to understand this role of SA, we identify the subset of SA-induced genes (SAIGs) that mediate early defense responses triggered by SA in Arabidopsis seedlings. One of these genes codes for a glutaredoxin (GRXS13) of nuclear and cytoplasmic localization. Mechanistic assays indicate that SA rapidly activates *GRXS13* transcription by a mechanism that partially requires NPR1 co-

activator and involves the subclass II of TGA transcription factors (TGA 2/5/6). This gene is also responsive to oxidative stress and to inoculation with the avirulent bacterium *Pseudomonas syringae* pv. tomato AvrRpm1. We have obtained Arabidopsis transgenic lines with different levels of *GRXS13* expression, by silencing and over-expressing the gene. Interestingly, lines over-expressing *GRXS13* show a more reductive basal redox state and less oxidative membrane damage induced by stress by high light radiation. These results suggest an important role for this gene in defense to oxidative stress. The function of this gene in the response to biotic and abiotic stress is currently being evaluated in our group. Supported by FONDECYT-CONICYT (grant Nº1060494) and Millennium Nucleus for Plant Functional Genomics (P06-009-F).

Alternaria genomics: Applications in plant and human health research

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The genus *Alternaria* as a whole has a dramatic impact on humans and human affairs, as it is associated with devastating plant diseases, mycotoxin contamination of food products, allergy, asthma, and opportunistic infection of immuno-suppressed patients. *A. brassicicola,* with a predicted genome size of 30 Mb, is the causal organism of black spot disease and is an economically important pathogen of Brassicas. Moreover, *A. brassicicola* is representative of a genus of pathogens that inflicts substantial damage worldwide, the necrotrophs. Importantly, *A. brassicicola*, which naturally infects cruciferous plants, has been used consistently as a necrotrophic pathogen of *Arabidopsis*. We have recently completed whole genome sequencing and annotation of the *A. brassicicola* genome and created a web-accessible genome annotation browser. In parallel, we have developed robust protocols and tools for molecular manipulation of the fungus (e.g. gene KO, overexpression, and protein fusion vectors). In this regard we have functionally analyzed approximately 150 genes in a search for novel plant virulence factors. In particular we have targeted kinases, transcription factors, putative secreted effectors, oxidative stress-associated genes and secondary metabolite biosynthetic genes and gene clusters. We have identified several novel virulence factors amongst each class of these genes. Lastly, we have used the *A. brassicicola* genome as a resource for identifying secreted proteins from the asthma and allergy associated fungus, *A. alternata,* that have pro-inflammatory activities towards human airway cells and in a mouse model of allergic airway inflammation.

Responses of _Arabidopsis thaliana_ to an infection and transformation with _Agrobacterium tumefaciens_

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Agrobacterium tumefaciens is a plant pathogen that causes disease by transferring and integrating bacterial DNA (T-DNA) into the plant genome. Despite its widespread use as biotechnological tool to transfer genes into plants, host responses to this pathogen are not well understood. Our studies focus on physiological changes and adaptations of *Arabidopsis thaliana* to an infection and transformation with *A. tumefaciens*. The profiles of metabolites and signaling molecules were compared with alterations in gene expression changes on a genome-wide level. Three different stages of interaction of *A. tumefaciens* with intact plants were analyzed: (i) Three hours after inoculation, (ii) six days after inoculation, when the T-DNA was integrated, and (iii) the fully developed crown gall. A comparison of host responses to an inoculation with the oncogenic strain C58 or the disarmed strain GV3101 allowed us to distinguish between signals derived from the bacterial pathogen on the one hand and the T-DNA on the other hand. Arabidopsis mutants with alterations in abiotic stress responses or pathogen defense reactions were used to study their impact on tumor development and to identify the defense pathway addressed by the microbe. Our data indicate that at early time points of infection the pathogen defense reactions are suppressed in the host plant, whereas after T-DNA integration and in fully developed tumors they are activated. From our observations we conclude that as soon as the oncogenes of the integrated T-DNA are expressed, the onset of crown gall development cannot be prevented any longer. But the extent of crown gall growth can still be restricted by the host. On the meeting we will present our recent findings on host responses to the T-DNA integration event by *A. tumefaciens*.

Destruction of the plant microtubule network by the bacterial phytopathogen, _Pseudomonas syringae_

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The eukaryotic cytoskeleton is essential for structural support and intracellular transport. Thus, manipulation of the cytoskeleton is a potent pathogenic mechanism utilized by animal pathogens to perturb multiple host pathways simultaneously. Despite the cytoskeleton playing a similar role in plant cells as well as being implicated in multiple facets of plant immunity, no phytopathogenic effector has yet been demonstrated to specifically target the plant cytoskeleton. Here we show that the *Pseudomonas syringae* type III secreted effector HopZ1a specifically interacts with tubulin heterodimers as well as polymerized microtubules. Furthermore, HopZ1a is an acetyltransferase and its enzymatic activity is activated by eukaryotic tubulin. Activated HopZ1a acetylates itself as well as tubulin, which leads to a dramatic decrease in the microtubule networks of *Arabidopsis thaliana* seedlings. HopZ1a-mediated cytoskeletal destruction may promote pathogen virulence by compromising host cellular integrity and dampening plant immune responses. Our results demonstrate that plant- and animal-pathogens share the cytoskeleton as a pathogenic target despite the architectural differences of their respective hosts.

Type III secretion and effectors in _P. syringae_ promote survival and growth on leaf surfaces

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Infection of plants by pathogens begins with a close association and communication between the organisms. The plant pathogen *P. syringae* pv. *syringae* B728a (*Psy*B728a) survives and/or multiplies on the leaf surfaces of host (*Nicotiana benthamiana*) and non-host (tomato) plants, a process called epiphytic growth. *Psy*B728a uses a type III secretion system (T3SS) to inject effector proteins into plant cells to facilitate the infection process. We report here three distinct behaviors of *Psy*B728a on host plants: first bacteria reside on leaf surfaces and survive without replicating (the adaptation phase), second they replicate on the surface and invade endophytic sites, third they form biofilm-like microcolonies on the surface. A T3SS was critical for the adaptation phase, as epiphytic survival of T3SS$^-$ bacteria on *N. benthamiana* was reduced prior to when wild-type bacteria replicate. An effector promoter-GFP reporter was induced in a subset of epiphytic bacteria during the adaptation phase. Activation of plant defenses caused a reduction of the epiphytic population on *N. benthamiana*. Interestingly, two effectors (HopZ3 and HopAA1) were recognized and restricted early epiphytic bacteria growth on *N. benthamiana* during the adaptation phase, whereas they promoted survival on tomato. These effectors localize to distinct sites in plant cells and likely have different mechanisms of action. HopZ3 may enzymatically modify host targets, as it requires residues important for the catalytic activity of other proteins in its family of proteases. Our results indicate that two effectors HopZ3 and HopAA1 are critical for regulation of epiphytic growth of *Psy*B728a and that activation of plant defenses also can restrict epiphytic bacteria.

Exploration of late blight quantitative resistance in potato diversity: QTL meta-analysis including the novel resistance sources _Solanum sparsipilum_ and _Solanum spegazzinii_

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The resurgence of late blight epidemics in potato (*Solanum tuberosum*) is due to the apparition of new *Phytophthora infestans* strains, resistant to chemicals and overcoming deployed R-genes. The alternative exploitation of polygenic resistance controlled by QTLs requires to inventory resistance sources and to get a better insight of the genetic architecture and diversity of quantitative resistance to late blight in potato. To find out novel resistance sources to late blight in the wild germplasm for potato breeding, we examined the polygenic resistance of *Solanum sparsipilum* and *Solanum spegazzinii* by a QTL analysis. The assessment of stem and foliage resistances made possible to identify 30 QTLs including a large-effect QTL region on chromosome X detected in both potato wild species. The mapping of literature-derived anchor markers suggested colinearities with published late blight QTLs or R-genes. We integrated the QTL results in a meta-analysis for late blight resistance in

potato together with the data of 19 other published studies. It consisted in constructing a consensus map of potato, on which we projected late blight resistance meta-QTLs. Results showed the resistance potential of *S. sparsipilum* and *S. spegazzinii* for late blight control, and highlighted some well-conserved QTLs in the potato related species. The relationships of late blight resistance meta-QTLs with R-genes and maturity QTLs were examined. [This work was supported by grants from the European BIOEXPLOIT project FOOD CT2005-513959.]

Normal 0 21 Functional characterisation of plant LysM-RLKs involved in the perception of bacterial symbiotic lipochitooligosaccharide signals

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Normal 0 21 The legume-rhizobia symbiosis is characterised by the ability of the bacteria to induce the morphogenesis of a new plant organ, the root nodule, in which they fix atmospheric nitrogen. The establishment of the symbiosis is initiated by rhizobial lipochitooligosaccharide signals called Nod Factors (NF). Genes involved in NF perception (*NFP* and *LYK3*) have been cloned in the model legume *Medicago truncatula*. These genes encode plant-specific transmembrane receptor-like kinases containing LysM motifs known to recognise NF related-oligosaccharides. *NFP* and *LYK3* were fused to coding sequences of various tags and stably expressed in *M. truncatula* roots and tobacco BY2 cells or transiently expressed in *Nicotiana benthamiana* leaves. NFP and LYK3 were found to be glycosylated *in planta* and LYK3 phosphorylated *in vitro*. We analysed the role of post-translational modifications in extracellular domains (disulfide bridges and N-glycosylation) or intracellular domains (phosphorylation) in NFP or LYK3 activities. The oligomerisation state of NFP and LYK3 was analysed using a two-hybrid split ubiquitin system in yeast and BiFC *in planta*. Using similar approaches, MtSYMREM, a soluble protein playing a role in rhizobial infection of *M. truncatula*, was found to interact and regulate stability of NFP and LYK3 at the plasma membrane in heterologous systems. In order to determine the function of LYK3 and NFP, whole BY2 cells, membrane extracts or purified proteins derived from plant tissues over-expressing the proteins, were used for NF factor binding assays with radiolabelled or photo-activatable modified NFs. This work is supported by the French Agence National de la Recherche (contract ANR-05-BLAN-0243-01 "NodBindsLysM") and the European Community (contract MRTN-CT-2006-035546 "Nodperception").

Evaluating the effect of tryptophan and phenylalanine supply on *S. scabiei* pathogenicity

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Members of the genus Streptomyces are mostly organisms present in soil as saprophytic bacteria. However, the plant pathogenic agent *Streptomyces scabiei* is the main causal agent of common scab, a disease causing severe economic losses for potato industry. Virulence of the bacterium mainly depends upon the production of a phytotoxin called thaxtomin A. This dipeptide is non-ribosomatically synthesized from nitrated tryptophan and phenylalanine. Interestingly, exogenous addition of these aromatic amino acids in liquid medium causes a drastic reduction of thaxtomin A biosynthesis. We evaluated severity of infection of *S. scabiei* in the presence of tryptophan or phenylalanine by inoculating radish, an alternative host to *S. scabiei*. Radish seedlings were grown in aseptic growth pouches containing water amended with tryptophan or phenylalanine and inoculated with *S. scabiei*. The presence of both aromatic amino acids reduced symptoms of infected radish roots when compared to seedlings grown in the absence of tryptophan or phenylalanine. Surprisingly, under certain conditions the presence of the aromatic precursors improved the growth of inoculated-seedlings compared to bacteria-free seedlings. Moreover, the plant growth hormone indole-3-acetic acid (IAA) can be synthesized by *S. scabiei* from tryptophan and was suspected to be involved in the growth improvement of inoculated hosts. These results suggest that the plant pathogen *S. scabiei* could under some specific conditions be considered as a plant growth promoting bacterium.

AtMYB30, a transcriptional regulator of the hypersensitive response, as a key component of a pro-cell death lipid signalling pathway?

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The molecular mechanisms underlying the Hypersensitive Response (HR), a form of programmed cell death commonly associated with plant disease resistance, are still poorly understood. *AtMYB30* which is a member of the plant MYB transcription factor family, has been identified as specifically, rapidly and transiently expressed during the very first steps of the HR in Arabidopsis. In addition, AtMYB30 has been shown to act as a positive regulator of the hypersensitive cell death programme (Vailleau *et al.*, 2002). By transcriptome analysis, together with molecular, genetic and biochemical studies, putative target genes of AtMYB30 have been shown to be involved in the very long chain fatty acid (VLCFA) biosynthesis pathway (Raffaele *et al.*, 2008). This suggests that AtMYB30 might modulate cell death-related lipid signaling by enhancing the synthesis of VLCFAs or VLCFA derivatives. In order to test this hypothesis, we have developed different approaches aimed at the identification of the putative lipid signals generated *via* the action of AtMYB30. These strategies aimed at (i) generating and phenotyping double mutants between *AtMYB30ox* and different lipid mutants, and (ii) identifying quantitative variations in lipids of plants subjected to pathogen attack and/or misexpressing AtMYB30 will be presented.

Quickly released peroxidase of moss (*Physcomitrella patens*) in defense against fungal invaders

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Mosses (*Bryophyta*) are non-vascular plants that constitute a large part of the photosynthesizing biomass and carbon storage on earth. Little is known about how this important portion of flora maintains its health status. This study assessed whether the moss, *Physcomitrella patens*, responds to treatment with chitosan, a fungal cell wall-derived compound inducing defense against fungal pathogens in vascular plants. Chitosan application to the liquid culture of moss caused a rapid increase in peroxidase activity in the medium. For identification of the peroxidase, MALDI-TOF/MS, other methods and the whole-genome sequence of *P. patens* were utilized. The peroxidase activity was due to a single secreted class III peroxidase designated as Prx34. The *Prx34* gene has two copies in the moss genome and was placed to a *P. patens*-specific phylogenetic cluster in an analysis that included the 45 putative class III peroxidases of *P. patens* and also those of *Arabidopsis* and rice. Induction of the peroxidase gene by chitosan treatment occurred later than the observed increase in peroxidase activity in culture medium, suggesting that Prx34 was released from storage in vacuoles and cell walls upon elicitation with chitosan. Both copies of *Prx34* were removed by targeted gene replacement. The knock-out line cultures did not show peroxidase activity upon chitosan treatment. Saprophytic and pathogenic fungi isolated from another moss killed the *Prx34* knock-out mutants but did not damage wild-type *P. patens*. The data of this study provided conclusive evidence that class III peroxidases in plants are involved in defense against hostile invasion by fungi. Results also singled out the first specific host factor that is pivotal for pathogen defense in a non-vascular plant.

Comparison of gene and protein expression profiles in leaves of susceptible and resistant *Eucalyptus grandis* exposed to *Puccinia psidii* Winter

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Eucalyptus is the most important arboreal genus for the Brazilian pulp and paper industries, producing around 11 million tonnes of cellulose pulp per year from 1.28 million hectares planted with eucalypt species (BRACELPA, 2006). Nevertheless, the production is affected by a number of diseases with one of the most important being eucalyptus rust caused by the neotropical rust fungus *Puccinia psidiii* Winter. To investigate and understand the molecular mechanism of response and interaction between *P. psidii* and *Eucalyptus*, SAGE libraries were made using resistant and susceptible individuals from segregating population. Forty and 70 genes were shown to be differentially

expressed in the susceptible and resistant libraries, respectively (Moon *et al*, 2007). We have developed a robust inoculation system under controlled conditions to follow the infection process and take samples at given time points after inoculation. We carried out more detailed expression analysis using qRT-PCR and identified genes induced in the presence of the pathogen and genes constitutively expressed. Proteomic approaches (2D-PAGE) were done to compare protein expression profiles in the same samples (with and without the presence the fungus) and now, we are undertaking more detailed analysis (trypsin digest and peptide samples sequenced using a Q-TOF API – Micromass- LC-MS/MS system) in an attempt to corroborate the gene expression data. Moreover we have also extracted proteins from the fungal spore before and after induced germination *in vitro* to look for proteins linked to the fungus penetration process. The data from the proteome and gene expression analyses will be compared in order to understand the molecular mechanism of the interaction between *P. psidii* and *E. grandis*.

Characterizing the *Arabidopsis thaliana* RPP13 resistance pathway using EMS mutagenesis

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Plant immunity is a complex web of resistance genes, signaling cascades, and cellular reprogramming. In *Arabidopsis thaliana* many of these resistance pathways depend on the downstream function of EDS1 or NDR1, either exclusively or additively, to mount a defense response to pathogen challenge. However, the *A. thaliana* CC-NBS-LRR resistance gene RPP13 confers race-specific resistance to the oomycete pathogen *Hyaloperonospora arabidopsis* independent of both these pathways. During infection, *H. arabidopsis* delivers the effector ATR13, whose presence is detected by RPP13, triggering a localized cell death and resistance response. In order to dissect this seemingly novel resistance pathway, we performed EMS mutagenesis on *Arabidopsis* transgenic lines containing both RPP13 and a dexamethasone-inducible ATR13 and screened for plants that survived after dex application. These survivors were further characterized by growth curve analysis using DC3000 delivering ATR13 via the type three secretion system. From this screen we have identified 36 putants from 23 different M2 pools. Using sequence analysis and semi-quantitative RT-PCR, we have shown several putants have non-mutated RPP13 expressed at the wildtype level. These results suggest our putants contain mutations in additional genes that are essential for RPP13-mediated resistance. We are currently attempting to identify these candidate genes using map-based cloning.

Quantitative proteomic analysis of *Gluconacetobacter diazotrophicus* interaction with sugarcane

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Gluconacetobacter diazotrophicus is a N_2-fixing bacterium found plants such as sugarcane and sweet potato. Its contributions to a profitable association with the host-plants include the induction of the synthesis of plant growth-promoting substances, and of a bacteriocin, whose activity inhibits growth of the sugarcane pathogen, *Xanthomonas albilineans*. To characterize the response of *G. diazotrophicus* to the presence of sugarcane plants two genotypes, SP-70 and Chunee, with distinct biological nitrogen fixation (BNF) abilities were used. A quantitative analysis of protein expression by the plants and bacterium was undertaken by using stable isotope ^{15}N protein labeling mass spectrometry. Sugarcane leafs and roots, bacterial cells separated from the plant culture media and secreted molecules were collected after 1 day of interaction. Proteins from the bacterium and plant tissues were separated by SDS-PAGE. Each gel was sliced into 10 segments and proteins in all samples were trypsin in-gel digested and then analyzed by Q-Tof mass spectrometry. We find that *G. diazotrophicus* differentially expressed regulatory proteins during interaction with SP70 (high BNF contribution), as an effort to adapt to the host environment. On the contrary, proteins involved in energy metabolism, are find upon interaction with the Chunee plant (low BNF contribution). Proteins up-regulated in SP70 plant in the presence of the bacterium are involved in growth and hormone-induced growth; those differentially expressed in Chunee have roles in bacterial recognition. These results showed the great potential of proteomics to describe events in the bacterium-plant interaction and showed for the first time that proteins from *G. diazotrophicus* and its plant-host can be selectively labeled in vivo.

Characterization of the elicitor structure of *Potexviruses* involved in the *Rx*-mediated resistance

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In nature, plants are continually challenged by various pathogens (bacteria, fungi, virus, nematodes…). Disease resistance in plants operates through multiple mechanisms to protect the host from potential pathogens. In particular, the interaction between a resistant host and an avirulent pathogen triggers a complex disease resistance response. The *Rx*-mediated resistance against *Potato virus X* (PVX) can be described in terms of such a gene-for-gene model (Flor 1971), in which the recognition of the viral elicitor, the PVX coat protein (CP) by the product of the *Rx* gene, a NBS-LRR (nucleotide binding site-leucine rich repeat) protein determines the outcome of the interaction (Bendahmane *et al.*, 1995). Previous studies have shown that the genetic background (potato versus *Nicotiana*) in which the *Rx* gene is expressed could influence the specificity or the intensity of the *Rx*-mediated response (Baurès *et al.*, 2008). Moreover the CP of 4 other *Potexviruses* (NMV, CymMV, WClMV and PepMV) were shown to induce the *Rx*-mediated resistance (Baurès *et al.*, 2008; Candresse *et al.*, submitted paper). Since the primary sequence of these different CPs reveals less than 40% of identity, the recognition is likely based on a particular structure rather than on specific amino acids. Our goal is to characterize the biochemical features of these elicitors. In a first step, a deletion study has allowed the identification of a minimal elicitor of 90 amino acids for the PVX, PepMV, NMV and WClMV CPs. In a second step, the study has been pursued for PVX and PepMV by the production of the minimal elicitors in heterologous expression system, from which these elicitor fragments will be purified in amounts suitable for structural physicochemical analysis by Dichroïsm, Fluorescence and NMR technologies.

Zinc-finger-nuclease-mediated resistance to plant viruses

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Zinc-finger nucleases (ZFNs) are artificial restriction enzymes capable of inducing site-specific breaks in double-stranded (ds) DNA genomes of various species. ZFNs have been used as a tool for genome targeting in plant and animal cells. ZFN-induced double-strand breaks are typically repaired by the plant's nonhomologous end joining DNA-repair mechanisms, leading to deletions/insertions and to site-specific mutagenesis at the repair site. We propose that ZFNs can also be used to battle extrachromosomal DNA fragments, such as those belonging to DNA viruses, by ZFN-mediated induction of site-specific mutagenesis or viral-genome degradation. We used the Bean dwarf mosaic virus (BDMV), an economically important Geminivirus, which is also capable of infecting and inducing disease symptoms in tobacco plants. BDMV is composed of genomic components DNA-A and DNA-B, and a pair of ZFNs was designed to target a specific sequence in the N-terminal region of the virus's AC1 replication gene (AC1 ZFNs). *In-vitro* digestion analysis, using *Escherichia coli*-expressed and purified ZFNs, demonstrated their activity against recombinant viral sequences. We validated the expression and activity of AC1 ZFNs in plant cells using an *in-planta* activity assay based on the reconstruction of a mutated *uidA* gene and monitoring for GUS expression. Overexpressing AC1-ZFN-transgenic tobacco plants were resistant to BDMV infection, as shown by the lack of disease symptoms and monitoring viral spread of the viral coat protein DNA by PCR analysis. Data on the development, validation and application of ZFN analysis for the purpose of restricting viral genome DNA movement in plant cells will be presented.

Arms race between the HopZ family of *Pseudomonas syringae* type III effector proteins and the *Arabidopsis* immune response: Recognition by a novel resistance protein, RAZ1, and targeting of host proteins

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The plant pathogen *Pseudomonas syringae* uses the type III secretion system to secrete and translocate effector proteins into the plant. The primary function of many of these effectors is believed to be the suppression of host defense

signaling; nevertheless, recognition of these effectors by resistance (R) proteins induces a defense response. The YopJ / HopZ family of effectors is a common and widely distributed class found in both animal and plant pathogenic bacteria. In previous work, we showed that the *P. syringae* HopZ family includes three major allele types (one ancestral and two brought in by horizontal gene transfer) whose diversification was driven by the host defense response (Ma et al., 2006). We further determined that virulence and defense induction phenotypes are strongly allele-specific (Lewis et al., 2008). In the current study, we screened a collection of R gene knockout lines to identify RAZ1 – the resistance protein responsible for HopZ1a-dependent immunity. RAZ1 is previously uncharacterized and functions independently of RIN4 and all other known R proteins. We also designed and performed a novel, high-throughput interactor screen using next-generation genomics technology to elucidate the RAZ1 resistance complex, the host targets of all the HopZ alleles, and the common interacting partners of RAZ1 and HopZ1a. This work addresses how differences in host target specificity within one family of type III effectors contributes to the host specificity in this important pathogen.

Resistance to *Botrytis cinerea* in wounded plant involves timely production of reactive oxygen species dependent on ABA

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Physical injury inflicted on living tissue makes it vulnerable to invasion by pathogens. Wounding of *Arabidopsis thaliana* leaves, however, does not conform to this concept and leads to immunity to *Botrytis cinerea*, the causal agent of grey mould. Many genes involved in the oxidoreductive metabolism were overexpressed in wounded plants in comparison to unwounded plant as observed by DNA microarray analysis. In fact, reactive oxygen species (ROS) accumulated within minutes after wounding. We are currently determining the importance of Atrboh-driven ROS accumulation for *Botrytis* infection. The relation between wounding, early ROS production and resistance was further tested in mutants. For example, variegated mutants did not produce the initial accumulation of ROS after wounding and lost the wound-induced resistance. Wound-induced defense to *Botrytis* was not dependent on salicylic acid, jasmonic acid or ethylene, but showed an interesting relationship towards abscisic acid (ABA). The mutants aba2 and aba3 were more resistant to *Botrytis* than the wild types, and produced an early accumulation of ROS after inoculation. More, after application of exogenous ABA, these mutants became susceptible as the untreated wild type plants and lost the wound-induced resistance. We hypothize that wound-induced resistance to Botrytis involves an early burst of ROS possibly under control of ABA.

Subcellular localization and role in plant defense of NADPH oxidase mediated ROS production

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Chemiluminescence detection of ROS triggered in tobacco BY-2 cells by the fungal elicitor cryptogein was previously demonstrated to be abolished in cells transformed with an antisense construct of the plasma membrane NADPH oxidase, NtrbohD. Here, using electron microscopy, it has been confirmed that the first hydrogen peroxide production occurring a few minutes after challenge of tobacco cells with cryptogein is plasma membrane located and NtrbohD-mediated. Furthermore, the presence of NtrbohD in Detergent Resistant Membrane fractions could be associated to the presence of NtrbohD-mediated hydrogen peroxide patches along the plasma membrane. Comparison of the subcellular localization of ROS in wild type tobacco and in plants transformed with antisense constructs of NtrbohD revealed that this enzyme is also responsible for the hydrogen peroxide production occurring at the plasma membrane after infiltration of tobacco leaves with cryptogein. Finally, the reactivity of wild type and transformed plants to the elicitor, and their resistance against the pathogenic oomycete Phytophthora parasitica were examined. NtrbohD-mediated hydrogen peroxide production does not seem determinant either for HR development or for the establishment of acquired resistance, but it is most likely involved in the signaling pathways associated with the protection of the plant cell.

Structure function analysis of BON1 and BAP1, two regulators of plant defense and programmed cell death

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The Arabidopsis BON1 and its functional partner BAP1 are negative regulators of plant defense responses and programmed cell death. BON1 has two C2 domains and one VWA domain while BAP1 has one C2 domain. Previous studies show that the VWA domain of BON1 is required and sufficient for BAP1 binding and the C2 domains of both proteins are capable of binding to phospholipids in Ca2+-dependent manner. Sequence analysis also predicts a myristoylation site at Gly2 in BON1. Here we show a structure-function analysis of BON1 and BAP1 to reveal the biochemical activities used for their biological functions. Mutations were made in BON1 and BAP1 to alter potential residues required for calcium binding, kinase activity, and myristoylation. In addition, scanning mutations were made in BAP1 to alter the conserved segments between BAP1 and its homolog BAP2. The mutant and wild-type BON1 and BAP1 genes were transformed to the bon1 and bap1 mutants respectively for complementation test. Mutations in some aspartate sites in the C2 domains and the myristoylation site in BON1 abolished BON1 function, indicating that the calcium-dependent lipid binding and myristoylation are essential for BON1 function. Mutations in BAP1 and the BON1 VWA domain that disrupt the BON1 and BAP1 interaction in the yeast two-hybrid assay were found to abolish their biological functions, indicating a full strength of physical interaction between BON1 and BAP1 is required for the BON1/BAP1 function. This structure- function analysis will enhance our understanding of the biochemical activities of these proteins and their roles in cell death and defense regulation.

The WRKY superfamily of transcription factors play an important role in plant defense and stress responses

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Plants are exposed to several biotic stresses in their environment. Induced defenses play a major role in plant disease resistance and are regulated by a network of interconnected signal transduction pathways with the plant hormones ethylene (ET), jasmonic acid (JA) and salicylic acid (SA) as crucial mediators. These specific hormone-mediated signaling cascades trigger distinct sets of defense-related genes leading to enhanced resistance to particular pathogens. In Arabidopsis, WRKY family of plant specific transcription factors (TFs) often involved in biotic stress signaling. WRKY TFs have been arranged in three distinct structural groups. Our earlier work defined WRKY70 as a central component mediating cross talk between SA and JA signaling in plant defense. Our research is focused on the contribution of WRKY54 and WRKY70 on gene expression and defense response in Arabidopsis. We examined the resistance of the wrky54 and wrky70 single as well as wrky54wrky70 double mutants to virulent bacterial pathogens, (E.c. carotovora and Pseudomonas syringae) and necrotrophic fungus (Botrytis and Sclerotinia). These data has suggested that the WRKY54 and WRKY70 directly contribute to bacterial disease resistance. In the other way, the compromised resistance to the necrotrophic fungus indicates that WRKY70 and WRKY54 might suppress the JA-induced defense. Oligonucleotide-microarrays have been used to study the target gene expression in mutants to investigate the WRKY70 and WRKY54 regulon. Moreover, we have also characterized the alterations in biotic and abiotic stress signaling using the WRKY single and the double mutants. Preliminary results suggest that downregulation of SA signaling promotes the abiotic stress responses for example to cold and drought.

Importance of ER quality control components for EFR function

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In PAMP-triggered immunity (PTI), plants detect pathogen-associated molecular pattern (PAMPs) via pattern-recognition receptors (PRRs). In Arabidopsis, the bacterial PAMPs flagellin and EF-Tu (or their surrogates peptides flg22 and elf18), are recognized by the leucine-rich repeat receptor kinase flagellin sensing 2 (FLS2) and EF-Tu receptor (EFR), respectively. However, the regulation and signalling pathways of these two receptors are poorly understood at the molecular level. To unravel EFR function, we performed forward genetic screens to identify Arabidopsis *elf18-insensitive* (*elfin*) mutants based on a seedling growth inhibition (SGI) assay. Notably, this screen revealed the importance of *UDP-glucose glycoprotein glucosyl transferase* (*UGGT)* and *calreticulin3* (*CRT3*) for EFR function. *Uggt* and *crt3* mutants were blocked in SGI, oxidative burst and MAP kinase activation upon elf18 elicitation. Intriguingly, *uggt* and *crt3* were however not impaired in FLS2-mediated responses, suggesting a differential requirement of these components for PRR function. C*rt3* mutants showed enhanced susceptibility to bacterial disease. UGGT and CRT3 are endoplasmic reticulum (ER) proteins involved in quality control (QC). Calreticulins (CRTs) bind

glucosylated N-glycans of nascent proteins in the lumen of the ER and participate in their folding. In case of incomplete folding, the protein can enter into a new folding cycle with CRT following its reglucosylation by UGGT. Consistently, we found that CRT3 is required for EFR protein accumulation. Our data demonstrate a physiological role for specific ER-QC components in controlling transmembrane receptors in plant innate immunity.

Analysis of proteome and oxidative responses of *Brassica napus* L. to *Sclerotinia sclerotiorum* and oxalic acid

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White mold (Sclerotinia stem rot) caused by the fungus *Sclerotinia sclerotiorum* is a serious disease of canola. Oxalic acid (OA) secreted by the pathogen has an important role in infection and disease progression. However, the molecular events associated with the canola response to *S. sclerotiorum* and OA are not completely understood. In this study, we performed a temporal investigation of the morphological and proteome-level changes taking place in *B. napus* as result of inoculation with *S. sclerotiorum* or treatment with OA. We identified 32 (12 up- and 20 down-regulated) and 37 (13 up- and 24 down-regulated) proteins whose abundance was altered in the *B. napus*/*S. sclerotiorum* and *B. napus*/OA interactions, respectively. These proteins were categorized into several groups including photosynthesis, protein processing, and redox homeostasis. Our morphological observations indicated that OA inhibits the oxidative burst, which is an early plant defense response. Based on the proteomic and morphological analyses, we investigated changes in transcript abundance and/or activities of proteins mediating oxidative stress responses in plants. Specifically, the activities of selected enzymes (catalase, peroxidase, and superoxide dismutase) and the transcript abundance of certain proteins (NADPH oxidase and oxalic acid oxidase) were validated in order to generate additional information regarding the observed suppression of the oxidative burst by OA. Our results may provide novel insights into plant defense responses to *S. sclerotiorum* and future, in-depth studies into the identified components may contribute to the development of integrated resistance to phytopathogens.

NADPH oxidases recruitment during Rhizobium infection

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Mutual recognition of legume plant hosts and Rhizobium bacteria is essential to establish a successful symbiotic relation. The exchange of biochemical signals appears to be crucial for both bacterial infection as well as the formation of the root nodular organ. The bacterial signalling molecules that set in motion these developmental changes are called Nod Factors (NFs). NFs are lipochito-oligosaccharides and able to trigger a specific signalling cascade, unravelled genetically, that ultimately leads to nodule formation without eliciting pronounced defence responses. Strikingly, one of both NF receptors - named LYK3 in Medicago - showed to be highly homologous to Chitin Elicitor Receptor Kinase (CERK) of Arabidopsis; a receptor that triggers innate immune responses upon perception of chitooligomers. We questioned whether during evolution a module of defence response genes that are transcriptionally activated upon chitin perception is also involved in NF induced symbiotic responses in legumes. Among the genes regulated by CERK NADPH oxidases are the most interesting because of their diverged functioning; including defense responses as well as root hair development. I studied the phylogeny of this family and identified legume specific diversification. Expression studies revealed that these genes are transcriptionally activated upon NF signalling. This together with promoter-reporter and protein localisation studies suggests a specific recruitment of this class of defense genes in NF induced signalling in legumes. This work is funded in part by the European Community's Sixth Framework Programme through a Marie Curie Research Training Network, contract MRTN-CT-2006-035546 "NODPERCEPTION".

Analysis of small GTPase OsRac1 activation in rice stably transformed with a FRET biosensor

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OsRac1 is a small GTP binding protein that alternates between the GTP-bound active form and GDP-bound inactive form. Previously we found that OsRac1 plays important role in plant defense by regulating metallothionein, mitogen-activated protein kinase 6 (MAPK6), cinnamoyl-CoA reductase, and NADPH oxidase and forming a complex with SGT1, Rar1, Hsp90 and RACK1A. In addition, OsRac1 is involved in pathogen-associated molecular pattern (PAMP)- and effector- triggered immunity against blast fungus, *Magnaporthe grisea*. However, the temporal dynamics of OsRac1 activation during PAMP- and pathogen effector- triggered signaling remain unknown. With the advent of fluorescence resonance energy transfer (FRET)-based biosensor, monitoring of the real-time activation of OsRac1 has become possible. We constructed protein termed Ras and interacting protein chimeric unit-Rac1 (Raichu-Rac1) that was designed to monitor the activation of OsRac1 in vivo and generated transgenic suspension cell cultures and plants that stably expressed Raichu-Rac1. Raichu-Rac1 consists of seCFP, CRIB domain of PAK1, OsRac1 and Venus. Upon activated, the binding of Rac1 to the CRIB domain of human PAK1 brings together the Venus and seCFP, allowing the occurrence of FRET. The expression of Raichu-Rac1 in the transgenic cells was confirmed by semi-quantitative RT-PCR and spectral imaging. The temporal dynamics of OsRac1 activation of these transgenic cell during immune responses are observed using FRET microscopy.

The stress-inducible promoter derived from defense-related gene *LsGRP1* of Oriental hybrid lily cv. Star Gazer confers effective gene expression in both monocot and dicot plants

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In plant breeding programs for disease resistance, a stress-inducible promoter is essential to avoid the cost of fitness. For the analysis of the mode of defense-related gene expression and the application in molecular breeding of disease resistance, a 868 base-pair promoter region of the salicylic acid and *Botrytis elliptica*-inducible gene, *LsGRP1*, was cloned with the adapter-primer method and named as P_{LsGRP1}. Agroinfiltration assay of transient expression of P_{LsGRP1}-driving β-glucuronidase gene in the leaves of *Nicotiana tabacum* W38 showed that β-glucuronidase activity was altered after the treatments of salt, heat, pathogen and salicylic acid. Expression analysis of P_{LsGRP1}-driving β-glucuronidase gene in the leaves of other plant species, such as lily, orchid, tobacco and arabidopsis by agroinfiltration revealed that P_{LsGRP1} has a high activity in both monocot and dicot plants, indicating that the stress-inducible P_{LsGRP1} has great potential as a tool of molecular breeding in different kinds of plants.

Molecular responses of the legume *Medicago truncatula* to wounding and caterpillar herbivory

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In plant-insect interactions, both plants and their insect herbivores have weapons against each other. Plants defend themselves against insect herbivores by recognizing factors in the insects' oral secretion or oviposition fluid. Insects, on the other hand, may interfere with these induced plant defenses. One strategy used by caterpillars to prevent plant defenses is believed to be the secretion of labial saliva which interferes with the induction of these defenses; however, the molecular mechanisms underlying this are unclear. We conducted a time course investigation of the molecular responses of the legume, *Medicago truncatula*, to four treatments: mechanical wounding, feeding by *Spodoptera exigua* caterpillars with or without impaired labial salivary secretions and undamaged controls. Transcript profiles of four genes, which encode a receptor-like protein kinase, ribulose-1, 5-bisphosphate carboxylase/oxygenase activase, a zinc ring finger protein and an unknown protein have been analyzed by quantitative real time-PCR.

Activation of an auxilin-like gene enhances Arabidopsis resistance to *Sclerotinia slcerotiorum*

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Sclerotinia sclerotiorum causes severe stem rot disease in many crops. To better understand mechanisms underlying its pathogenesis, we use Arabidopsis as a model plant to study the molecular plant-pathogen interaction through functional genomic approach. After screening 50,000 activation-tagged lines, we obtained an Arabidopsis mutant line showing enhanced resistance to *S. sclerotiorum*. Further genetic analysis showed that the phenotype with enhanced resistance in the mutant is caused by overexpression of a gene encoding an auxlin-like protein (ALP), which is also induced after inoculation in wild type plants. Both the activation-tagged mutants and ALP-overexpression mutants are hypersensitive to ABA, JA and salt, but not to ET. The genes known to be involved in JA and ABA signal

pathways such as *AtMYB2*, *AtMYC* and *RD22* are upregulated in the activation-tagged line. Our preliminary results suggest that the ALP enhances Arabidopsis resistance to *S. slcerotiorum* possibly through modulating ABA and JA signal pathways.

Pseudomonas syringae: Genome characterization and information management

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Significant advances are being made in characterization of the gene repertoire, functional properties, expression patterns, and genome structure of *Pseudomonas syringae*, with particular emphasis on those genes involved in the interaction of *P. syringae* pv *tomato* DC3000 (*Pto* DC3000) with its plant hosts. New sources of data include ongoing experimental characterization of the Hop effectors, confirmation of expression by transcript and peptide sequencing, identification of promoters and other extragenic features, and identification of inter-genomic variability associated with uncharacterized genes potentially involved in strain-specific phenotypes. However, effective application of genomic data to understanding the host-pathogen interaction requires that the vast amounts of information being generated are efficiently captured for ready access by interested researchers. To this end, *P. syringae* genome analyses are posted on the *Pseudomonas*-Plant Interaction (PPI) website (http://www.pseudomonas-syringae.org/), new information communicated to relevant outside databases, and strategies for systematic information capture developed. One such strategy involves development of Gene Ontology (GO) terms to describe the gene products critical to host interaction. To date, 46 hop genes and eight helpers have been comprehensively annotated with GO terms describing their cellular locations, molecular functions, and the biological processes in which they engage. This information together with supporting evidence and interacting host products and processes can be accessed through the PPI website. Efforts are also being directed toward development of an enhanced format for genome annotation records at NCBI so as to better accommodate the full range of features being characterized.

Construction and analysis of a wheat leaf rust, _Puccinia triticina,_ EST database

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We have assembled an EST library and database for the obligate wheat pathogen *Puccinia triticina* (wheat leaf rust). 45000 ESTs were sequenced from a variety of asexual life cycle stages such as urediniospores, germ tubes, appressoria, *in-planta* infection mycelia, isolated haustoria, and teliospore structures. In addition, sequences were sampled from the sexual cycle stages including pycnio- and aecio-spores arising during infection of the alternate host *Thalictrum speciosissimum* (meadow rue). Processing and assembly resulted in approximately 13000 non-redundant sequences. BLAST searches indicated that roughly 55% of these sequences had homologies in various databases (est_others, NR, fungal; $e \leq 10^{-5}$) while 31% were similar to genes of known function ($e \leq 10^{-5}$). Comparison to the set of 20657 predicted *P. graminis tritici* proteins resulted in 2838 matches ($e \leq 10^{-5}$), whereas 3116 were homologous to genomic sequences ($e \leq 10^{-5}$). This resource is currently being employed for gene discovery and expression analysis. We isolated a leaf rust mitogen-activated protein kinase which was shown to allow complementation of the deleted homolog in the related fungus *Ustilago maydis* (Hu et al., MPMI 20:637, 2007). Such a heterologous expression system allows functional analysis of rust genes which is difficult in the intransigent rust system. Sets of sequences from the various life cycle stages have been mined for transcripts unique to a given stage or group of stages. Cursory levels of gene expression have been monitored via frequencies of redundant input sequences. This EST database is also an important component in the ongoing *P. triticina* genome project where EST sequences will be mapped to their positions on the forthcoming genome assemblies (see poster by Bakkeren).

A polygalacturonase-inhibiting protein (PGIP) from _Citrus_ spp. inhibits the enzymatic activity of an endopolygalacturonase from the oomycetous pathogen _Phytophthora parasitica_

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Our previous study has discovered a multigene family (*pppg1-pppg10*) encoding endopolygalacturonase (endoPG) in *Phytophthora parasitica*, an oomycetous plant pathogen known to cause severe disease in a wide variety of plant species. Further analysis indicated that expression of some of these genes is induced in the process of plant infection, suggestive of their importance in the pathogenesis of *P. parasitica*. To find out if polygalacturonase-inhibiting proteins (PGIP) contain an inhibitor activity for endoPGs from *P. parasitica*, we cloned PGIP genes from two *Citrus* species. Nucleotide sequences of PGIPs obtained from these *Citrus* spp. appeared to be identical, and we named one of these clones *cgpgip*. Southern hybridization using *cgpgip* as a probe revealed the presence of a single copy gene in *C. grandis*, while two bands showed up in *C. sinensis*. Analysis of the deduced amino acid sequence indicated that *cgpgip* encodes a protein of 327 amino acid residues, with a predicted molecular mass of 36.1 kD and a pI of 8.28. Moreover, it contains a signal peptide in the N-terminus, the leucine rich repeat (LRR) domain, and six potential *N-glycosylation* sites. Phylogenetic analysis indicated that PGIPs from *Citrus* spp. were clustered into a group distinct from PGIPs of *Phaselous* spp., which are well characterized and known to have an inhibitor activity toward endoPGs of fungi. To characterize the function of *cgpgip*, recombinant proteins of *cgpgip* and some *pppgs* were expressed by using a yeast expression system, and used to analyze the inhibitor activity of *cgpgip*. The results indicated that the recombinant protein of *cgpgip* contained an inhibitor activity toward *pppg2*. We will continue to investigate the role of PGIPs in plant defense against *Phytophthora* pathogen.

RIN4 functions with plasma membrane H^+-ATPases to regulate stomatal apertures during pathogen attack

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Pathogen perception by the plant innate immune system is of central importance to plant survival and productivity. The *Arabidopsis* protein RIN4 is a negative regulator of plant immunity. In order to identify additional proteins involved in RIN4-mediated immune signal transduction, we purified components of the RIN4 protein complex. We identified six novel proteins that had not previously been implicated in RIN4 signaling, including the plasma membrane (PM) H^+-ATPases AHA1 and/or AHA2. RIN4 interacts with AHA1 and AHA2 both *in vitro* and *in vivo*. *RIN4* overexpression and knockout lines exhibit differential PM H^+-ATPase activity. PM H^+-ATPase activation induces stomatal opening, enabling bacteria to gain entry into the plant leaf; inactivation induces stomatal closure thus restricting bacterial invasion. The *rin4* knockout line exhibited reduced PM H^+-ATPase activity and, importantly, its stomata could not be re-opened by virulent *Pseudomonas syringae*. We also demonstrate that RIN4 is expressed in guard cells, highlighting the importance of this cell type in innate immunity. These results indicate that the *Arabidopsis* protein RIN4 functions with the PM H^+-ATPase to regulate stomatal apertures, inhibiting the entry of bacterial pathogens into the plant leaf during infection.

The application of Mach-Zehnder transmission-light interference microscopy as a tool for analysis of _Phakopsora pachyrhizi_ (Asian soybean rust) infection structures

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Mach-Zehnder transmission-light interference microscopy allows sophisticated observation of living cells without the draw-backs of fixation or staining. By measuring optical path differences the dry mass and water content of cells can be determined with extreme accuracy using the refractive index increment. This method, originally developed in the 1960s, has been used in forensic medicine did not achieve wide acceptance because of the complexity of the data analysis and the need for purpose built microscopes. Recent refinement of the method using software assisted analysis, has again drawn attention to Mach-Zehnder interference microscopy. Here, we present the first application of this method in a phytopathological context. Specifically, we are interested in the infection process of the Asian soybean rust fungus, *Phakopsora pachyrhizi.* Germinating Uredospores penetrate the epidermal host cell directly, which is uncommon among the majority of rust fungi where penetration via stomata is the rule. Using Mach-Zehnder-Interferometry we estimated the osmotic potential (ψ_s) in *P. pachyrhizi*

appressoria which supports the hypothesis that the mechanical force generated by the appressoria would be sufficient to facilitate penetration into host epidermal cells.

Monitoring barley root endophytic colonization by the potential biocontrol agents *Fusarium equiseti* and *Pochonia chlamydosporia*

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Fusarium equiseti and *Pochonia chlamydosporia* are two soil-inhabiting fungal species which colonize plant roots with promising properties for biological control of fungal root pathogens and nematodes. Furthermore, *P. chlamydosporia* promotes plant growth. Root endophytism would supply a stable source of inoculum to sustain the populations of potential biocontrol organisms in the rhizospheric soil. In this work, new tools were developed to study the endophytic development of *F. equiseti* and *P. chlamydosporia* in barley roots. These were applied to monitor host colonization patterns by both species. Molecular beacons for both species were designed and used in Real time-PCR quantification of fungal populations in roots. Genetic transformation of isolates with the GFP gene was carried out by an *Agrobacterium*-mediated transformation protocol, and spatial patterns of root colonization were investigated by Laser confocal microscopy. Quantification of endophytes by real time-PCR in roots of barley gave similar results for both fungi, and were more accurate than culturing methods. Conversely, monitoring of root colonization by GFP-expressing transformants showed differences in the endophytic behavior of both species, and provided evidence of modulation of plant responses to endophytic colonization. Both *F. equiseti* and *P. chlamydosporia* colonized endophytically barley roots, in spite of host defenses. These results support the hypothesis of a balanced antagonism between the virulence of the colonizing endophyte and the plant defense response. Development of real time-PCR techniques and GFP-transformants of these fungal species will facilitate future work to determine the role of endophytism in their biocontrol capacities.

Induction of novel defense metabolites in tomato plants by the bacterial pathogen *Pseudomonas syringae*

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Because plants cannot escape from their potential stress agents they have developed a staggering and sophisticated battery of defense mechanisms against microbial attacks. Among them, synthesis of natural antimicrobial phenylpropanoid products is a common response in both compatible and incompatible plant-microbe interactions. The role of some of these metabolites in plant disease resistance has been well-established in mutant and transgenic plants with altered expression of phenylpropanoid genes or modified levels of phenylpropanoid metabolites. The main objective of this work is to identify metabolites that potentially play a role in tomato plant defense. For this purpose, we have studied the synthesis of hydroxycinnamic acid amides (HCAA), and other metabolites from the phenylpropanoid pathway in tomato (*Solanum lycopersicum* cv. Rutgers) plants upon challenge with the bacterial pathogen *Pseudomonas syringae* pv. *tomato*. Here, we report the identification and characterization of several HCAA, together with the flavonoid rutin and chlorogenic acid, as being induced in Rutgers tomato plants upon exposure to *P. syringae*. Their structural elucidation has been carried out using MS-HPLC and ^{1}H-NMR techniques as well as organic synthesis. In this plant-pathogen interaction, the hydroxycinnamic acids, p-coumaric and ferulic, are conjugated to β-phenylethylamine-alkaloids tyramine, dopamine, octopamine, and noradrenaline forming the corresponding HCAA. We identify feruloylnoradrenaline and feruloyloctopamine as physiological compounds present in tomato for the first time. The synthesized amides were tested for their antioxidant activity using *in vitro* studies. Rutin and chlorogenic acid were found to be good inducers of pathogenesis-related defense genes in tomato.

Transcriptional and hormonal profiles of tomato plants colonized by arbuscular mycorrhizal fungi

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Arbuscular mycorrhizal (AM) symbioses are mutualisitc associations between soil fungi and most vascular plants. As a result of the symbiosis, AM fungi improves plant fitness by providing plants with water and essential nutrients, allowing the host plant to cope with both biotic and abiotic stresses (Harrison, 2005). On the other hand, AM funi depend on the carbon provided by their plant host to complete their life cycle. During AM establishment a fine-tuned communication between the host plant and fungal partners is required for full functionality. This communication is very complex and involves signaling by several phytohormones such as jasmonates, ethylene, auxin, abscisic acid and a new class of phytohormones called strigolactones (Bouwmeester et al., 2007; Hause et al., 2007; Herrera-Medina et al., 2007). Transcript and hormone profiling of tomato plants colonized by the AM fungi Glomus mosseae and G. intraradices revealed that mycorrhization implies important changes both in the roots and the shoots, affecting key elements in signaling. Our results provide evidences of the requirement of new hormones in the roots for a successful mycorrhizal establishment. In particular, systemin - a peptidic hormone - appears to play a key role in the AM symbiosis. Supporting this finding, tomato plants silenced in systemin (McGurl et al., 1992) are unable to establish AM associations. The relationship of systemin with other phytohormones such as jasmonates and strigolactones is analyzed. Finally, the potential implication of these hormonal changes onMycorrhiza Induced Resistance will be discussed. ArArbuscular mycorrhizal (AM) symbioses are mutualisitc associations between soil fungi and most vascular plants. As a result of the symbiosis, AM fungi improves plant fitness by providing plants with water and essential nutrients, allowing the host plant to cope with both biotic and abiotic stresses (Harrison, 2005). On the other hand, AM funi depend on the carbon provided by their plant host to complete their life cycle. During AM establishment a fine-tuned communication between the host plant and fungal partners is required for full functionality. This communication is very complex and involves signaling by several phytohormones such as jasmonates, ethylene, auxin, abscisic acid and a new class of phytohormones called strigolactones (Bouwmeester et al., 2007; Hause et al., 2007; Herrera-Medina et al., 2007). Transcript and hormone profiling of tomato plants colonized by the AM fungi Glomus mosseae and G. intraradices revealed that mycorrhization implies important changes both in the roots and the shoots, affecting key elements in signaling. Our results provide evidences of the requirement of new hormones in the roots for a successful mycorrhizal establishment. In particular, systemin - a peptidic hormone - appears to play a key role in the AM symbiosis. Supporting this finding, tomato plants silenced in systemin (McGurl et al., 1992) are unable to establish AM associations. The relationship of systemin with other phytohormones such as jasmonates and strigolactones is analyzed. Finally, the potential implication of these hormonal changes onMycorrhiza Induced Resistance will be discussed.

Control of type III secretion in the plant pathogen *Xanthomonas campestris* pv. *vesicatoria*

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The Gram-negative bacterial plant pathogen *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) is the causal agent of bacterial spot disease on pepper and tomato. Pathogenicity depends on a type III secretion (T3S) system, which translocates bacterial effector proteins into the eukaryotic host cell. The T3S system is a highly complex protein transport machine that spans both bacterial membranes and is associated with an extracellular pilus and a predicted channel-like translocon in the plant plasma membrane. Efficient secretion of at least eleven effector proteins requires the cytoplasmic control protein HpaB. HpaB acts as a global T3S chaperone and guides effector proteins to the ATPase HrcN, which is associated with the secretion apparatus and dissociates effector-HpaB complexes in an ATP-dependent manner. The contribution of HpaB to effector protein secretion is presumably regulated by the virulence factor HpaA, which is secreted by the T3S system and translocated into the plant cell. HpaA promotes the secretion of pilus, translocon and effector proteins and therefore acts as a general control protein of the T3S system. Experimental evidence suggests that binding of HpaA to HpaB within the bacterial cell favours secretion of pilus and translocon proteins.

Green peach aphid-*Arabidopsis thaliana* interaction

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Green peach aphid [GPA; *Myzus persicae* (Sülzer)], the most polyphagous aphid, utilizes its slender stylet to feed continuously from sieve elements. The model system consisting of *Arabidopsis thaliana* and GPA allows us to

characterize the interaction between GPA and its plant host. Previously, we had demonstrated that the Arabidopsis *PHYTOALEXIN DEFICIENT4* (*PAD4*) gene, which exhibits homology to acyl hydrolases, is an important modulator of antixenosis (feeding deterrence) and antibiosis (affect aphid fecundity) against GPA (Pegadaraju et al. 2005, 2007). PAD4-dependent resistance against GPA was accompanied by elevated expression of the *SENESCENCE ASSOCIATED GENES* (*SAG*). The PAD4 protein contains the conserved Ser, Asp and His residues that form the catalytic triad of many α/ß fold acyl hydrolases. Arabidopsis plants expressing mutant versions of PAD4 [PAD4(S118A) and PAD4(D178A)] supported higher numbers of GPA as compared to wild type (WT) plants in no-choice tests. However, PAD4(S118A) and PAD4(D178A) mutations did not impact antixenosis activity in choice tests and the PAD4-modulated expression of *SAG* in GPA infested plants, suggesting that the antixenosis and antibiosis activity of PAD4 are determined by separate regions of PAD4. The *MPL1* (*MYZUS PERSICAE INDUCED LIPASE1*) gene is another critical component of Arabidopsis defense against GPA. Like PAD4, *MPL1* expression is induced in response to GPA infestation and MPL1 contains the conserved Ser-Asp-His residues found in α/ß fold acyl hydrolases. However, unlike PAD4, MPL1 is not required for antixenosis. MPL1 is only required for antibiosis against GPA. Since, *MPL1* encodes a bona-fide lipase we propose that a lipid(s), or a product thereof, is involved in Arabidopsis antibiosis to GPA.

Horizontal transfer of genomic island PPHGI-1 between races of *Pseudomonas syringae* pv. *phaseolicola*

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PPHGI-1 is a 106kb genomic island present in the bean pathogen *Pseudomonas syringae* pv. *phaseolicola* (*Pph*) strain 1302A. Its loss is driven by exposure to the stress imposed by the plant's resistance reaction, the hypersensitive response (HR). We now show that this genomic island is able to transfer horizontally *in planta* between strains of *Pph* by transformation after co-inoculation. Transformation occurs at high frequency in the resistant host, bean cultivar Tendergreen (TG) but is reduced in susceptible tissues. Transfer has also been achieved in the non-host plants, Tobacco and Arabidopsis. Limited transformation has been observed *in vitro*, under various conditions including at very low temperatures and in apoplastic fluid, the highest frequency being observed in apoplastic fluid extracted from TG leaves undergoing the HR. Our results suggest that both excision of the island and competence for transformation are enhanced within the microenvironment of the plant compared to culture. Transfer of PPHGI-1 leads to a change in virulence of the recipient strain and could have other implications to its fitness as PPHGI-1 contains gene clusters encoding type IV pili, photosensory and chemotactic proteins and enzymes involved in DNA repair and recombination. Our findings show that pathogen evolution can occur by the simplest process of horizontal gene transfer and is particularly prevalent during host defence when the pathogen is in greatest need to acquire potentially new genetic traits to alleviate the antimicrobial stress imposed by the resistant host.

A nematode venom allergen protein interacts with a cathepsin-like cysteine protease in the host and is required for plant parasitism

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Animal and plant parasitic nematodes have the capability to remain within the host for a long time. To do so, they have evolved immunoevasive and immunosuppressive strategies. Secretory proteins produced in the esophageal glands of parasitic nematodes likely include suppressors of plant innate immunity. A venom allergen protein from *Globodera rostochiensis* (Gr-vap1) was identified, by cDNA-AFLP, as being strongly up-regulated in invasive J2s. In situ hybridization microscopy showed specific expression of Gr-vap1 in the subventral esophageal glands. Gr-vap1 codes for a secretory protein, including a single SCP/CAP domain. Temporal expression analysis of Gr-vap1 in different developmental stages revealed up-regulation in the motile J2s and adult males. Knocking-down Gr-vap1 expression, by RNA interference, significantly reduced the infectivity of nematodes on host plants. Protein interaction studies using Gr-vap1 and a tomato root cDNA library, in a yeast-two-hybrid screening, resulted in the identification of various interacting host proteins associated with plant immunity. A pull-down assay confirmed the physical interaction of Gr-vap1 with Rcr3, an extracellular cathepsin-like cysteine protease from tomato. Others have shown that Rrc3 is required for disease resistance to fungi and oomycetes in plants. However, heterologous expression of nematode VAPs in *Arabidopsis thaliana* causes enhanced susceptibility towards diverse plant pathogens. We hypothesize that VAPs are important modulators of innate immunity and as such interfere with different host defense response pathways.

Identification of ToxA-interacting proteins suggests a possible role for pathogenesis-related protein 1 (PR-1) in mediating *Stagonospora nodorum*-wheat interactions

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ToxA is a proteinaceous host-selective toxin produced by the wheat fungal pathogens *Stagonospora nodorum* and *Pyrenophora tritici-repentis*. Sensitivity to the toxin and susceptibility to the fungus are both controlled by a single dominant gene (*Tsn1*) in the host. Map-based cloning has revealed that *Tsn1* encodes a protein belonging to the NBS-LRR family of plant disease resistance proteins (Faris et al., unpublished). The pathway(s) connecting activities of ToxA and *Tsn1* is still unknown although it has been reported that ToxA may interact with two unrelated chloroplastic proteins (Manning et al., 2007; Tai et al., 2007). By using the yeast two-hybrid system, we have identified two additional ToxA-interacting proteins (TaPR-1a-1 and TaPR-1a-2) that are members of the pathogenesis-related protein 1 family known to be extracellularly located and associated with hypersensitive response/disease resistance pathways. TaPR-1a-1 and a-2 proteins are highly similar (89% identity) and both contain the conserved SCP-like extracellular protein domain and an N-terminal signal peptide. Preliminary experiments indicate that TaPR-1a-1 and a-2 are expressed in uninfected wheat with expression levels differing between susceptible and resistant lines. More interestingly, in the early stages (12/24h after inoculation) of infection by a ToxA-producing isolate, TaPR-1a-1 and a-2 appear to be up-regulated in susceptible wheat but not in resistant wheat. These findings raise the possibility that PR-1a-1 and a-2 may play important roles in mediating host-pathogen interactions by binding directly to ToxA. The characterization of ToxA-PR-1a-1/2 interactions and downstream pathways will help us answer the question of how a plant disease resistance gene like *Tsn1* governs disease susceptibility in the *S. nodorum*-wheat interaction.

Nucleotide binding and intra- and inter-molecular interactions in R proteins

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Resistance proteins (R) are involved in pathogen recognition and subsequent initiation of defence responses aimed at restriction of pathogen growth. We focus on three R proteins: Mi-1 conferring resistance to Meloidogyne incognita, aphid and white fly, and Rx and I-2 conferring potato virus X and Fusarium oxysporum resistance, respectively. These R proteins belong to the nucleotide binding site-leucine rich repeat (NB-LRR) family. Our previous research supports a model for a switch function of NB-LRR proteins, in which their activity is regulated by their nucleotide binding state. We are interested in three aspects of the proposed model: i) nucleotide binding and hydrolysis, ii) intra- and iii) intermolecular interactions. For the first aspect heterologously produced R proteins, and various mutants, are analysed for their ATPase activity and nucleotide binding in vitro. To study intramolecular interactions, trans-complementation of the Mi-1 N-terminus with the rest of the R protein was investigated using Agrotransformation. The physical interactions between both domains are examined by co-immunoprecipitation. To get insight in dynamics of these interactions, different mutants imitating various stages of Mi-1 activation are included. Alongside, the composition of R proteins signalosomes is studied by analysing the interaction with putative I-2 interacting partners identified with yeast two-hybrid. Functional involvement of these proteins in I-2 mediated defence is analysed using stable silenced tomato lines.

The *Sinorhizobium meliloti* symbiosis regulator ExoR is a target of periplasmic proteolysis

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Sinorhizobium meliloti ExoR regulates the production of succinoglycan and flagella through its interactions with ExoS, the sensor of the ExoS/ChvI two-

component regulatory system, which is important for the *S. meliloti*-alfalfa symbiosis. It has been proposed that the ExoR-ExoS interaction suppresses ExoS so that the disassociation of ExoR from ExoS should lead to ExoS activation. To better understand the molecular mechanism that regulates the reversible ExoR-ExoS interaction, we have expressed and purified ExoR to generate ExoR specific polyclonal antibodies for characterizing ExoR in the wild type strain Rm1021, the *exoR95* mutant, and the newly constructed *exoR108* mutant. In addition to the previously identified precursor and mature forms of ExoR, we discovered an 18 KD form of ExoR. By analyzing tagged ExoR, we showed that the 18 KD ExoR is derived from the proteolysis of the mature form of ExoR in the periplasm. This proteolysis can be disrupted by a single amino acid change in the region of the digestion and by the change of C-terminus in the ExoR95 protein. Altogether, our findings suggest that the ability of ExoR to suppress ExoS through the formation of ExoR-ExoS complex is regulated by the proteolysis of the mature form of ExoR in the periplasm.

Genetic and diversity of sugarcane associated *Burkholderia* and characterization of gene related to biological control of *Pectobacterium carotovora* and bacteriocin production

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The genetic and physiological diversity of endophytic and rhizobacteria *Burkholderia* spp from sugarcane plants grown in Brazilian fields were evaluated and a mutant library was obtained to study the genes related to biological control of *Pectobacterium carotovora* and bacteriocin production. The study of this microbial diversity may be an important alternative to improve the performance and sustainability of these crops, since the physiological characteristics are related to the plant growth promotion. The sequences analyses of the 16S rRNA and *gyr*B genes demonstrated the main affiliation of isolates to the *Burkholderia cepacia* complex. Although the clear separation driven by niche occupation was not observed, this work demonstrates the broad association of this group with sugarcane, and highlights the possible applications that these bacteria, which can nitrogen fixation, production of siderophore, solubilization of phosphor, produce IAA and inhibit fungal pathogens, in a way to achieve a more sustainable cultivation of this crop. These *Burkholderia* spp. were characterized as excellent bacteriocin producers and also, we observed that some strains is able to control *P. carotovora* symptoms in *Orchids.* We observed that this control is not due the pathogen inhibition, since these *Burkholderia* were not able to inhibit this pathogen *in vitro.* Therefore, we used random mutation by transposon insertion to obtain a mutants library, from which we are screening mutants defective for biological control and bacteriocin production.

Molecular characterization of AVR2 function of *Fusarium oxysporum* f. sp. *lycopersici*

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Many plant pathogens secret effector proteins to modulate plant defense and promote host colonization. The Effector Avr2 of *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) has been identified by a proteomics approach in xylem sap of infected tomato plants. The Avr2 and I-2 pair represents the first complete gene-for-gene pair from a plant and a xylem invading fungal pathogen. Avr2 carries virulent and avirulent functions on susceptible and resistant plants, respectively. Point mutations in Avr2, resulting in single amino acid changes, prevent its recognition by *I-2* when both genes are co-expressed in leaves of *Nicotiana benthamiana*. Although the hypersensitive response (HR) is not observed following natural *Fol* infection of *I-2* tomato plants, HR was observed in stems and leaves, after PVX mediated expression of Avr2 in *I-2* tomato plants. AsAvr2 is expressed without signal peptide, and hence remains localized inside the plant cell when expressed in *Nicotiana benthamiana* leaves, this indicates that Avr2 activates I-2 intracellularly. Because *Fol* secretes Avr2 into tomato xylem vessels, and Avr2 functions inside cells, this implies uptake of Avr2 by the host cells.

A rapid bioassay to evaluate virulence of *Clavibacter michiganensis* subsp. *michiganensis* in tomato leaves

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Clavibacter michiganensis subsp. *michiganensis* (Cmm) is an important bacterial pathogen of tomato, on which it causes bacterial wilt and canker. Under greenhouse conditons, it may take several weeks after inoculation of Cmm on tomato plants before disease symptoms develop and this makes it difficult to screen Cmm strains for virulence on tomato plants. Here we report an easy and rapid bioassay to evaluate virulence of Cmm in tomato leaves. Tomato (cv. Trust F1) plants were grown under greenhouse conditions (22/18°C (d/n) temperature, 16/8 h photoperiod and 75% RH). For bioassay studies, leaflets of mature leaves were detached and pressure infiltrated (15-20 µL per spot of 10 fold dilution of 48 h culture) with the two strains of Cmm using a 1 mL syringe. The leaflets were placed in Petri plates containing moist filter paper, wrapped with parafilm and incubated at room temperature. The leaflets were observed regularly for disease symptom development. When the symptoms were clear, leaflet sections (away from the infection site) were removed, and Cmm were isolated using NBYA and semi-selective medium (SCM). When the isolated Cmm when pressure infiltrated into *Mirabilis jalapa* leaves, they caused a hypersensitive reaction within 48 h. Our results show that Cmm 930 is more virulent than Cmm ATCC7629. Our results also demonstrate that the detached tomato leaf bioassay is a rapid method to evaluate virulence of Cmm strains.

Competitive index analysis in *Pseudomonas syringae* pv. *phaseolicola* reveals virulence attenuation for many type III effector mutants

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Pseudomonas syringae pv. *phaseolicola* is a gram-negative pathogen that depends on the Hrp type III secretion system (TTSS) to cause the halo blight disease in bean plants and trigger a hypersensitive response (HR) in incompatible hosts. The TTSS is responsible for the secretion and translocation of effector proteins into the plant cell, where they modulate different cellular processes. The specificity of the TTSS-mediated interaction is based on the set of delivered effectors translocated by each strain, called the secretome. In recent years, many effectors have been identified in the sequenced strain of *P. syringae* pv. *phaseolicola* 1448a (Pph 1448a), although the contribution to virulence of most of them remains unclear. Functional redundancy between type III effectors has been proposed as a factor hindering the virulence analysis of effector mutants, since the virulence phenotypes of most mutants are usually very slight or undetectable using regular virulence assays. We have previously shown that competitive index assays (CI), are a more sensitive and accurate method to detect differences in growth within the plant than standard assays, allowing detection of otherwise undetectable virulence phenotypes. Thus, we were able to detect virulence attenuation in tomato for a DC3000 *avrPto* mutant. In this work, we set the basis for the virulence analysis of Pph 1448a effector mutants at different stages of the infection process, using CI assays, after inoculation by either infiltration or dipping. We generate many Pph 1448a-derivative strains carrying mutations in type III effectors, and analyse their virulence in bean plants, demonstrating growth attenuation for many of them.

Expression analysis of *Sinorhizobium meliloti* protocatechuate transport and catabolic genes in alfalfa

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Aromatic acids accumulate in soil as originating primarily from plant-derived lignin, and are catabolized by saprophytic bacteria via the beta-ketoadipate pathway. Protocatechuate (PCA) catabolism appears to be universal in all species of rhizobia (*Rhizobium, Bradyrhizobium, Mesorhizobium, Sinorhizobium*) examined to date, suggesting the presence of selective pressures acting to maintain this phenotypic trait. Although PCA catabolism has been studied in the nitrogen-fixing rhizobia, these studies focus upon free-living cells, and there is little data relating to the role of *pca* genes in regards to plant-microbe symbioses. The alfalfa microsymbiont *Sinorhizobium meliloti* encodes PCA metabolic enzymes within two operons (*pcaDCHGB* and *pcaIJF*) and we have recently identified a PCA-inducible transport system that we infer is involved in the uptake of PCA. In this work, we examine whether the *pca* metabolic and transport genes are required for the establishment of nitrogen-fixing root nodules through inoculation of a host (*Medicago sativa*) with *pca* mutant strains of *S. meliloti*. As a means of monitoring *pca* expression *in planta*, we employed fusions to the reporter gene *gusA* and performed beta-glucuronidase assays upon mature and senescing root nodules. As well, we stained longitudinal sections of nodules for reporter enzyme activity to investigate whether expression was localized to particular regions of the nodules. Our results indicate a low level of *pca* gene

expression that is not required for the establishment of alfalfa nodules. We conclude that whereas PCA catabolism may be important to the ecological success of soil-inhabiting rhizobia, this metabolic pathway does not play an essential role in the plant-microbe symbiosis.

Induction of resistance and accessibility in the compatible and the incompatible rice-blast fungus interactions

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To study rice-blast fungus interactions *in planta* under confocal laser scanning microscopy, the transformants of *Magnaporthe oryzae* expressing enhanced green fluorescence protein (EGFP) and red fluorescence protein (Dsred2) were used. Several rice cultivars were inoculated with the transformants in the compatible (S) or incompatible (R) interactions. In S-interaction, invasive hyphae (IH) rapidly spread into epidermal cells of leaf sheath; 300–600 µm in length and 20–40 µm in depth at 48 hpi, 600–900 µm in length and 40–56 µm in depth at 72 hpi, respectively. In R-interaction, penetration was strongly inhibited, the growth of IH was limited in the attacked cell and the host cell was browned, granulated and clearly auto-fluorescenced under 460–490 nm wavelength excitation. When rice leaf sheaths were pre-inoculated with the incompatible race of *M. oryzae* 20 hrs prior to the challenging inoculation, invasive growth of the compatible race of *M. oryzae* was significantly suppressed. Strong auto-fluorescence on the cell wall together with hypersensitive reaction (HR) was observed in the cells after challenged with the compatible race, suggesting that resistance reaction was induced. This strong suppression and HR were limited around 500 µm from the site of pre-inoculation with the incompatible race and, moreover, RT-PCR analysis revealed strong expression of PBZ1/PR10 gene around the induced cells, implying that the resistance was induced locally rather then systemically. On the other hand, pre-inoculation of leaf sheath with the compatible race 20 hrs prior to the challenging inoculation suppressed resistance and allowed the incompatible race to infect the cells. However, induced accessibility to incompatible race was limited in the pre-inoculated or adjacent cells.

Probing nuclear activity of MLA immune receptors by ChIP-Seq

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The polymorphic barley MLA CC-NB-LRR-type proteins recognize isolate-specific *Blumeria graminis* f. sp. *hordei* effector proteins. The recognition of pathogen effectors induces nuclear associations between MLA10 and WRKY1/2 transcription factors (TFs) which act as repressors of MAMP-triggered defense responses. MLA appears to interfere with the WRKY repressor function, thereby de-repressing MAMP-triggered defense (Shen et al., 2007). However the mode of MLA function in the nucleus remains unknown. We hypothesize that MLA binds to specific chromatin sites containing the WRKY TF bound to DNA. To test this hypothesis, we have initiated a chromatin immunoprecipitation and sequencing (ChIP-Seq) approach to detect genome-wide protein DNA associations. Presumed DNA-MLA complexes were pulled-down from nuclear fractions of transgenic barley lines containing a single copy transgene expressing functional epitope-tagged MLA driven by their native promoters (Bieri et al., 2004). Samples were collected after powdery mildew conidiospore inoculation with virulent and avirulent isolates as well as from non-challenged plants. In total, 1.4 million of PCR-amplified co-immunoprecipitated DNA fragments were sequenced by 454 pyrosequencing technology, which cover 6% of the haploid barley genome. Repetitive sequences were excluded based on the *Triticeae* repeat sequence database followed by contig assembly of unique sequence reads. An in-house bioinformatics pipeline assembled 8,873 contigs from 345,479 unique reads and identified 366 contigs consisting of high coverage of sequences reads from pathogen challenged conditions. In addition to validation of the identified potential binding sites by ChIP-PCR, the expression profile of genes adjacent to the binding sites will be examined.

***Agrobacterium*-mediated transformation in *Fusarium* species as an alternative to control fungal diseases**

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Fusarium species are one of the most dangerous soil fungi which cause illness on plants. *Fusarium oxysporum* and *Fusarium solani* are phytopathogen, the former of pea, cucurbits and banana, the latter of tomato. All cultivars are economically important to Mexico. *F. solani* species are ubiquitous in soil and are decomposers of decaying plant material. *F. oxysporum* attacks root of plant making damage on internal tissues that leads to plant wilting. The method for controlling this phytopathogen is only chemical, but it causes environmental problems. An alternative could be the usage of *Agrobacterium*-mediated transformation in order to interrupt virulence genes or genes involved in sexual reproduction. *F. solani* sp. and *F. oxysporun* were isolated from the root of mamey (*Pouteria sapota*) and tomato respectively. The mycelium of this fungus was grown on *Aspergillus* minimal medium agar for three days. A loopful of this culture was taken and diluted in 9 ml of salt solution. Cocultivation was performed using 100µl of the fungus solution (it was equivalent to 350 viable colonies) mixed with 100µl of *Agrobacterium* (OD_{660nm} = 0.15), grown in minimal medium containing 5mM glucose with and without 200µM acetosyringone. We obtained 21 resistant colonies which showed that *F. solani* was transformed using *Agrobacterium tumefaciens* EHA101 with AS in the cocultivation medium for 24 h. The concentration of hygromicine used as selector was 400µg/ml. The use of cellulose filters was suitable for this protocol and the time of cocultivation (24 h) was enough in order to avoid an excessive growth of *F. solani.* This protocol was supported by PCR using 35S and TNOS primers. We could obtain an easy protocol for transforming *F. solani* and *F. oxysporum,* which is a powerful tool in functional genomics.

Patterns of genetic variation in Mlo among Vitis spp. and interspecific hybrids

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Non-functional alleles of Mlo have been shown to confer resistance to powdery mildews on barley, Arabidopsis, and tomato. To characterize patterns of variation in Mlo sequence in Vitis spp., we have initiated studies of size and sequence polymorphism in the USDA-ARS Vitis germplasm collection. Minor size polymorphisms (insertions and deletions smaller than 10 bp, as assayed by capillary electrophoresis) were frequent in 19 arbitrarily selected accessions, with one Mlo candidate gene having 12 unique fingerprints. However, only minor indels were detected, all of which were found only in introns. To discover variation in protein-coding sequence, we assayed one exon of two Mlo candidate genes in 95 accessions. After observing no size variation in these exons, we sequenced eleven accessions representing diverse North American, Asian, and Mediterranean gene pools, finding a SNP rate of only 0.0018 and a missense mutation rate of 0.0002. Due to the strong conservation of Mlo exon sequence observed in Vitis spp., we are currently applying ecoTILLING as an efficient technique for identifying the rare genetic variation in candidate resistance genes, with follow-up haplotype sequencing to detect functional mutations in protein coding sequences.

Differential and multiple host susceptibility (S) genes control the interaction of *Xanthomonas oryzae* pv. *oryzae* with the host plant rice (*Oryza sativa* L.)

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Xanthomonas oryzae pv. *oryzae* delivers members of type III transcription activator-like (TAL) effectors into rice cells, where the proteins mediate gene/effector specific host gene expression. The pathogen is highly dependent on major TAL effectors, which mediated their effect through alternate targeted host susceptibility (S) genes. *X. oryzae* pv. *oryzae* contains 19 genes for TAL effectors and uses additional TAL effectors concomitantly as major, moderate or minor virulence effectors. These additional effectors target additional and specific host genes including Os*TFX1*, which encodes a bZIP transcription factor and *TFIIAγ1*, which encodes the core transcription factor TFIIAγ, or small, subunit. The roles of the plant genes in the host susceptibility are under investigation. The results indicate that *X. oryzae* pv. *oryzae* utilizes different yet related TAL effectors for multiple and concerted effects on host gene expression, and, as a consequence, host physiological responses as a strategy for virulence. Evidence regarding the different specificities of the TAL effectors in individual strains of the pathogen using immunological detection will be presented and discussed.

Tobacco NPR1 is a strong transcriptional activator that is sensitive to salicylic acid

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In tobacco (Nt), NPR1 is involved in the resistance response to Tobacco mosaic virus (Liu et al., 2002). Despite a high overall similarity (52% identity, 68% similarity), NtNPR1 does not contain the nuclear localization signal and critical cysteine residues found to be engaged in monomer to oligomer transition, nuclear translocation and transcription activation of Arabidopsis (At) NPR1 (Mou et al., 2003; Rochon et al., 2006; Tada et al., 2008). To understand the function of NtNPR1, we have analyzed its biochemical activities in yeast. Like AtNPR1, NtNPR1 interacts with TGA transcription factors and NIMIN1 and NIMIN2 proteins (Zwicker et al., 2007). Accumulation of NtNPR1:GFP fusion proteins occurs, however, exclusively in the nucleus. Furthermore, NtNPR1 contains a cryptic transactivation domain in its N-terminal moiety which exceeds transcription from the strong viral activator VP16 by a factor of 3.5. This domain can be partially uncovered by salicylic acid (SA). Our data strongly suggest that SA acts directly on NtNPR1, and that NtNPR1 undergoes a conformational switch mediated by SA and possibly NIMIN2 proteins, resulting in its activation and expression of PR genes. Consistently, an SA-responsive hinge region was mapped in NtNPR1. Moreover, albeit likewise involved in the defense response against pathogens, Nt and AtNPR1 may function differentially at the molecular level.

Poplar-*Melampsora* rust interaction: JAZ genes participate in defense responses against Melampsora

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Jasmonate ZIM-domain (JAZ) transcriptional repressors were recently identified as key negative regulators of jasmonate (JA) responses in Arabidopsis. In the presence of JA and the bacterial mimic coronatine, JAZ proteins are degraded and JA signalling is de-repressed. This subsequent JA signalling activates transcription of JAZs via a regulatory feedback loop, which likely attenuates the JA response and ensures strict control of defence responses. Recent transcriptome analyses from our lab have shown that several JAZ genes are up-regulated during infection by *Melampsora* rust. The poplar genome encodes 14 JAZ genes. We will present transcript profiling by real-time quantitative PCR (RTqPCR) of the poplar JAZ family, which shows that the JAZ genes exhibit different expression profiles. Although all 14 JAZ genes are up-regulated by mechanical wounding, only five are up-regulated by rust infection. This up-regulation of JAZ transcripts after rust infection indicates that JA responses are part of the poplar defence response against *Melampsora* and is consistent with other data from our lab. Experiments are also underway to identify JAZ-interacting proteins, which should provide insights regarding the signalling pathways that are regulated by JAZ proteins and also could indicate if the five rust-responsive JAZs have distinct protein interactions.

Poplar-Melampsora rust interaction: Kunitz protease inhibitors are not only anti-herbivore genes

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Kunitz-type trypsin inhibitors (KTIs) are among the best described plant gene families involved in defence against insects. The poplar KTI family consists of at least 22 members, many of which are up-regulated after insect attack and wounding. However, a subgroup of eight poplar KTIs (group C) has not been reported to be up-regulated by insect challenge or wounding. Recent transcriptome analyses from our lab showed that several group C KTIs are up-regulated after Melampsora rust infection. By contrast, a separate transcriptome analysis reported that KTIs from groups A and B, which are those responsive to wounding and insects, were down-regulated after rust infection. Using real-time quantitative PCR, we have confirmed that all group C KTIs are strongly up-regulated by rust infection, while representative KTIs of the rest of this family are not. Very few plant KTIs have been reported to have anti-microbial activity. We are now functionally characterizing the poplar group C KTIs and will compare the activity of these inhibitors to that reported for other poplar KTIs. We are also conducting experiments to determine whether the group C KTIs negatively affect the pathogenesis of Melampsora rust. If so, the poplar KTI family represents a phenomenal example of divergence of this family of defense proteins to protect against challenge from both insects and pathogens.

Is *Phy-P5* a major broad-spectrum resistance QTL conferring resistance to *Phytophthora* in Solanaceae?

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Blight caused by the pathogen *Phytophthora* spp. (Oomycete) is one of the most damaging diseases of Solanaceae crops. Contrary to monogenic resistances that are easily overcome by virulent strains, polygenic resistances generally confer a durable control of disease severity. Despite the potential importance of polygenic disease resistances, little is known about their molecular bases in plants and the mechanisms of action of the genes controlling them. A major-effect QTL region that confers resistance towards *P. capsici* was detected on pepper chromosome P5 from 5 partially resistant parents and with 11 different isolates. We were not able to determine whether this QTL region, that we called *Phy-P5*, corresponds to the same QTL in all populations because of the lack of common markers between maps. Moreover, comparative mapping suggested colinearities between *Phy-P5* and resistance QTLs to *P. infestans* on potato chromosome IV and on tomato chromosome T4. To determine whether *Phy-P5* is a broad-spectrum resistance QTL to *Phytophthora* and to identify candidate genes, we anchored the pepper genomic region harbouring *Phy-P5* (i) within the published pepper maps, (ii) with the other members of the Solanaceae family and (iii) with the model plants by developing bridge markers. Up to now, our results showed that *Phy-P5* was conserved across 5 genetic backgrounds in pepper and controlled 4 *P. capsici* isolates. We also determined the colinear region of *Phy-P5* in tomato, potato and *Arabidopsis* making a step forward the research of functional orthologs of *Phy-P5* in those species.

Impact of tailing sand on the establishment of the *Frankia* sp.-alder symbiosis

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In Alberta, the land area disturbed by tailing sands (TS) of the oil sands industry covers over 420 Km^2 and if exploitation activities hold their current rate of expansion, they will have produced 1 billion m^3 of TS by 2025. TS are the waste part of the oil sand exploitation activities. TS are devoid of nutrients, and they contain phytotoxic naphthenic acids (NA) (60ppm), NaCl (40mM) and Na_2SO_4 (2mM). Parallel to this situation, alders, which are native to Alberta, have proven useful in land reclamation strategies. One advantage they hold is their root symbiosis with *Frankia* sp. These bacteria fix atmospheric N_2 saprophytically or symbiotically and support alder growth by supplying 70–100% of plant nitrogen requirements. Our research in hydroponic conditions shows that alders are able to survive and grow in the presence of the components of TS, as well as same for *Frankia* sp. *in vitro.* Nonetheless, *Frankia* sp. need to be able to establish the symbiosis with the alders in this challenging conditions and fix atmospheric nitrogen in root nodules to contribute to reforestation. Results on the efficiency of the symbiosis establishment and the N_2 fixation rate by the alder nodules will be shown. In this work, river alder (*Alnus incana* (L.) Moench ssp. *rugosa),* and green alder *(Alnus viridis* (Chaix) DC. ssp. *crispa)* were studied. *Frankia alni* strain ACN14a was chosen for its capability to enter symbiosis with alder, and since it's genome is sequenced. This was important to perform the other axis of this study: evaluating the impact of NA on the protein expression of *Frankia alni* ACN14a. We will discuss the significance of the five proteins up-regulated in the presence of the NA and will present the rates of N_2 fixation by the saprophytic microorganism in the presence of NA.

SERK1 is a critical component of the Mi-1 resistance signalling pathway

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Mi-1 is an atypical *R*-gene that confers broad range resistance in tomato. How *Mi-1* triggers plant defense to pests as diverse as parasitic root-knot

nematodes and phloem feeders like potato aphids and whiteflies is still poorly understood. To identify candidate genes involved in *Mi-1*-mediated resistance, we have undertaken a large-scale EST library screen using tobacco rattle virus to induce gene silencing (TRV-VIGS) in *Nicotiana benthamiana*. The *Mi-1*-mediated resistance in tomato is partly characterized by a hypersensitive response (HR) consisting of localized cell death. Transient expression of *Mi-DS4*, encoding a constitutively active form of Mi-1 that induces HR in *N. benthamiana* leaves, was used to phenotype the silenced plants. To decipher whether the absence of HR was specifically related to *Mi-DS4*-mediated HR, plants were also assayed with *Pto* L205D, encoding a constitutively active form of the tomato Pto R-protein that confers resistance to *Pseudomonas syringae* pv. *tomato*. Over 2,000 clones were screened and about 2% were specifically involved in *Mi-DS4*-mediated HR, among them a LRR-protein-like kinase (P47-G3). We identified and cloned the tomato homologue of the *N. benthamiana* P47-G3. DNA blot analysis indicated that it represents a unique gene in tomato, which was named *SlSERK1* by sequence homology to other plant LRR-kinases. Silencing *SlSERK1* in tomato using TRV-VIGS attenuates *Mi-1*-mediated resistance to potato aphids. Further characterization of the *SlSERK1* is undergoing to gain insights into its role in the Mi-1 resistance signalling pathway.

Characterization of four antagonistic bacteria belonging to the genera *Pseudomonas* and *Serratia* for the control of post-harvest fungal diseases of pome fruit

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Pseudomonas fluorescens isolates 1-112, 2-28, 4-6 and *Serratia plymuthica* isolate 6-25 isolated from the rhizosphere of legumes exhibited significant antagonistic activity against the three major post-harvest pathogens of pome fruit, *Penicillium expansum*, *Botrytis cinerea* and *Mucor piriformis*. These isolates were screened for their ability to suppress fungal decay *in vitro* on plates and *in vivo* on pome fruit under cold storage conditions. The isolates consistently inhibited the growth of the fungal pathogens (up to 74% control) on both apple and pear fruit under cold storage conditions. The cell-free supernatant of *Serratia* isolate 6-25 also significantly inhibited fungal growth. The purpose of this study was to examine the mechanism of their antagonistic behaviour by characterization of the four bacteria isolates. The isolates were characterized by their 16S rRNA sequences, carbon utilization patterns, siderophore production and by their growth patterns on various media under different temperatures and pHs. All of the bacteria grew well between 1 and 28°C. *Pseudomonas* isolate 4-6 consistently increased the pH of the medium while *Serratia* isolate 6-25 decreased the pH. Green fluorescent protein (GFP) was introduced into the bacterial isolates. The stability of the GFP construct and growth of the transformed bacteria was compared with the wild-type. Survival and colonization patterns on wounded apples are being monitored using GFP markers via confocal and epi-fluorescent microscopy. These data provide greater insight into the mechanisms by which the *Pseudomonas* and *Serratia* isolates inhibit blue mold (*Penicillium expansum*), grey mold (*Botrytis cinerea*) and Mucor rot (*Mucor piriformis*).

The impact of the stringent response on the biocontrol activity of *Pseudomonas* spp. DF41 and *Pseudomonas chlororaphis* PA23

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Pseudomonas spp. DF41 and *Pseudomonas chlororaphis* PA23 are rhizosphere-associated bacteria that have demonstrated *in vitro* and *in planta* inhibition of *Sclerotinia sclerotiorum* (Lib.) de Bary. The biocontrol activity of these strains is attributed to secondary metabolite production. Several factors affect the production of these metabolites such as quorum sensing and the stationary phase sigma factor RpoS. In *Erwinia carotovora* and various *Streptomyces* species, the production of secondary metabolites is mediated by a stress response known as the stringent response. Upon stringent response activation, the alarmone (p)ppGpp is generated by the synthetase enzyme, RelA, and is hydrolyzed by a second enzyme, SpoT. Under certain conditions, SpoT may synthesize (p)ppGpp. To determine the impact of the stringent response on the biocontrol activity of DF41 and PA23, DF41*relA*, DF41*relAspoT*, PA23*relA*, and PA23*relAspoT* mutants were generated. These mutants exhibited increased antifungal activity against *S. sclerotiorum in vitro* compared to their respective wild-type strains. Transcriptional fusions of genes associated with biocontrol were monitored in the aforementioned strains. Expression of genes encoding autoinducer synthase and RpoS were markedly decreased in the stringent response mutants of DF41 and PA23. In DF41, the stringent response negatively regulates the transcription of genes involved in synthesis of a cyclic lipopeptide antibiotic. Conversely, in PA23, phenazine and pyrronitrin biosynthetic gene expression was unaffected by this stress response. These findings indicate that, depending on the bacterial strain under investigation, the stringent response differentially regulates factors involved in biocontrol.

Influence of biotic and abiotic stresses over gene expression in habanero pepper plantlets

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Yields of habanero pepper (*Capsicum chinense* Jacq.) cultures, as well as in other crops in the Yucatan Peninsula, are reduced by different types of biotic and abiotic stresses. Among abiotic stress, the most important are caused by drought, high temperatures, and soil poorness. On the other side, viral infections are the most studied in the field and fungal and oomycete infections are poorly investigated, yet the second one is lethal to Capsicum species. The generation of genetically-modified plants with resistance genes could be an environmental-friendly alternative for the control of microbe-associated diseases. In the present work, we analyzed the effects of different types of stress over the expression of three genes that could mediate the responses against biotic (protein PR10 and NPR 1) and abiotic (glutathione peroxidase) stress. To doing so, we measured the modifications in transcript levels when 4-week plantlets were inoculated with a compatible strain of *Phytophtora capsici*, or were subjected to different types of abiotic stress: osmotic (mannitol, NaCl), temperature (cold), mechanic (wound), and chemical (salicylic acid, jasmonic acid, ethylene). Results will be discussed in terms of gene protection roles and their potentiality to be used in genetic transformation assays. This project is supported by CONACYT grant P-54831.

The HrpN protein, which participates to Type III secretion translocation of the plant pathogen Erwinia amylovora, triggers plant basal defense response

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Pathogenicity of the plant pathogen Erwinia amylovora relies on a functional type III secretion system (TTSS) which allows translocation of a number of effectors into the plant cell. Injected effectors manipulate the plant cell metabolism to allow successful bacterial colonization. Among those, the DspA/E effector efficiently suppresses plant basal defense response such as callose deposition. Surprisingly, an E. amylovora *tts* mutant is unable to trigger callose deposition indicating that this plant basal defense response is triggered either by another type III effector or by the TTS apparatus. Genetic analysis indicates that the TTS protein HrpN, which is involved in DspA/E translocation, is required to trigger callose deposition. The purified HrpN protein is inducing a faint callose accumulation. HrpN could be injected into the plant cell as shown by analysis of translocation of HrpN1-200::CyaA fusion. Translocation of HrpN1-200-CyaA or HrpW1-200-CyaA fusions is impaired in a *hrp*N background indicating that HrpN involvement to the translocation process is not restricted to DspA/E translocation. Transgenic apple seedlings expressing a HrpN protein directed to the membrane exhibit callose deposition without pathogen challenge. All these data indicate that HrpN participate to the translocation pore and that translocation is a process which activates plant defense response.

Biochemical and phospho-proteomic approaches to dissect oligogalacturonides signalling pathway in plant defence

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During the infection of plant tissue by pathogens, homogalacturonan is broken down in lower size fragments called oligogalacturonides (OGs) by the action of fungal polygalacturonases. The interaction between polygalacturonases and their inhibitors (polygalacturonase-inhibiting proteins: PGIPs) in the plant cell wall favours the formation of OGs with degree of polymerization from 10 to 15 that can activate the plant innate immune response. Although the effects of OGs in plant defence are well recognised, the perception/transduction mechanisms of these elicitors are still not completely understood. A phospho-

proteomic approach on apoplastic, plasma membranes, and cytosolic proteins is being used to study the role of protein phosphorylation in the OGs signalling pathway. We have also prepared fluorescent (f-OGs) and biotinylated OGs (b-OGs) that maintain biological activity as tools to dissect the OG signalling pathway. f-OGs have been used to study directly *in vivo* the dynamic of the OG perception by confocal microscopy in tobacco and *Arabidopsis* leaves. b-OGs have been chemically cross-linked to PGIP2 of *P. vulgaris*, known to bind pectin and OGs, and are being used to isolate and identify plasma membrane associated high-affinity binding sites. Moreover, we are developing a highly sensitive assay to detect OGs in plant tissues using b-OGs; the assay utilizes proteins that have OG-binding sites and Surface Plasmon Resonance (SPR) analysis.

Profiling of symbiosis related miRNAs in *Lotus japonicus*

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Micro RNAs (miRNAs) are short, non-protein-coding RNAs that play key roles in the regulation of diverse biological processes in eukaryotes. In plants, they have been related to physiological and developmental processes, as well as defense responses to pathogens, but their involvement in mutualistic symbioses with soil microbes is poorly understood. We use the model legume *Lotus japonicus* (*Lotus*) to profile miRNAs in the context of root endosymbiosis with nitrogen-fixing rhizobia. To trace relative abundances of miRNA precursor transcripts in symbiotic and non-symbiotic tissues, we analyzed cDNA microarray data (Radutoiu *et al*., submitted). Hybridization samples stem from *Lotus* wild type and 8 mutant genotypes impaired in the establishment or regulation of root symbiosis with the rhizobial symbiont of *Lotus*, *Mesorhizobium loti*. For direct profiling of miRNAs in symbiotic and non-symbiotic tissues, and to identify novel miRNAs specific to *Lotus* or its lineage, we are sequencing small RNA populations of infected or non-infected *Lotus* wild type and mutant tissues using SOLEXA technology.

A transcriptomic approach to study the response of different species of citrus plants to the infection with *Phytophthora citrophthora*

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Plants have evolved a series of mechanisms to counteract the attack of plant pathogens. Some of them are constitutive, others are activated following a specific recognition of the microorganism and lead to the establishment of a complex defensive response characterized by the transcriptional activation of a great number of genes. The oomycete *Phytophthora citrophthora* is the causal agent of a disease of great importance in Spain characterized by the appearance of root rot and gummosis in trunk, leading to important losses in fruit quantity and quality, and causing decay, or even death, of the tree in severe infections. To elucidate the molecular mechanisms determining the resistance to this phytopathogenic fungus we have undergone the transcriptional analysis of the response of several citrus species showing different degree of resistance to the fungus. To achieve this goal roots of *Citrus aurantifolia* (very resistant), *Citrus aurantium* (semi-resistant) and *Citrus sinensis* (highly susceptible) plants were infected with spores of *P. citrophtora*. cDNA microarrays developed by the Citrus Functional Genomics Project, containing 12000 unigenes from citrus, were hybridized with samples taken 48 hours after infection. Our preliminary results show that the transcriptome of *C. aurantifolia* do not suffer significant changes due to infection, suggesting a constitutive resistance of this species. However, the response of *C. aurantium* plants to infection lead to the deregulation of a great number of genes, suggesting that the resistance observed in these plants would be of inducible matter. Interestingly, gene repression seems to predominate in *C. sinensis* plants, and would be the cause of their susceptibility to the fungus. In this work, we show and discuss these results in higher detail.

Transcriptome profiling analysis of the *Pectobacterium*-potato interaction

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Pectobacterium species are enterobacterial pathogens that cause soft rot in a wide host range of plants including potato. Previously, we compared the genome sequence of *P. carotovorum* subsp. *carotovorum* (*Pcc*), *P. carotovorum* subsp. *brasiliensis* (*Pcb*) and *P. atrosepticum* (*Pa*). These *Pectobacterium* differ in host range and represent diverse lineages. Most of the genes known to be involved in virulence, including plant cell wall degrading enzymes (PCWDE) and the type II and III secretion system were conserved among these species. However, *Pcc* and *Pcb* were significantly more aggressive when compared to *Pa* on potato tubers. In this study, we used a microarray-based approach to identify additional putative virulence genes highly expressed in *Pcc* during potato infection. Bacterial RNA was isolated from inoculated tubers incubated at 26°C and hybridized to a tiled microarray designed from a draft *Pcc* sequence obtained with 454 sequencing technology. A *trans* regulator and associated structural genes not present in *Pa* were among the most highly expressed genes. PCWDE such as *pelB*, *pehA, celV*, *pelA*, *pelC, svx* and genes belonging to the flagellar system were also highly expressed during bacterial log phase growth *in planta*. Additionally, we found that several genes specific to the *Pcc* linage were highly expressed, including an ABC transporter, permease, hemolysin and several hypothetical genes. The structural and regulatory type III secretion system genes were among those with the very lowest level of expression in tubers. These results indicate that lineage specific genes and newly identified regulators may play an important role in bacterial adaptation to the host environment.

Subcellular localisation of proteins fused to GFP in *Xanthomonas axonopodis* pv. citri: Targeting the division septum

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Xanthomonas axonopodis pv. citri (Xac) is the causal agent of citrus canker, a disease that affects citrus worldwide. Although the genome of Xac has revealed several biochemical traits, ~37% of the annotated ORFs remain hypothetical. In order to start a systematic characterisation of novel proteins encoded by this bacterium, we developed integrative vectors for GFP-tagging and subcellular localization of proteins by fluorescent microscopy in Xac. Our GFP expression vectors contain the xylose promoter, an artificial RBS, the *gfp* gene, and a fragment of the *amy* gene of Xac, for vector integration into its chromosome. Here we show that our vectors can be stably integrated into the *amy* locus of Xac without altering its pathogenicity. To test their functionality, we produced a Xac mutant expressing the ortholog of *B. subtilis* (Bsu) ZapA protein, which stimulates the assembly of FtsZ polymers in Bsu. GFP-ZapA^{Xac} was successfully expressed in Xac and detected in Western blot experiments. Furthermore, we show, for the first time, the subcellular localisation of a GFP-tagged protein in *Xanthomonas*. GFP-ZapA^{Xac} generated a localisation pattern similar to that observed for GFP-ZapA^{Bsu}: a bar/ring-like structure perpendicular to the longitudinal axis of the cell and located in the middle of the rod, where the division septum develops. Treatment of the Xac mutant with inhibitors of protein synthesis completely abolished the localisation pattern described above, showing that the GFP signals were not an artefact. Our results corroborate the use of our vectors for protein localisation in *Xanthomonas*, and constitute a new and useful tool for the characterisation of proteins expressed in this bacterium.

Ethylene Response Factor is required for AAL-toxin-induced programmed cell death

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AAL-toxin is a pathogenicity factor of *Alternaria alternata* f. sp. *Lycopersici*, a pathogen of tomato stem canker disease. Little is known about the signaling pathway of AAL-toxin leading to programmed cell death. To dissect the biological function of genes involved in AAL-toxin-induced cell death, we used *Nicotiana umbratica* that is sensitive to the AAL-toxin and effective in virus-induced gene silencing (VIGS). We knock-downed various key genes for phytohormone signaling pathways and showed that ethylene signaling plays a pivotal role in AAL-toxin-induced cell death. Silencing *EIN2* and *EIN3*, key components of ethylene signaling, compromised AAL-toxin-induced cell death. Furthermore, to hunt components, which function in ethylene downstream signaling, we silenced some *Ethylene Response Factors* (*ERFs*). *ERFs* are major transcription factors in plants and regulate many genes in relation to ethylene downstream signaling. We identified NuERF4 as a component for the cell death signaling. In NuERF4-silenced plants, AAL-

toxin-induced cell death and disease symptom of *A. alternata* f. sp. *Lycopersici* were suppressed. Next, we investigated regulatory mechanisms of NuERF4 for transcription using GAL4 *cis*-element and minimal 35S promoter, indicating that NuERF4 positively activated the transcription. Overexpression of NuERF4 did not induce cell death in N. umbratica, however, accelerated AAL-toxin-induced cell death and disease symptom by the pathogen. These results suggest that NuERF4 positively regulates genes involved in AAL-toxin cell death.

Potato – late blight interaction: What is behind the poor correlation between foliar and tuber resistance?

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The economic value of potato crop resides in its tubers, but most research has focused on foliar resistance to late blight (*Phytophthora infestans*). Recently, *R1* and *Rpi-phu1* (Park et al. 2005; Sliwka et al. 2006) conferring resistance to *Phytophthora infestans* were shown to confer foliar and tuber resistances whereas *R3a* and *Rpi-abpt* were foliage specific. Millett and Bradeen (San Diego 2005) showed that *RB* gene was constitutively expressed in leaf and tuber but conferred only foliar resistance. These results suggested that not all *R*-genes can confer both foliar and tuber resistances. In order to trigger a hypersensitive responses (HR), an *Agrobacterium tumefaciens* Transient Assay (ATTA) in the tubers was developed. Upon infiltration of *IPIO1*, *Avr2*, *Avr1* and *Avr3a* HRs were triggered in transgenic tubers expressing *Rpi-blb1*, *Rpi-blb3*, *R1* or *R3a*. This result showed that all *R*-genes, conferring tuber resistance or not, could trigger a HR upon effector infiltration. Moreover, during *P. infestans* infection, *IPIO1*, *Avr2*, *Avr1* and *Avr3a* were expressed in leaves and tubers. Brugmans et al. (2008) performed NBS profiling using cDNA from leaf, stem and root showing different *R*-gene expression patterns between tissues and between individuals within a particular tissue. We studied the expression patterns of *Rpi-blb1*, *Rpi-blb3*, *R1*, *R3a* and *Rpi-vnt1.1* by QRT-PCR in leaf and tuber tissues. Comparing the *R*-gene expression with ability to trigger HR using ATTA, as well as the ability to display resistance to a compatible isolate, we hypothesize that the expression level of a given *R*-gene may be determinant for tuber resistance. The implications of these results for GMO applications will be discussed. This work was financially supported by the FP6 programme BioExploit Food-CT-2005-513959.

OsPti1a-mediated protein kinase cascade is involved in disease resistance

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OsPti1a, which is a functional ortholog of tomato Pti1, negatively regulates both basal resistance and *R*-gene mediated resistance in rice (Takahashi et al., 2007). To find signaling components required for OsPti1a function, yeast two-hybrid screening was carried out. OsPti1a interacting kinase1 (Pik1), thus obtained, belongs to AGC protein kinase VIIIb and directly targets OsPti1a for its phosphorylation. PIK1 was transiently phosphorylated in response to ROS (H_2O_2). Overexpression of *Pik1* enhanced resistance against a compatible race of the fungus. Further, phosphorylation site in OsPti1a that is a target by Pik1 was required for basal resistance against bacterial pathogen, *Xanthomonas oryzae* pv. *oryzae*. These results suggest that Pik1 positively regulates disease resistance through suppression of OsPti1a-mediated negative regulation. In addition, it is known that AGC kinases are regulated by 3-phosphoinositide dependent protein kinase1 (Pdk1) that is a regulator of phospholipids signaling in animal and plant. Indeed, Pik1 interacted with Pdk1 and was phosphorylated by Pdk1. Further, Pdk1 was activated by fungal elicitor treatment and overexpression of *Pdk1* enhanced resistance against a compatible race of the rice blast, suggesting that Pdk1 probably regulates Pik1 through protein phosphorylation in basal resistance. Therefore, the phosphorylation relay Pdk1-Pik1-OsPti1a is assumed to be involved in defense signaling.

BAK1, a regulator of cell death control

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The leucine-rich repeat receptor-like kinases (LRR-RLKs) represent the largest group of receptor-like kinases in *Arabidopsis*. LRR-RLKs serve diverse functions in plant development, hormone perception and additionally in recognizing non-self derived molecules the so called pathogen/microbe-associated molecular patterns (PAMPs/MAMPs), as shown for FLS2 and EFR. The LRR-RLKs consist of extracellular leucine-rich repeats, a transmembrane and an intracellular kinase domain and show striking structural similarity to LRR-type-receptors involved in innate immunity of insects and animals. In microarray analyses, among other LRR-RLKs, BAK1 (brassinosteroid-associated receptor kinase 1) was identified to be differentially regulated after bacterial infection and PAMP/MAMP treatment. This kinase was first found to act as a co-receptor for the brassinosteroid insensitive 1 (BRI1) receptor in plant development. BAK1 was also found to be a co-receptor for the flaggelin receptor FLS2. We observed that BAK1 mutants show enhanced susceptibility towards the necrotrophic fungus *A. brassicicola* and also enhanced symptom development upon infection with the bacterial pathogen Pto DC3000. We have provided experimental evidence that this phenomenon is due to a brassinosteroid independent role of this co-receptor in cell death control. To further investigate the role of BAK1 in cell death control, interaction studies were performed. Co-immunoprecipitation experiments were conducted with BAK1-GFP expressing plants with and without cell death triggering infection caused by *A. brassicicola*. A number of interacting proteins were identified via liquid chromatography tandem mass spectrometry analyses. A characterization of these BAK1-interacting proteins will be presented.

Genome structure of *Cassava brown streak virus* reveals novel features for the family Potyviridae

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Cassava brown streak virus (CBSV, genus Ipomovirus; Potyviridae) damages cassava crops in East Africa. Comparison of the coat protein (CP) encoding sequences of eight isolates from the Lake Victoria zone to isolates from Indian Ocean coastal lowlands indicated only 75.8–77.5% nucleotide (nt) and 87.0–89.9% amino acid identity and existence of two genetically different and geographically separated populations of CSBV (Arch. Virol. 154:353-359, 2009). The complete sequence of a highland isolate of CBSV was determined. It was smaller (9096 nt) than genomes of other ipomoviruses, *Sweet potato mild mottle virus* (SPMMV, 10818 nt), *Squash vein yellowing virus* (SqVYV, 9836 nt) and *Cucumber vein yellowing virus* (CYVV, 9734 nt). These (+)ssRNA viruses encode a large polyprotein proteolytically processed to mature proteins. The N-terminus of CBSV polyprotein was structurally novel for Potyviridae. While it contained the expected serine proteinase P1, it was lacking the next protein, helper-component proteinase (HC-Pro). Also CVYV and SqVYV lack HC-Pro but contain two diversified P1 proteins of which P1b was related to the P1 of CBSV. The P1 of CSBV was an efficient RNA silencing suppressor as reported previously for the P1b of CVYV. Furthermore, CBSV genome contained an insert in the 3'-proximal part between the NIb and CP encoding sequences. No similar sequence is known in any virus and there is no precedent of structural variability in the 3'-proximal part of virus genomes in Potyviridae. The novel sequence, flanked as expected by proteolytic cleavage sites, encodes a HAM1-like protein. In microbes, plants and animals HAM1 is implemented in intercepting non-canonical NTPs from being incorporated to DNA. Its function in CBSV remains to be shown.

LUPI, a *Medicago truncatula* E3 ligase of the U-box family which interacts with symbiotic receptors

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The establishment of the nitrogen-fixing root symbiosis between legumes and rhizobia generally requires perception of rhizobial lipochitoooligosaccharidic molecules, called Nod factors (NFs), by the plant host. Genetic studies of the NF perception pathway in *Medicago truncatula* have identified the crucial

role of three Receptor-Like-Kinases: NFP, LYK3 (LysM-RLKs) and DMI2 (a LRR-RLK). So far, the regulation of these receptors and the direct signaling events that they activate is poorly understood. In this study, we searched for protein partners of these receptors using the yeast two-hybrid technology. Screens were made in a GAL4 system using a NF elicited root hair cDNA library and the soluble kinase domains as baits. LUPI, an E3 ligase protein of the U-box/armadillo repeats (ARM) sub-family was identified in both LYK3 and DMI2 screens. Thus these receptor proteins may be regulated by ubiquitinylation or alternatively, the E3 ligase activity of LUPI could be modulated by phosphorylation. Characterization of these interactions is currently underway using proteins transiently expressed in *Nicotiana benthamiana* leaves or purified from *Escherichia coli*. The gene expression pattern of *LUPI* is studied using both qRT-PCR and promoter::GUS fusions. The functional role of this candidate in the NF signaling pathway is being assessed in *M. truncatula* by RNA interference, by analysis of TILLING and Tnt1 insertion mutants, and by overexpression. This work is funded in part by the European Community's Sixth Framework Programme through a Marie Curie Research Training Network, contract MRTN-CT-2006-035546 "NODPERCEPTION".

Characterization of type III secretion proteins HrcU and HrpP in pathogenesis by *Erwinia amylovora*

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Translocation-associated type III secretion (T3S) systems can be divided into three structural components: the basal body, the pilus, and the translocon. The basal body transports T3S effectors across the bacterial cell envelope. In plant-pathogenic bacteria, the pilus functions as a conduit across the plant cell wall. The translocon is a pore-forming complex at the distal end of the pilus. Together these components function to deliver effector proteins directly into the host cytoplasm. The enterobacterium *Erwinia amylovora* is the casual agent of fire blight of apple and pear. *E. amylovora* Ea1189 utilizes a T3S system for effector translocation that is required for pathogenesis. We are particularly interested in two T3S proteins in Ea1189, HrpP and HrcU. Homologs of these proteins in enteric mammalian pathogens function to regulate pilus length and comprise a T3S system substrate specificity switch that signals secretion of proteins comprising the translocon. We constructed ΔhrpP and ΔhrcU mutants in Ea1189. Both of these mutations resulted in loss of pathogenicity indicating vital roles for both genes in disease development. Yeast-two hybrid analyses have revealed a strong interaction between HrpP and the cytoplasmic tail of HrcU. The secretome of ΔhrpP and ΔhrcU strains was analyzed using mass spectrometry to identify differences in secretion profile. Regulation of T3S pilus length in phytopathogenic bacteria is also unknown. Using Ea1189 ΔhrpP strains, we plan to visualize T3S pili via transmission electron microscopy and probe for ΔhrpP effects on pilus morphology. To date HrpP is the first HrcU-interacting protein identified in *E. amylovora*.

Molecular basis of disease chlorosis caused by *Pseudomonas syringae* pv. *tomato* strain DC3000 infection in *Arabidopsis thaliana*

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A major symptom of many plant diseases, caused by diverse pathogens, is the development of tissue chlorosis. However, the molecular mechanisms underlying disease-associated chlorosis are poorly understood. *Arabidopsis thaliana* plants infected with the bacterial pathogen *Pseudomonas syringae* pv. *tomato* strain DC3000 (DC3000 herein after) or the fungal pathogen, *Alternaria brassicicola* become chlorotic by 60 hours post-infiltration (hpi) or 10 days post-infection (dpi), respectively. An EMS-mutagenized *Arabidopsis thaliana Col-gl1* population was screened for mutants that do not develop chlorosis when inoculated with DC3000. One mutant was identified and named *noc1* (for *no chlorosis 1*). Interestingly, *A. brassicicola* also failed to induce chlorosis on *noc1* mutant plants, suggesting a shared molecular process in the development of tissue chlorosis in bacterial and fungal diseases. The *noc1* mutation has been mapped to the long arm of chromosome 4 in a region that includes the gene *NON-YELLOWING1* (*NYE1*:At4g22920), *nye1* mutants do not develop chlorosis during dark-induced senescence. We found that the *NYE1* transcript is induced 24 hours after infection with DC3000. Sequencing of the *NYE1* gene in the *noc1* mutant revealed an amino acid change from aspartate to tyrosine at position 88. Complementation of *noc1* with *NYE1* fully restores DC3000-induced chlorosis. Thus, we have identified a key regulator of a major disease symptom caused by bacterial and fungal pathogens.

Symbiosis *Medicago truncatula-Sinorhizobium meliloti*: NO production and bacterial response

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Nitric oxide (NO) is a signalling and defence molecule of major importance in living organisms. The role of NO is crucial in plant-pathogen interactions during which it participates in plant defence response and resistance. Indications for the presence of NO during symbiotic interactions have also been reported. In the model legume *Medicago truncatula*, it has been recently demonstrated that NO production is localized in the bacteroid-containing cells of the nodule fixation zone, although enzymatic pathway(s) responsible for its synthesis are yet unknown. The role of NO in symbiosis is far from being elucidated as is the response to NO of the symbiont bacterium *S. meliloti.* Here we show that NO is not only produced in the fixation zone but also during early steps of the symbiosis, and we propose that NO might have different functions along the course of the symbiotic process. Using a combination of biochemical and genetic approaches we show that the plant partner contributes to up to 80% of NO production in the nodule, probably via the activity of nitrate reductase. As a first approach to decipher the bacterial response to NO present in nodules, we identified the *S. meliloti* NO stimulon in culture, using a transcriptomic approach. We identified ~ 100 bacterial genes whose expression is up-regulated in presence of NO, *via* two major regulatory pathways involving the FixLJ and NnrR regulators. The symbiotic phenotype of mutants of some of the NO-regulated genes is being studied. The *hmp* gene encoding a flavohemoglobin is of special interest, since a *hmp* mutant displays a reduced nitrogen fixation efficiency. As flavohemoglobins are known to detoxify NO, this result is the first indication of the importance of bacterial NO response in symbiosis.

Virus induced gene silencement of a germin like protein from *Capsicum chinense* BG-3821 based on Pepper Huasteco Yellow Vein Virus (PHYVV)

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Geminiviruses are plant pathogens characterized by a geminated capside and one or two circular single strand DNA molecules. They are spread by insect vectors. Pepper Huasteco Yellow Vein Virus (PHYVV) and Pepper Golden Mosaic Virus (PepGMV) both from Begomovirus genus, are the most important virus pathogens affecting pepper culture (*Capsicum sp*) in Mexico. Every year, an important decrease of pepper production ranging from 20 to 100% occurs due to geminiviruses infections. In previous studies it was demonstrated that interaction of BG-3821 wild habanero pepper (*Capsicum chinense* BG3821) from Yucatán, with PHYVV and PepGMV shown resistance to both simple and mixed infections. Based on suppression substraction hybridization approaches (SSH), it was isolated a *Capsicum chinense* EST, which presented a high similarity to a Germin Like Protein (GLP). To demonstrate the role of this protein in resistance of *C. chinense* to Begomoviruses, we designed CP:NC93, a VIGS based on "A" component of PHYVV. Two stages of phenological development of pepper plants (4-6 true leaves and flowering) were evaluated for silence-ment. The analysis of silencing of GLP was enhanced with exogenous application of 10 mM salicylic acid in plants of *C. chinense* BG-3821, according to previous studies. So far, RT-PCR analysis of silenced plants displayed absence of GLP transcript in *C. chinense* BG-3821 at the first phenological stage (4-6 true leaves, 30 dpi). Their phenotypes were susceptibility to simple and mixed infections of these viruses, compared with control plants, which did not show any symptoms of these infections. At flowering stage, those silenced plants, remained silenced, but with a phenotype less susceptible to infections caused by these viruses, in agreement with the scale of symptoms severity.

Tomato MAPKKK epsilon is a positive regulator of cell death associated with disease resistance

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The Gram-negative bacteria *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) and *Pseudomonas syringe* pv. *tomato* (*Pst*) are the causal agents in tomato of spot and speck diseases, respectively. Control of these diseases by cultural practices or chemicals is not effective and genetic sources of resistance are only limited to certain *Pst* and *Xcv* strains. To isolate genes involved in resistance to *Pst* and *Xcv*, we used a functional screen based on virus-induced gene silencing (VIGS) techniques. Silencing of a MAP kinase kinase kinase, *SlMAPKKK epsilon*, in tomato plants compromised resistance to both bacterial pathogens but did not affect cell death and bacterial growth associated with disease susceptibility. In addition, silencing of *SlMAPKKK epsilon* in *N. benthamiana* plants significantly inhibited the hypersensitive cell death mediated by different pairs of resistance genes and corresponding avirulence genes. Interestingly, overexpression of *SlMAPKKK epsilon* in *N. benthamiana* leaves activated a pathogen-independent cell death. By overexpressing *SlMAPKKK epsilon* and suppressing expression of various MAPKK and MAPK genes by VIGS, we dissected MAPK cascade activated by SlMAPKKK epsilon. These results reveal a role for SlMAPKKK epsilon in the elicitation of cell death and elucidate a novel MAP kinase cascade involved in disease resistance.

Multiple approaches to isolate the soybean cyst nematode resistance gene *Rhg1* of *Glycine max*

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We are working to identify the *Rhg1* gene. Soybean cyst nematode (*Heterodera glycines*, SCN) is the most economically important soybean pathogen, annually causing greater than $1 billion of soybean crop loss in the U.S. Once SCN is present in a field it cannot be eradicated feasibly. Over 95% of SCN-resistant varieties carry the *Rhg1* locus derived from a single source: PI88788. *Rhg1* is a "major QTL" - it has the strongest impact on SCN of any known locus but it is not the only gene affecting SCN resistance. Because soybean is very difficult to transform we have refined assays that test SCN resistance in transgenic roots generated with *A. rhizogenes*. The *Rhg1* and *Rhg4* genomic regions were previously sequenced by others and LRR-kinase genes were proposed as the candidate SCN resistance genes. Our experiments expressing the LRR-kinase from the Peking *Rhg1* locus in susceptible test lines (with or without the resistant allele at *Rhg4*) have provided no evidence for any increase in SCN resistance. Recent fine-mapping of PI88788 *Rhg1* (collaboration with Myungsik Kim, David Hyten and Brian Diers) implicates a small genomic region that does not include the LRR-kinase gene. Soybean genome sequence is available only from SCN-susceptible varieties, but we are now testing candidate genes at *Rhg1* by gene silencing in a PI88788-derived resistant background. We developed a new binary vector for this purpose that carries a GFP-intron marker and a soybean *GmUbi III* promoter to drive gene-specific amiRNA constructs designed into the Arabidopsis miR319a structure. We are also investigating inter-genic regions at the *Rhg1* locus of PI88788 to identify and test any segments that may be absent from the susceptible, sequenced Williams 82 genome.

Analysis of temperature effects on gene expression in tropical Race 1 biovar 3 and temperate Race 3 biovar 2 strains of *Ralstonia solanacearum*

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Ralstonia solanacearum is a soil-borne plant pathogen that causes lethal bacterial wilt disease in many plant species. Most strains of *R. solanacearum* are tropical, but one group, Race 3 biovar 2 (R3b2), is adapted to cooler environments and can attack plants in temperate zones and tropical highlands. Because of its cold adaptation and unknown pathogenicity factors, R3b2 poses a severe threat to the American potato industry is listed as a Select Agent pathogen in the U.S. We compared the growth and virulence of two *R. solanacearum* strains, GMI1000 (Race 1, tropical) and UW551 (R3b2, temperate) at different temperatures. The two strains grew similarly in both rich and minimal media at 20°C and 28°C. At 28°C, both strains wilted tomato plants rapidly in a naturalistic soil-soak virulence assay. In contrast, at 20°C UW551 was much more virulent on tomato than GMI1000, indicating that interaction with plants is required for the temperate epidemiological trait of R3b2. We used custom-designed microarrays to study whole genome gene expression patterns of the two strains at cool (20°C) and warm (28°C temperatures. In rich medium, the expression profiles of the two strains at different temperatures differed with respect to both strain and temperature. In the four conditions, we found differential expression of genes involved in cell wall/membrane synthesis and function, carbohydrate transport and metabolism, transcription, replication, recombination and repair, and diverse unknown functions. Among the temperature-responsive genes were some that are specific to R3b2.

Shared regulatory mechanisms in plant defense responses to necrotrophic fungi and insect herbivory

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Plant defense responses to necrotrophic fungi are regulated by a complex set of interacting pathways. We studied mechanisms underlying plant responses to necrotrophic fungi and their interrelationship with other response pathways. Genetic components of plant pathways regulating responses to necrotrophic infection were identified. Among these, the *tomato protein kinase 1* (*TPK1b*) gene encodes a receptor-like kinase localized to the plasma membrane. Reducing *TPK1b* gene expression through RNA interference (RNAi) increases tomato susceptibility to the necrotrophic fungus *Botrytis cinerea* and to feeding by larvae of tobacco hornworm (*Manduca sexta*). *TPK1b* RNAi plants show no altered responses to the bacterial pathogen *Pseudomonas syringae.* Interestingly, *TPK1b* RNAi seedlings are impaired in ethylene (ET) responses. Susceptibility to *B. cinerea* and insect feeding is correlated with reduced expression of *proteinase inhibitor II* in response to *B. cinerea* and 1-aminocyclopropane-1-carboxylic acid, the natural precursor of ET, but wild-type expression in response to mechanical wounding and methyl-jasmonate. TPK1b functions independent JA biosynthesis and response gene normally required for resistance to *B. cinerea*. These data reveal a signaling role for TPK1b in an ET-mediated shared defense mechanism for resistance to necrotrophic fungi and herbivorous insects. In parallel, we identified the tomato *Abscisic acid Induced MYB1 (SAIM1)* transcription factor as an important regulator of plant responses to necrotrophic fungi. *SlAIM1* is also required for responses to abscisic acid and Na^+ homeostasis suggesting the role of *SlAIM1* in defense is dependent on ABA. Together, our studies provide new insight into the genetic control of plant responses to necrotrophic fungi and cross-talk with other response pathways.

Regulatory control of the *Fusarium graminearum* transcriptome in different plant hosts

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The fungal plant pathogen *Fusarium graminearum* is capable of infecting a variety of grass species with markedly different outcomes. In wheat (*Triticum aestivum*, cv. Bobwhite) the fungus is extremely aggressive, can cause cell death throughout the inflorescence from a single point inoculation and result in the accumulation of high levels of the trichothecene mycotoxin, deoxynivalenol (DON). On the other hand, rice (*Oryza sativa* spp. *japonica*, cv M201) plants inoculated with the same strain of the fungus exhibit only localized necrotic lesions and accumulate 100-fold less DON than in wheat. To identify fungal gene expression patterns that could result in differences in toxin accumulation and symptom development, the transcriptome of *F. graminearum* in both hosts was assessed 48, 96 and 192 hours after inoculation using a custom *F. graminearum* Affymetrix GeneChip. Genes expressed only in wheat or rice were identified and were most significantly enriched in the MIPS Functional Category 99: Unclassified Proteins. Nevertheless genes for several predicted transcription factors were among those differentially expressed in the different hosts and these genes have been targeted for mutagenesis. Non-coding regions upstream of genes expressed exclusively in wheat or rice revealed enrichment for particular nucleotide sequences suggesting the potential for unified regulatory control in response to particular host plants.

Analysis of kinase activity and phosphorylation of pattern recognition receptors

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In plant immunity the recognition of bacterial flagellin and the elongation factor EF-Tu by the Arabidopsis receptor kinases FLS2 (flagellin sensing 2) and EFR (EF-Tu receptor) became a paradigm for PAMP (Pathogen-Associated Molecular Pattern) perception. Both receptors are membrane proteins which consist of an extracellular leucine-rich-repeat domain and an intracellular serine/threonine kinase domain. This structure is reminiscent to the structure of some animal receptor kinases and suggests a similar model of receptor activation: ligand binding to the ectodomain of the receptor induces a conformational change which in turn activates kinase activity in the cytoplasm to initiate the signaling. However, so far the relevance of the kinase activity of FLS2 and EFR in PAMP signalling has not yet been proven. Moreover, the kinase substrate of these receptors still needs to be identified. To address these points, "kinase dead" versions of FLS2 and EFR were prepared and expressed in Arabidopsis *fls2* and *efr* mutants in order to analyze their functionality in signal transduction. Additionally, in vivo phosphorylation pattern of FLS2 in response to flg22 perception is studied by immuno-precipitation and mass spectrometrical analysis. Finally, the biological relevance of the newly identified phosphosites is investigated by studying complementation of the *fls2* plants with mutant forms affecting these sites. In immunoprecipitates we also identified potential new partners of FLS2, which we will confirm using reverse genetics and co-immunoprecipitation approaches.

Ethylene modulates the PAMP-stimulated oxidative burst

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One of the immediate early defense responses is the generation of reactive oxygen species (ROS), referred to as the oxidative burst, which is mounted upon perception of pathogen-associated molecular pattern (PAMPs). In Arabidopsis, recognition of bacterial flagellin (flg22) is mediated by the receptor kinase FLS2, which actives the respiratory burst oxidase homologues D (RbohD) responsible for ROS production. To date, only little is known about the components that are underlying the PAMP-triggered oxidative burst. We discovered that mutants impaired in ethylene signaling are compromised flg22-elicited ROS production. Nevertheless, expression levels of *FLS2* and flg22 activation of MAP kinases remained normal. Ethylene signaling mutants typically exhibit elevated levels of ethylene even in the absence of any trigger, and chemical reduction thereof lead to the recovery of the flg22-triggered oxidative burst. We will propose a model of the interaction between ethylene signaling and ROS production. Furthermore, we will discuss the role of hormonal cross-talk in the regulation of the oxidative burst.

Cin1, a novel repeat protein secreted during the early stages of infection of apple by *Venturia inaequalis*

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Venturia inaequalis is a hemibiotrophic ascomycete that causes apple scab. Germ tubes, from conidia or ascospores, penetrate the leaf or fruit surface directly via appressoria-like swellings. Subsequently the hyphae divide laterally to form a stroma between the cuticle and the outer wall of the epidermal cells. Stromata lack the tubular morphology of hyphae and resemble pseudoparenchymal cells. Stromata can be induced by growing the fungus *in vitro* on cellophane discs. We have used growth on cellophane as a model to identify genes up-regulated during biotrophic infection *in planta*. One of these genes, *cin1*, was identified from a differential screen of mycelia grown on cellophane, and qRT-PCR showed that it was up-regulated over a thousand fold in infected apple leaves compared with liquid culture. The predicted Cin1 protein possesses a putative signal peptide for secretion but has no similarity to sequences in publicly available databases. Cin1 has seven or eight repeats of about 60 amino acids, with four conserved cysteine residues per repeat. *cin1* has been knocked down using RNAi, and these transformants are non-pathogenic. The phenotype of the RNAi knockdowns and the analysis of *cin1* expression using enhanced-yellow fluorescent protein fusions will be presented.

Evaluating the use of germinating zoospores for the identification of effectors from the biotrophic Oomycete *Plasmopara viticola*, the causal agent of grapevine downy mildew

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Grapevine downy mildew caused by the Oomycete *Plasmopara viticola* is one of the most important diseases affecting *Vitis* spp. The current strategy of control relies on the use of chemical fungicides. An alternative to the use of fungicides is the use of downy mildew resistant varieties, which is cost-effective and environment friendly. The identification of pathogen effectors as putative avirulence genes is a necessary step to understand the biology of the interaction as well as to choose the most efficient combination of resistance genes in a strategy of pyramiding. Based on knowledge from other Oomycetes, *P. viticola* effectors can be identified using a candidate gene strategy based on data mining of genomic resources. Public genomic resources of *P. viticola* are very limited, not to say inexistent. A recent search at NCBI/EMBL databases produced 79 *P. viticola* entries (10 ESTs and 69 core nucleotides), the majority sequences of mitochondrial or ribosomal origin. As an obligate biotrophe, *P. viticola* can only grow on living tissues and the pathogen biomass in the invasive stages of infection is quite low compared to the plant biomass. Nevertheless, zoospores are easily obtained by washing off sporangia from infected leaves; furthermore, the first stages of pathogen development (growth of germinative tubes and vesicle formation) can be reproduced in vitro. To explore the suitability of such material for the identification of pathogen effectors, we created a cDNA library from in vitro germinated spores and obtained 1500 ESTs. Preliminary sequence analysis revealed the presence of 40 ESTs from genes putatively involved on pathogenicity. A detailed sequence analysis of the ESTs as well as the expression profile of candidate effector genes will be presented.

Are bacterial virulence factors involved in initial host colonization processes responsible for host specificity?

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Bacteria belonging to the genus *Xanthomonas* are grouped in pathovars defined on the basis of their host range and type of symptoms. Within a pathovar strains are highly specialized on a restricted host range. Currently, the genetic basis of host specificity for pathogenic bacteria remains poorly understood. We hypothesize that virulence factors which are involved in the initial stages of host colonization play a role in host specificity. Candidate host specificity factors we selected are methyl accepting chemotaxis proteins (MCPs) and adhesins. Actually, nothing is known about the repertoires of MCPs and adhesins in strains of the genus *Xanthomonas*. We have determined the distribution of 30 MCPs and 13 adhesins among 180 strains belonging to 18 pathovars of *X. axonopodis* and 3 pathovars of *X. campestris*, as well as phylogenetic relationships of the strains based on polymorphism analyses of housekeeping genes. The selected strains were isolated from different host plants and various geographical origins. Some pathovars were polyphyletic while others were monophyletic. Repertoires of MCPs and adhesins were polymorphic among strains and displayed both ubiquitous and variable genes. Among the pathovars and genetic lineages tested, thirteen were distinguished by their distinct suites of MCPs and adhesins. The other pathovars and genetic lineages remained difficult to differentiate: five groups with unique repertoires were constituted. Polyphyletic pathovars displayed essentially homogeneous repertoires. These results show that events leading to host specificity occur as early as initial steps of chemotactic attraction and adhesion on host tissues.

Regulators involved in the coordinated expression of virulence genes during *Erwinia chrysanthemi 3937-Arabidopsis* interaction

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Successful infection of a pathogen into its host relies on the coordinated expression of numerous virulence factors encoding genes. In plant-bacteria

interactions, this control is very often achieved by the integration of several regulatory circuits controlling cell-cell communication or sensing environmental conditions. *E. chrysanthemi* (*Ech*), the causal agent of soft rot on many crops and ornamentals, provokes maceration of infected plants mainly by producing and secreting a battery of plant cell wall-degrading enzymes but several other virulence factors have also been characterized. During Arabidopsis infection, most *Ech* virulence gene transcripts accumulated in a coordinated manner at the moment in the infection process where, in the absence of maceration, intensive bacterial multiplication stops and the bacterial population stabilizes in the leaf (Lebeau et al, 2008). In a search for the regulatory proteins involved in this control, effects of mutations in regulatory genes on disease expression and *in planta* virulence gene expression were analyzed. The AHL-based ExpI-ExpR quorum-sensing system (Reverchon et al, 1998) is not required for virulence or the coordinated expression of virulence genes during infection. In contrast, the global regulator PecS recently shown to be at the top of a regulation network (Hommais et al, 2008), participates to this control since a *pecS* mutant exhibits an early expression of virulence genes during infection. This premature expression requires a functional GacA-GacS system to proceed. *rpoS* and *fis* (Lautier et al, 2007) mutants, inactivated in either the sigma factor-S or the nucleoid associated protein Fis, are altered in virulence. The effects of these mutations on virulence gene expression *in planta* are currently investigated.

AVR-Pia of _Magnaporthe oryzae_ encodes a protein with a secretion signal sequence

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AVR-Pia in *Magnaporthe oryzae* is an AVR gene that exhibits avirulence toward the rice *Pia* resistance gene. In order to clone and analyze the *AVR-Pia*, we have already reported that *AVR-Pia* is located inthe 1.2-kb DNA region (Vm), which has several ORFs. The longest ORF (255 bp) expressed by *TrpC* promoter didn't complement the avirulence in the mutant Ina168m95-1. Then, Vq, a 702-bp region containing the longest ORF and its 5′ region was cloned and revealed to contain *AVR-Pia*. Frame shift mutations were then introduced into this region by inserting a total of six single bases to cover all the putative ORFs. Only the two fragments with mutations in the 255-bp ORF showed virulence to Aichiasahi (*Pia*). It was finally concluded that *AVR-Pia* is the 255-bp ORF, which contains a predicted 19-amino acid signal peptide and was found to have a weak similarity with the bacterial cytochrome c family protein. The expression of *AVR-Pia* during the infection was assessed by quantitative realtime PCR using RNA extracted from leaves of Aichiasahi inoculated by strain Ina168. The expression of *AVR-Pia* was detected in 24 hours after inoculation, indicating that the *AVR-Pia* is expressed during appressorium formation.

Ralstonia solanacearum extracellular polysaccharide is a specific elicitor of defense responses in wilt-resistant tomato plants

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Ralstonia solanacearum, which causes bacterial wilt of diverse plants, produces a copious extracellular polysaccharide (EPS), which is a major virulence factor. The function of EPS in wilt disease development is uncertain. It has been variously hypothesized that EPS itself obstructs plant water transport and that EPS cloaks the bacterium from host plant recognition. We found that tomato plants infected with *R. solanacearum* race 3 biovar 2 strain UW551 upregulated genes in both the ethylene and salicylic acid defense signal transduction pathways. However, in response to *R. solanacearum* infection the horizontally wilt-resistant tomato line Hawaii7996 activated expression of these defense genes faster and to a greater degree than did susceptible cultivar Bonny Best. Interestingly, results suggest different roles of EPS in resistant and susceptible host responses to *R. solanacearum* infections. An EPS-deficient mutant of UW551 induced significantly lower defense responses in Hawaii7996 than the wild-type strain, weakening the "cloaking" hypothesis. In contrast, insusceptible plants the wild-type strain induced a lower defense response than did the *eps* mutant, supporting the idea that EPS can shield *R. solanacearum* from recognition by susceptible hosts. The *eps*- mutant also induced noticeably less accumulation of the defensive reactive oxygen species hydrogen peroxide in resistant tomato leaves, despite attaining similar cell densities *in planta*. In addition, cell-free purified EPS from UW551 triggered significant defense gene expression in resistant but not in susceptible tomato plants. Collectively, these data suggest that the wilt-resistant tomato line Hawaii7996 specifically recognizes EPS from *R. solanacearum*.

Comparative analysis of metabolic networks provides insight into the evolution of pathogenic and non-pathogenic lifestyles in _Pseudomonas_

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Bacteria in the genus *Pseudomonas* are ubiquitous inhabitants of the plant environment. However, individual strains and species adopt diverse lifestyles and colonise different plant-associated environments. The association of different species of *Pseudomonas* with different environments and different host interactions indicates that each species possesses distinct environment and lifestyle specific adaptations. In this study we have used the metabolic pathway prediction tool Rahnuma and complementary bioinformatic and phylogenetic analyses to assess the metabolic similarity across nine genome-sequenced *Pseudomonas* strains belonging to five distinct species by comparing the distribution and function of metabolic reactions annotated in the KEGG database. The results show that strains from the two pathogenic species, the opportunistic human pathogen *Pseudomonas aeruginosa* and the plant pathogen *Pseudomonas syringae* display a relatively high level of intra-species similarity compared to strains from the non-pathogenic species *Pseudomonas fluorescens,* which is consistent with experimental analyses of nutrient utilisation by these bacteria. We use parsimony analysis and reaction neighbourhood analysis to model the evolution of metabolic pathways in *Pseudomonas* and to infer the pathways and reactions present in the common ancestor of *Pseudomonas*.

A novel Arabidopsis mutant showing the abnormality for chitin elicitor signaling

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The detection of pathogens based on the perception of microbe/pathogen-associated molecular patterns (MAMPs/PAMPs) plays important role on basal resistance in plants. Chitin oligosaccharides represent a major MAMP elicitor of fungi and have been known to induce various defense responses in *Arabidopsis* and rice. Concerning to the receptor for the chitin oligosaccharides, we recently identified two essential components for chitin perception and transduction, CEBiP (Chitin Elicitor Binding Protein) and CERK1 (Chitin Elicitor Receptor Kinase1), in rice and Arabidopsis, respectively. To clarify downstream signaling cascade of these receptors, we developed a high-throughput screening method for signaling mutants based on the measurement of elicitor-induced ROS generation. We screened the activation tagged T-DNA insertional mutants for those suppressed for chitin response using this method, resulting in the finding of a novel mutant that almost completely lost chitin elicitor responsiveness. In the following generations of the mutant, however, even increased response to the chitin elicitor was observed. This apparently inconsistent phenomenon suggested a possibility of the overexpression of a negative regulator for defense responses in the original mutant and following silencing. Southern blotting analysis revealed that this mutant had at least two T-DNA insertion sites. We identified a candidate gene responsible for this mutant by IPCR and are analyzing the function of this gene.

OsTGAP1, a bZIP transcription factor, regulates cluster-scale inducible gene expression for momilactone biosynthesis in rice

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Plants attacked by pathogenic microorganisms respond with a variety of defensive reactions, including the production of antimicrobial secondary metabolites known as phytoalexins. Momilactones and phytocassanes are known as major phytoalexins in rice. We recently showed that the momilactone biosynthetic genes are clustered on rice chromosome 4 and are coexpressed after treatment with a chitin oligosaccharide elicitor. Genes for

most metabolic pathways in plants are not organized in gene clusters. So, it is interesting to elucidate the mechanism(s) regulating the expression of such clustered genes. To understand the regulatory mechanism of expression of the momilactone biosynthetic gene cluster, we carried out the promoter analysis of *OsKSL4* and searched transcription factors regulating *OsKSL4* expression using microarray analysis. As a result, we found that OsTGAP1, an elicitor-inducible bZIP transcription factor, regulates expression of *OsKSL4*. Interestingly, OsTGAP1 also regulates the expression of the genes in the cluster beside *OsKSL4*. The knockout mutant for *OsTGAP1* had almost no expression of the clustered genes or momilactone production upon elicitor treatment. On the other hand, OsTGAP1 over-expressing lines exhibited enhanced expression of the clustered genes and hyper-accumulation of momilactones in response to the elicitor. These results suggest that OsTGAP1 functions as a key regulator of inducible transcription in momilactone biosynthesis, and may indicate the presence of a unique cluster-scale transcription strategy for inducible defense mechanisms in rice. We are now performing a search for the OsTGAP1-binding sites in the momilactone biosynthetic gene cluster by ChIP-on-chip analysis using custom tiling array spanning the cluster region.

The role of NPR1 in Induced Systemic Resistance (ISR)

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Salicylic acid-activated NPR1, conferring resistance to biotrophic pathogens, is known to be associated with the crosstalk-restriction of the jasmonic acid (JA) pathway. However, in induced systemic resistance (ISR), triggered by the plant growth-promoting rhizobacterium, *Pseudomonas fluorescens* WCS417r, resistance is conferred by the JA and ethylene pathways and yet *requires* NPR1. To better understand the function of NPR1 in ISR, we utilized the *npr1* mutant and NPR1 transgenic plants. Using a Dex-inducible system, we found that nuclear accumulation of NPR1 is required for the full induction of resistance and that ISR is a nuclear function of NPR1. Since JA treatment can mimic pathogen attack and turns on a subset of ISR-responsive defense genes that are not normally targets of NPR1, we examined the activation of these JA-responsive genes in the *npr1* mutant. The ISR-specific priming of JA-responsive genes, both after JA treatment and pathogen challenge, is compromised in the *npr1* mutant suggesting that NPR1 is important for priming of defense genes in ISR. Interestingly, basal expression of a gene, known to be a target of NPR1 in SAR, is upregulated in an NPR1-dependent manner in ISR-expressing plants suggesting a potential involvement of this target gene with NPR1 in priming in ISR. We are currently utilizing whole genome microarray analysis to identify NPR1-regulated genes in ISR.

Nematodes as primary causal agent of gradual yield decline, debilitation and sudden death of tea bushes in some selected low grown areas of Sri Lanka, compunded by poor agronomic practices

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Reduction of yield, bush debilitation, eventually leading to death was reported from many tea (*Camellia sinensis* L.) holdings in Ratnapura district of Sri Lanka. The present study investigated probable biotic and abiotic causal factors of sick soils in the affected tea fields in comparison with a healthy condition. The problem resulting in bush debilitation, yield decline and death was different to already known and reported conditions in tea and plantation crops; plant parasitic nematodes act as a primary attribute amongst various biotic and abiotic factors causeing the disease complex. Hence, the significantly changed distribution and incidence pattern of *Pratylenchus loosi* and *Radopholus similis* warrant mandatory integrated nematode management strategies. An average disease severity index (ADSI) was developed with scores ranging from 0 – 4: 0 for apparently unaffected bushes and 4 for very severely affected bushes using visual observations. The mean root growth after two months from planting of 8 month old tea (cultivar TRI 2026) in sick soils of 2 locations were significantly low compared to healthy soils. Root growth in plants grown in original soils was low than that of sterilized soils indicating a biological factor causing failures in overall plant growth, infestation by in varying levels *P. loosi* of which was identified as the primary attribute in field studies. The nematode populations in different root sizes and fragments were significantly (Pr> 0.0001) different at different severity levels. The root fragments exhibited significantly (Pr.> 0.0111) greater nematode numbers than that of other root sizes.

Symbiotic efficiency of *Mesorhizobium*, *Rhizobium* and *Sinorhizobium* bacteria nodulating wild *Lotus* sp. in South area of Tunisia

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Twenty-two rhizobial strains, belonging to *Mesorhizobium, Rhizobium* and *Sinorhizobium* genera and nodulating *Lotus creticus*, *L. pusillus*, and *L. arabicus* on the arid soils of Tunisia, were examined for symbiotic properties In previous work, we reported for the first time *Sinorhizobium* sp. as a new microsymbiote of *Lotus* sp. The objective of this work wqs to determine the symbiotic effectiveness of different isolates with the aim of obtaining selected strains to re-introduce as inoculants in *Lotus* pastures. Symbiotic results showed that ten isolates can be considered as efficient (relative effectiveness ≥ 70%): one isolate belonging to *Mesorhizobium* sp., four to *Rhizobium* and five to *Sinorhizobium*. Since *Lotus* species are able to grow under stress conditions, the inoculation of seeds and seedlings with these appropriate native rhizobia resistant to salinity, to acidity and to high temperature would guarantee root nodulation, enhance plant performance, and reintroduce these microorganisms in the soil.

Nitric oxide: A signaling molecule during the Russian wheat aphid resistance responses in wheat?

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The involvement of nitric oxide (NO) in the Russian wheat aphid (RWA), *Diuraphis noxia*, resistance responses was investigated in the resistant (cv., *Tugela DN* containing the *Dn1* resistance gene) and near-isogenic susceptible (cv., *Tugela*) wheat (*Triticum aestivum* L.) plants. Induction of NO due to sodium nitroprusside (SNP, a NO donor) treatment consequently led to induction of the secondary defense related enzyme activities (intercellular ß-1,3-glucanase and peroxidase). These enzymes are used as markers of resistance during RWA resistance. Salicylic acid (SA) content induction in SNP-treated (root application) plants gives an indication that NO may be acting upstream SA. Inhibition studies using tungstate further substantiated this proposition. Symptom analysis scoring results provided evidence that NO leads to a reduction in RWA feeding symptom appearance, indicating that NO is involved in RWA resistance. Results also show that seed dressing with SNP is more effective than root application. This was evidenced by a significant decrease in aphid populations and less aphid attraction bn the leaves of the infested susceptible plants where seed treatment was performed than where root treatment was undertaken.

Visualising the transcription of *Ralstonia solanacearum* key pathogenicity genes *in planta*

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During infection, pathogens deploy a tightly regulated genetic program to overcome the host natural defences and mobilise metabolic resources to their benefit. This program leading to visual disease symptoms is unknown for most pathosystems, although many genes involved in infection have been characterised in *in vitro* cultures. *Ralstonia solanacearum* – a soilborne vascular pathogen that causes bacterial wilt – is one of the organisms where regulation of pathogenicity genes is best known. We present here the setting up of a system to visualise bacterial promoter output *in planta* and its use to determine the exact expression timing of key transcriptional regulators during infection. This system allows a simple and rapid way to measure the activity of selected promoters on their very natural environment. Our promoter probing toolbox is based on versatile suicide plasmids that carry the promoter of interest fused to fluorescent or luminescent reporter genes flanked by transcription terminators. For stable integration, the fusions are targeted specifically to a permissive site in the chromosome through double recombination. The modular nature of all DNA constructs enable easy replacement of the promoters as well as the reporter genes. A miniaturised pathosystem inoculating on tomato plantlets grown on agar has also been developed for detection of bacterial gene expression *in planta*. Using these novel tools we have been able to detect expression from monocopy gene fusions in the bacterial genome during xylem colonisation. Data will be presented showing the expression of key pathogenicity genes controlling expression of the type III secretion system or exopolisaccharide production at different stages of tomato infection.

Analysis of ROS production at early stages of the *Phaseolus vulgaris*-*Rhizobium etli* symbiosis

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Reactive oxygen species (ROS) are produced in plants as a result of aerobic metabolism during their development or in response to abiotic and biotic stress. ROS are key actors in stomatal closure, cell death, defense response, and root hair development. During the early stages of a legume-rhizobia interaction, an accumulation of ROS has been observed in alfalfa. Inhibition of ROS production prevents root hair curling and formation of infection threads. Recently, in our group, fast and transient ROS changes were observed in root hair cells, seconds after treatment with specific Nod factors. All these data support the notion that ROS production is needed for the legume-rhizobia interaction. To further explore the participation of ROS in the early events of the bean-rhizobia symbiosis, the content of H_2O_2 in bean roots infected with rhizobia 5 days post inoculation (dpi) was analyzed in comparison with uninoculated roots. The results indicate that there is an important increase in H_2O_2 levels after rhizobium inoculation. The O_2^- anion production was also examined in nodule primordium 5 dpi and we found that this is prevented with DPI but not with NaN_3 (apoplastic peroxidase inhibitor), suggesting the participation of NADPH oxidases (RBOH). To investigate the possible role of RBOH in ROS production, we determined the size of the *Rboh* gene family by Southern blotting. *PvRboh* gene family consist of at least three members. A profile of transcript accumulation in different bean tissues was determined for each *PvRboh* and no evident changes in the transcript accumulation patterns were after bacterial inoculation. Experiments are in progress to unravel the phenotype of knockdown *Rboh*s in bean transgenic roots inoculated with rhizobia.

The ThPG1 endopolygalacturonase is required for the *Trichoderma harzianum*-plant beneficial interaction

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There are well known the biocontrol mechanisms of *Trichoderma* species such as mycoparasitism, nutrient competition, and antibiosis. In the last years, the *Trichoderma* studies are aimed to analyze the *Trichoderma*-plant relationship. Considering the complexity of this interaction at the plant rhizosphere, we used proteomic, genomic and transcriptomic approaches to study a novel *Trichoderma* gene coding for a plant cell wall (CW) degrading enzyme in an *in vivo* system. We carried out a proteome analysis using a three-component (*Trichoderma*-tomato plantlets-pathogen) system, and a differentially expressed *T. harzianum* endopolygalacturonase (endoPG) was identified. Spot 0303 remarkably increased only in the presence of the soilborne pathogens *R. solani* or *P. ultimum*, and corresponded to an expressed sequence tag (EST) from a *T. harzianum* T34 cDNA library, that was constructed in the presence of plant CW polymers and used to isolate the *Thpg1* gene. Compared to wild type strain, *Thpg1*-silenced transformants showed lower PG activity, less growth on pectin medium and reduced capability to colonize tomato roots. These results were combined with microarray comparative data from the transcriptome of *Arabidopsis* plants inoculated with the wild type or a *Thpg1*-silenced transformant. The endopolygalacturonase-encoding gene was found to be required for active colonization of the roots and induction of plant defense responses by *T. harzianum* T34.

Mutational dissection of the pepper Bs3 resistance pathway in Arabidopsis

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The Gram-negative bacterium *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) is the causal agent of bacterial spot disease in pepper and tomato. *Xcv* uses a type III secretion system to inject effector proteins into the host cytoplasm. AvrBs3, a transcription activator like effector (TAL) protein was shown to induce virulence-associated plant transcripts in susceptible plants. Previously, we showed that AvrBs3 binds to and transcriptionally activates the promoter of the pepper Bs3 resistance gene. Bs3 encodes a YUCCA-like flavin monooxygenase and is thus a structurally novel type of resistance protein. In order to mutationally dissect this novel type of resistance pathway we generated transgenic Arabidopsis plants expressing *avrBs3* under control of an inducible and *Bs3* under control of its native (AvrBs3-inducible) promoter. These transgenic plants undergo systemic cell death upon application of the inducing compound and provide the basis of a mutational screen aimed at identifying components of the Bs3 resistance pathway. Results on the mutational dissection of the Bs3 pathway will be presented.

The catalytic domains of the diversified alleles of the *Pseudomonas syringae* type III effector HopZ1 determine their distinct recognition specificities in plant hosts

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Pathogens and hosts are engaged in an endless and deadly warfare. Type III secretion system (T3SS) and secreted effectors (T3SEs) are essential virulence factors of gram negative bacterial pathogens. During the arms race with the pathogens, plants have evolved resistance (R) genes to detect specific pathogen T3SEs and induce defense responses. However, this immunity can be efficiently defeated by pathogens through effector sequence variation. In the plant pathogen *Pseudomonas syringae*, HopZ1 is a member of the widely distributed YopJ T3SE family. HopZ1 is under strong diversifying and positive selections, most likely imposed by the plant R gene-associated defense. HopZ1a, most resembling the ancestor allelic form, triggers defense responses in *Arabidopsis thaliana*, *Nicotiana benthamiana* and soybean. However, HopZ1b, derived from a HopZ1a-like ancestor, evades the recognition in *Arabidopsis* and soybean. It has been suggested that sequence diversification has allowed HopZ1b to evade detection while retaining virulence functions. In this study, we employed domain shuffling and site-directed mutagenesis to determine the key regions and amino acid residues in HopZ1 alleles that confer the allelic specificity. Our data demonstrated that the central domain containing the predicted catalytic core determined HopZ1 recognition specificity. Moreover, key residues within the central domain required for triggering plant defense responses were identified. These key residues were specific to the plant host and differed between HopZ1a and HopZ1b. These findings support the hypothesis that HopZ1 evolution, driven by sequence diversification, resulted in altered host substrate-binding specificity, and thus led to recognition evasion.

Dissection of allelic variation in barley identifies master regulators of defense against stem rust

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In order to characterize the transcriptomic response to stem rust invasion in a genetic context, Barley1 GeneChips were used to measure gene expression in each member of the Q21861 x SM89010 doubled haploid population (QxSM) after treatment with *Puccinia graminis* f. sp. *tritici* isolate TTKS (*Pgt* TTKS), commonly referred to as Ug99. For comparison, global gene expression in the QxSM lines was also measured after mock inoculation as part of the same experiment. By analyzing the changes in genomic distributions of expression Quantitative Trait Loci (eQTL) between *Pgt* TTKS -inoculated and mock-inoculated treatments, major alterations in the regulation of steady-state and inoculation-responsive mRNA levels were uncovered. Notably, five *trans*-eQTL hotspots were identified and appear to regulate the expression of hundreds of inoculation responsive genes scattered around the genome. Interestingly, none of them are associated with the *Rpg5*/*rpg4* locus at which Q21861 carries an allele that recognizes *Pgt* TTKS. However, one of these *trans*-eQTL hotspots is coincidently located with an enhancer of *R* gene-mediated resistance that was discovered through a parallel effort to identify QTL alleles that confer resistance to *Pgt* TTKS. The positional identification of *trans*-eQTL hotspots demonstrates that transcriptome-wide induction and suppression of defense genes is tightly coordinated by regulators that are genetically tractable.

Salicylic acid causes alteration in ABA signaling and induces ABA insensitivity in lesion mimic mutants

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It has been reported that a number of *Arabidopsis* lesion mimic mutants show alterations in abiotic stress responses as well as pathogen resistance. Of these, the *Arabidopsis* mutants *cpr22* and *ssi4*, that have mutations in a cyclic nucleotide-gated ion channel and a TIR-NBS-LRR type *R* gene, respectively, are typical lesion mimic mutants; they accumulate elevated levels of salicylic acid (SA) and exhibit spontaneous cell death, constitutive expression of defense-related genes, and enhanced resistance to various pathogens. Despite the fact that the mutations are in different genes, in both mutants the aforementioned immunity-related phenotypes are suppressed under high relative humidity conditions and enhanced by low relative humidity. To investigate environmental effects on these mutants, we have conducted genome wide transcriptome analysis. Interestingly, the expression of a number of genes that are related to abscisic acid (ABA) signal transduction was significantly altered in both mutants compared to wild type plants. Endogenous ABA measurement and the characterization of ABA-related phenotypes provided additional support for the involvement of ABA. Furthermore, double mutant analysis with *NahG* plants, that degrade SA, indicated that these alterations in ABA signaling were attributable to the elevated SA levels. Long-term drought stress did not induce activation of ABA signaling in *cpr22*, but rather suppressed the drought-induced increase in ABA levels. These result suggested a new mechanism by which ABA signaling during long-term stress can be suppressed upstream of ABA synthesis.

Characterization of the flagellin interaction sites in the extracellular LRR domain of FLS2

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Plants like Arabidopsis and tomato can detect bacterial flagellin with a receptor kinase termed FLS2. The 22 aa peptide flg22, representing the most conserved domain within the N-terminal part of flagellin, acts as a ligand for FLS2 and activates immune responses at subnanomolar concentrations. Receptor activation occurs according to the address-message concept, with binding of the N-terminal part of flg22 (address) as a first step, and activation by the C-terminal part of flg22 (message) as a second step. Here, we aim at mapping the sites on the extracellular leucine rich repeat (LRR) domain of FLS2 that interact with the address and message part of the flg22 ligand. One type of approach makes use of species-specific differences in perception of flg22-derivatives observed for FLS2 from Arabidopsis (AtFLS2) and tomato (LeFLS2). AtFLS2 and LeFLS2 are structurally conserved and both have 28 leucine rich repeats (LRRs) with 54% identity in their aa sequence. Constructing a first series of hybrid At/Le-FLS2 receptors with swaps of LRR-subdomains allowed attribution of address and message function to separate parts in FLS2. Currently, with a series of smaller swaps and point mutations we attempt to fine map these sites. In a complementary, biochemical, approach flg22 was modified, either at its N- or C-terminus, by conjugation with a biotin group and a photoactivatable crosslinker group. Both types of modified ligands are fully active as ligands and they specifically crosslink to FLS2. For analysis of the sites of crosslinking by affinity purification and MS/MS we currently try to increase the number of binding sites by overexpressing the ectodomain of FLS2 (eFLS2). When expressed in plant cells first results show that eFLS2 is a soluble protein that binds flg22 with all the characteristics found with intact FLS2.

Identification of virulence / pathogenicity genes of *Colletotrichum graminicola*

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A previously developed Agrobacterium tumefaciens-mediated transformation (ATMT) protocol for the corn pathogenic fungus *Colletotrichum graminicola* led to high rates of tandem integrations, especially of the whole Ti-plasmid, and was therefore considered unsuitable for identification of pathogenicity and virulence genes in this pathogen. We used a modified ATMT protocol with acetosyringone present only during co-cultivation of *C. graminicola* and A. tumefaciens. Analysis of 105 single-spore isolates randomly chosen from a collection of approx. 2,000 transformants indicated that almost 70% of the transformants had single T-DNA integrations. Of 500 independent transformants tested, 19 exhibited attenuated virulence in infection assays on whole plants. Microscopical analysis primarily revealed defects at different pre-penetration stages of infection-related morphogenesis. Cytorrhizis experiments showed that appressorial penetration defects were not due to reduced turgor in appressoria. To date, in seven transformants T-DNA integration sites were identified by amplification of genomic DNA ends after endonuclease digestion and polynucleotide tailing. Mostly 5' flanks of genes encoding hypothetical proteins were disrupted by the T-DNA, one transformant showed integration into the coding region of a putative gene of other Ascomycota. One transformant was affected in establishment of compatibility with its host plant, as it elicited papilla formation. To characterize candidate genes functionally and to confirm the results obtained by ATMT, the entire genes were deleted. Expression profiling and GFP tagging will address the role of these genes during specific steps of the infection process.

Diffusible signals from arbuscular mycorrhizal fungi stimulate plant growth in land plants

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Arbuscular Mycorrhization (AM) refers to a mutualistic association between plant roots and *Glomeromycota* fungi. AM fungi have existed in symbiosis with plant roots for over 460 million years, since the appearance of the first land plants. More than 80% of present-day plant species can form beneficial mycorrhizal associations. Studies in model legumes have identified the existence of a signal exchange between AM fungi and host plants. Diffusible signals secreted by AM fungi have been shown to promote lateral root development and induce a symbiosis-specific *MtENOD11* expression in the model legume *Medicago truncatula.* They also induce a transient cytosolic calcium elevation in soybean cells. Our results indicate that treatment with the supernatant of germinating AM spores triggers an efficient stimulation of plant growth, especially lateral root development, in monocots in the absence of AM fungi. Similar results have been observed in *Medicago truncatula*. Effects of this treatment on the root architecture of symbiotic mutants in *Medicago truncatula* and rice have been studied. Stimulation of growth in *Physcomitrella patens* have also been observed indicating that this mechanism is very well conserved across land plants.

Towards Arabidopsis immune system protein-protein interaction network

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Plant innate immunity operates through cell-surface localized pattern recognition receptors (PRR) and cytosolic receptor NB-LRR proteins (nucleotide-binding, leucine-rich repeat). Bacterial type III effectors are delivered directly into the host cell and can modulate subsequent immune responses by targeting various host proteins. An enabling step for understanding type III effectors-host target interactions is mapping networks of physical protein-protein interactions. We generated 115 clones for *Pseudomonas syringae* type III effectors from 15 different diverse strains, pathogenic on an evolutionarily widespread set of hosts. We also generated 180 kinase domain clones from receptor like kinase (RLKs; a sub-class of PRR) and N-terminal clones of coiled-coil (CC)/Drosophila Toll or mammalian Interleukin 1 receptor (TIR) domains from 140 NB-LRR proteins. Using a stringent, high-throughput and semi-automated Y2H system, we pairwise tested interactions among 115 bacterial type III effectors, 180 RLKs' kinase domains, 140 N-termini of NB-LRR proteins and the products of 9,500 Arabidopsis clones. Among the 4.6 million tested combinations, we obtained 671 interactions between 177 baits and 376 preys. The major functional categories of interactors include transcription factors, enzymes, F-box proteins and proteins of unknown function. This first plant immunity interactome includes examples, where an Arabidopsis protein is targeted by multiple type

III effectors and also interacting with kinase domain of RLK or N-termini of NB-LRRs, consistent with our previous findings. We will also provide data for functional relevance of key host target proteins found in our interactome. An initial draft of the Arabidopsis immunity interactome consolidating our all findings will be presented.

Characterization of *Rfb*: A novel form of resistance to *Pseudomonas syringae* pv. *tomato*

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Immunity to *Pseudomonas syringae* pv. *tomato* in Solanum species is governed by recognition of the type III effector proteins AvrPto and AvrPtoB by members of the Pto family of kinases and the NBS-LRR protein Prf. Investigation of the interactions between AvrPtoB and two Pto family members, Fen and Pto, has resulted in the following evolutionary model exemplifying the molecular arms race between a pathogen and its host. In the absence of R proteins, the N-terminal region of AvrPtoB (ApB-N) suppresses PAMP-triggered immunity leading to enhanced virulence. Under disease pressure, Fen evolved to mimic the target(s) of ApB-N and elicit effector-triggered immunity (ETI) through the typical R protein Prf. To evade ETI, ApB-N acquired an E3 ubiquitin ligase domain that targets Fen for degradation via the 26S proteasome pathway once more allowing disease to ensue. Pto is recalcitrant to ubiquitination by AvrPtoB and is proposed to have evolved to counter ETI suppression by AvrPtoB. We hypothesized that a natural variant of Fen might also be recalcitrant to ubiquitination by AvrPtoB. We screened a subset of wild tomato accessions encompassing 11 *Solanum* species for recognition of full-length AvrPtoB but not AvrPto. Accessions displaying this phenotype were analyzed for sequences related to *Pto* and were found to contain four intact *Pto* family members, two of which interacted with full-length AvrPtoB in yeast. We propose that a Fen variant is responsible for this novel form of resistance termed *Rfb* (Recognition of Full-length AvrPtoB).

Natural variation in wild relatives of tomato for resistance to *Pseudomonas syringae* pv. tomato

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The bacterial pathogen *Pseudomonas syringae* pv. tomato (*Pst*) causes speck disease on wild relatives of tomato and mediates virulence through the deployment of effector proteins via the type III secretion system. Effector-triggered immunity (ETI) to *Pst* in tomato is conferred by the recognition of two effectors, AvrPto and AvrPtoB, by the protein kinases Pto and Fen, which are dependent on the NBS-LRR protein Prf for ETI signaling. In *Solanum pimpinellifolium* these three genes are encoded within an ~60kb region that also includes four additional Pto-like kinases, which likely arose through duplication. Two resistance phenotypes mediated by Pto family members have been described previously; recognition of AvrPto and AvrPtoB (Pto) and recognition of AvrPtoB lacking its E3 ubiquitin ligase domain (Rsb). To further investigate the natural variation in resistance to *Pst*, accessions from 11 *Solanum* species were screened for the ability to recognize AvrPto or AvrPtoB. In addition to the Pto and Rsb resistance phenotypes, four additional phenotypes were predicted; recognition of only AvrPto (Raa), recognition of only AvrPtoB (Rfb), no resistance (none), and resistance independent of AvrPto and AvrPtoB recognition (non-Pto). Preliminary evidence indicates that all six phenotypes exist among the wild relatives of tomato. Sequence analysis of the Pto-like kinase family is being used in conjunction with the phenotypes to gain insight into the evolution of *Pst* resistance in tomato species.

Involvement of *Magnaporthe oryzae* chitin-binding protein gene, *CBL1*, in appressorium differentiation

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Magnaporthe oryzae is the causal agent of rice blast disease. Like many other fungal pathogens, *M. oryzae* forms appressorium, a specialized infection structure. Appressorium formation of *M. oryzae* could be induced by environmental signals, including both physical and/or chemical cues. We previously isolated chitin-binding protein gene (*CBP1*) from *M. oryzae* (Kamakura *et al*. 2002). Since *cbp1* disruption mutants showed abnormal appressorium differentiation only on artificial surfaces, *CBP1* seemed to play an important role in appressorium formation induced by physical factors. The mutant maintained the ability to form appressoirum by chemical inducers. In the present work, we found another homologous chitin deacetylase gene MGG_09159, named *CBL1* (*CBP1* like gene 1), showing strong homology to *CBP1* at the domain level in the genome database of this fungus. To investigate the relationship *CBP1*and *CBL1* and the function of the chitin deacetylases in *M. oryzae*, we have generated the *cbl1* disruption mutants and *cbp1cbl1* doubleknock out mutants by targeted gene disruption. Disruption of *cbl1*caused no defect in colony growth or morphology during appressorium differentiation. However, double disruption of *cbp1* and *cbl1* caused decrease in the conidiation ability, adhesion of germling, and levels of appressorium formation. Furthermore, activation of cutin monomer 1,16-hexadecandiol, which is reported as a chemical inducer of appressorium formation, couldn't completely restore the defect of appressorium formation in double disruption mutant, while it induced appressorium formation of *cbp1* mutant at 6 hr inoculation. These data suggest that homologous chitin deasetylase protein genes *CBP1* and *CBL1* play important roles in differentiation process of *M. oryzae* infecting host plant.

***Pseudomonas syringae* manipulates host auxin physiology to promote disease**

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Pseudomonas syringae is a bacterial plant pathogen that uses manipulation of plant hormone signaling as one of its virulence strategies. Several lines of evidence indicate that modulation of auxin physiology is important in *P. syringae* disease development. Levels of indole-3-acetic acid (IAA), the major form of auxin, increase in plant tissue during infection. Treatment with exogenous 1-naphthaleneacetic acid (NAA), a synthetic auxin analog, causes an increase in disease symptom severity during infection. Also, the *P. syringae* Type III-secreted effector protein AvrRpt2 modifies auxin physiology in *Arabidopsis thaliana* during susceptible interactions (Chen et al., PNAS, 2007). Treatment with flg22, a MAMP that induces basal defenses, down-regulates auxin signaling through a miRNA-mediated pathway, suggesting that suppression of auxin-mediated responses is an important component of defense (Navarro et al., Science, 2006). The excess auxin that accumulates during infection may be synthesized by the pathogen, or the pathogen may stimulate the plant host to synthesize it. We are investigating potential pathways that *P. syringae* strain DC3000 may use to synthesize IAA. Disruption of genes predicted to encode enzymes in the indole-3-acetamide pathway for IAA synthesis resulted in no measurable change in the amount of IAA produced in culture by DC3000. Other potential IAA synthesis genes are currently being examined to determine if another pathway is used. We will also report on our efforts to elucidate the roles of IAA during pathogenesis.

Effect of colonization of a bacterial endophyte, *Azospirillum* sp. on disease resistance against rice blast fungus in rice

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Agriculturally important grasses contain numerous diazotrophic bacteria, which interaction is known to have some other benefits to host plants. In this study, we analyzed the effect of a bacterial endophyte, *Azospirillum* sp., on disease resistance of host plants. Rice inoculated with *Azospirillum* sp. exhibited enhanced resistance against rice blast disease caused by a virulent fungus, *Magnaporthe grisea*, and rice bacterial blight caused by a virulent bacterial pathogen, *Xanthomonas oryzae*. The inoculation with this bacterium did not induce salicylic acid (SA) accumulation, the expression of pathogenesis-related genes, and any visible morphological affects in the host rice plants. These results indicated that the *Azospirillum* sp. were able to induce disease resistances in rice by activating the mechanism different from SA-dependent defense signaling. The effects of this bacterium have been also examined in rice fields, which indicated that the inoculated rice plants exhibited moderate disease resistance against rice blast.

Role of Lipoxygenases in plant defense responses

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Oxylipins are synthesized by the introduction of molecular oxygen, by Lipoxygenases (LOX) at either the C-9 (9-LOX) or C-13 (13-LOX) of

polyunsaturated fatty acids. The 9- or 13-hydroperoxides produced are precursors of several biologically active compounds including jasmonates, which have hormone like regulatory and defense-related roles. The 9-hydroperoxides are converted to compounds whose physiological actions are largely unknown; although research suggests that they have antimicrobial properties, induce hypersensitive response and function in cell wall modifications required for lateral root development and pathogen arrest. Oxylipins are also produced by several filamentous fungi, yeast and oomycetes and are thought to function as intra-kingdom signals. In our laboratory we utilize Arabidopsis as a model system to study various aspects of plant defense responses. Our research has shown that the Arabidopsis LOX1 and LOX5 genes, which encode 9-LOXs, play an important role in defense against a hemibiotrophic fungal pathogen, *Fusarium graminearum*. Interestingly, the lox5 mutant also displays resistance against a generalist aphid, *Myzus persicae*, and is also compromised in systemic acquired resistance (SAR). Based on these observations, we believe that the LOX pathway in plants plays an important role in modulating plant responses against various biotic stresses.

The *Zea mays* ChitA chitinase and its modification by secreted proteins from fungal ear rot pathogens

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Zea mays (maize) seeds contain ChitA, a chitinase protein that contains a small, N-terminal domain (hevein) that allows the chitinase to bind insoluble chitin polymers with high affinity. We have discovered that maize ChitA chitinase is modified by a class of secreted fungal proteins termed chitinase modifying proteins (cmps). Fungal cmps are secreted by the ear rot pathogens *Bipolaris zeicola* (Holomorph = *Cochliobolus carbonum*) and *Stenocarpella maydis* (syn. *Diplodia maydis*). Biochemical experiments have demonstrated that fungal modified ChitA retains chitinase activity but has lost the ability to bind insoluble chitin. A previous report classified ChitA as an antifungal protein. This classification was based on the ability of purified ChitA to inhibit the growth of some fungal plant pathogens in agar plate assays. We hypothesized that fungal modified ChitA, which no longer binds chitin, would have reduced potency in agar plate assays. We found that ChitA's ability to inhibit fungal growth in agar plate assays was unaffected by cmp facilitated modification and loss of chitin binding affinity. In summary, cmp proteins secreted by fungal ear rot pathogens modify maize ChitA chitinase resulting in loss of chitin binding abilities. Agar plate assays demonstrate that this modification does not alter ChitA antifungal activity and suggest that the *in planta* function of ChitA is more complex than the antifungal activity demonstrated in agar plate assays.

Control of pattern-recognition receptors by an ER protein complex in plant immunity

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Perception of pathogen-associated molecular patterns (PAMPs) by patternrecognition receptors (PRRs) plays an important role in plant innate immunity. In Arabidopsis, the leucine-rich repeat receptor kinases (LRR-RKs) EFR and FLS2 mediate recognition of the bacterial PAMPs EF-Tu and flagellin, respectively. Only limited knowledge exists on how EFR and FLS2 function at the molecular level. Here, we identify by forward genetics the Arabidopsis stromalderived factor 2 (SDF2) as being required for responses mediated by EFR and FLS2. *Sdf2-2* mutants are more susceptible to bacteria fungi, indicating that other, yet unknown PRRs also require SDF2. SDF2 resides in the endoplasmic reticulum (ER) in a complex with ERdj3B and the luminal binding protein (BiP). ERdj3B and BiP are components of the ER-quality control (ER-QC), and EFR accumulation is impaired in the *sdf2* mutant. *Erdj3b* mutants were independently identified in our screen, and loss of ERdj3B function leads to compromised EFR and FLS2 responses. Additionally, *sdf2* and *erdj3b* mutants were impaired in the unfolded protein response induced by the drug tunicamycin. Our results demonstrate that several surface-exposed PRRs require the ER complex SDF2- ERdj3B-BiP for their function, and provide the first demonstration of a physiological requirement for ER quality control in trans-membrane receptor function in plants.

The influence of the bacterial secondary metabolite 2,4–diacetylphloroglucinol on induced systemic resistance in *Hordeum vulgare*

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The inoculation of the roots of different plants with bacteria can lead to the establishment of Induced Systemic Resistance (ISR) where the aerial plant parts are 'primed' to resist pathogen challenge. A variety of bacteria including many *Pseudomonas fluorescens* strains have been shown to elicit the ISR response, which has been studied almost exclusively in dicots. One bacterial factor which is known to trigger ISR in *Arabidopsis* and rice is the antifungal compound 2,4-diacetylphloroglucinol (DAPG). The mechanism(s) through which DAPG exerts these effects is not fully understood however. The aim of our work was to establish whether DAPG is capable of establishment of ISR in barley (*Hordeum vulgare*) effective against the seedling blight pathogen *Fusarium culmorum*, and to begin to address the molecular basis of this influence of DAPG through analysis of plant gene expression. The DAPG-producing wild type strain *P. fluorescens* F113 and a DAPG-deficient derivative were inoculated into soil into which seeds of barley (*Hordeum vulgare* cv *Lux*) were planted. Following a growth period of 10 days, the seedlings were then challenged with the fungus *Fusarium culmorum*. At ten days post-infection, plants inoculated with wild type *P. fluorescens* F113 showed reduced disease symptoms compared to those inoculated with the non-DAPG producing mutant, which were in turn less than those seen in plants without bacterial treatment. Micro-array and RT-PCR analyses showed significant changes in expression of a number of genes including those encoding transcriptional factors, lipid transfer proteins and leucine-rich repeat proteins, which may be implicated in ISR.

The two virulence determinants of AvrPto are functionally conserved in diverse homologs

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Pseudomonas syringae type III effector AvrPto promotes bacterial virulence in susceptible plants by suppressing the host plant's PAMP-triggered immunity (PTI). We have demonstrated previously that AvrPto has two distinct virulence determinants with similar and additive effects: the CD-loop and the C-terminal phosphorylation sites (Ser147 and 149). In resistant tomato, the resistance protein complex Pto/Prf recognizes AvrPto via the direct interaction of the CD-loop and Pto. This recognition event leads to effector-triggered immunity (ETI). We have discovered that AvrPto is also recognized by another resistance gene (Rpa) that detects only the phosphorylated C-terminal region. We will present results that address the potential conservation of these two virulence determinants in diverse AvrPto homologs from P. syringae pathovars infecting a wide range of host plants.

Dual promoter vectors for high-throughput gene function analysis by RNA silencing

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RNA silencing offers potent and flexible tools to explore gene function in the post-genomics era. We previously showed that the RNA-silencing vector, pSilent-Dual1 (pSD1) carrying two convergent promoters, *Aspergillus nidulans TrpC* (PtrpC) and *gpd* (Pgpd), was applicable for large scale loss of functional analysis in *Magnaporthe oryzae* (Nguyen *et al*., 2008). In this study, we constructed a new RNA silencing vector pSilent-Dual2 (pSD2) with two convergent *TrpC* promoters, and assessed the efficacy of RNA silencing induced by the dual promoter vectors in detail. The size of a target gene inserted into the vectors greatly affected the efficacy of gene silencing. In general, a smaller fragment induced gene silencing more efficiently either with pSD1 or pSD2 in *M. oryzae* even though a minimum of a 0.2kb fragment was necessary for inducing stable silencing. The pSD2 vector showed a little higher silencing efficacy than did pSD1 especially when insert size was relatively small. Correlation of gene silencing between transcriptionally-fused genes in pSD1 was examined using the xylanase (MGG01542) or calcium channel (MGG05643) gene fused to an eGFP fragment. Analyses of

enzymatic activity and quantitative PCR revealed that silencing of the target genes was moderately correlated (R=0.6-0.7) with that of the eGFP gene at the transcriptional and phenotypic levels.

A MAPK cascade is a molecular switch for jasmonic acid biosynthesis during biotrophic challenge

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Cytochromes P450 are one of the largest superfamilies of proteins and are ubiquitously distributed in all organisms. Allene oxide synthase (*AOS*) is an atypical P450 of the CYP74A gene family. This enzyme plays a key role in the biosynthesis of jasmonic acid (JA), a plant hormone which is involved in plant defence reactions and development. Phytohormone biosynthesis and signalling are good examples of the complexity of positive and negative regulation among cross-linked signalling pathways. Moreover, establishing an appropriate defence reaction depends upon a complex array of interlinked biological processes. MAPK cascades are molecular switches that activate appropriate cellular responses based on endogenous signals or environmental cues. These phospho-cascades are implicated in JA biosynthesis and signal transduction, but the mechanism by which the MAPK-JA pathway interaction occurs is still unclear. Using two different species of the biotrophic rust fungus *Melampsora*, we found that JA accumulates in poplar (*Populus nigra x P. maximowzcii*) leaves following infection with *Melampsora medusae* f. sp. *deltoids* (*Mmd*). We consequently monitored transcript accumulation of poplar *AOS* paralogs *PtAOS1* and *PtAOS2* as well as of *PtMPK3*, the poplar ortholog of *AtMPK3* and *NtWIPK* from *Arabidopsis thaliana* and *Nicotiana tabacum* respectively. In addition, a possible protein interaction between PtMPK3-1 and PtAOS1 was tested in yeast and *in planta*. Taken together, our results suggest that JA participates in the poplar defence response against rust fungi and link JA biosynthesis to a MAPK cascade via direct interaction between the PtMPK3-1 and PtAOS proteins.

Does PEN1 control endocytosis required for innate immunity?

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Innate immunity is vital for establishing an effective host defence against pathogens. In plants, this is exemplified by the pre-invasive penetration resistance against "non-host" powdery mildew fungi. This innate immunity is under control of the plasma membrane localised PEN1 syntaxin (SYP121) which accumulates at the site of attack. Syntaxins are SNARE proteins playing a central role in the exocytotic process of vesicle traffic. PEN1 is thought to confer penetration resistance by secreting material into the papillae yet PEN1 is not a prerequisite for papillae formation. Through our work, we have discovered that PEN1-dependent penetration resistance is sensitive to a number of endocytosis inhibitors leading to a delay in papillae formation as seen for mutants in PEN1. Moreover, inhibition of sphingolipid- and sterol biosynthesis, as well as depletion of sterols from the plasma membrane, also hampers PEN1-dependent penetration resistance. This suggests that PEN1 is associated with endocytotic processes required for timely papilla formation as well as for penetration resistance. Involvement of PEN1 in early signalling is in agreement with its general effect on papilla timing, rather than on papilla formation *per se*. We think that PEN1 is responsible for establishing a plasma membrane environment that facilitates an endocytosis subsequent to recognition of fungal-derived cues.

Identifying *Pseudomonas syringae* Type III effector proteins that modulate auxin signaling in *Arabidopsis thaliana*

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Plant hormones act in a complex network where their pathways regulate and interact with each other to control different responses. This cross talk between hormones can be exploited by pathogens to suppress plant defense responses and thereby increase their growth. *Pseudomonas syringae* pathogenicity is reliant on a Type III secretion system (TTSS) that acts as a specialized injection apparatus to deliver virulence proteins, known as type III effectors (TTEs), into the plant cell cytosol. A few well characterized TTEs, such as AvrPtoB, HopAM1 and AvrPto, have been shown to modulate *Arabidopsis thaliana* hormone signaling pathways. In this study, we have screened hormone promoter::*uidA* transgenic *Arabidopsis thaliana* lines against a *P. syringae* TTE library in order to identify TTEs involved in the perturbation of hormone signaling *in planta*. The screening follows a quantitative and a qualitative approach using different transgenic *Arabidopsis* GUS lines. We have identified three *P. syringae* TTEs, capable of inducing auxin signaling using transgenic *IAA1::uidA* or *DR5::uidA* seedlings exposed to these TTEs. Whether these effectors are directly or indirectly manipulating the auxin signaling pathway remains to be elucidated.

The type III effectors HsvG and HsvB determine host specificity of *Pantoea agglomerans* by acting as plant transcription factors

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Pantoea agglomerans pv. *gypsophilae* (*Pag*) elicits galls on gypsophila and HR on beet, whereas *P. agglomerans* pv. *betae* (*Pab*) induces galls on beet and gypsophila. HsvG and HsvB are two paralogous type III effectors present in both *Pag* and *Pab* but determine host specificity on gypsophila and beet, respectively. Their structure is distinguished by the presence of two direct repeats (71 and 74 aa) in HsvG as opposed to one repeat in HsvB. Domain-switching experiments determined that the repeat domain plays a crucial role in host specificity. Both effectors are localized to the nucleus, bind DNA via their helix-turn-helix domain and act as transcription factors in yeast via their repeat domain. A binding site selection procedure followed by a 3′ Race method was employed to isolate an HsvG target gene from gypsophila cuttings. An ORF encoding a predicted protein of 244 amino acids was isolated and exhibited the highest homology (50%) with hypothetical DnaJ protein from the genome of *Oryza sativa* (*japonica* group) lacking the J-like domain, which is essential for chaperon activity. Motif scan identified classical zinc finger C2H2-type motif and basic-leucine zipper motif that could imply a transcription factor function. By comparing qRT-PCR on gypsophila cuttings inoculated with *Pag*824-1 (wt) with its *hsvG*$^-$ mutant, it was shown that HsvG induced its target gene *in planta* by 2 fold at 2 hrs post inoculation, reaching a peak of 6 fold at 4 hrs with a progressive decline to 3.5 and 2.3 fold at 4 and 6 hrs, respectively. Results suggest that HsvG acts as a plant transcription factor, which might determine host specificity by activating a putative host transcription factor. Similar results are being obtained for HsvB on beet.

Transposon mutant library of *Clavibacter michiganensis* subsp. *sepedonicus,* and characterization of HR negative transposon mutants

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Clavibacter michiganensis subsp. *sepedonicus* (*Cms*) is a Gram-positive bacterium and the causal agent of bacterial ring rot of potato. *Cms* and other actinobacterial phytopathogens are relatively poorly characterized. However, molecular biological studies of *Cms* and related species have gained greater feasibility due to the recent availability of genomic sequences and genetic tools for several related taxa. Here we report construction and characterization of a transposon mutant library of *C. michiganensis* subsp. *sepedonicus* strain R10. To evaluate the effectiveness of Tn*1409*Cβ for mutagenesis of R10, junction sites of 275 transposon mutants were sequenced. Transposon insertions were distributed throughout the chromosome and within the two plasmids, pCSL1 and pCS1. Many of the insertions were located within relatively low GC regions of the genome indicating a biased, nonrandom insertion of Tn*1409*Cβ. The library was screened for mutants unable to induce a hypersensitive response (HR) on non-host plant, as HR negative phenotype has previously been associated with lack of virulence in *Cms*. Two HR negative mutants were isolated, both containing insertion in the same CDS, CMS2989 (*chp-7*), although at distinct locations. *chp-7* is a member of a protein family homologous to the pathogenicity determinant and putative serine protease *pat-1* of *C. michiganensis* subsp. *michiganensis,* and it is conserved also in several other Gram-positive and Gram-negative phytopathogens. Both *chp-7* mutants were reduced in virulence, but were not affected in their ability to multiply *in planta*. Complementation with *chp-7* restored virulence as well as HR, demonstrating a role for Chp-7 in *Cms*-plant interactions.

Patterns and receptors in *Arabidopsis* innate immunity

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Microbe-derived pattern recognition is a prerequisite for the activation of innate immunity in multicellular organisms. Bacterial peptidoglycans trigger immunity-associated responses in various plants. We have identified by reverse genetics a gene encoding an *Arabidopsis* peptidoglycan-binding protein. Inactivation of this protein resulted in a strong reduction of peptidoglycan-inducible plant responses and significantly enhanced virulence to bacterial pathogens. Members of the superfamily of necrosis and ethylene-inducing peptide 1 (Nep1) like proteins (NLPs) are widely found in bacteria, fungi and oomycetes, and have been shown to trigger plant innate immunity-associated responses. We have determined the crystal structure of NLP_{Pya} from *Pythium aphanidermatum,* to 1.35Å resolution. The protein fold exhibits structural similarities to cytolytic toxins that are produced by marine organisms, and computational modelling of the 3D structure of NLPs from *Phytophthora parasitica* and *Pectobacterium carotovorum* revealed significant fold conservation. We show that NLPs are virulence-promoting phytotoxins that facilitate plasma membrane destabilization, cytolysis and host infection. Hence, NLPs constitute evolutionary and functionally conserved toxins whose wide taxonomic distribution is unique among phytopathogenic microbes. NLP-mediated phytotoxicity and plant defenses also share identical fold requirements, suggesting that NLP-induced interference with host integrity signals the activation of plant defenses. Damage-associated activation of innate defenses in plants is reminiscent of microbial toxin-induced inflammasome activation in vertebrates and thus constitutes another conserved element in eukaryotic innate immunity.

NPR1 and MAPK genes are differentially expressed when defense against *Phytophthora capsici* is induced in habanero pepper

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Phytophthora blight, a lethal disease caused by the soilborne *Phytophthora capsici* oomycete, affects all species in the genus Capsicum. Nowadays, there is neither a true-resistant variety nor the cause of susceptibility is known. By using an ethylene-based strategy, we have induced resistance against *P. capsici* in *Capsicum chinense* Jacq. (habanero pepper). The gaseous phytohormone ethylene participates in some mechanisms by which plants react to pathogen attack. In this study, 5 mM ethephon (an ethylene releasing compound) was sprayed onto one-month *C. chinense* seedlings, 24 h before they were inoculated with the oomycete. We then analyzed the expression of *C. chinense* cDNAs that code proteins with different metabolic functions. Ethephon application consistently induced some genes coding pathogenesis-related proteins and specially NPR1, and down-regulated two MAPKs genes. These MAPKs share high sequence similarity with *Solanum lycopersicon* MAPK whose expression was enhanced by different types of environmental stress. However, our results suggest that these *C. chinense* MAPKs might regulate in a negative manner the effective establishment of a defense against *P. capsici*, and that NPR1 could participate in a positive way. RNP has a CONACYT PhD fellowship number 208245; this project is supported by CONACYT P54831.

Functional characterisation of Type VI secretion system of the potato pathogen *Pectobacterium atrosepticum*

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Soft rot and blackleg of potato are economically important plant diseases worldwide. *Pectobacterium atrosepticum* is one of several closely related pathogens causing these diseases. Main virulence factors of soft rot bacteria are plant cell-wall degrading enzymes. Recently a novel secretion system, Type VI secretion (T6SS), has been identified from several pathogenic or symbiotic bacteria. T6SS has been found to be involved in plant-microbe interaction between *P. atrosepticum*, *Agrobacterium tumefaciens*, *Rhizobium leguminosarum* and their respective host plants (Bladergroen et al. 2003, Mol Plant Microbe Interact 16:53-64; Liu et al. 2008, PLoS Pathog 4e:1000093; Wu et al. 2008, J Bacteriol 190:2841-2850). *P. atrosepticum* carries a large gene cluster encoding the putative secretion machinery components and the putative secreted effector proteins, Hcp and VgrG. Additional homologues coding for the secreted proteins are presented in several loci in the chromosome. *P. atrosepticum* T6SS genes are induced by host extract and *in planta*, mutations in T6SS secretion genes and over-expression of the T6SS machinery components or secreted genes affects virulence (Mattinen et al. 2007, Proteomics 7:3527-3537; Mattinen et al. 2008, Microbiology 154:2387-2396). In order to understand the function of the T6SS cluster, two cluster mutants were compared with the wild type strain in microarray analysis. The results suggested that mutations in secretion genes reduced the expression of the putative effector genes and affected global gene expression. Functional characterisation of the T6SS components, the T6SS effectors and the globally regulated genes is in progress.

Expressionof hrpG and activation of response regulator HrpG are controlled by distinct signal cascades in *Ralstonia solanacearum*

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The transcriptional regulator HrpB activates the entire *hrp* regulon in theplant pathogen *Ralstonia solanacearum*. Through a complex multigene regulatory cascade PrhA-PrhR/PrhI-PrhJ-HrpG, expression of *hrpB* is induced in a *hrp*-inducing, nutrient-poor medium and in response to contact between the bacterium and plant cell. In this study, we analyzed the expression levels of these regulatory genes and hrpB using *lacZ* reporter strains grown in three different conditions: in a nutrient-rich or nutrient-poor medium; and co-cultivated with *Arabidopsis thaliana* seedlings. We found that *prhA* and *prhIR* were expressed constitutively. Expression of *prhJ* and *hrpG* was PrhA-dependent in all three conditions. Despite the high levelof *hrpG* expression in all cases, *hrpB* was induced only when the bacteria were co-cultivated with *A. thaliana* seedlings or grown in nutrient-poor medium. A mutation in the predicted phosphorylation site of *hrpG* greatly reduced the function of HrpG. From these results, we conclude that the *prhA*-dependent regulatory cascade controls the expression of *hrpG*, and a new cascade, which is induced by a signal from plant cells, activates HrpG by phosphorylation. Only when both signal cascades are effective is full expression of *hrpB* induced. We speculate that the metabolic status of the bacteria in the nutrient-poor medium also contributes to the second cascade.

Hormone signaling in the *Arabidopsis-Leptosphaeria maculans* pathosystem

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Hormones like salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) largely affect outcomes of host-pathogen interactions in numerous pathosystems, yet their contributions to host resistance have not been established in the *Arabidopsis* and the hemibiotrophic fungus *Leptosphaeria maculans* interactions. In *Arabidopsis*, major resistance to *L. maculans* is controlled by the resistance locus *RLM1*. No clear influence on disease resistance by SA, JA, or ET had been observed in the *RLM1* background. Here, roles of these hormones in the *Arabidopsis-L. maculans* pathosystem were re-evaluated in the *rlm1* background through genetic and chemical manipulations of hormone signaling. In the absence of the *RLM1* and phytoalexin camalexin, *coi1* mutation caused significant susceptibility, indicating the role of the JA signaling in host defense. The marked susceptibility conferred by *coi1*, however, was compromised in the *ein2coi1* line, suggesting a negative effect of the ET signaling on host resistance. Consistently, the *rlm1* plants treated with the ET generator 2-chloroethylphosphonic acid showed increased susceptibility to the fungus, while application of the ET synthesis and reception inhibitors $CoCl_2$ and $Ag_2S_2O_3$, respectively, reduced disease severity. Treatment of benzothiadiazole carbothioic acid, which has been reported to induce resistance to *L. maculans* in *Brassica napus*, enhanced susceptibility of the *rlm1* line. The *NahG* transgene, on the other hand, did not affect *Arabidopsis* resistance significantly. In summary, our observations shed light on the host-pathogen interactions controlled by the interplays among JA, ET, and SA signaling below the *RLM1*-mediated resistance. It appears that the host ET signaling is required for successful pathogenic development of *L. maculans*.

Identification of a novel gene cluster in rice for diterpenoid phytoalexin biosynthesis

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Antimicrobial diterpenoid compounds such as momilactones and phytocassanes are a major phytoalexin produced in rice upon pathogen attack. Recently, we reported that the momilactone biosynthetic genes are clustered in rice chromosome 4 and are coexpressed after the elicitation. Although there are some examples of gene clusters for plant defense compounds, the rice momilactone biosynthetic genes cluster is known as the only cluster to be inductively regulated by pathogen invasion. In this report, we demonstrate that a novel gene cluster for phytocassanes biosynthesis is organized in rice chromosome 2, which consists of two diterpene cyclases genes for phytocassane (*OsCPS2* and *OsKSL4*) and six uncharacterized cytochrome P450 monooxygenases (P450) genes. Using genetic approaches, we first investigated functions of the *CYP71Z7* gene, one of the *P450* genes in the middle of the cluster. The knockdown of *CYP71Z7* specifically suppressed the elicitor-inducible production of phytocassane A, B, and D (those of C-2 oxygenated phytocassanes), whereas phytocassanes C, E, and a metabolic intermediate 1-deoxyphytocassane C (those of C-2 non-oxygenated phytocassanes) accumulated to levels more than 10 times higher than those in the wild-type plants. These results suggest that the CYP71Z7 probably catalyzes C-2 hydroxylation of possible metabolic substrates phytocassane C and/or 1-deoxyphytocassane C to yield phytocassanes A, B, and D, and that phytocassanes biosynthetic genes are clustered in chromosome 2 like the momilacone biosynthetic gene cluster.

Identification and functional analysis of type III effector proteins in Mesorhizobium loti

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Mesorhizobium loti MAFF303099, a microsymbiont of the model legume Lotus japonicus, possesses a cluster of genes (tts) that encodes a type III secretion system (T3SS). In the presence of heterologous nodD from Rhizobium leguminosarum and a flavonoid naringenin, we observed elevated expression of the tts genes and secretion of several proteins to culture medium. Inoculation experiments with wild type and T3SS mutant strains revealed that the presence of T3SS affected nodulation either positively (L. corniculatus subsp. frondosus; L. filicaulis) or negatively (L. halophilus and two other species). By inoculating the L. halophilus with mutants of various type III effector candidate genes, we identified an ORF mlr6361 as a major determinant for the nodulation restriction by L. halophilus. mlr6361 encodes a protein of 3,056 amino acids with 15 repetitions of a sequence motif of 40 to 45 residues and a shikimate kinase-like domain at its carboxyl-terminus. Homologues with similar repeat sequences are present in the hypersensitive-response and pathogenicity regions of several plant pathogens including strains of Pseudomonas syringae, Ralstonia solanacearum and Xanthomonas species. These results suggest that L. halophilus recognizes Mlr6361 as potentially pathogen-derived, leading to a block in the infection process.

Anti-programmed cell death control in *Phaseolus vulgaris* root nodules

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The *Phaseolus vulgaris*-rhizobia symbiotic interaction is a highly specific and coordinated process that culminates with the formation of a new root-derived organ (nodule) specialized in nitrogen-fixation. Mutual recognition of these symbionts depends on the exchange of particular signals: the root exuded flavonoides and the rhizobial Nod factors. After recognition, plant cell wall degradation by the rhizobia and the subsequent penetration of root hair cells by means of an infection thread occurs. Simultaneously, a nodule meristem is induced underneath in the root cortex. Bacteria are released from the infection threads into the replicating cells by endocytosis, and become surrounded by a plant-derived membrane, the peribacteroid membrane (PBM), which creates a physical barrier between rhizobia and the cell's cytoplasm. Bacteria inside the PBM multiply, enlarge, and finally differentiate into their specialized N_2-fixing form, the bacteroid. This process gives rise to the infected cells which may contain more than 20 000 bacteroids per cell, these cells are intercalated with uninfected cells in the nodule's central zone. Amazingly, these infected cells survive until nodule natural senescence process, implying that several plant pro-survival signals are active during the symbiotic interaction. We present evidence that novel nodulin proteins are required to block plant cell death in the infected and uninfected cells in bean nodules. This work was partially supported by grants IN214909 and CONACYT 83324.

Regulation of the Hrp type III secretion system in *Pseudomonas syringae* pv. *phaseolicola*

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In plant pathogenic bacteria, the type III secretion system (TTSS) is essential for its virulence. The expression of TTSS genes (known as *hrp* genes) is tightly regulated. In *P. syringae*, the regulatory cascade involves four positive regulators. HrpR and HrpS, that interact directly and, together with σ^{54} factor, activate the expression of *hrpL*. HrpL is an alternate sigma factor that binds to the promoters of the TTSS genes, activating their expression. Additionally, HrpA, a major component of the Hrp pilus, is required for full expression of all TTSS genes, somehow affecting levels of *hrpRS* mRNA. The *hrp* regulon is also subjected to negative regulation, through the action of HrpV and the Lon protease. Expression of *hrp* genes is induced within the plant, and has been shown to respond to different host and environmental factors. The *hrp* genes are repressed in rich media and can be induced in the laboratory using minimal media that presumably reproduces conditions encountered *in planta*. However, the nature of the environmental signal(s) responsible for the induction of expression is debatable, since different studies have used variations on the minimal medium composition and/or growing conditions, achieving induction of the *hrp* genes expression in all cases. Moreover, these different studies have been carried out using mainly *in vitro* inducing conditions and different pathovars. In this work, we have analysed the roles of some of these regulators, within the same pathovar, under identical experimental settings, and more importantly, including analysis within the plant. We apply real-time PCR, competitive indices, and other *in vitro* and *in planta* assays, to the analysis of single and double mutant strains, as well as strains ectopically expressing these negative regulators at different levels.

LjCLE-RS1* induces systemic expression of beta-1.3-glucanase that responds to abscisic acid in *Lotus japonicus

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Hostlegumes control nodule number by autoregulation of nodulation (AUT), in which the presence of existing root nodules inhibits further root nodule formation. AUT is clearly observed by the split-root system. In this system, the tip of the main root of a seedling is removed resulting in the emergence of lateral roots. The two roots are then individually incubated on an appropriate medium. One root is inoculated first with rhizobia, then the second root some days later. The nodulation on the second root is strongly inhibited. AUT will consist of at least two long-distance signals, a root-derived infection signal and a shoot-derived signal that inhibits nodulation. In *Lotus japonicus*, HAR1 mediates AUT and, LjCLE-RS1 and LjCLE-RS2 are strong candidates as the infection signal. We employed split root system of *Trifolium repens* and *L. japonicus* to investigate plant molecules involved in inhibition of nodule formation in AUT. In the split-root system of *T. repens*, AUT was partially canceled on the second root treated with abamine, the specific inhibitor of abscisic acid (ABA) biosynthesis. The application of exogenous ABA to one root did not inhibit nodulation on the second root. These suggest that increased concentration of endogenous ABA inhibits nodule formation on the second root. The repression of *LjGlu1*, an ABA-responsible beta-1,3-glucanasegene of *L. japonicus*, enhanced nodule formation and symbiotic nitrogen fixation. In the split-root system of *L. japonicus*, the inoculation of one root with *Mesorhizobiumloti* induced systemic expression of *LjGlu1* in the second root. In the plants of *L. japonicus* with hairy root expressing *LjCLE-RS1*, *LjGlu1* was induced systemically. These results suggest that ABA and LjGlu1 are involved in AUT.

A microRNA involved in bacterial innate immunity

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In plants and animals, microRNAs (miRNAs) regulate a variety of cellular processes including developmental patterning, hormone signaling, nutrient homeostatsis and defense against biotic and abiotic stresses. Here we present evidence that a bacteria -inducible miRNA play a role in plant defense. During our analysis of small RNA sequences obtained from small RNA profiling using bacteria-challenged *Arabidopsis*, we encountered elevated levels of several miRNAs. Experimental analysis revealed that the abundance one of the miRNAs was specifically and strongly induced by *Pst* avrRpt2. Detailed functional analysis revealed that this miRNA has multiple targets, which include two protein kinases and a component in small RNA pathway. Interestingly, the two protein kinases were targeted at the mRNA level by the miRNA, whereas another target gene, which is a component in the small RNA pathway, was regulated by the miRNA via translational repression. These genes are all involved in plant defense responses. In conclusion, we have identified bacteria-inducible miRNAs that regulate anti-bacterial immunity by negatively modulating the expression of their targets.

Salicylic acid and MAMP-mediated transcriptional regulation of secretory pathway in Arabidopsis immunity

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Systemic Acquired Resistance (SAR) is an inducible form of plant defense, which confers broad spectrum immunity throughout the plant. Activation of the SAR pathway requires signal molecule salicylic acid (SA), accumulation of pathogenesis-related (PR) proteins and function of the NPR1 protein (Non-expressor of *PR* genes). Apart from modulating PR1 expression, NPR1 also regulates another set of SAR response genes, which contain *TL1* elements in their regulatory regions. We identified a novel *TL1* Binding Transcription Factor (TBF1), whose expression is up-regulated in both local and systemic tissues upon SA treatment or *P. syringae* infection. We showed that TBF1 specifically binds to BiP2 (an ER-resident gene) promoter in *TL1* motif-dependent manner in Y1H. Using an Electro-Mobility Shift Assay, we showed that the binding capacity of *tbf1* mutant plant protein extract to *TL1* element is severely reduced compared to that of Col-0. Furthermore, we demonstrated that SA-dependent induction of several secretory pathway genes was reduced or delayed in *tbf1* mutant suggesting their regulation by TBF1. Moreover, *tbf1* mutant plants are impaired in their ability to secrete PR1 upon SA treatment. The *tbf1* mutant displayed enhanced susceptibility to both avirulent and virulent *P. syringae* strains and failed to establish SAR. Both NPR1 transcript and protein levels are altered in *tbf1* suggesting an existence of a feedback mechanism between these two molecules. Intriguingly, *npr1* plants failed to induce TBF1 expression when treated with SA but exhibited wild-type TBF1 transcript upon treatment with elf18. We also found that *tbf1* mutant plants were specifically impaired in elf18 but not flg22-induced resistance indicating a novel role of TBF1, independent of NPR1 defenses.

Elucidation of molecular mechanisms of defense responses and orogrammed cell death mediated by EDR1 and EDR2

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Plants have evolved very sophisticated mechanisms to defend themselves against pathogen infection. Fine regulation of defense responses is critic for plants to effectively defend a wide variety of pathogens. EDR1 and EDR2 are negative regulators in plant defense responses and programmed cell death and mutations in EDR1 or EDR2 lead to enhanced resistance to the powdery mildew pathogen *Golovinomyces cichoracearum*. Genetic analyses have shown *edr1* and *edr2*-mediated resistance is SA dependent, but JA and ethylene independent. However, how EDR1 and EDR2 regulate defense responses and cell death is not clear. To identify other components in EDR1 and EDR2 pathways, we mutagenized *edr1* and *edr2* seeds with ethylmethane sulfonate (EMS). In the first round of screen, we have identified a number of suppressors for *edr1* and *edr2* mutants and currently, we are mapping and cloning the responsive genes. Interestingly, we have identified multiple alleles of known components in SA pathway in the *edr2* mutant screen, including four *pad4* alleles, two *npr1* alleles and one *sid2* allele, suggesting the suppressor screen is very efficient. To identify EDR1and EDR2 interacting proteins, we screened yeast two hybrid library using different domains of EDR1or EDR2. In those screens, we identified several positive interacting proteins, and we are now taking genetic and biochemical strategies to test the biological significance of those interactions. Further Analyses of EDR1 and EDR2 function and identification of other components in EDR1 and EDR2 pathways will provide significant insights into defense responses and programmed cell death in plants.

Ferrous iron uptake but not siderophore mediated iron uptake is required for virulence of *Xanthomonas oryzae* pv. *oryzae*

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Xanthomonas *oryzae* pv. *oryzae* causes bacterial blight, a serious disease of rice. A mutation in *X. oryzae* pv. *oryzae* homolog of the *feoB* gene (encodes major bacterial ferrous ion transporter) causes a severe virulence deficiency. This mutant is deficient for growth on low iron medium and exhibits constitutive production of siderophores. We have identified and characterized a seven gene operon in *X. oryzae* pv. *oryzae* that encodes proteins required for biosynthesis, export and uptake of siderophore. Five of these genes, designated *xssABCDE* (Xanthomonas siderophore synthesis) and *xsu* (Xanthomonas siderophore utilization), are homologous to *Vibrio parahaemolyticus* genes involved in production and utilization of vibrioferrin siderophore. This iron-regulated operon is expressed as a single polycistronic mRNA. Mutants for *xssA, xssB* and *xssE* genes are siderophore deficient and growth restricted under iron limiting conditions but are virulence proficient. A *xsu* mutant displayed impairment in utilization of exogenous siderophore indicating that Xsu acts as a specific receptor for Ferric-siderophore complex. Histochemical and fluorimetric assays with *gus* fusions indicate that, during *in planta* growth, the *feoB* gene is expressed and that the *xss* operon is not expressed. This study represents the first report describing the role of *feoB* in virulence of any plant pathogenic bacterium and first functional characterization of siderophore biosynthetic genes in any xanthomonad.

Mode of action of a non pathogenic *Fusarium oxysporum* strain against *Verticillium dahliae*

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Verticillium wilt is a devastating disease of a wide range of plant hosts, incited by *Verticillium dahliae*. Since there are no chemical treatments to control the pathogen, management strategies are focused on preventive measures. In a previous study, it has been reported the efficacy of a non pathogenic *Fusarium oxysporum* strain, F2, isolated from a suppressive compost amendment, to reduce Verticillium wilt symptom development in eggplants. In the present study, we examined the mode of action of F2 against *V. dahliae*. For this purpose, we transformed the F2 and the *V. dahliae* isolate with the EGFP and DsRed2 reporter genes, respectively, so to visualize their presence on the root surface of eggplants. In addition, the endophytic presence of both fungi was monitored by QPCR analysis. It was shown that F2 colonizes the root surface along the intercellular junctions excluding *V. dahliae* from the same ecological niche. QPCR analysis showed that application of the F2 reduces the levels of *V. dahliae* endophytically along with the disease severity. While, in a split root experiment it was revealed that F2 does not trigger the defense mechanisms of eggplants against *V. dahliae*. Therefore, competition for space or nutrients on the root surface constitutes the main mechanism of action of the F2 against *V. dahliae*.

Characterization and functional analysis of a grapevine F-box and its *Arabidopsis* homolog during plant defense response

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A differential display approach revealed several grapevine genes which are regulated upon *Botrytis cinerea* infection. Among them, the complete sequence of *BIG-24.1* (for Botrytis induced grapevine) gene was characterised. *BIG-24.1* codes for a F-box protein with a kelch-beta-propeller. F-boxes are ubiquitous in Eucaryotes and most of them are part of the SCF complex (Skip, Cullin, F-box) which is involved in the degradation of cellular regulatory proteins via the ubiquitin / 26S proteasome pathway. To further investigate the regulation of *BIG-24.1*, we studied its expression pattern by real-time quantitative PCR in response to biotic or abiotic stresses. Like for *B.*

cinerea, *Pseudomonas syringae* pv. *pisi*, UV-C, cold stress, salicylic acid or methyl jasmonate treatments induce *BIG-24.1* expression. The expression pattern of the gene homolog *At-24.1* was analysed in response to these stresses in wild type *Arabidopsis thaliana* and in mutants affected in different signalling pathways. *At-24.1* and *BIG-24.1* exhibit different regulations: *At-24.1* is, for instance, preferentially regulated by jasmonate. They have both a photoperiod-dependent regulation. These results correlate well with the two promoter sequences analyses. Functional analysis of the *At-24.1* mutant in response to various pathogens was also investigated. Preliminary data suggest that *At-24.1* is not involved for the resistance to *B. cinerea*, *Peronospora parasitica* or *P. syringae* pv. *tomato* DC3000.

The *BIG-102* gene regulation is linked to biotic and abiotic stresses in *Vitis vinifera* and *Arabidopsis thaliana*: A possible role in defense?

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Using suppression substractive hybridization, several grapevine genes up-regulated in leaves infected by *B. cinerea* were identified. Among them, *BIG-102* (for Botrytis induced grapevine) was strongly up-regulated in this interaction. No function has been found for this gene in databases. To further investigate the regulation of *BIG-102*, we studied its expression pattern by real-time quantitative PCR in response to different biotic or abiotic stresses. Like for *B. cinerea*, *Pseudomonas syringae* pv. *pisi*, UV-C, cold stress, salicylic acid or methyl jasmonate treatments induce *BIG-102* expression. A gene homolog was identified in *Arabidopsis thaliana* and named *At-102*. The expression pattern of *At-102* was analysed in response to biotic and abiotic stresses in wild type and in mutants affected in different signalling pathways. *At-102* and *BIG-102* exhibit similar regulations for most of these stresses. The regulation of the two genes preferentially involve salicylic acid pathway. These results correlate well with the promoter sequences analyses. Functional analysis of *Arabidopsis At-102* mutant in plant development and in response to various pathogens and stresses was also investigated. Interestingly, we found that the *At-102* mutant is more susceptible than the wild type plants to *B. cinerea* infection. In contrast, the mutant is not susceptible to *Peronospora parasitica* or *P. Syringae* pv. *tomato DC3000* infections. These results suggest that At-102 protein is involved in plant defense against *B. cinerea*. The production of BIG-102 and At-102 proteins is currently completed in order to test a potential antifungal activity.

CaPIR1 is a pepper RING-finger E3 ligase protein that negatively regulates plant basal resistance

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The RING finger family of proteins is known to possess ubiquitin ligase activity and play pivotal role in protein degradation. Here, we show that the CaPIR1 gene, which encodes a pepper RING-finger E3 ligase, is involved in innate immunity. Expression of CaPIR1 was specifically induced during the hypersensitive response (HR) in response to both non-host and host pathogens and treatments of plant hormones, methyl jasmonate and ethylene. Plants expressing the GUS under the control of CaPIR1 promoter confirmed specific induction of GUS expression in leaf mesophyll cells and vascular tissue under pathogen infection. We studied the function of CaPIR1 gene in hypersensitive response (HR) and basal resistance using an Agrobacterium-mediated transient expression system in Nicotiana benthamiana leaves. Overexpression of CaPIR1 led to a significantly enhanced susceptibility to Pseudomonase singae pv. tabaci. and also marked promotion of HR cell death induced by various R/avr interactions and Bcl2-associated X (Bax) protein. We further demonstrate that expression levels of a number of defense-related genes are reduced and delayed in CaPIR1 expressed leaves during pathogen infection. These results suggest that a pepper CaPIR1 may have a role as a negative regulator in plant innate immunity.

HrpH, a lytic transglycosylase associated with the type III secretion system of *Pseudomonas syringae* can suppress basal innate immune responses in *Nicotiana benthamiana*

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Lytic transglycosylases (LT) are associated with the type III secretion systems (T3SS) of animal and plant pathogens. HrpH is an LT that is encoded and expressed with the *P. syringae hrp*/*hrc* gene cluster and contributes to the translocation of effectors into plant cells. Three properties of HrpH suggest activities in addition to its putative role in T3SS penetration of the peptidoglycan layer: HrpH is more than two times larger than typical T3SS-associated LTs; HrpH is translocated into plant cells by the T3SS; HrpH contributes to effector translocation in planta but not to secretion in culture. Given that HrpH may be one of the earliest proteins translocated into host cells during infection and that a primary function of the T3SS is delivering suppressors of basal innate immunity (PAMP-triggered immunity; PTI), we asked whether HrpH could suppress PTI. *P. fluorescens* cells carrying pLN18 or other derivatives of the *P. syringae* pv. *syringae* 61 *hrp*/*hrc* cluster previously cloned in pHIR11 elicit PTI in inoculated tissue within 6 h unless the bacteria also express and translocate a PTI-suppressor, such as the model effector AvrPto. *P. fluorescens* (pLN18) expressing the *P. syringae* pv. *tomato* DC3000 HrpH suppressed PTI based on several assays with *Nicotiana benthamiana*, where PTI suppression was indicated by: dye uptake into small veins in inoculated tissue; elicitation of the hypersensitive response by DC3000 challenge-inoculated 6 h after the *P. fluorescens* (pLN18/pHrpH) inoculation; production of colonies visible by confocal microscopy following challenge inoculation with GFP-labeled DC3000; translocation of AvrPto-Cya by challenge-inoculated DC3000; and suppression of callose formation and reactive oxygen generation. We are now investigating how HrpH suppresses PTI.

AtSIZ1, SUMO E3 ligase: A negative regulator of salicylic acid-mediated disease resistance by SP-RING domain in *Arabidopsis thaliana*

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Covalent attachment of small ubiquitin-like modifier (SUMO) to proteins participate in diverse cellular processes in the eukaryotic cell. The *Arabidopsis* SUMO E3 ligase, SIZ1 is an ortholog of mammalian PIAS and yeast Siz SUMO E3 ligases. Here, we show that the *Arabidopsis* SUMO E3 ligase (AtSIZ1) negatively regulates salicylic acid-mediated innate immunity by the SP-RING domain of the protein. *siz1* plants exhibit constitutive systemic acquired resistance (SAR) that is characterized by accumulation of salicylic acid (SA), and constitutive pathogenesis-related (PR) gene expression. In *siz1* plants expressing SA hydroxylase *nahG* (*nahG siz1-2*), disease resistance is linked to elevated SA levels. To investigate putative functional domains of the protein in innate immunity, we used site-directed mutagenesis to change predicted critical residues in each important AtSIZ1 domain as follow; SAP (LXXVL to LXXAA), PHD (C4HC3 to C3YHC3), PINIT (PIIT to PAAT), SP-RING (C2HC3 to CAHC3), or SXS (SXS to AXA). Each separate domain mutant was constructed under the control of the *AtSIZ1* promoter (3650-bp upstream region of *AtSIZ1*) and transformed into *Arabidopsis siz1-2* mutant plants. The function of SP-RING domain of SIZ1 in the salicylic acid-mediated disease resistance will be discussed in the meeting.

Transcriptional profiling of rice leaves undergoing a hypersensitive response like reaction induced by *Xanthomonas oryzae* pv. *oryzae* cellulase

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Plants have powerful innate immune responses that help them ward off most potential pathogens. We have recently reported that a secreted cellulase of *Xanthomonas oryzae* pv. *oryzae* (Xoo) induces potent innate immune responses in rice including a hypersensitive response (HR) like reaction. In order to understand gene expression changes during cellulase induced HR in rice leaves, we have conducted a microarray analysis after 12 hr following enzyme treatment. BLAST analysis performed for the 267 (152 up and 115 down regulated) rice genes differentially expressed (>2 fold) following cellulase treatment indicated that a number of defense and stress response functions are upregulated while a number of functions involved in metabolism and transport are down regulated. A significant proportion of the differentially expressed genes (42/267) are predicted to encode transcription factors. Several upregulated genes, including some key enzymes and transcription factors, were found to be related to functions involved either in jasmonic acid biosynthesis or those that respond to jasmonic acid. We further showed by using real-time PCR that coinfiltration with wild type Xoo suppresses the

cellulase induced expression of two transcription factors, *OsRERJ1* and *OsAP2/ERF* that are predicted to be involved in the jasmonic acid mediated defense pathway. Agrobacterial mediated transient transfer of *OsAP2/ERF* into rice cells induces defense responses such as a programmed cell death reaction in roots and callose deposition in leaves.

The *Arabidopsis* CAMTA3 transcription activator is a suppressor of pathogen defence responses

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In a mutant suppressor screen, we identified two (semi)dominant mutants rescuing the lesion-mimic phenotype of the syntaxin syp121 syp122 double mutant and mapped both to the same chromosome region. Map-based cloning showed that they were mutated in separate sites of a Calmodulin-binding domain of CAMTA3, a CaM-binding transcription activator. The CAMTA3 T-DNA loss-of-function mutant in Col-background showed a weak lesion-mimic phenotype, increased PR1-expression and increased resistance toward bacterial and fungal pathogens. Introducing the loss-of-function mutant into the syntaxin syp121 syp122 double mutant made the lesions even more severe, showing that our dominant CaM-domain mutants have the opposite effect of knocking out the gene. In line with that, the dominant mutations in the CaM-domain led to increased disease susceptibility in wild-type background. Taken together, the phenotypes of the two types of mutations in CAMTA3 show that the function of the transcription activator is to suppress pathogen defence signalling and that this suppression becomes constitutive by mutations in the CaM-domain. The weak lesion-mimic phenotype of the loss-of-function mutant can be rescued by combining it with mutations in genes acting as positive regulators of defence signalling. Likewise, we combined the dominant CaM-domain mutant with other suppressors of syntaxin-related death in the syp121 syp122 background and found that this always led to further rescue of the lesion-mimic phenotype. This indicates that CAMTA3 acts at a basal level in defence signalling.

Exploring the role of pathogen effectors in the suppression of hormone-regulated defense signaling pathways

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Plants are equipped with an arsenal of defense mechanisms. These mechanisms enable the plant to defend itself against a multitude of attackers. However, since defense activation involves costs as well, it often has a negative effect on growth and or development. Therefore, plants have to carefully regulate their defenses depending on the pathogen encountered. Salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) are phytohormones crucial for orchestrating plant defense. SA-mediated responses are generally involved in defense against biotrophic pathogens, while JA- and ET-mediated defense responses are mainly involved in defense against necrotrophic pathogens and insects. Cross-communication between defense-signaling pathways has been well documented and helps the plant to tailor its defense response to the attacker encountered. Interestingly, specialized microbial pathogens have acquired the ability to manipulate hormone-regulated defense signaling pathways to evade host immunity. The downy mildew pathogen Hyaloperonospora arabidopsidis has a biotrophic life style and depends on the ability to suppress host immunity for its survival. H. arabidopsidis produces a large number of putative effector proteins, many of which contain an N-terminal RxLR motif. This motif is thought to target the effectors to the host cell. To investigate the hypothesis that RxLR-containing effectors of H. arabidopsidis play a role in the suppression of SA-dependent defenses, we started to study transgenic Arabidopsis lines that overexpress these putative H. arabidopsidis effectors.

Dissecting the mechanism of resistance against *Plasmopara viticola* induced by *Trichoderma harzianum* T39 in grapevine

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Some non-pathogenic microorganisms can reduce plant disease symptoms through activation of a plant-mediated defense mechanism known as induced systemic resistance (ISR). ISR provides protection against various types of pathogen and it promise to be an attractive biocontrol strategy. However, scarce information is available on the efficacy, activation mechanisms and metabolic costs of ISR in non-model plants. We analyzed the *Plasmopara viticola*/grapevine pathosystem after treatments with the biocontrol agent *Trichoderma harzianum* T39 in comparison with benzothiadiazole (BTH). T39 activates a systemic plant-mediated resistance and reduces downy mildew symptoms at a level comparable to treatments with BTH, which is known to activate the systemic resistance pathways mediated by salicylic acid. If only treated leaves are considered, T39 induces a lower resistance level and the persistence of the effect is shorter than BTH. Moreover BTH treatments entail metabolic costs, which strongly reduce grapevines growth, probably because of the allocation of resources into the defense mechanism. On the other hand, repeated T39 treatments do not affect photosynthesis and plant growth (number of leaves and leaf area, dry weight of shoots and roots). These results suggest the activation of different defense pathways by BTH and T39. Expression of some known defense-related genes involved in the various induced resistance phenomena was evaluated (real-time RT-PCR) in untreated and T39 or BTH treated plants, before and after pathogen challenge. Surprisingly no differences were seen between BTH and T39 treated plants. Thus, a genome-wide gene expression analysis could help in the identification of the key genes involved in the grapevine self protection induced by T39.

Nodule metabolism in two actinorhizal systems

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There are two phylogenetically related root nodule symbioses between plants and nitrogen-fixing soil bacteria, legume-rhizobial symbioses and actinorhizal symbioses between a diverse group of mostly woody dicotyledonous plants from 25 genera within 8 families and *Frankia* strains. The root nodule is a complex metabolic system because it comprises two evolutionary very different symbionts. Both partners co-evolved to develop an intricately intertwined metabolism in infected cells. Relatively little data about nodule metabolism in actinorhizal plants have been gathered. Metabolic enzymes are relatively conserved between plant species which makes it possible to use heterologous microarrays to study the system. We chose to compare gene expression pattern in nodules *versus* roots of the actinorhizal plants *Datisca glomerata* and *Alnus glutinosa* on an Affymetrix genechip containing probes from the legume *Medicago truncatula* and its microsymbiont *Sinorhizobium meliloti.* The results will be discussed in respect to nodule metabolism.

***Alternaria alternata* prevents the sporulation of *Plasmopara viticola* on grapevine: Characterization of the mechanism**

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Downy mildew (cause by the obligate parasite *Plasmopara viticola*) is one of the most relevant diseases worldwide. Endophytes are good candidates for use as biocontrol agents due to the fact that many of them are capable of inducing defense mechanisms in host plants. They often occupy the same microhabitat as pathogens, and some of them can produce toxins against insects, as well as growth hormones that are useful to their plant hosts. The grapevine endophyte *Alternaria alternata* strain VP12A5B was isolated from grape leaves with atypical downy mildew symptoms (small oil spots, delimited by veins and showing weak sporulation). It fully inhibits the sporulation of *P. viticola* on artificially inoculated grapevine (*Vitis vinifera*) leaf discs. In this study, we aimed to determine if *A. alternata* cells, applied with or without the metabolites present in their growth media, are active against *P. viticola*, as well as the optimal timing of these applications with respect to the *P. viticola* infection. *A. alternata* applied with its own culture broth (metabolites) suppressed downy mildew, while *A. alternata* mycelia applied without this broth did not. The greatest inhibition of sporulation was seen with the *A. alternata* treatment applied 24 hours after *P. viticola* inoculation. Repeated applications affected sporulation, but not the accumulation of pathogen DNA inside plant tissues. For cases in which *P. viticola* sporulation was inhibited, transmission electron micrographs confirmed *A. alternata*'s induction of severe alterations in *P. viticola* mycelia and the death of haustoria inside *A.*

alternata-treated grapevine tissues. The unique post-infection activity of this *A. alternata* stain and its metabolites represents a new challenge for grapevine downy mildew biocontrol.

Characterization of *Bradyrhizobium japonicum* genes that are highly expressed in determinate root nodules

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The symbiotic interaction of *Bradyrhizobium japonicum* and its soybean host plant is a highly regulated process eventually leading to the formation of root nodules harboring intracellular, nitrogen-fixing bacteroids. Global transcription profiling of *B. japonicum* in determinate soybean nodules showed that 34% of its genes were expressed above background level in bacteroids. In contrast, 66% were expressed under aerobic, free-living conditions, suggesting that many genes are not needed during symbiosis where *B. japonicum* focuses its energy expenditure on important symbiotic functions. For mutant analysis and further characterization, we have chosen two transcriptional units that are highly expressed and up-regulated during symbiosis compared to free-living aerobic cultures. Importantly, expression of these genes is directly controlled by the key transcriptional regulators of symbiosis, NifA and RpoN. The first gene encodes a transcriptional regulator of the LuxR family (Blr1880), which is usually involved in cell density-dependent activation of symbiosis-related genes. The *ahp* genes (bll1776-1777) on the other hand encode subunits of the anti-oxidant alkylhydroperoxidase AhpCD complex. Mutant strain analysis showed that *B. japonicum* AhpCD is involved in scavenging of hydrogen peroxide; however, it is not essential for symbiosis. In contrast, the *luxR* mutant strain formed soybean nodules with decreased nitrogen fixation activity (about 50%) compared to those formed by the wild type. Moreover, this mutant showed an 8-fold higher sensitivity to 3 mM H_2O_2, indicating that this LuxR-type regulator also plays a role in the defense against reactive oxygen species. Microarray analyses will be performed in order to define the cell-density dependent regulon of LuxR.

The complete genome sequence of *Xanthomonas albilineans* provides new insights into the reductive genome evolution of the xylem-limited *Xanthomonadaceae*

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The *Xanthomonadaceae* includes two xylem-limited plant pathogenic bacteria, *Xanthomonas albilineans* and *Xylella fastidiosa.* The complete genome of *X. albilineans* was sequenced, providing not only strong clues to identify new pathogenicity factors in this pathogen causing sugarcane leaf scald disease, but also new insights into the evolution of *Xanthomonadaceae*. Previous phylogenetic analysis suggested that the *Xanthomonas* and *Stenotrophomonas* genera of *Xanthomonadaceae* form a coherent group excluding *X. fastidiosa*. Surprisingly, phylogenetic analysis using *X. albilineans* genomic sequences resulted in a different tree in which *X. fastidiosa* belongs to the *Xanthomonas* group. Based on this latter tree, *X. albilineans* and *X. fastidiosa* are derived from the progenitor of the *Xanthomonas* genus which itself is derived from the progenitor of *Stenotrophomonas*. Comparative genomic analysis identified 551 ancestral genes which are present in both *Xanthomonas axonopodis* pv. *vesicatoria* and *Stenotrophomonas* but absent in both *X. fastidiosa* and *X. albilineans*, revealing that these two latter species experienced a similar reductive genome evolution during their descent from the progenitor of the *Xanthomonas* genus. This degenerative evolution is probably driven by adaptation to the nutrient-poor xylem elements and to the cloistered environmental niche of xylem vessels. Comparable genomic erosion is observed among intracellular animal bacteria and is associated with a mutualistic life style. Adaptation of *X. albilineans* and *X. fastidiosa* to a xylem-limited life style is also illustrated by the unique characteristics of the enzymes involved in cellulose degradation, and the absence of a type III secretion system of the Hrp1 and Hrp2 injectisome families.

***In vivo* localization and oligomerization studies of *Medicago truncatula* Nodulation Factor receptors**

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Legumes develop a symbiosis with nitrogen-fixing soil bacteria, collectively known as rhizobia. Exchange of molecular signals between the two organisms involves rhizobial lipochito-oligosaccharides, called Nod Factors, required for activation of plant infection and nodulation pathways. The exact molecular mechanism by which legume plants perceive these LCO signals is not yet fully understood, although recent genetic studies identified several putative receptors that could be involved in the process. For the model legume *Medicago truncatula* these receptors include the lysin motif receptor-like kinases (LysM RLKs) encoded by the NFP (*Nod Factor Perception*) and LYK3 (*LysM domain containing receptor-like kinase* 3) genes. Using the FRET-FLIM technique we aim to identify the localization of these receptors and their ability to form homo- and hetero- dimers *in vivo*. Fusions of NFP and LYK3 to fluorescent proteins were transiently expressed in *Nicotiana benthamiana* epidermal cells and the oligomerization states of these receptors were probed. By including point mutations of selected residues important for kinase activity and deletions of receptor domains, structure-function information was obtained in these localization and interaction studies. The effect of application of *Sinorhizobium meliloti* bacteria and purified Nod Factors on the interactions of these receptors was also studied. This work was supported in part by the European Community's Marie Curie Research Training Network action (contract number MRTN-CT-2006-035546 "NODPERCEPTION").

Gene expression profiling following *Xanthomonas oryzae* pv. *oryzae* infection of susceptible and resistant lines of the elite indica rice variety, Samba Mahsuri

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Genome wide changes in rice gene expression were monitored using Affymetrix microarrays after *Xanthomonas oryzae* pv. *oryzae* (Xoo) infection in compatible and incompatible interactions in susceptible Sambha Mahsuri (a fine quality indica rice variety) and a resistant three gene (*Xa*21, *xa*13 and *xa*5) pyramid line in Samba Mahsuri background. Differentially expressed genes ($\geq$2.0 fold, $P \leq 0.05$) were identified by PLIER and RMA algorithms. A total of 448 genes are up regulated upon bacterial infection in the compatible interaction while 529 genes are down regulated. Genes involved in signal transduction and protein synthesis/turnover were present in greater proportion amongst upregulated genes while metabolism and transport related genes are present in greater proportion amongst down regulated genes. In the incompatible interaction a total of 218 genes are up regulated upon bacterial infection whereas 144 genes are down regulated. Genes encoding functions involved in defense response are present in a greater proportion in the upregulated group whereas energy and signal transduction related genes are present in a greater proportion amongst the down regulated genes. Surprisingly, a total of only 41 genes are differentially modulated upon bacterial infection in a comparison of compatible vs. incompatible interactions, 24 of which are upregulated, while 17 are down regulated. The very low number of differentially expressed genes in the comparison of compatible and incompatible interactions indicates that common networks/pathways are shared in these two kinds of interactions. Overall, this study provides a wealth of information on plant-bacterial interaction that will accelerate follow-up molecular, biochemical and comparative studies.

Coordinated expression of the Type III secretion system and other *Ralstonia solanacearum* pathogenicity factors by HrpG and PrhG

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The type III secretion system is an essential pathogenicity determinant of the plant pathogen *R. solanacearum* which is required for the injection of effector proteins into plant cells. A regulatory cascade, ending with the regulator HrpB, controls the transcription of the genes encoding this secretion system. The master regulator of this cascade is named HrpG which answers to plant

signals and minimum medium signals. HrpG regulates the expression of two subsets of genes: (i) the type III secretion system genes, via HrpB, and (ii) other genes involved in the pathogenicity of the bacterium but that are controlled independently from HrpB. We have identified in *R. solanacearum* GMI1000 a protein, called PrhG, that shows a very high similarity (72% of global identity at the amino acid level) with the master regulator HrpG. A *prhG* mutant appears to be impaired in pathogenicity, but remains able to induce some wilting symptoms, contrary to the completely avirulent *hrpG* mutant. We will present evidence that *prhG* and *hrpG* respond differently to environmental signals: *prhG* only responds to minimum medium signals but not to the plant cell signals while *hrpG* expression is specifically activated in response to plant cell signals. Despite the high similarity between the DNA binding domains of both proteins (96% identity), transcriptomic analyses show that PrhG only controls the expression of HrpB and of the type III secretion system genes, but shares no other common targets with HrpG. This suggests that HrpG and PrhG recognize and bind to different promoter sequences.

Role of cytokinin signalling in regulating *Medicago truncatula* root and nodule organogenesis

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Legumes are characterized by their ability to develop two types of lateral organs from primary roots depending on environmental conditions: lateral roots (common to all plants) and nitrogen-fixing symbiotic nodules. Physiological and genetic data indicate that development of these organs is coordinated and involves common regulatory pathways, including phytohormonal controls. Recently, cytokinin signalling mediated by the receptor *MtCRE1* has been identified as crucial for both organogeneses in the *Medicago truncatula* model legume (1). We characterized symbiotic and non-symbiotic root phenotypes of *M. truncatula* mutants affected in cytokinin signalling genes, obtained through the TILLING platform developed in the frame of the Grain Legumes EEC project (GLIP; C. LeSignor and R. Thompson, INRA-Dijon; D. Baker and J. Clarke, JIC-Norwich). In addition, a 12 bp consensus sequence specifically bound by the MtRR1 transcription factor, a B-type Response Regulator involved in cytokinin signalling was identified. This result, coupled with a combination of transcriptomic and *in silico* analyses, allowed identifying new cross-talk elements between nodulation and cytokinin signalling pathways as well as new cytokinin response gene. These analyses allowed us to refine the involvement of this cytokinin signalling pathway in legume root and nodule development.

Recovery and functional analysis of a potential nucleoside hydrolase gene that may affect parasitic fitness in the Dutch elm disease fungus *Ophiostoma novo-ulmi*

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The ascomycete fungus *Ophiostoma novo-ulmi* is the causal agent of the current pandemic of Dutch elm disease. We used insertional mutagenesis to tag genes that may contribute to parasitic fitness in this fungus. Several transformants were recovered that were significantly less virulent than wild-type reference strain H327 on both Golden Delicious (GD) apples, used for preliminary pathogenicity screening of *O. novo-ulmi* strains, and elm saplings. Analysis of a genomic library from one of these transformants, designated KP78, allowed us to identify six genes next to the mutation site. In order to assess if differences in the expression of these genes were correlated with differences in the ability of strains H327 and KP78 to colonize elm tissue, both strains were grown on elm sapwood agar. Real time quantitative PCR analysis indicated that two genes were significantly downregulated in mutant KP78. One gene had homology with a nucleoside hydrolase (*urh1*), and the other with an ubiquinol-cytochrome C reductase (*ucr1*). Several knockdown mutants of strain H327 obtained by RNA interference (RNAi) targeting the *urh1* gene were less virulent on GD apples. Complementation of mutant KP78 with a wild-type *urh*1 allele restored high levels of transcript accumulation but not high virulence on GD apples. Ongoing work includes the assessment of virulence of selected *urh1* RNAi mutants and complemented strains on elm saplings, as well as further characterization of the role of *urh1* and neighboring genes and motifs in parasitic fitness of *O. novo-ulmi*.

Regulation of BAK1 complex formation

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An *Arabidopsis* reverse genetic screen for resistance-related phenotypes of pathogen inducible leucine-rich repeat receptor kinases has revealed BAK1, the previously identified brassinosteroid receptor BRI1-associated kinase, as a component of pathogen induced cell death control. BAK1-deficient plants develop spreading necrosis upon infection and this phenomenon is not dependent on brassinosteroid signaling. In collaboration with Chinchilla et al., who have shown physical interaction of BAK1 with the flagellin receptor FLS2, further evidence was provided that BAK1 is a general regulator of LRR-RLKs. The multiple functions of BAK1 are most likely based on interaction with different ligand-binding receptors. Our recent work focuses on the identification of BAK1-interacting proteins that are necessary for the regulation of plant cell death control. Potential interactors were found by a yeast-two-hybrid screen and stimulus dependent co-immunoprecipitations of *in vivo* BAK1 complexes and MS-based analyses of the interaction partners. A subfamily of RLKs was identified as BAK1 interaction partners and the interaction was confirmed by independent methods (yeast-2-hybrid, FRET-FLIM and directed co-ip). The family members show differential antagonistic changes in expression upon infection with bacterial pathogens and mutants show antagonistic phenotypes concerning BAK1 controlled pathways. We think that this group of newly identified RLKs may control the complex formation of BAK1 with its respective interacting ligand-binding receptors.

Transcriptomic, proteomic and mutant analyses demonstrate that tomato fruit susceptibility to *Botrytis cinerea* depends on the activation and progression of ripening

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Development can alter susceptibility to pathogens. Tomato fruit ripening is an example of development that coincides with increased susceptibility to *Botrytis cinerea*. Unripe tomato fruit are largely resistant and ripe fruit are susceptible. Fruit ripening in tomato is regulated independently and cooperatively by ethylene and transcription factors, including *NOR* and *RIN*. Mutations in *NOR* or *RIN* or interference with ethylene perception and production prevent fruit from ripening and thereby, could influence susceptibility. We show that ripe fruit susceptibility to *Botrytis* depends on *NOR* but only partially on *RIN* or ethylene perception, leading to the conclusion that some, but not all, ripening processes make fruit susceptible. By simultaneously analyzing fungal and infected unripe and ripe fruit proteomes, we identified >94 *Botrytis* proteins; 60% of these proteins were the same regardless of the ripening stage, suggesting that whether *Botrytis* infects a fruit depends largely on fruit processes. Disassembly of the fruit cell wall is crucial for infection since without the polygalacturonase and expansin expressed during ripening, susceptibility of ripe fruit is substantially reduced (Cantu et al., 2008). Histochemical, microarray and proteome analyses demonstrate that green fruit respond to *Botrytis* by limiting pathogen growth and expansion but paradoxically by also precociously expressing ripening genes, indicating that *Botrytis* promotes events that facilitate infections once fruit ripen. Thus, the plasticity of plant responses to pathogens during development is demonstrated in green fruit where responses provide resistance before ripening is complete and in ripe fruit where susceptibility is promoted, by some but not all ripening regulators.

Towards the positional cloning of *MtTRS1*, a QTL of resistance to the root pathogen *Ralstonia solanacearum* in *Medicago truncatula*

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Ralstonia solanacearum, is the causal agent of bacterial wilt, one of the most important bacterial diseases worldwide. A pathosystem with *Medicago*

truncatula was set up, A17 line being susceptible and F83005.5 line resistant when challenged by the sequenced strain GMI1000. Macroscopic infection process studies revealed modifications of cortical cell wall leading to increased autofluorescence and lignification of the endodermis at significantly higher rates in the resistant line compared to the susceptible line. We demonstrated that the resistance was controlled by the root. A genetic analysis of recombinant inbred lines (RILs) enabled us to identify a major Quantitative trait locus (QTL) on the top of chromosome 5 (*Medicago truncatula* Tolerance to *Ralstonia solanacearum* locus 1, *MtTRS1*) in F83005.5 line. The positional cloning of this locus is now underway by two complementary approaches: i./ linkage analysis using RILs and Heterozygous inbred families (HIFs) to reduce the zone of interest, ii./ association mapping, exploiting natural genetic diversity of *M. truncatula* lines for high resolution mapping.

Whole genome profiling: A new method for sequence based whole genome physical mapping

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Whole genome sequences are a very important tool to identify the genes that are responsible for important traits in commercial plant breeding, such as pathogen resistances. However, the investments necessary to develop a comprehensive whole genome physical map and corresponding sequence assembly are economically unfeasible for many of these crops. Therefore we have developed a new cost effective method to construct high quality sequence-based physical maps, called Whole Genome profiling (WGP). Such maps are constructed by sequence-based fingerprinting of a 10x BAC library, pooled as individual BAC clones in a multi-dimensional format, followed by sequencing of short 30 bp tags using the Illumina Genome Analyzer II. Resulting in several dozen sequence tags spaced ~2-3 kb across each BAC clone. Subsequently the BAC clones are ordered into contigs by using overlapping regions with identical sequence profiles. Unlike other profiling methods the WGP map has ordered sequence-based anchor points. The availability of a sequence-based map allows very efficient and low cost Whole Genome Sequencing (WGS) of the organism of interest whereby the quality of the WGS assembly dramatically increases. Following proof of principle in Arabidopsis (125 Mb), we have successfully applied WGP to melon (450 Mb) in combination with WGS, and are currently constructing a BAC map for a 2.6 Gb plant genome. Initial results indicate that WGP is also applicable to larger genomes. Clearly WGP offers an array of applications all of which are geared towards identifying and characterizing economically important genomic regions or genes in crops that often have large complicated genomes. Keygene N.V. owns patents and patent applications covering its whole genome technologies.

Activation of PAMP-triggered immunity in *Arabidopsis thaliana* and wheat by a polysaccharide isolated from a non-pathogenic rhizobacteria: An alternative to pesticide to control plant diseases

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Plant innate immunity relies on the perception by plant of non-self (PAMP, MAMP) and self-modified (MIMP) conserved patterns triggering local and/or basal plant defense and a response extended to the whole plant. This systemic reaction ensures a better resistance to further pathogen invasions. Elicitors are environmentally friendly alternatives to chemical pesticides in plant disease control. We have studied the potential of a polysaccharide Soligel®, isolated from a nonpathogenic rhizobacteria in plant resistance induction. We showed using cytochemistry experiments that Soligel® locally induced a transient production of H_2O_2, but no HR in *Arabidopsis thaliana*. A systemic acquired resistance-like response was activated upon Soligel® application, by the induction of systemic expression of *NPR-1* dependent -*PR-1* defense gene. We also showed an activation of camalexin and ethylene pathways. Soligel® did not induce the jasmonate dependent defense genes. Bioassays on *A. thaliana* wild-type and mutants confirmed these results at the whole-plant scale with transient protection of plants against *Pseudomonas syringae* DC3000 and *Botrytis cinerea*. We also studied the response of a cereal, *Triticum aestivum*, to Soligel® in the control of *Septoria tritici* leaf blotch, a major disease caused by *Mycosphaerella graminicola.* Plant immunity of dicotyledonous plants is better documented than is the defense response of monocotyledonous species. A single application of Soligel® decreased significantly wheat infection by *M. graminicola* as compared to water-treated plants. A cytomolecular approach showed that plant response to Soligel®, restricted the pathogen to stomata and inhibited further invasion process. Field experiments using Soligel® to control *Septoria tritici* will also be presented.

Exploring the Frankia alni genome for effectors in the actinorhizal symbiosis

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The genome of Frankia alni was determined (Normand et al., 2007) in order to identify the effectors involved in symbiosis with its host plant. No canonical nod genes were identified even though a few genes with limited similarity with nodB and nodC were found dispersed in the genome. Furthermore, using Nimblegene expression arrays, these were found not to have their expression level significantly modified during symbiosis. The root hair deforming factor (Ceremonie et al., 1999) thus cannot be the product of the nod genes as in Rhizobium and a different pathway must thus exist. One effector known to play a role in symbiosis is phenyl-acetate (PAA). PAA is present as a substituent on the hopanoid lipid that provide a barrier to oxygen diffusion on vesicles. It is an auxin and has been found to induce cell differentiation in animal cells. The possible biosynthetic pathways has been explored. Other effectors that could play a role in the first step of attachment are lectins. Several such genes are present on the genome of Frankia. These could be involved in determining host-specificity.

Structure-function analysis of the RPS5 disease resistance protein

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The *Arabidopsis* RPS5 protein belongs to the nucleotide binding site-leucine-rich repeat (NBS-LRR) family of disease resistance proteins. RPS5 mediates detection of the type III effector protein AvrPphB, a cysteine protease produced by specific strains of *Pseudomonas syringae.* Activation of RPS5 by AvrPphB requires a second *Arabidopsis* protein, PBS1, which is a substrate of AvrPphB. Transient co-expression of RPS5, PBS1 and AvrPphB in *Nicotiana bethamiana* leaves induces programmed cell death (PCD). RPS5 contains a predicted N-terminal myristoylation motif (MGGCFS), and localizes to the plasma membrane. Substitution of the two glycine residues with alanine abolished PCD triggered by an autoactive mutant form of RPS5 containing a D266E substitution, which suggests that myristoylation of RPS5 may be required for engaging downstream signaling proteins after activation. However, co-expression of PBS1 and AvrPphB with the myristolyation motif modified RPS5 or D266E protein induced a slightly delayed PCD compared to wild type RPS5. One possible explanation is the presence of overexpressed PBS1, which contains an N-terminal palmitoylation site, and/or AvrPphB, which contains an N-terminal myristoylation motif, can partially compensate for the loss of myristoylation in RPS5 since AvrPphB, PBS1, and RPS5 co-immunoprecipitate.

The ER as activator of vacuole-mediated programmed cell death induced by the mutualistic fungus *Piriformospora indica* in *Arabidopsis* roots

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The basidiomycete fungus *Piriformospora indica* is well-defined for the beneficial effects it transfers to various hosts, including improved plant peformance, increased yield, elevated abiotic stress tolerance as well as local and systemic resistance against pathogens. *P. indica* belongs to the *Sebacinales* and is thought to be an orchid mycorrhiza (Schäfer and Kogel 2009). The fungus is able to colonise a broad spectrum of plants including *Arabidopsis*. Interestingly, plant colonisation was found to be dependent on programmed cell death as demonstrated by cytological and genetic studies. However, colonised roots do not show physiological impairments like tissue necrotisation (Schäfer and Kogel 2009). Recent studies even indicate an initial biotrophic phase preceding the cell death phase (P. Schäfer, unpublished). Based on genetic, molecular, and TEM-based studies, we dissected the cell death program associated with *Arabidopsis* root colonisation by the mutualist. In mammalian systems, the ER is well-known for its ability to induce mitochondria-dependent and –independent PCD. A similar role of the ER in plant PCD has not been assigned. We identified the ER as starting point for a

novel type of plant PCD triggered during root cell colonisation by *P. indica*. This type of PCD is thought to be independent of mitochondrial proapoptotic components and signalling. In addition, vacuolar collapse was found to be central to ER-induced cell death. Results are presented that decipher this type of PCD and display its biological significance for *Arabidopsis* root colonisation by *P. indica.*

Plant-growth promoting *Pantoea agglomerans,* a endophytic bacterium, expressing heterologous gene cry by and its potential on biocontrol of *Diatraea saccharalis*

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The Gram-positive bacterium *Bacillus thuringiensi* is a pathogen of insect larvae which produces highly specific crystal inclusions during sporulation. These parasporal crystals consist predominantly of protoxin molecules known as d-endotoxins, Cry toxins. Despite the success of conventional *B. thuringiensis*-based products, they have several disadvantages as bioinsecticides. In the case of the sugarcane borer *Diatraea saccharalis*, a widespread sugarcane pest, these include instability in the environment and on the surface of sugarcane, as well as difficulty in reaching the internal regions where the larvae feed. The use of recombinant DNA technology has provided solutions to the problems: genetically modified microorganisms that are able to colonize and to live inside of sugarcane. For this reason, the *cry1Ac7* gene from *B. thuringiensis* strain 234 was previously introduced into a endohytic bacterium *Pantoea agglomerans* isolate 33.1. This bacterium is able to colonize and to promote a growth of sugarcane in green house assay. The plasmid pJTT carrying the gene *cry1Ac7* was previously constructed and gently ceded by K. J. Downing. Toxicity bioassays indicated that *D. saccharalis* larvae fed of artificial diet added the strain 33.1: pJTT showed a mortality rate significantly higher than *D. saccharalis* larvae fed of artificial diet added the strain 33.1 wild type. The larval development of *D. saccharalis* was also impaired when added the strain 33.1:pJTT. These results showed that the use of an plant-growth promoter endophytic bacterium could be a possible solution to the problem of inaccessibility of conventional *B. thuringiensis*-based products to the interior regions of the plant.

RNAi silencing of a receptor-like kinase in *Phaseolus vulgaris* transgenic roots unravels its potential role in regulating vascular bundle formation in nodules

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Receptor-like kinases (RLKs) are signaling proteins that in plants are involved in the regulation of development, in pathogen responses, and in recognition events, among others. Forward genetics and map-based cloning approaches have driven the identification of RLKs as essential players involved in nodule morphogenesis. Specifically, RLKs with leucine-rich repeat domains, are required for the early responses to Nod factors and nodule initiation, as well as for bacterial internalization in cortex cells during symbiosome formation in model legumes. This LRR-RLK protein is also required for arbuscular mycorrhiza (AM) establishment. Recently, it has been reported that this receptor defines a common genetic basis for plant root endosymbiosis not only with AM fungi and rhizobia but also with Frankia bacteria. To gain insight into the function of *Phaseolus vulgaris* LRR-RLK (PvRLK), transgenic *P. vulgaris* roots with different down-regulated *Pv*RLK levels were obtained and analyzed. The results show that there is impairment in nodule formation and also the nodule development is altered, especially in the path regulating vascular bundle formation. In wild type roots and nodules, PvRLK subcellular localization was done by immunolocalization during the nodulation process using antibodies raised against the extracellular region. PvRLK is located mostly in the root central cylinder, in vascular bundles and in the nodule parenchyma. It is interesting to note that PvRLK was also localized in other tissues, but its transcript is detected only in roots and nodules. Supported by DGAPA, UNAM IN204907.

Gene duplication and copy number variation drives the evolution of *Phytophthora* RXLR effectors

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Gene duplications and copy number variation (CNV) provide a major source for genetic and phenotypic adaptability in eukaryotic organisms. Recently, we identified RXLR effectors of *Phytophthora sojae* that correspond to avirulence genes *Avr1a*, *Avr3a*, and *Avr3c*. Each of these *Avr* genes occurs in a tandem array of duplicated DNA segments. Comparison of *P. sojae* strains indicates there is CNV for *Avr1a* and *Avr3a*; whereas *Avr3c* did not display CNV but showed evidence of sequence exchanges between adjacent copies. To determine the extent of duplication within the superfamily of RXLR effectors in the *P. sojae* and the *P. ramorum* genomes, gene copy numbers were estimated by counting trace file matches from whole genome shotgun sequences. The results indicate that multiple, identical or near-identical copies of RXLR effector genes are prevalent in oomycete genomes. Species-specific differences in the pattern and extent of duplication of RXLR effectors were noted, between *P. sojae* and *P. ramorum*. These differences have likely been molded by host range and specialization. There is massive redundancy of particular RXLR effector genes in *P. sojae*, such as *Avh426* with approximately 54 copies per haploid genome. We predict that specialization of *P. sojae* towards soybean has driven amplification of the high-copy effector genes. Our findings that *Avr* genes occur in tandem arrays, display CNV, and that gain-of-virulence mutations may spread to adjacent copies provides new evidence of the plasticity of the RXLR effector family. Thus, plant immune systems and pathogen effector systems mirror each other mechanistically, by relying on gene clusters, CNV, and associated sequence exchange mechanisms to provide rapid and novel changes to genes that control immunity and virulence.

Using genomic environment to mine for *Phytophthora infestans* genes that contribute to host adaptation

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Phytophthora infestans is the most devastating pathogen of potato and a model organism for the oomycetes. It also exhibits high evolutionary potential due among other factors to its ability to rapidly adapt to host plants. The *P. infestans* genome experienced a repeat-driven expansion relative to other *Phytophthora* (74% repeats vs. < 40%) and shows an unusual discontinuous distribution of gene density. Effector genes, such as members of the RXLR and Crinkler families, localize to expanded, repeat-rich and gene-sparse regions of the genome. Because this distinct genomic environment is thought to enable evolutionary plasticity and host adaptation, we used it as a novel criterion to mine for new candidate effectors. We used *in silico* predictions to identify the *P. infestans* secretome and classify secretome genes based on several criteria, such as large intergenic distances, conservation across *Phytophthora* species, and expression during an infection time course on potato. This approach revealed a number of novel families of secreted protein genes, termed "Robinsons", that co-opted the same genomic environment as effector genes and are rapidly evolving in *Phytophthora*. We speculate that many Robinson proteins function as effectors and/or contribute to host adaptation and specificity. We will summarize and discuss the data that address this hypothesis.

Translocation of secreted effector proteins from flax rust and their recognition by host resistance proteins

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Translocation of pathogen effector proteins into the cytosol of host cells has been shown to be a key determinant for the pathogenicity of many bacterial and oomycete plant pathogens. *Avr* genes isolated from flax rust (*Melampsora lini*) have been found to encode secreted proteins that are expressed in haustoria and translocated into host cells by an as yet unknown mechanism. Here we show that *M. lini* AvrM and AvrL567 translocation across the plant plasma membrane can occur in the absence of the pathogen. We also demonstrate that this translocation depends on signal sequences located in the N terminal region of these proteins. To study the *in vivo* interaction between translocated Avr and plant R proteins in the flax/flax rust pathosystem, we used the Bimolecular Fluorescence Complementation assay. Our results show that AvrL567 and L6 interaction takes place in both the cytosol and nucleus of plant cells.

Cloned rice blast resistance gene *Pi-k^h* confers broad spectrum resistance to *Magnaporthe oryzae*

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Rice blast caused by *Magnaporthe oryzae* is one of the most devastating and widespread diseases of rice throughout the world. None of the rice cultivars possesses durable resistance because of highly variable nature of the pathogen in the North Western Himalayan region of India. Therefore, intensive efforts are required towards developing blast resistance cultivars to achieve economic, eco-friendly and sustainable management of the disease. Many blast resistance genes have been cloned till date, but none of them offers any solution alone to the problem in this region. Blast resistance gene *Pi-k^h* was tagged and fine mapped on the rice chromosome 11 in indica rice line Tetep and the gene was subsequently cloned by our group. In this study, the candidate gene *Pi-k^h* was functionally validated by cloning in plant transformation vector and a gene construct was made along with the native promoter of the gene. A blast susceptible *japonica* rice line Taipei 309 was transformed with *Pi-k^h* gene by using biolistic approach and confirmed by PCR and Southern blotting. These transgenic plants containing the gene *Pi-k^h* confers resistance to 4 distinct isolates of *M. oryzae* collected from different parts of India. Histochemical analysis of inoculated transgenic lines showed increased callose deposition on the cell wall. Biochemical analysis of these transgenic resistant plants showed increased activity of defense related enzymes in case of incompatible interaction when inoculated with *M. oryzae*. Having been functionally validated and found to be directly involved in defense response to blast, this gene encoding NBS–LRR class of resistance protein is a promising candidate for developing blast resistant cultivars and understanding the basic mechanism involved in host-pathogen interaction.

Control of nuclear and nucleolar localization of the potyviral NIa protein

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Potyviruses (genus *Potyvirus*; family Potyviridae) have a monopartite single-stranded, positive-sense RNA genome and belong to the supergroup of "picorna-like" viruses. They are the largest and the most damaging group of plant viruses infecting many agriculturally important plant species. The multifunctional NIa protein of potyviruses constitutes of an N-terminal VPg and a C-terminal proteinase domain separated by a suboptimal proteolytic cleavage site. The majority of NIa accumulates in the nucleus of virus-infected cells, but the role of the nuclear pool of the protein is not understood. In this study, we have investigated the control of nuclear and nucleolar localization *of Potato virus A* (PVA) NIa and its possible roles in virus infection. Two regions at the N-proximal VPg domain were shown to be responsible for the nuclear and nucleolar localization of NIa. Amino acid substitutions in both NLS I (residues 4-9) and NLS II (residues 41-50), but not only in one of them, prevented nuclear localization. However, amino acid substitutions in either of them were sufficient to abolish nucleolar localization. All mutations in the both NLS regions reduced PVA virulence, as shown by reduced virus replication in protoplasts, accumulation in infected plant tissues and/or systemic movement in plants.

***Dalbergia pervillei*, a legume refractory to *Rhodococcus fascians* infection, contains new prenylated isoflavones repressing the expression of bacteria loci required for symptom development**

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Rhodococcus fascians is an Actinomycete phytopathogen with a wide range of hosts including monocotyledonous and dicotyledonous plants. A variety of symptoms is induced by this bacterium on infected plants such as simple leaf deformations, witches' brooms (etiolated stems with malformed leaves), root growth inhibition, fasciation (thickened and fleshy stems) and leafy galls (a plant hyperplasia consisting of aborted leaves and multiple dormant axillary meristems). The capacity of *R. fascians* to induce leafy gall formation depends on several loci (mainly, fas and att), required for full or optimal bacterial pathogenicity, and located on a linear plasmid. Induction of leafy galls following *R. fascians* infection of *Dalbergia pervillei* (Vatke), an endemic plant of Madagascar, proved to be difficult. Testing the effect of *D. pervillei* extracts on *R. fascians* pathogenicity gene expression revealed that prenylated isoflavones contained in *D. pervillei* bark inhibit the expression of *R. fascians att* and *fas* loci, both being required for symptom development, even in optimal *in vitro* expression conditions. The chemical structure of one of these prenylated isoflavones has been resolved. This new isoflavonoid, named perbergin, is shown to affect *R. fascians* gene expression at nanomolar concentrations and has no effect on bacteria viability. The identification of these prenylated isoflavones shed light on a new level of regulation of the virulence loci in the phytopathogen *R. fascians*.

Influence of treatment of rice seeds with siderophores producing fluorescent pseudomonades on morphological and physiological characteristics

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Fifty rice fields of different locations in provinces Mazandaran, Golestan and Guilan in north of Iran were chosen for collection of rice rhizosphere samples. The bacterial cultures were isolated and strains of fluorescent pseudomonades were selected under UV light (260 nm). Basing on morphological, physiological and biochemical tests 111 isolates were identified and evaluated for their ability to produce siderophores by CAS and CASAD method. Twenty eight isolates with the largest production of siderophores (with rates halo diameter/colony diameter more than 3 on average) were selected and tested in 4 replications. Then six isolates with the largest rate of siderophore production were used for inoculation of rice seeds of three varieties in pot culture experiments. Inoculation with all six strains increased significantly grain yield, weight of 1000 seeds, panicle number and spikelet number per panicle, number of seeds per spikelet, number of tillers, chlorophyll a and chlorophyll b contents and nitrate reductase activity in leaves of rice (average data of four replications; $P = 0.05$, according to Duncan's Multiple Range Test). These characteristics significantly dependent on the strain peculiarities, rice varieties and interaction between them.

OCP3, a new regulator of the Induced Systemic Resistance

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In *Arabidopsis*, selected strains of nonpathogenic rhizobacteria from the genus *Pseudomonas* are able to trigger an Induced Systemic Resistance (ISR) that is highly effective against a broad-spectrum of pathogens. This pathogen-induced ISR functions independently of salicylic acid (SA) but requires responsiveness and intactness of the jasmonate and ethylene signal transduction pathway. Furthermore, albeit independent of the SA pathway, the activation, full expression and execution of the ISR response *in planta* requires the normal function of the NPR1 disease regulator and in particular its associated cytosolic function. In this work we describe the functional implication of OCP3 in the ISR response to different life-style pathogens such as *Pseudomonas syringae* and *Hyaloperonospora parasitica*. OCP3 was previously identified as a nuclear transcriptional regulator that is pivotal to mount an effective disease resistance to necrotrophic fungal pathogens and controls essential aspects of drought stress adaptation responses in *Arabidopsis*. In addition, we investigated a possible role of this transcriptional regulator in controlling critical aspects of the cytosolic function of NPR1 and also in the modulation of a SA/JA cross-talk in the context of plant-pathogen interactions.

The usage of Chitin and Chitosan against fungal infection of *Colletothrimchum capsici* on Papaya crops in Yucatan State

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Papaya cultivars are native from south of Mexico and Central America; this plant has been among the major production crops in tropical countries along last years. The main problem on yield production is due to fungi infection on fruit by *Colletothrimchum* which cause an illness named Antracnosis.

Although *Colletothrimchum gloesporoides* has been reported as principal responsible of postharvest disease on papaya fruit in Yucatan, *Colletothrimchum capsici* has been found more virulent. The use of fungicides has been the only solution until now; however, it represents environmental damage. This is the reason for searching new strategies which does not include the application of toxics chemical; chitosan has been successful used for protecting some fruits due to its bacteriostatic and fungistatic properties. Our goal is the application of chitin and chitosan as protective alternative against *Colletrotichum capsici*. We tested different concentrations: 100, 200, 300, 400, 600, 1000 µl ml^{-1} of both chitin and chitosan, in order to find the minimum concentration to avoid fungus growth. Furthermore, the physiological effect of chitin and chitosan was observed in microculture. Also, it was evaluated *in vivo* the inhibitory activity of both agents, using post-harvest condition. We could not observe inhibitory activity on petri dishes using either Chitin or Chitosan, but results obtained in vivo assay showed inhibitory effect of Chitin against *Colletrotichum capsici*. Chitosan does not have inhibitory effect neither *in vitro* nor *in vivo* assay.

Bacterial growth in the apoplast is limited by nutrient availability

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Saprophytes and type-III secretion system (TTSS) mutants of phytopathogenic bacteria exhibit little growth in the apoplastic environment. The presence of a functional TTSS is known to be a requirement for growth inside plant tissues. However, a major increase in population can be attained when saprophytes and mutants of phytopathogens defective in TTSS are co-infiltrated into plants with a variety of carbon sources. Sugars such as fructose and sucrose can induce high population levels comparable to that of wild type *Pseudomonas syringae* pv. *syringae* B728a in compatible bean plants, in a dose-dependent manner. This increase was nevertheless dependent on the ability of the strain to metabolize the exogenous carbon source, since the saprophyte *Pseudomonas fluorescens* 55 did not multiply when co-introduced with sucrose, a sugar it cannot metabolize. Similarly, high bacterial levels were found when water congestion in the apoplast was maintained after inoculation, even without nutrient addition. Our experiments suggest that inaccessible pre-existing nutrients in the apoplast are made more available to bacteria by introduction of water to the intercellular spaces. This effect is transient in plants in which the introduced water is allowed to dry. Wetting of the apoplast is thus sufficient to allow bacterial multiplication although some additional release of nutrients into the apoplast during the wetting process cannot be ruled out. We thus propose that endophytic growth in leaves is limited by apoplastic nutrients and that TTSS-proficient pathogens can overcome this limitation with effectors that release water and/or possibly also nutrients into the apoplast.

Molecular evolution of Avr-R interactions in the flax / flax rust pathosystem

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Disease resistance in the flax (*Linum usitatissimum*) / flax rust (*Melampsora lini*) pathosystem is determined by a gene-for-gene recognition where members of the *AvrL567* gene family in flax rust interact with members of the *L* gene family (*L5*, *L6*, and *L7*) in flax. Amino acid differences between AvrL567 variants determine their recognition specificities, and many of these polymorphisms occur in residues found on the surface of the protein. Site-directed mutagenesis of these residues has confirmed that single substitutions compromise AvrL567 recognition without disrupting protein stability. This effect is cumulative with increased numbers of substitutions, and supports the hypothesis that multiple contacts occur between AvrL567 and L-family proteins. Flax *R*-genes are predicted to encode TIR-NBS-LRR class resistance proteins, and most of the nucleotide variation between alleles occurs in the LRR domain. As there is no crystal structure for the LRR of L5, a random mutational approach has been applied to identify amino acids with important roles in recognition specificity. Directed evolution using error-prone PCR has generated random sequence variation in the LRR of L5, and has resulted in L5 variants with novel recognition specificities. Additionally, investigations into *Avr* evolution in flax rust isolated from natural populations of the Australian native *Linum marginale* have been undertaken. Initial results indicate that *AvrP123* and *AvrP4* variants are recognized by as-of-yet uncharacterized *R*-genes in natural flax populations, and that patterns of *AvrP123* and *AvrP4* recognition vary both between populations and within populations from year to year.

Defense response of moth bean (*Vigna aconitifolius*) against fungal (*Macrophomina phaseolina*) pathogen: Altered peroxidase activity and total proteins

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Plant-pathogen interaction results in a number of biochemical changes in the host plant in order to withstand pathogen attack. The oxidative burst is an early response to pathogen attack leading to the production of reactive oxygen species (ROS) including hydrogen peroxide. Changes in the peroxidase activity and total proteins were determined in two varieties viz. RMO-40 and FMM-96 and two age groups i.e. one week and one month old plants of moth bean [*Vigna aconitifolia* (Jacq.) Marechal]. The experiments were performed in control and fungal pathogen (*Macrophomina phaseolina*) inoculated plants using *in vitro* i.e. excised stem and leaf portions, and *in vivo* i.e. intact plant systems. The peroxidase activity and total proteins were found to be higher in the pathogen inoculated plants compared to control for both the varieties and age groups. The presence of H_2O_2 was also detected histochemically in control and pathogen inoculated leaves of moth bean plants using 3,3-diaminobenzidine (DAB). The gel documentation based profile of the total proteins separated by SDS-PAGE revealed the presence of a number of bands and increase in the concentration of proteins after pathogen inoculation. A probable identification of PR-1, PR-2, PR-3, PR-4, PR-5, PR-8, PR-9 and PR-11 class of pathogenesis related proteins could also be done. The data indicate a distinct role of peroxidase and total proteins in the defense response of moth bean plants, an economically important kharif crop of Rajasthan and other semiarid regions.

cDNA-AFLP analysis of interactions between *Verticillium* and tomato

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Verticillium spp. are soil-borne plant pathogens that are responsible for devastating wilt diseases in over 200 dicotyledonous plant species, including many economically important crops. Despite the economical importance of *Verticillium* spp., little is known about the molecular basis of *Verticillium* pathogenicity and host resistance against this fungus. To identify crucial components involved in compatible and incompatible interactions between *Verticillium* and tomato, a comparative transcript profiling was performed on susceptible (MM-S) and resistant (MM-R) tomato lines of the cultivar MoneyMaker. The MM-S plants were inoculated with a race-1 (MM-S1) or a race-2 (MM-S2) isolate of *Verticillium*, while MM-R plants were inoculated with a race-1 (MM-R1) isolate only. The complete cDNA-AFLP analysis of the incompatible and compatible interaction was performed using 176 selective primer combinations. This allowed the visualization of 11,500 transcript-derived fragments (TDFs). Of these, 1434 TDFs (12.5%) showed differential regulation. Two classes of differentially expressed TDFs (DE-TDFs) were defined; I) TDFs up-regulated in the incompatible interaction (302 TDFs) and II) TDFs down-regulated in the compatible interaction (634 TDFs). The DE-TDFs being up-regulated specifically in resistant tomato cultivar (MM-R1) may be involved in the host resistance response following *Verticillium* perception, and these TDFs will be functionally analyzed using virus-induced gene silencing (VIGS). This study will help to identify crucial components involved in *Verticillium* pathogenicity and host plant resistance. This research is supported by the Dutch Technology Foundation STW, applied science division of NWO and the Technology Program of the Ministry of Economic Affairs.

RPB1* and *SGT1a* are required for atypical activation of jasmonic acid-dependent defense responses in clubroot-resistant *Arabidopsis thaliana

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Clubroot disease of *A. thaliana* and other crucifers is caused by *Plasmodiophora brassicae*, a yet poorly understood, obligate biotrophic protist. A HR-like, isolate-specific resistance reaction can be observed in the *A. thaliana* ecotypes Tsu-0, RLD, Ze-0 and Ta-0. Resistance is not affected by a *npr1-1*, *jar1-1*, *ein3-1*, *ein4*, *etr1-1*, *rar1-13* or *sgt1b-3* mutant background, but is suppressed in a *sgt1a-1* mutant background, while other *SGT1*-dependent specific resistances known so far depend on *SGT1b*, which can only be partially complemented by overexpression of *SGT1a*. We have identified and cloned *RPB1* (Resistance to *Plasmodiophora brassicae*) and shown its expression by RACE. Transgenic expression of *RPB1* under the

control of its native promoter in the susceptible ecotype Col-0 confers resistance to *P. brassicae*. Two functional copies (*RPB1a* and *RPB1b*, which encode identical amino acid sequences) have been found in Tsu, Ze and Ta, and only one copy (*RPB1a*) in RLD. Furthermore we identified 5 putative *RPB1-like* genes in *A. thaliana* and one in *B. rapa*. *RPB1* encodes a small protein with three predicted trans-membrane domains and no other known conserved domains. Transcriptome analysis of *P. brassicae* infected roots of the resistant ecotype Tsu-0 provides neither evidence for a SA- nor an ethylen-dependent defense response. Down-regulation of JA-biosynthesis genes and *JAZ* genes in infected roots of resistant plants indicate lower jasmonate levels in comparison to control roots. In contrast, the expression of PDF1.2 and other common markers for JA-dependent defense responses is strongly induced. We propose a model, in which *RPB1* and *SGT1a* are crucial components of a signaling network that links specific pathogen recognition with JA-dependent defense responses in an atypical manner.

Comparative functional genomics to elucidate virulence factors in *Erwinia amylovora* and biocontrol features in *Pantoea agglomerans*

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Fire blight is a devastating disease of rosaceous plants that has economic importance as a disease of apple and pear trees worldwide. The enterobacterial plant pathogen *Erwinia amylovora* is the causal agent of the disease. The related species *Pantoea agglomerans* includes effective commercial biocontrol strains against fire blight. The objectives of this work were to elucidate the virulence factors in *E. amylovora* and biocontrol features in *P. agglomerans* using bioinformatics tools. Comparison of the *E. amylovora* genome to the our sequenced and annotated genome of *P. agglomerans* revealed candidates for virulence regions in *E. amylovora* including three type III secretion systems, a non-ribosomal peptide synthase and putative genomic islands. Biocontrol features were identified in the genome of *P. agglomerans*, including a genomic island carrying pantocin A biosynthetic genes, several operons putatively involved in competition for metabolites in the plant habitat and an enterobactin siderophore biosynthesis and uptake cluster. An additional comparison of the *E. amylovora* genome to that of the avirulent species *E. tasmaniensis*, allowed the identification other relevant differences that might explain the virulence of *E. amylovora*. Candidates were found for future detailed investigation of the virulence factors in *E. amylovora* and the relevant biocontrol features in *P. agglomerans*. Current work includes the generation of knockout mutants of identified features.

Identification and functional analysis of *Magnaporthe oryzae* effectors

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The ascomycete *Magnaporthe oryzae* causes "blast disease" affecting several cereals in particular rice. During the compatible interaction, the first phase of *M. oryzae* infection cycle is biotrophic. *M. oryzae* is suspected to secrete, as bacterial and oomycete pathogens, an arsenal of effector proteins that disrupt the activation and execution of plant defenses. In order to identify *M. oryzae* effectors, a search for putatively secreted proteins during rice infection was performed from different sequence datasets such as, genome annotation data, transcriptome data of blast fungus-infected rice leaves, and proteomic data of blast proteins released in culture media. A list of 370 candidate effectors expressed during rice infection and possessing a putative signal peptide for secretion was generated. Among them, 82 where shown to be secreted in vitro in proteomic analyses, while the expression of 10 was found to be down regulated in the *M. oryzae bip1* mutant impaired in a b-zip transcription factor essential for the pathogenesis of the blast fungus. These 10 candidate effectors were selected to validate their effector-like activities in plant by transient transformation of rice leaves using particle bombardment technique. First results of the functional analysis of some candidate effectors will be presented, for example their capacity to activate HR *in planta*, or to suppress HR induced by *M. orysae* avirulent proteins.

Physiological changes in the tomato apoplast during infection by *Pseudomonas syringae*

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The plant pathogenic bacterium *Pseudomonas syringae* spends most of its parasitic life in the plant apoplast. *P. syringae* is able to colonize this niche by suppressing and manipulating plant defences through the secretion of proteins and toxins. Genomic analyses and nutritional assays indicate that the tomato pathogen *P. syringae* pv. tomato DC3000 is well adapted for growth using the nutrients present in the tomato leaf apoplast and shows nutritional specialization for growth in this environment relative to non-plant pathogenic *Pseudomonas*. This supports the hypothesis that some of the keys for successful colonisation of a susceptible host reside in bacterial adaptation to and modulation of the nutritional and physiological characteristics of the plant apoplast. However, the nutritional component of plant pathogenesis is relatively unexplored. We are examining the effect of *P. syringae* on apoplast composition and the effect of apoplast composition on *P. syringae* growth and virulence using a combination of metabolomic and microbiological approaches, with the goal of identifying and understanding metabolic changes in the tomato apoplast during infection.

Evaluation of pattern recognition receptors for durable disease control in crops

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Plants detect conserved molecules referred to as PAMPs (pathogen-associated molecular patterns) by pattern recognition receptors (PRRs). PAMPs are essential conserved molecules that cannot be mutated or lost, and so PRRs could potentially offer durable resistance to pathogens. Arabidopsis thaliana provides an excellent model system to study PAMP-triggered immunity (PTI), and detects a variety of PAMPs from bacteria and fungi. The related LRR receptor kinases (LRR-RLKs) FLS2 and EFR are the PRRs for bacterial flagellin and EF-Tu, respectively whereas CERK1 is required for response to chitin, the main component of fungal cell walls. However, PRR responses in agricultural crops have not been characterised. Based on our proof-of-concept findings that PRRs can be transferred across plant families and confers broad spectrum resistance, we have also transformed EFR and CERK1 into wheat to test whether their function is preserved. In addition, we have initiated an investigation into PAMP-perception and PRR function in wheat, barley and Brassica. We are evaluating the range of PAMP-mediated responses in the diverse accessions of these key crop species, and testing how the environment influences their activity. Our research will enable us to evaluate whether PRRs can be developed in agriculture to develop broad spectrum disease resistance.

Patterns of coevolution between *Phytophthora infestans* effectors and *Solanum* disease resistance genes revealed by effectoromics

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The genome sequence of *P. infestans* revealed a large and complex repertoire of RXLR effectors, and these can be implemented in high-throughput screens to discover effectors with avirulence activities. Recently this approach proved successful and resulted in the identification of *Avr-blb1* (Vleeshouwers et al, 2008), and here we report on an expanded effectoromics screen with 95 *P. infestans* candidate effectors and 130 *Solanum* genotypes. Our study revealed notable trends in the recognition of effectors which could be related to the phylogenetic and geographic origin of *Solanum*. One example is the high rate of responses to one RXLR effector by genotypes of the phylogenetic clades *Demissa* and *Longipedicellata*, which consist of natural hosts of *P. infestans* in Mexico. Close inspection of the results matrix suggested the effector is also recognized by cultivars containing *R2* family genes (Lokossou et al, 2009). Analysis of the genome sequence of *P. infestans* revealed 18 genes with similarity to the RXLR effector and the recently described *PiAvr2*. Co-expression of non-redundant *PiAvr2* family members and *R2* family members in *N. benthamiana* revealed a complex pattern of specificity. We hypothesize that the high degree of variation observed for the *R2* genes in *Solanum* and the *PiAvr2* family in *P. infestans* may have been driven by a co-evolutionary arms-race between host and pathogen.

Auxin affects the response to pathogen by interfering with plant defence

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Auxin is a well studied hormone involved in many aspects of plant development. Recently, our laboratory (Navarro et al. 2006) as well as others (Wang et al, 2007) showed that auxin also influences the ability of the plant to respond to pathogens. We hypothesized that auxin impacts the balance between salicylic acid (SA) and jasmonic acid (JA) signalling. Using transgenic plants with high auxin signalling (35S:AFB1) or low auxin signalling (35S:miR393, arf mutants) we clearly demonstrated that high auxin signalling is correlated with an increase susceptibility toward biotrophic pathogens whereas low auxin signalling is correlated with higher resistance against biotrophs and higher susceptibility against necrotrophs. As expected we found that after inoculation with Pseudomonas syringae pv. tomato DC3000, 35S:AFB1 plants accumulated less SA compared with the WT. Consistent with these data, PR1 expression was suppressed by auxin. Therefore auxin suppresses the SA signalling pathway. However, this suppression is independent of JA. Surprisingly, after inoculation with Pseudomonas syringae pv. tomato DC3000 35S:miR393 plants did not accumulate more SA, indicating that the higher resistance observed is independent of SA signalling. The nature of this pathway will be discussed further.

The transcriptional repressors WRKY18 and WRKY40 modulate key early *Arabidopsis* responses required for susceptibility towards powdery mildew

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Two related *Arabidopsis* repressors, WRKY18 and WRKY40, play a major role in PAMP-triggered basal defense. The transcriptional reprogramming induced by the powdery mildew *G. orontii* during early events of fungal establishment was monitored with respect to the role of WRKY18/40, in this interaction. Expression of >1600 *Arabidopsis* genes were differentially altered already 8hpi, suggesting rapid occurrence of pre-penetration signaling between the pathogen and the host. We found that WRKY18/40 negatively affect pre-invasion host defenses and deduced a subset of genes that appear to be under WRKY18/40 control. A mutant lacking the WRKY18/40 repressors executes pathogen-dependent but exaggerated expression of a subset of defense genes leading, for example, to strongly elevated levels of camalexin. This implies that WRKY18/40 act in a feedback repression system controlling basal defense. Moroever, using chromatin immunoprecipitation (ChIP), direct *in vivo* physical interactions of WRKY40 to promoter regions containing W-box elements of the regulatory genes *EDS1* and AP2/ERF transcription factor, *RRF1* were demonstrated. Our data support a model in which WRKY18/40 negatively modulate the expression of positive regulators of defense such as *CPY71A13*, *EDS1* and *PAD4*, but positively modulate the expression of some key negative regulatory genes of JA-signaling. Furthermore, based on previous studies (Shen et al. Science 315, 1087, 2007), we hypothesize that specific associations with an unidentified resistance (R) protein, modulate the activities of Arabidopsis WRKY18 and WRKY40. As a first approach, we used a targeted Y-2-H assay to identify candidate CC-NB-LRR protein interactors. Several CC-NB-LRR proteins have been isolated and are currently under investigation.

Screen for synthetic cis-regulatory elements responsive to PAMPs that trigger basal defense mechanisms in plants

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The identification of cis-acting DNA elements responsive to biotic/abiotic stresses are of importance both for basic gene regulation studies and for applied purposes. Delineation of in vivo cis-regulatory elements is tedious and requires extensive functional dissection of promoter regions. In silico analyses are equally difficult due to the small size of such elements. We have developed a novel screening method that allows the isolation of synthetic cis-regulatory elements responsive to biotic/abiotic stresses. This method exploits the fact that a phosphorylated form of the RNA polymerase II (RNApol-II) correlates with the transition from transcriptional initiation to elongation and mRNA capping (Genes Dev. 14, 2452, 2000). Using an available monoclonal antibody specific for the phosphorylated form of RNApol-II, fragments of cross-linked RNApol-II-chromatin, representing actively transcribed genes can be immunoprecipitated. Randomized oligonucleotide libraries were constructed linking synthetic elements upstream of a minimal promoter driving LUC expression. By this means, actively transcribed elements were immunoprecipitated with the RNApol-II antibody from a population of cells transformed with the randomized library following PAMP treatment. Library screens of several 1x106 population of synthetic elements has been performed using parsley cell lines elicited with Pep25, a Phytophthora sojae-derived peptide PAMP. Libraries and pools of synthetic elements containing putative PAMP-responsive DNA elements have been sequenced using Illumina technology. The data are currently being analyzed by bio-informatic tools to identify known and novel DNA elements involved in PAMP signal transduction. Selected unique elements will be validated in transfected protoplasts as well as in planta.

A novel nodulin with a zinc-finger like domain is degraded via proteosome

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The symbiotic association between common bean and rhizobia is a highly specific and coordinated process that culminates with the formation of an active nitrogen-fixing nodule. Mutual recognition of these symbionts at the root surface leads to plant cell wall degradation by the rhizobia and the subsequent penetration of root hair cells by means of an infection thread. Simultaneously, a nodule meristem is initiated in the outer cortex of the root; these structure matures into a spherical nodule mainly by cell enlargement. Besides, rhizobia differentiate into specific nitrogen-fixing bacteroids, that are released from infection threads into the cytoplasm of the plant cells enclosed by a peribacteroid membrane to form the symbiosome. All these processes require a precise spatio-temporal expression of specific genes. A novel nodulin with a zinc-finger like domain was identified in *Phaseolus vulgaris* root nodules induced by *Rhizobium etli*. This protein is accumulated to a very low extent in mature nodules, however the transcript levels are highly abundant suggesting an extrict post-translational regulatory mechanism. Here we evidence the specific degradation of this protein via the ubiquitin/26S proteosome pathway. This event is preceded by phosphorylation and the role of a MAPK is suggested. The biological function of this protein is unknown, nevertheless recent data suggest an implication in cell cycle regulation. Yeast-two hybrid screenings are under way for identifying putative protein ligands and gaining insight into the biological function of this nodulin in the differentiation of infected and uninfected cells in bean nodules. This work was partially supported by grant IN203807.

Generation of a resistance gene that combines multiple recognition specificities for AvrBs3-like proteins

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The Gram-negative bacterium *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) is the causal agent of bacterial spot disease of pepper and tomato. *Xcv* injects via the type III secretion system the effector protein AvrBs3. AvrBs3 binds to and transcriptionally activates plant gene promoters that contain an *UPA* (upregulated by AvrBs3) box. The pepper *Bs3* resistance gene contains also an UPA box and is transcriptionally induced by AvrBs3. By contrast, the allelic variant *Bs3-E* contains a 13-bp insertion in the *UPA* box and is induced by the AvrBs3 deletion derivative AvrBs3Δrep16 but not by AvrBs3 (Römer et al. 2007). Using single nucleotide exchange mutagenesis we defined the boundaries of the *UPA* boxes in the *Bs3* and the *Bs3-E* promoter. We also demonstrated that the *UPA* box retains its function at different positions within the *Bs3* as well as in the tomato *Bs4* promoter. Thus, the *UPA* box works irrespective of the promoter location as well as the promoter context. In addition, we placed the *Bs3* coding sequence under transcriptional control of a complex promoter consisting of three distinct *UPA* boxes and demonstrated that each of the matching AvrBs3-like proteins triggers a cell death reaction with this construct. This demonstrates that we can functionally combine

distinct *UPA* boxes into one complex promoter. Our data provide the basis to generate durable plant disease resistance to xanthomonads that contain multiple AvrBs3-like proteins.

Development of a model system to elucidate *Xylella fastidiosa* pathogenesis

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Pierce's disease of grapes and almond leaf scorch are devastating diseases caused by the bacterium *Xylella fastidiosa* (Xf). To date, progress in determining the mechanisms of host plant susceptibility, tolerance or resistance has been slow, due in large part to the long generation time and limited available genetic resources for grape, almond and other known hosts of Xf. The long generation time and limited genetic resources for *Xylella fastidiosa* compound the problem. The model plant *Arabidopsis thaliana* is an ideal system for rapid progress in genetic and pathological studies. There are many publically available genetic resources for Arabidopsis and it has a short generation time. Here we report work evaluating Arabidopsis as a host for Xf. A pin-prick inoculation method has been developed and Xf can be detected by microscopy and PCR. Xf has also been re-isolated from infected Arabidopsis tissue. Timcourses following Xf growth and changes in pathogenesis-related gene expression are in progress, as is the testing of various pathogenesis-related mutants and Arabidopsis ecotypes. Additionally, a larger plant, *Nicotiana benthamiana*, is being evaluated as a potential plant host for Xf and many of the same studies are being conducted. On the pathogen side, *Xanthomonas campestris* pv *campestris* (Xcc) is closely related to *Xylella fastidiosa* and both bacteria colonize the host plant xylem. Examination of potential shared virulence factors between the two bacteria is in progress.

A small family of NPP1-like proteins from *Magnaporthe oryzae* elicits cell death in monocot as well as dicot plants

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Magnaporthe oryzae, the causal agent of rice blast disease, is one of the most economically important plant pathogens. Bioinformatic analysis reveals that its genome encodes over 700 secreted proteins which may facilitate the fungal pathogenesis in rice plants. Recently, we have isolated and characterized a small family of four genes encoding 25-34 kD secreted proteins which share sequence homology with NPP1 (Necrosis-inducing *Phytophthora* protein 1) and other NPP1-like proteins (NLPs) from bacteria, oomycetes and fungi. Among the four *M. oryzae* NLP proteins (MoNLPs), MoNLP1 and MoNLP2 show the highest sequence identity to *Pythium* PaNie (53%) and *Fusarium* Nep1 (51%), respectively. To evaluate their potential role *in planta* as the fungal pathogen-associated molecular patterns (PAMPs), all four MoNLP genes were cloned and placed individually under the control of CaMV *35S* promoter in a binary vector (pCambia 1301S) and introduced into *Agrobacterium tumefaciens* strain EHA 105. Agrobacterium-mediated transient assays demonstrated that *MoNLP1* and *MoNLP2* were capable of inducing cell death in tobacco leaves at 30–48 hours post-inoculation whereas *MoNLP3* induced cell death within 60–72 hours. In contrast, the *MoNLP4* gene did not elicit cell death even after three to seven days. When introduced into rice protoplasts, the *MoNLP1*, *MoNLP2* and *MoNLP3* genes caused significant cell death, as evidenced by reduction in the GUS activity. In addition, MoNLP1 purified from *E. coli* was shown to rapidly induce cell death on tobacco (dicot), rice, maize and sorghum (monocot) leaves within 18 hours. Unlike other NLPs which were reported to cause cell death only in dicots, our data suggest that MoNLPs may act as PAMPs and are capable of eliciting cell death in both dicot and monocot plants.

An *Agrobacterium tumefaciens* mediated T-DNA insertion in *Epichloë festucae* disrupts the mutualistic interaction of this endophyte with its host perennial ryegrass

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The mutualistic symbiosis between the fungal endophyte *Epichloë festucae* and its host, *Lolium perenne*, is an ideal experimental system to study the signalling required for symbiosis maintenance. In wild-type symbiota, hyphal growth is tightly synchronized with growth of the leaves. Mutants of *E. festucae* that disrupt this pattern of growth give rise to a stunted host phenotype. The aim of this study is to identify additional fungal genes required for symbiosis maintenance by screening T-DNA insertional mutants that cause a stunted host phenotype. In contrast to wild-type, growth of mutant TM1066 in leaves was unregulated with multiple hyphae in the intercellular spaces as well as the vascular bundles, a tissue rarely colonized by the wild type. However, epiphyllous growth of this mutant was dramatically reduced. Tail PCR was used to rescue sequences flanking the LB junction of this single copy T-DNA insertion mutant. Sequence analysis revealed homology to a pseudouridine synthetase. Further molecular analysis identified a 1.1-kb deletion associated with the T-DNA insertion, resulting in truncation of the 3' region of the pseudouridine synthetase as well as the 3' region of an adjacent MAPKK, homologous to the *S. cerevisiae* Mkk2, a component of the cell wall integrity/cell cycle MAPK pathway. We are currently testing the hypothesis that deletion of the MAPKK was responsible for the symbiotic phenotype by analyzing a targeted disruption of the *mkk2* and by testing whether the wild-type gene will complement the original insertion/deletion mutation associated with TM1066.

Both salicylic acid and jasmonic acid are required for PAMP signaling in potato

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Defense reactions induced in potato by Pep-13, a pathogen-associated molecular pattern from *Phytophthora*, include the accumulation of salicylic acid (SA) and jasmonic acid (JA), an oxidative burst, the activation of defense genes and hypersensitive-like cell death. Accumulation of JA and hydrogen peroxide as well as hypersensitive cell death are SA-dependent since they do not occur in *NahG* plants expressing a salicylate hydroxylase. To assess the importance of the signaling compound JA, RNA interference constructs targeted at the JA biosynthetic genes allene oxide cyclase and 12-oxophytodienoic acid reductase were expressed in transgenic potato plants. In addition, expression of the F-box protein COI1 was reduced by RNA interference. Plants expressing the RNA interference constructs failed to accumulate the respective transcripts in response to wounding or infiltration of Pep-13 nor did they contain significant amounts of JA after elicitation. In response to infiltration of Pep-13, the transgenic plants accumulated less hydrogen peroxide and exhibited a reduced hypersensitive cell death. The ability of the JA-deficient plants to accumulate SA suggests that SA accumulation is upstream of JA accumulation. Thus, SA and JA do not act antagonistically, but are both required for PAMP-induced defense in potato.

Quorum sensing genes *rpfF* and *xanB2* are not essential for albicidin production nor sugarcane colonization by *Xanthomonas albilineans*

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Xanthomonas albilineans (Xa) produces albicidin, a unique and specific toxin that causes foliar symptoms of sugarcane leaf scald disease. In *X. campestris* pv. *campestris*, a cluster of *rpf* (for regulation of pathogenicity factors) genes and *xanB2* are involved in control of various cellular processes. *rpfF* and *xanB2* encode DSF (diffusible signal factor) and DF (diffusible factor), respectively, which are two quorum sensing signalling molecules. Both quorum sensing systems appear to be used by Xa, since mutation of *rpfF* in Florida strain XaFL07-1 resulted in reduced protease production, and mutation of *xanB2* resulted in loss of xanthomonadin (yellow pigment) production. Mutations of *rpfF* and *xanB2* were verified by PCR analyses. Mutations of *rpfF* and complementation *in trans* were also verified by use of an *X. campestris* DSF reporter strain. Sugarcane cultivar CP80-1743, moderately susceptible to leaf scald, exhibited pencil line symptoms indicative of albicidin production on emerging leaves and colonization of leaf vessels after inoculation of stalks by the decapitation method with all mutants, including separate deletion mutations of *rpfG* and *rpfC* (encoding two sensor components of the DSF system). Preliminary experiments indicated that several *rpfF* and *xanB2* mutants colonized sugarcane stalks as efficiently, both spatially and in intensity, as wild type Xa. Additional inoculation experiments are in progress to assess disease severity caused by *rpf* mutants, including deletion of the entire *rpfGCF* region. However, our preliminary data showed that neither DSF nor DF is essential for albicidin production or sugarcane colonization by Xa. Therefore, albicidin production and sugarcane colonization by Xa may not be controlled by quorum sensing or may involve another system.

Understanding the role of the *Thctf1* transcription factor of *Trichoderma harzianum* in plant-*Trichoderma* interactions

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Species of the soil-borne fungus *Trichoderma* are used in the biological control of a wide variety of plant pathogenic fungi. It is clear that the abilities of *Trichoderma* to inhibit the growth of other fungi are due to the combined action of cell-wall degrading enzymes (CWDEs) and secondary metabolites produced by *Trichoderma*. One of the first volatile antifungal compounds isolated from *Trichoderma* species was 6-pentyl-alpha-pyrone (6PP), and it is well known that it produces the typical yellow pigmentation developed by some *Trichoderma* strains grown on plate and it can be involved in the inhibition of plant pathogens such as *Rhizoctonia solani*. We have cloned and characterized the *Trichoderma harzianum Thctf1* gene, which shows high sequence identity with a transcription factor gene of *Fusarium solani* f. sp. *pisi*. In *T. harzianum* T34, disruption of the *Thctf1* gene by homologous recombination gave rise to transformants that did not show the yellow pigmentation observed in the wild-type strain in plate experiments. Chromatographic and spectroscopic analyses revealed that the disruptants did not produce two secondary metabolites, derived from 6PP and not previously described in *Trichoderma* genus, that are present in wild-type culture filtrates. The antifungal ability was analyzed *in vitro* in the disruptant and wild-type strains. It was observed that *Thctf1* null mutants of *T. harzianum* displayed a reduced antimicrobial capacity. *In vivo* assays are also being carried out in order to know the role of *Thctf1* in *Trichoderma*-plant interactions, in the absence or presence of a pathogen. A tomato microarray approach supports the changes in the plant transcriptome during these interactions.

The symbiotic interaction between tomato and arbuscular mycorrhizal fungi affects root metabolism and nutrient acquisition from nutrient rich soil patches

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Roots interact with the soil from which plants acquire nutrients, but soils are heterogeneous and contain nutrient patches due to either localized microbial decomposition or fertilizer banding in agricultural soils. Arbuscular mycorrhizal fungi associate with roots, increase the absorptive surface of the plant root system, and influence growth and community ecology. Thus, nutrient patches are likely to affect mycorrhizal roots differently than nonmycorrhizal roots. The main aim of this project was to study the changes in transcription of mycorrhizal and a non-mycorrhizal mutant of tomato (rmc) roots after encountering patches of NH_4^+ under field conditions. $^{15}NH_4^+$ was injected into soil patches and within 24 hours it was found in wild-type and rmc mutant (reduced mycorrhizal colonization) plant roots and shoots. Microarray and qRT-PCR analysis identified numerous genes differentially regulated by the NH_4^+ treatment in wild-type and rmc mutant roots. These included nitrogen metabolism and amino acid biosynthesis genes, cell growth and cell wall biosynthesis genes, and hormone biosynthesis genes. Additionally, the NH_4^+ treatment also resulted in significant changes in the expression of phosphate metabolism genes indicating cross-talk between N and P metabolism and a possible link to mycorrhizal-regulated phosphate metabolism. This work enhances our understanding of root acquisition of nitrogen, and the interaction between roots and symbiotic fungi. A deeper understanding of nutrient uptake in mycorrhizal roots could potentially lead to strategies for decreased fertilizer usage and enhanced nutrient capture from soils.

Elicitation of hypersensitive responses in *Nicotiana glutinosa* by the suppressor of gene silencing protein P0 from poleroviruses

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Plant resistance (R) proteins that function in gene-for-gene resistance against viruses have been found that recognize gene products with diverse functions in viral replication. Additionally, viral proteins that function as suppressors of host RNA interference (RNAi), the plant's innate line of defense against viral pathogens, have been shown to elicit recognition by plant R proteins and determine avirulence. Here we report inheritance of a novel, dominant gene designated *Rpo1* encoding resistance in *Nicotiana glutinosa* directed against the polerovirus beet western yellows virus (BWYV). The BWYV P0 protein, a suppressor of RNAi, was found to elicit a hypersensitive response (HR) that co-segregated with BWYV recognition. We show that induction of HR requires a functional P0 protein, as a P0 construct with mutations in the F-box motif that abolish suppressor activity was unable to elicit HR. P0 proteins from two additional poleroviruses, the cucurbit aphid-borne yellows virus and the potato leaf roll virus, were also found to activate HR in *N. glutinosa*. Broad recognition of polerovirus P0 proteins and the requirement for F-box protein function suggest that *Rpo1*-encoded resistance relies on detection of P0 activity in host cells and could provide durable field resistance to poleroviruses.

Coordination of multi-layered immunity and abiotic stress responses through protein quality control in the ER

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A subset of membrane-localized receptors in plants involves pattern recognition receptors that trigger immune responses upon the detection of microbe-associated molecular patterns (MAMPs), termed MAMP-triggered immunity (MTI). They include the Leu-rich repeat receptor-like kinase EFR that recognizes bacterial elongation factor-Tu and its elicitor-active epitope elf18. MTI not only represents the first layer of inducible defense against pathogenic microbes but also functional links to other layers of immunity. Plants mount these immune responses through the coordination with other physiological processes such as growth and abiotic stress responses. We describe Arabidopsis "*priority in sweet life*" (*psl*) mutants that show de-repressed sucrose-induced flavonoid accumulation in the presence of elf18. One class of these *psl* mutants, consisting of at least 7 complementation groups including novel *efr* alleles, are impaired in the generation of functional EFR. *PSL1* and *PSL2* respectively encode calreticulin3 and UDP-glucose:glycoprotein glycosyltransferase that act in concert in the endoplasmic reticulum (ER) for protein quality control (ERQC). *PSL4*, *PSL5*, and *PSL6* encode other predicted ERQC components, underpinning a critical role of ERQC in EFR maturation. Unexpectedly, SA-induced, but EFR-independent, defense is also compromised in *psl2*, *psl4*, *psl5* and *psl6* plants. This suggests that another ERQC client than EFR exists for this immune response, and that different combination of ERQC components defines client-specific biogenesis routes. Furthermore, some of the *psl* plants are also altered in several abiotic stress responses. We propose that ERQC becomes engaged in different biotic/abiotic stress responses through the biogenesis of membrane-localized receptors/regulators, thereby providing a basis for their coordination.

***Magnaporthe oryzae* effector gene function analyzed by gene disruption and overexpression**

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To study function of candidate effector genes of *M. oryzae*, we carried out targeted gene disruption of selected ~100 putative secreted protein genes that are expressed during rice infection. A total of 10 genes caused phenotypic changes after gene disruption. Targeted gene disruption of a gene *MC69* caused remarkable reduction in penetration rate and infectious growth in rice leaf sheath cells as well as reduction in blast symptoms in rice and barley. MC69 is a putative secreted protein comprising 54 amino acids with a signal peptide. This phenotype caused by *MC69* disruption was complemented by re-introduction of an intact copy of *MC69*. These results suggest that MC69 is a virulence effector. When *M. oryzae* strain expressing MC69::EGFP fusion protein driven by the native *MC69* promoter was used for inoculation of rice sheath cells, strong GFP fluorescence was detected in appressoria, at the hyphal tips and the cap structure of invading hyphae. When *M. oryzae* harboring *MC69p::MC69::EGFP* was cultured in liquid medium, MC69::EGFP protein was detected in culture filtrate, indicating that MC69 is indeed secreted from the fungus. Further functional analysis of MC69 is underway. In parallel to gene disruption study, we are studying virulence

changes of *M. oryzae* after overexpression of ~50 effector candidate genes. Overexpression of 3 genes caused reduction and 2 genes caused enhancement of virulence.

A yeast *STE11* homologue *CoMEKK1* expression is regulated by cAMP signaling pathway and essential for pathogenesis related morphogenesis in *Colletotrichum orbiculare*

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Several signal transduction pathway including MAP kinase pathways are involved in appressorium development in *C. orbiculare,* the causal agent of cucumber anthracnose disease. In this study, we isolated T-DNA inserted mutants defective in appressorium development. *CoMEKK1*, a yeast MAPKK kinase *STE11* homologue, was identified as disrupted gene in a T-DNA inserted mutant. The phenotype of *comekk1* targeted disruption mutants was similar to *cmk1* mutant, *a Saccharomyces cerevisiae Fus3/Kss1* MAP kinase homologue gene disruptant; reduction of conidiation and germination, defect in appressorium formation and invasive growth in host cucumber leaves. Moreover, *comekk1* and *cmk1* mutant were sensitive to high osmotic stress and salinity stresses, indicating that Comekk1p/Cmk1p signal transduction is involved in stress tolerance. *CoMEKK1-4,* a constitutively active allele of *CoMEKK1,* introduced transformants in wild-type and *comekk1* mutants showed slower hyphal growth and abnormal form of appressoria, but those in *cmk1* disruptant did not. To investigate whether signal transduction via Comekk1p controls Cmk1p activation, we observed Cmk1p-GFP intracellular localization. The frequency of nuclear localization of Cmk1p-GFP fusion protein induced by salt stress decreased in *comekk1* mutants. These results indicated that Comekk1p functions upstream of Cmk1p. Finally, salt stress and plant surface exudates induced expression of *CoMEKK1* gene. This expression was also induced by addition of exogenous cyclic AMP, but not in a cAMP signaling mutant *cac1* defective in adenylate cyclase gene. These results indicate that *CoMEKK1* response to environmental cue undergoes via gene expression under the control of cAMP signaling pathway.

Interaction between arbuscular mycorrhiza fungus and *Fusarium oxysporum* on okra and jute mallow

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The interaction between Arbuscular Mycorrhiza (AM) fungus *Glomus mosseae* and *Fusarium oxysporum* and the subsequent effect on the growth of jute mallow and okra plants were investigated in a greenhouse. At different ages (4, 6, and 8 weeks for jute mallow; 2, 4, 6 and 8 weeks for okra), inoculation with *F. oxysporum* significantly reduced the mean values of the growth parameters of okra and jute mallow. In contrast, the growth response and biomass of jute mallow and okra plants inoculated with *G. mosseae* was significantly higher than that of non-mycorrhizal plants, both in the presence and absence of the pathogens. Plants inoculated with *G. mosseae* had a lower incidence of necrosis, defoliation and wilting and eventual death than non-mycorrhizal ones in okra plants. Jute mallow showed a relative level of resistance against the pathogenic effect, with 8 weeks more resistant. The pathogen and mycorrhiza inoculated either simultaneously or dually into the okra and jute mallow plants suppressed the effect of the pathogen. The susceptibility of the vegetables to the incidence and susceptibility of the pathogen was age mediated as it reduces as the age of plants increases. There was a general sequential increase in the mean values of the growth parameters of okra and jute mallow. Mycorrhiza had a positive effect on the stem height, leaf surface area, number of leaves and the stem girth of jute mallow and okra plants than the other treatments. Results obtained showed that inoculation of *Glomus mosseae* before *F. oxysporum* inoculation into okra and jute mallow seedlings increased their resistance and suppressed the pathogenic infection.

The plant growth-promoting fungus *Aspergillus ustus* alters root architecture through a hormone signaling mechanism and induces resistance against foliar pathogens in *Arabidopsis thaliana*

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Due to health and environmental issues, there is a need to reduce the use of agrochemicals to enhance plant growth and control diseases in crops. This has raised the need to study alternatives, such as plant growth promoting microorganisms and biocontrol agents. We have identified an *Aspergillus ustus* isolate that promotes growth and induces developmental changes in *Arabidopsis* thaliana seedlings. *A. ustus* inoculation on *A. tahliana* roots induced an increase in shoot and root growth, lateral-root and root-hair number. Assays to measure reporter gene expression from auxin induced/repressed or cell cycle controlled genes (*DR5*, Tir1 and *CycB1*) with the *uidA* reporter gene, showed an enhanced GUS activity of *DR5* and *CycB1*, while a lower activity was observed in Tir1 lines when compared with mocked-inoculated seedlings. In order to determine the contribution of phytohormone signaling pathways in the effect elicited by *A. ustus*, we evaluated the response of a collection of hormone mutants of *Arabidopsis* (*aux1-7*, *axr4-1*, *eir1-1*, *etr1-3*, *ahk2-2*, *ahk3-3*, and *abi4-1*), defective in auxin, ethylen, cytokinin or abscisic acid signaling respectively, to inoculation with this fungus. All mutant lines tested showed increased biomass production when inoculated with *A. ustus*. In addition, *A. ustus* induced the expression of defense genes in *Arabidopsis* Col-0 seedlings, which induced systemic resistance against *Botrytis cinerea* and *Pseudomonas syringae*. Taken together, our results suggest that the increase in plant growth and root architecture changes are induced by auxin-like molecules produced by *A. ustus* different from those molecules that do not produce an effect on the *Arabidopsis* lines we used in this work. In addition, this fungus is able to protect plants against plant phytopathogens.

FLI1, a novel component of PAMP-triggered immunity in *Arabidopsis*

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Recognition of pathogen-associated molecular patterns (PAMPs) constitutes one of the first layers of active plant defense. The pattern recognition receptor kinases FLS2 and EFR are responsible for the perception of bacterial flagellin (flg22) or elongation factor-Tu (elf18), respectively. FLS2, together with its co-receptor BAK1, and EFR stimulate a plethora of defense responses that restrict bacterial infection, e.g. *Pseudomonas syringae* pv. *tomato* (PtoDC3000). Surprisingly, to date relatively little is known about additional components of PAMP-triggered immunity. In a forward genetic approach, we identified *fli1* as flagellin insensitive. Immediate early responses such as the oxidative burst appear unaffected by *fli1*, but deposition of callose in response to flg22 and elf18 is severely impaired. Importantly, *fli1* allows enhanced proliferation of PtoDC3000 to almost similar levels than *fls2* mutants. Moreover, *fli1* exhibits enhanced resistance to oomycete infection of *Hyaloperonosopora parasitica* cv. Cala2. Molecular characterization revealed no alterations of both, FLS2 and BAK1 sequences and accumulation. Hence, we have identified a novel component of PAMP-triggered immunity in *Arabidopsis*. We will discuss phenotypic analysis of *fli1* and its genetic isolation.

Analyzing the role of Pto kinase activity in recognition of bacterial effectors and signaling by a chemical genetic approach

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The tomato (*Solanum lycopersicum*) protein kinase Pto confers resistance to *Pseudomonas syringae* pv. *tomato* bacteria expressing the AvrPto and AvrPtoB effector proteins. Pto specifically recognizes both effectors by direct physical interactions triggering activation of immune responses. Here, we used a chemical genetic approach to sensitize Pto to analogs of PP1, an ATP-competitive small-molecule inhibitor. By using PP1 analogs in combination with the sensitized Pto (Pto^{as}), we examined the role of Pto kinase activity in effector recognition and signal transduction. Strikingly, while PP1 analogs efficiently inhibited kinase activity of Pto^{as} *in vitro*, they enhanced interactions of Pto^{as} with AvrPto and AvrPtoB in a yeast two-hybrid system. In addition, in the presence of PP1 analogs, Pto^{as} bypassed mutations either at an autophosphorylation site critical for the Pto-AvrPto interaction or at catalytically essential residues, and interacted with both effectors. Moreover, in the presence of the PP1 analog 3MB-PP1, a kinase-deficient form of Pto^{as} triggered an AvrPto-dependent hypersensitive response *in planta*. Thus, rather than phosphorylation *per se*, a conformational change likely triggered by autophosphorylation in Pto and mimicked by ligand binding in Pto^{as} is a prerequisite for recognition of bacterial effectors. Following recognition, kinase activity appears to be dispensable for Pto signaling *in planta*. The

chemical genetic strategy applied here to develop specific small-molecule inhibitors of Pto represents an invaluable tool for the study of biological functions of other plant protein kinases *in vivo*.

Rhamnolipids elicit plant defense responses in *A. thaliana* and enhance resistance against *B. cinerea* and *P. syringae* pv. tomato DC3000

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In the course of their life, plants have to face a broad range of microorganisms which are potentially pathogens. To counter these attacks they have evolved a large set of defense responses. These defenses include pre-existing physical and chemical barriers, as well as inducible responses that are activated after pathogen perception (Hammond-Kosack and Jones, 1996). This recognition step can be achieved by the means of molecules common to many classes of microbes known as microbe-associated molecular patterns (MAMPs) or general elicitors (Mackey and McFall, 2006). They are involved in non-specific immunity and the associated resistance is effective against a broad range of pathogens (Bent and Mackey, 2007; Jones and Dangl, 2006). MAMPs belong to different families including proteins, glycans and lipids. Rhamnolipids (RLs) produced by *Pseudomonas aeruginosa* are amphiphilic molecules described as potent biosurfactants with application in the fields of agriculture, food industry, oil recovery and environment protection technologies. Recently, we showed that RLs not only inhibit spore germination and mycelium development of *Botrytis cinerea* but also are perceived as MAMPs by grapevine plant cells. In addition, RLs also confer grapevine protection against grey mold disease (Varnier et al., 2009). In order to gain more insights on the elicitor/antifungal parts in RLs protective effect, we used *Arabidopsis thaliana* signalisation mutants. Here, we show that RLs protection of *A. thaliana* against the necrotrophic fungus *B. cinerea* mainly involve a *npr1*-independent-SA pathway. Moreover, in the *Pseudomonas syringae* pv. tomato-*A. thaliana* interaction, RLs affect motility of the hemibiotrophic bacterium and enhance plant resistance against the pathogen.

Long-chain sphingoid bases are important intermediaries in the maize defense response to *Fusarium verticillioides* infection

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Fusarium verticillioides is a fungal plant pathogen commonly associated with maize (*Zea mays*) and can infect the plant at various developmental stages. Most *F. verticillioides* isolates produce fumonisin B1 (FB1) that is a potent inhibitor of the sphinganine N-acyltransferase, leading to a depletion of complex sphingolipids and an accumulation of long-chain sphingoid bases (LCB). The role that FB1 plays in pathogenicity has been controversial but recent genetic evidence showed that it is required for seedling disease development. Because the fungus can survive in soil and seed, it is able to infect during germination and seedling establishment. Thus a suitable model to study the association among FB1 production, LCB accumulation and defense response, are germinating maize embryos exposed to the mycotoxin and/or the fungus. Maize embryos germinated in presence of 20 µM FB1 readily accumulated LCBs; sphinganine levels reached its maximum (18-fold) at 24 h germination whereas phytosphingosine showed a 4-fold accumulation at 15 h and sphingosine a 3.5-fold increase over control. In order to associate LCB build-up with defense response in germinating maize, we determined glucanase activity. Glucanases belong to pathogenesis-related protein 2 (PR-2) group. Low glucanase activity was observed during early germination (6 – 18 h) and a two-fold induction in enzymatic activity was detected at 30 h germination. Our results point out that FB1 causes LCBs accumulation and that these intermediaries are responsible for glucanase activation.

Novel insights into the complex regulatory role of ABA signaling pathway in *Arabidopsis thaliana* resistance to necrotrophic pathogens

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Plant resistance to necrotrophic pathogens depends on the interplay of different signaling mechanisms, such as those mediated by the hormones SA, JA and ET. In addition to these well-characterized pathways, other plant hormones are emerging as novel regulators of plant resistance to pathogens. The role of ABA signaling in the regulation of plant innate immunity is complex and still not well understood. In Arabidopsis resistance to necrotrophic pathogens, such as *Plectosphaerella cucumerina*, a positive effect of the constitutive activation of ABA pathway has been described (Hernandez-Blanco *et al*. 2007). However, ABA biosynthetic (*aba1*) and signaling (*abi1*) defective mutants were found to be more resistant to necrotrophs than wild-type plants, suggesting a negative role of ABA in the regulation of plant resistance to this type of fungi. Comparative transcriptomic analysis of *aba1* mutant and wild-type plants upon *P. cucumerina* infection led to the identification of some defensive responses that were constitutively activated in the *aba1* mutant. Meta-analysis profiling demonstrated that *aba1* up-regulated genes were predominantly regulated by JA and SA. In addition, genetic analysis demonstrated that JA biosynthetic pathway is necessary for full resistance of *aba1* mutant to necrotrophs. Recent advances on the crosstalk among ABA, JA, SA and ET signaling pathways in the regulation of Arabidopsis immune response to necrotrophic fungi will be presented.

Differential and combinatorial functions of antimicrobials in Arabidopsis non-host resistance to biotrophic and necrotrophic fungi

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One defense pathway contributing to non-host resistance in Arabidopsis to biotrophic fungi involves the targeted delivery of indole glucosinolate-derived metabolites at pathogen contact sites. We have examined whether this secondary metabolite pathway is also rate-limiting for colonization by necrotrophic fungi, which generally have a broad host range. The immune response of Arabidopsis mutants blocked in indole glucosinolate hydrolysis or other defense signaling pathways was analysed upon inoculation with adapted or non-adapted isolates of the necrotrophic fungus *Plectosphaerella cucumerina*. We found that the accumulation/delivery of indole-derived metabolites, which is blocked or impaired in *cyp79B2B3*, *pad2*, or *pen3* mutant lines, is required for full Arabidopsis non-host resistance to non-adapted *P. cucumerina*, as evidenced by enhanced fungal growth on leaves. In contrast to interactions with biotrophic fungi, camalexin or 4-methoxy-indol-3-ylmethylglucosinolate, which is generated by the P450 monooxygenase CYP81F2 and hydrolyzed by PEN2 myrosinase, is dispensable for growth restriction of non-adapted *P. cucumerina*. This suggests a contribution of other yet unknown tryptophan-derived metabolites in defence responses to non-adapted *P. cucumerina*. These data imply differential contributions of anti-microbials in non-host resistance to necrotrophic and biotrophic pathogens.

A chemical genetics approach to identifying novel components in plant immunity signalling

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The recognition of specific pathogen effector molecules by plant resistance (R) proteins instigates signalling events which activate defence responses that protect plants from disease. Despite our knowledge of this general framework many of the molecular pathways that connect the inputs to the outcomes of plant immunity have not been characterised. One of the most diagnostic indicators of R-protein mediated immunity is rapid cell death local to the site of initial pathogen incursion, a phenomenon known as the hypersensitivity response (HR). We are using the occurrence of the HR in a chemical genetics screen to identify novel components involved in R-protein mediated immunity. When Arabidopsis thaliana cells, which posses the R-protein RPM1, are treated with the incompatible pathogen Pseudomonas syringae pv tomato DC3000 carrying the avrRpm1 effector gene (Pst-avrRpm1) cell death occurs within 24 hours and can be quantified by staining the cells with Evans blue. Addition of the phytohormone salicyclic acid (SA), which is characteristically up-regulated during the defence response, substantially increases the intensity of the cell death in the presence of the bacterial pathogen. We are screening both synthetic combinatorial and natural product libraries for inhibitors of the HR in A. thaliana cells after treatment with Pst-avrRpm1 and SA. Compounds that elicit a $\geq$ 25% decrease in cell death in the first round of screening will be subject to a second round of testing in A. thaliana leaves to confirm inhibition of the HR in planta. Following this the site and mode of action of the hit compounds will be characterised by genetic and biochemical methods.

Insight into the antagonism between auxin and oligogalacturonides

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Alpha 1-4-linked oligogalacturonides (OGs) derived from plant cell walls are well known elicitors of defence responses. Transcriptome analysis shows that more than 95% of the early OG-regulated genes are also regulated in the same direction by the pathogen-associated molecular pattern Flg22; OG and Flg22 transcript changes however, diverge over time. OGs also regulate growth and development of plant cells and organs, due to an auxin-antagonistic activity. The molecular basis of this antagonism is still unknown. In Arabidopsis, OGs inhibit adventitious root formation induced by IAA in leaf explants, as well as the expression of several IAA-up-regulated genes. We have performed genetic, biochemical and pharmacological experiments aimed at elucidating whether the OG/auxin antagonism requires the molecular signals ethylene, jasmonic acid, salicylic acid, NO and ROS, and whether it involves any of the following mechanisms: 1) alteration of auxin homeostasis; 2) stabilization of auxin-response repressors; 3) decrease of auxin receptor levels through the same microRNAs (miR393) that is responsible for the repression of auxin signalling exerted by Flg22.

Genome characterization and discovery of novel QXLR effector motif in the cucurbit downy mildew pathogen *Pseudoperonospora cubensis*

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The cucumber downy mildew pathogen, *Pseudoperonospora cubensis*, is an economically important pathogen of cucurbit species, including cucumber, melon, and squash. In recent years, resistance to *P. cubensis* in the United States has been overcome, either through introduction of a new pathotype or through pathogen evolution. The genetics and cell biology of the cucumber-*P. cubensis* pathosystem has yet to be characterized in detail despite its increasing economic importance. Sequencing of the *P. cubensis* genome using GS-FLX sequencing has yielded significant insight into the effector repertoire of this pathogen. Using a bioinformatics approach and comparative data mining based on previously identified oomycete effector proteins we have identified candidate effector proteins from *P. cubensis* containing characteristic N-terminal RXLR and dEER motifs. Interestingly, we have also identified a QXLR motif that replaces the RXLR motif in nearly half of our identified putative effectors sequences. The newly sequenced genome of *P. cubensis*, identification of candidate effector proteins and the novel QXLR motif will serve as a basis for further characterization of the *P. cubensis*-cucumber interaction through the analysis of effector protein function and gene expression using laser capture microdissection and digital gene expression.

Suppression of PAMP-triggered immunity by the orchid mycorrhiza *Piriformospora indica* and its significance for *Arabidopsis* roots colonisation

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The basidiomycete *Piriformospora indica* transfers beneficial traits to its hosts, including improved plant performance, increased yield, abiotic stress tolerance as well as systemic resistance against pathogens (Stein et al. 2009). *P. indica* belongs to the *Sebacinales*, a fungal order that houses mycorrhizal fungi others than arbuscular mycorrhizas. Within this order the fungus displays closest relationships to orchid mycorrhizas as revealed by phylogenetic and cytological investigations (Schäfer and Kogel 2009). The fungus is able to form a mutualistic symbiosis with a broad spectrum of monocot and dicot plants including *Arabidopsis*. *P. indica* displays a cell death-dependent colonisation, which is now known to be preceded by an initial biotrophic growth phase. Hence, colonisation of *Arabidopsis* roots by *P. indica* apparently bases on a more sophisticated plant-microbe cross-talk. Our studies revealed a complete reversion of flg22-induced growth retardation in *P. indica*-colonised *Arabidopsis* and identified a strong activity of the fungus in suppressing PAMP-triggered immunity. In subsequent studies, we could show the effectiveness of activated flg22-activated PTI in restricting colonisation of *Arabidopsis* roots by the beneficial fungus by up to 60%. We will discuss at which layer of PTI-signalling *P. indica* might interfere and present results on plant hormones that participate in defense suppression and repression of flg22-induced growth retardation by *P. indica*. The current findings outline the mutualistic *P. indica*-*Arabidopsis* interaction as a defined system to study innate immunity in plant roots.

The *U. maydis* effector Stp1 suppresses defense responses of the host plant maize

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The basidiomycete fungus *Ustilago maydis* is a facultatively biotrophic pathogen, that causes smut disease in its host plant maize. *U. maydis* penetrates the plant cuticle, proliferates inter- and intracellularly within the plant and finally induces characteristic tumours. During intracellular growth the fungus is surrounded by the plant plasma membrane and is thus never in direct contact with the cytoplasm of host cells. Recently, we showed that novel secreted proteins play crucial roles during establishment of this biotrophic interaction (Kämper et al., 2006). One of those secreted proteins, Stp1, was characterized in detail during this study. *stp1* deletion mutants are non-pathogenic due to a block of growth directly after penetration of plant cells. This coincides with a hypersensitive response of the infected plant cell, accompanied by enhanced autofluorescence, papillae formation and H_2O_2 accumulation. Gene expression profiling by maize microarrays revealed that in contrast to wild type strains the Δstp1 mutant induces a heavy plant defense response in infected maize plants. A Stp1-mCherryHA fusion protein localized to the apoplast of the infected plant tissue. However, transient expression of Stp1 lacking the N-terminal signal peptide in *N. benthamiana* and *Z. mays* revealed, that the protein localizes specifically to subcompartments of the nucleus. This could indicate that Stp1 is transferred from the apoplast to the plant cell after infection. Results from ongoing experiments, where a Stp1-mCherry fusion that includes the signal peptide is transiently expressed in maize will also be provided. Additional evidence for the function of Stp1 in the plant cell nucleus comes from two nuclear plant proteins that are interacting with Stp1 in a yeast-two hybrid screen.

Identification of virulence loci in smut fungi by genome comparison

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Ustilago maydis and *Sporisorium reilianum* are closely related smut fungi that parasitize on maize. After plant penetration, hyphae of both fungi grow initially intracellularly and then ramify extensively in the apoplast of the plant. Although their presence is recognized by the plant, both fungi can efficiently colonize plant tissue, presumably because they actively suppress plant defense mechanisms. We previously identified secreted virulence effectors by screening the *U. maydis* genome for gene clusters encoding secreted proteins (Kämper et al., 2006). However, deletion analysis revealed that seven of the twelve identified gene clusters did not contribute to virulence. In order to increase the chance of indentifying genomic regions containing virulence determinants, we hypothesized that these factors would be under evolutionary pressure for rapid change to avoid recognition and defense reactions of the plant host. Therefore, we performed a gene-by-gene comparison of the syntenic genomes of *U. maydis* and *S. reilianum* and identified chromosomal regions of low-conserved genes. Strikingly, these "divergence regions" included all the previously identified clusters of secreted proteins with an effect on *U. maydis* virulence, while half of the clusters without effect on virulence were excluded. Furthermore, deletion of four of six novel divergence regions also affected virulence of *U. maydis*. Intriguingly, one of these novel virulence loci did not encode secreted proteins. This indicates that biotrophic fungi use a panoply of rapidly evolving secreted effector proteins to facilitate efficient plant colonization. Our results show that a whole genome comparative approach allows reliable prediction of effectors important for virulence.

Stenotrophomonas – from plant microbial ecology to efficient biocontrol agents

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Stenotrophomonas rhizophila, a recently described bacterial species (Wolf et al 2002), is a promising seed inoculant. It displays broad antifungal activity in vitro (Wolf et al. 2002) and promotes the growth of a variety of crops in saline

soils (Egamberdiyeva, unpublished results). However, little is understood how this plant growth promoting bacterium colonises and affects different plant species and how it is influenced by salinity. We have studied root colonisation and the direct effect of *S. rhizophila* DSM 14405^T on different plant species in gnotobiotic systems. Application of *S. rhizophila* DSM 14405T decreased root length and the number of root tips of tomato and cotton; the number of leaves in tomato was slightly increased. High doses of *S. rhizophila* DSM 14405^T (10^8 cells per seedling) had deleterious effects on tomato and cotton. Contrarily, development of oilseed rape was unaffected by *S. rhizophila* DSM 14405^T even at high doses. The bacterium established high populations on the roots, stems and leaves of the host plants (10^8-10^9 cells per g fresh weight in tomato and oilseed rape, 10^6-10^8 cells per g fresh weight in cotton). Confocal laser scanning microscopy of tomato plants treated with DsRed-tagged *S. rhizophila* DSM 14405^T showed that *S. rhizophila* preferentially colonises the inside of root hairs. Further experiments will elucidate how salinity and the soil microflora affect root colonisation and plant growth promotion of *S. rhizophila* DSM 14405^T; furthermore the interaction with pathogenic fungi will be investigated.

Effectors of the powdery mildew fungus

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Plant pathogens utilize small effector proteins to manipulate their hosts thereby interfering with defense pathways, transcription and vesicle trafficking. Using a bioinformatic approach potential effector proteins of the barley powdery mildew pathogen Blumeria gramins f. sp. hordei (*Bgh)* were identified. Upon transient expression in barley, a subset of theses candidates increased the penetration efficiency of Bgh. A yeast-two-hybrid screen of a barley cDNA-library identified anADP-ribosylation factor (ARF)–GTPase-activating protein (GAP) and an ubiquitin-conjugating enzyme as potential interactors of the candidate effector MH4. ARF-GAPs are key components of coatmer protein (COP) I-coated vesicle trafficking and cargo loading at the Golgi, while ubiquitin-conjugating enzymes function in protein modification leading to proteasomal degradation, DNA-repair transcriptional regulation or endocytosis. GFP-tagged MH4 protein weakly associates with the plant endomembrane system when transiently expressed in barley epidermal cells. Real time PCR analysis of another effector candidate, MH2, revealed increased transcript levels during appressorium formation. We identified and cloned a homologous gene from a powdery mildew fungus (*Golovinomyces orontii*) that is virulent on the dicot reference species *Arabidopsis thaliana*. Stable expression of this gene in Arabidopsis and subsequent challenge with the adapted fungus will unveil whether powdery mildews employ conserved infection strategies on monocots and dicots.

Comparative transcriptome and genome sequencing of *Phytophthora infestans* and closely related species reveals a polymorphic RXLR effector that modulates host processes

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The process of speciation and its relationship to host adaptation in oomycete plant pathogens remains a major unknown. It is thought that some oomycete species arise as a consequence of host-jumping, followed by adaptation, specialization and finally speciation on the new host plant. One expected outcome is a modified effector repertoire adapted to subvert defence signalling of the new host. The potato late blight pathogen *Phytophthora infestans* encodes a large repertoire of effector genes that evolve rapidly through birth-and-death evolution and typically exhibit adaptive selection. We hypothesized that effectors with species-specific polymorphisms are important for host adaptation. We applied Illumina technology to sequence the transcriptomes of *P. mirabilis* and *P. ipomoeae*, two species very closely related to *P. infestans* but that infect non-solanaceous hosts. Comparative analyses revealed *PexRD35a12*, a novel RXLR effector present in both *P. ipomoeae* and *P. mirabilis* but annotated as pseudogene in the *P. infestans* genome due to a homozygous 5-bp deletion. We expanded our analyses and identified a large number of *P. infestans* isolates with heterozygous full length and mutated *PexRD35a12* allele configurations, whereas an intact allele is always present in the sister species. To study PexRD35a12 function we examined localisation and activities of PexRD35a12 variants in *Nicotiana benthamiana*. Our results revealed that some but not all PexRD35a12 variants associate with endoplasmic membranes, suppress cell death, and alter apoplastic protein content indicative of interference with host protein secretion. Our study highlights the value of identifying species-specific effector configurations using comparative genomics. We now aim to connect the identified effector activities to a role in host adaptation.

High-throughput investigation of the *Arabidopsis thaliana-Pseudomonas syringae* pathosystem in a liquid assay format

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The interactions between plants and their pathogens involve a complex array of molecular events that remain to be fully elucidated. In order to investigate the *Pseudomonas syringae*-Arabidopsis pathosystem in a high-throughput manner, we devised a liquid assay using standard 96-well plates. We demonstrated that Arabidopsis seedlings incubated with *P. syringae* in liquid culture display a macroscopically visible "bleaching" symptom within five days of inoculation. Bleaching is associated with a loss of chlorophyll from cotyledonary tissues and is correlated with bacterial virulence. Based on this symptom, we initiated a chemical screen to identify small molecules that alter the susceptibility of *Arabidopsis* to infection by *P. syringae*. In addition, we are screening transposon disruptants of *P. syringae* pv. *maculicola* strain ES4326 in an effort to identify novel virulence-associated genes. For both screens, we have established a set of experiments through which screening hits can be classified and further characterized. This analytical workflow is illustrated for the compound sulfamethoxazole identified by chemical screening, and for the *P. syringae* gene *FleN* identified by transposon screening, which encodes a putative regulator of flagellar number. We demonstrate that the liquid pathosystem provides a rapid, economical, and biologically relevant surrogate for studies using soil-grown plants.

Identification and characterization of novel genes involved in the virulence of *Xanthomonas campestris* pv. *vesicatoria*

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The Gram-negative plant pathogenic bacterium *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) is the causal agent of bacterial spot disease on pepper and tomato. Xcv enters the plant tissue via natural openings or wounds and multiplies in the intercellular spaces. In *Xcv*, essential pathogenicity and many virulence-associated genes are induced upon contact with the plant cells by two known regulatory proteins, HrpG and HrpX. Both proteins regulate the expression of the *Xcv* type III secretion system, an essential pathogenicity factor. Genome sequencing of *Xcv* strain 85-10 revealed 4,854 genes in the chromosome and four native plasmids. High-throughput sequencing of cDNAs from *Xcv* strain 85-10 and derivatives and bioinformatic approaches identified novel genes, some of which are HrpG/HrpX dependently expressed. Genetic studies resulted in the discovery of new *Xcv* virulence factors involved in post-transcriptional regulation of gene expression.

The BAK1-FLS2 receptor complex: Towards understanding activation of the flagellin receptor

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Plants can sense potential microbial invaders via the perception of molecular associated molecular patterns (MAMPs) by pattern-recognition receptors (PRRs). In Arabidopsis, the LRR-receptor kinases (LRR-RKs) FLS2 and EFR form the best characterized PRRs, recognizing respectively the bacterial flagellin and EF-Tu. Flagellin and EF-Tu perceptions are important for defence against bacterial pathogens but little is known about the molecular mechanisms of receptor activation. Previously we demonstrated that another LRR-RLK called BAK1 (BRI1-Associated Kinase 1) interacted with FLS2 at a step after ligand binding and that *bak1* mutants were much less responsive to flagellin and EF-Tu. In the present work we refine our kinetic analysis of receptor oligomerization and show that FLS2 can interact with BAK1 within few seconds after stimulation of Arabidopsis cells with flg22, clearly before the onset of physiological responses. In vitro phosphorylation assays performed on immuno-precipitates of FLS2 and BAK1 show that FLS2 is phosphorylated specifically in presence of BAK1. This confirms our results of in vivo phospho-labelling (see Schulze et al., XIV MPMI congress). Similar observations are done for EFR and BAK1 which are capable of complex

formation in response to EF-Tu. To further examine the molecular basis of the FLS2-BAK1 interaction and the biological significance of BAK1 in PRR regulation, we follow a reverse genetics approach to express diverse forms of BAK1 in Arabidopsis plants. In summary, our results show that binding of the ligand flg22 leads to very rapid oligomerization of FLS2 with BAK1, followed by fast phosphorylation of the RKs (in vivo and in vitro). This suggests a role of these phosphorylations for activation of downstream signaling.

Dynamics of phosphorylation of the FLS2/BAK1 complex in response to flagellin perception

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PAMP-triggered immunity, the first line of plant defense, is activated by recognition of pathogen associated molecular patterns (PAMPs). These are perceived by highly specific receptors at the plasma membrane, such as the flagellin receptor FLS2 (Flagellin sensing 2). Upon stimulation with the peptide flg22, the characteristic epitope of bacterial flagellin, FLS2 associates with its co-receptor BAK1 (BRI1 associated kinase 1). While FLS2 is responsible for ligand binding, the kinase domains of both FLS2 and BAK1 are believed to be activated by phosphorylation leading to cellular signal transduction. Using in vivo labeling with [^{33}P]phosphate, we characterized de novo phosphorylation events on FLS2 and BAK1 and followed the stability of the phosphorylated proteins over time. In Arabidopsis cell cultures both, FLS2 and BAK1, are phosphorylated within 15 s of treatment with flg22. This supports the hypothesis of phosphorylation being crucial for receptor activation and signal transduction. Funding by the SNF (31003A-120655 and 31003A-105852) and a post-doctoral grant from the Deutsche Akademie der Naturforscher Leopoldina (BMBF-LPD 9901/8-152) to BS are gratefully acknowledged.

Functional testing of crop plant and Brachypodium distachyon UDP-glucosyltransferases in yeast: identification of a barley UGT inactivating the Fusarium mycotoxins deoxynivalenol and nivalenol

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Fusarium graminearum produces either deoxynivalenol (DON) or nivalenol (NIV), both chemotypes coexist in field populations. The protein biosynthesis inhibitors DON and NIV seem to as act as defense suppressors, which can be detoxified to a variable extent into glucosides in different host plants and genotypes. This seems to be the molecular basis for a Fusarium resistance QTL (Qfhs.ndsu-3BS) in wheat [1]. Modification of the toxin structure may be relevant to escape detoxification. We have started to test candidate UDP-glucosyltransferase (UGT) genes by heterologous expression in yeast. Attempts to identify the relevant gene(s) of crop plants by sequence similarity with the DON (but not NIV) inactivating UGT73C5 from Arabidopsis [2] were unsuccessful. Using the cDNA-AFLP method a wheat UGT fragment TF108 was identified [3], which was highly induced by Fusarium infection in the resistant cultivar. The cloning of a full length TF108 cDNA by RACE PCR turned out to be extremely difficult, only cDNAs with a few sequence deviations from TF108 could be cloned. Diploid grass genomes contain about 200 UGT genes (≈ 600 in hexaploid wheat!), and the C-terminal cDNA-AFLP fragment is located in the highly conserved UDP-glucose binding part. Therefore also candidate rice UGT cDNAs (from a cluster of 4 tandemly repeated genes) were expressed in yeast. No increased resistance was observed. More successful was testing of several candidate barley genes, one UGT gene inactivating both DON and NIV could be identified. Recently we have initiated systematic testing of the UGT genes of the grass model species Brachypodium distachyon.

Differential effect of a novel *BAK1* allele on brassinosteroid, innate immunity and cell death signalling

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The bacterial pathogen-associated molecular patterns (PAMPs) elf18 and flg22 are recognised by the Arabidopsis leucine-rich repeat receptor kinases (LRR-RLKs) EFR and FLS2, respectively. To elucidate novel components of PAMP-triggered immunity (PTI) we performed a forward genetic screen to identify *elfin* (*elf18-insensitive*) Arabidopsis mutants. Out of 103 non-*efr elfin* mutants, one was clearly impaired in elf18, as well as in flg22 responsiveness. Map-based cloning of the mutated gene revealed a single point mutation in the kinase domain of the LRR-RLK BAK1. BAK1 forms heretodimers with FLS2 and the LRR-RK brassinosteroid receptor BRI1 to control PTI and BR signalling, respectively. In addition, BAK1 is also involved in cell death signalling. Unexpectedly, detailed phenotypic characterization revealed that the novel recessive *bak1-5* allele is more impaired in PTI signalling than null *bak1* alleles, and is clearly more susceptible to a range of adapted and non-adapted pathogens. Surprisingly, while previously described bak1 alleles are hyposensitive to BRs, *bak1-5* displayed a mild hypersensitive phenotype, revealing that the single amino acid change in BAK1-5 has differential effects on PTI and BR signalling. We will present the detailed genetic, physiological and biochemical comparative analysis of *bak1-5*.

An NB-LRR binding protein: A switch for transcriptional reprogramming in plant immunity

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Plant pathogenic bacteria use the type III secretion system to deliver a suite of effector proteins inside host cells to promote parasitism. To cope with pathogen attack, plants use the so called disease resistance (R) proteins that directly or indirectly recognize pathogen effectors referred to as avirulence (Avr) proteins. This recognition is highly specific and often elicits a localized programmed cell death known as the hypersensitive response (HR). When expressed in transgenic rice, the maize *Rxo1* gene, which encodes an NB-LRR R protein, confers a strong HR against pathovars of the rice pathogen *Xanthomonas oryzae* expressing the type III effector gene, *avrRxo1*. Despite many genetic and biochemical studies, downstream signaling components that translate effector-induced NB-LRR protein activation into transcriptional reprogramming and defense execution remain elusive. Here we show that a rice putative transcription factor (TF) interacts with RXO1 in yeast-two-hybrid assays and is required for *Rxo1*-mediated HR. Two homologs of this TF are present in rice. Computational analysis of the genome indicates that these TFs might regulate the expression of 328 gene targets with 49 transposable elements and 132 genes coding for unknown proteins. Among the remaining 147 genes, approximately 40% encode signaling molecules including three NB-LRR proteins and one RLK, reminiscent of plant immune "sensors", as well as 15 transcriptional regulators belonging to distinct families of TFs to name only a few. Our data suggest that this RXO1 interacting protein is a key switch for transcriptional and signal transduction cascades that mediate plant innate immunity.

Identification of haustorium specific genes of wheat leaf rust (*Puccinia triticina* Eriks.) that are expressed during early stages of infection

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Wheat (*Triticum aestivum* L.) is severely affected by the biotrophic fungus *Puccinia triticina* (leaf rust) which causes substantial losses over the year. Even though there are resistant varieties, the fungus tends to overcome resistance very quickly. Disease resistance in plants is often controlled by the interaction between a single dominant plant resistance gene (R) and an effector/avirulence factor (Avr) from the pathogen. This interaction typically activates a hypersensitive type response at the site of infection. The goal of this research is to identify Avr genes from *P. triticina*. Leaf rust haustoria were extracted by sucrose gradient and ConA affinity chromatography and cDNA libraries were generated. Considering the presence of signal peptide, 26 clones were selected as potential candidates and timing of expression was evaluated by RT-PCR of infected leaf tissue and germinated spore samples. Twelve clones were selected as candidates and are being validated by transient expression experiments using co-bombardment with GFP into leaf

tissue of leaf rust resistant isogenic lines. The results of this work will be discussed.

Does TIR1-like gene has a role in resistance of tomato roots to root-knot nematodes?

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TIR1 protein is one of the main components in regulating Auxin signaling pathway. It has been shown that higher expression of TIR genes is necessary to provide proper hormonal condition for *Pseudomonas syringae* (a tumorogenic pathogen) to induce disease in Arabidopsis. Root-knot nematodes have the ability to manipulate tomato root cells fate and produce giant cells in susceptible plants. How the *Mi* genes confer resistance to nematodes is not known yet. In our research, we have found that TIR1-like sequences are present in the *Mi* gene cluster. In a population segregating for resistance to nematodes, quantitative real-time PCR results showed that expression of TIR1-like gene is significantly higher in the root of susceptible plants than the resistant ones. In order to reveal the relationship between the *Mi* gene and TIR1-like gene, *Mi* was silenced by using Virus-induced gene silencing (VIGS) and the expression of TIR1-like gene was checked. Results showed that the expression of TIR1-like gene was not changed upon the successful silencing of the silencing of *Mi* gene. Altogether our data suggest a role for TIR1-like gene in resistance to nematode most probably independent from *Mi* gene.

Identification of an Rsm system in *Pseudomonas chlororaphis* PA23 and its role in biocontrol

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Pseudomonas chlororaphis strain PA23 is able to inhibit the fungal pathogen *Sclerotinia sclerotiorum*. This bacterium produces the antibiotic compounds pyrrolnitrin, phenazine, and HCN, together with proteases and siderophores. These antifungal compounds are regulated by a two-component global regulatory system (GacS/GacA) as well as a novel LysR-type transcriptional regulator called PtrA (Pseudomonas transcriptional regulator). In *Pseudomonas fluorescens* CHA0, regulation of secondary metabolites via the Gac system occurs at the posttranscriptional level and involves the Rsm (Regulation of secondary metabolism) system. The Rsm network consists of the RNA-binding proteins, RsmA and RsmE, and small regulatory RNAs, RsmY, RsmZ, and RsmX. Genes homologous to *rsmZ*, *rsmA* and *rsmE* have been identified in PA23. To investigate the role of the Rsm system in relation to GacS/GacA and PtrA, an *rsmZ-lacZ* transcriptional fusion was constructed together with plasmids overexpressing *rsmZ*, *rsmA*, and *rsmE*. Each of these constructs was introduced into PA23, PA23-314 (*gacS*$^{-}$), and PA23-443 (*ptrA*$^{-}$). We discovered that *rsmZ* transcription is significantly reduced in the *gacS* and *ptrA* mutants and overexpression of *rsmZ* can partially complement both mutants. Addition of *rsmA* or *rsmE* on multicopy plasmid restored phenazine and quorum-sensing signal production in the *ptrA* mutant. Conversely, these genes had no impact on the *gacS* mutant phenotype. Our findings indicate that the Rsm network, PtrA and the Gac two-component system form part of an interconnected regulatory cascade controlling secondary metabolite production in *P. chlororaphis* PA23.

Functional characterization of the salicylic acid-induced MATE transporter *EDS5*

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When plants are invaded by microorganisms (e.g. bacteria, fungi and/or oomycetes) or exposed to abiotic stress (UV light), an increase in the level of salicylic acid (SA) is observed. Mutants impaired the SA biosynthesis such as *ics1* and *eds5* have been previously shown to be unable to induced defense responses and become more susceptible to pathogens, highlighting the importance to understand the SA signaling pathway. SA is synthesized *de novo* mostly from chorismate that is converted to isochorismate by the activity of *isochorismate synthase 1* and *2* (*ICS1/2*). Isochorismate is then converted to SA by a yet unknown step. *ICS1/2* are localized in chloroplasts indicating that most of the SA biosynthesis takes place in these organelles. Transgenic plants expressing the *salicylate hydroxylase* gene (*NahG*) do not accumulate SA and are impaired to induced resistance. Since *NahG* is residing in the cytosol, SA produced in the chloroplast must be transported to the cytosol, however the elements involved in this transport are still unknown. In this study, we will present evidence based on localization and function that *EDS5*, a member of the MATE transporter family, could be a potential transporter of SA from the chloroplast to the cytosol.

Linear and circular forms of PMUs (potential mobile units) in the Aster Yellows phytoplasma strain Witches Broom (AY-WB) genome and their role in adaptation to plant and insect hosts

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Phytoplasmas are insect-transmitted plant pathogenic bacteria that continuously cycle back and forth between their plant and insect hosts. These bacteria have a reduced genome that lack genes for basic metabolic pathways making these bacteria recalcitrant to *in vitro* cultivation so far. However, the chromosomes contain numerous large repeated units of ~ 20 kb in size that resemble integrative and conjugative elements (ICEs). These units were named potential mobile units (PMUs) (Bai et al. 2006). Their abundance and conserved gene order in the phytoplasma chromosomes lead to the hypothesis that they have a positive contribution to phytoplasma fitness (Bai et al. 2006). Here we demonstrate that PMU1 of the Aster Yellows phytoplasma strain Witches Broom (AY-WB) exists as linear (L-PMU1) and circular (C-PMU1) forms of which the latter is more abundant during insect infection. All genes encoded in PMU1, except the insertion sequences *tra5*, are more abundantly expressed in insects relatively to plants. This corresponds to the presence of predicted promoter sequences upstream of the first gene, *sigF*, in C-PMU1 but not in L-PMU1. Thus, PMUs are apparently part of a phase-variation mechanism allowing phytoplasma adaptation to their insect hosts.

Systemic acquired resistance in Arabidopsis: A terpenoid is involved in long-distance signaling

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Systemic acquired resistance (SAR) is an inducible defense mechanism that is induced throughout a plant that was previously exposed to a localized infection by a pathogen. SAR protects the plant against subsequent attacks by a variety of pathogens. Activation of SAR requires the translocation of a signal molecule(s) from the pathogen-inoculated organ through rest of the plant, where this factor(s) is perceived resulting in the elevated expression of salicylic acid (SA)-dependent defenses. In recent years significant progress has been made on identifying plant defense activating molecules that are systemically translocated through the plant. Studies carried out by Park *et. al.* (2007) identified methyl salicylate (MeSA) as a potential SAR signal in *Tobacco mosaic virus*-inoculated tobacco (*Nicotiana tabaccum*). Another study by Truman *et al*. (2007) suggested that jasmonic acid (JA) is a vasculature translocated factor that is potentially involved in the activation of SAR in *Arabidopsis thaliana*. We had previously reported that petiole exudates (PE) collected from avirulent (Avr) pathogen-inoculated leaves of wild-type (WT) Arabidopsis, when applied to leaves of WT plant, systemically induced SAR (Chaturvedi et al. 2008). In contrast, similar Avr PE collected from the SAR-defective *sfd1* mutant lacked this SAR-inducing activity. We report the purification of a terpenoidal compound from Avr PE of WT plants that induces SAR in WT and *sfd1* plants. SAR induction by this terpenoidal compound required the *DIR1*-encoded apoplastic lipid-transfer protein and SA signaling through NPR1.

The involvement of lipids signaling in EIX induced defense responses

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Plant-microbe interactions involve numerous regulatory systems essential for plant defense against pathogens. An ethylene-inducing xylanase (EIX) of Trichoderma viride is a potent elicitor of plant defense responses in specific cultivars of tobacco (Nicotiana tabacum) and tomato (Solanum esculentum).

The EIX receptors (LeEix1 and LeEix2) were isolated in our lab. Structural analysis of these receptors suggests that they belong to a class of leucine-rich repeat cell-surface glycoproteins with a signal for receptor-mediated endocytosis. Both receptors are able to bind EIX while only LeEix2 mediates defense responses. The main purpose of the present work is to investigate the molecular mechanisms which allow plants to specifically activate defense responses after EIX elicitation. Lipids are critical components of plant cell membranes and have been previously shown to play an important role in various plant defense responses. N-Acylethanolamines (NAEs) are lipids found in both plants and animals. NAEs are derived from the hydrolysis of the membrane phospholipids and involved in endocannabinoid signaling. NAE levels are known to increase significantly within minutes post elicitation in plants. We have found that addition of NAE to tobacco cell suspensions as well as to plants treated with EIX inhibited elicitor-induced cell death / HR. Furthermore, over-expression of tomato FAAH (NAE hydrolase) stimulated defense responses. Interestingly, NAE appears to affect the cellular vesicle content. *equal contribution.

TGA1 clade transcription factors regulate aspects of the defense response in a manner distinct from NPR1

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Upon pathogen recognition, *Arabidopsis* initiates transcriptional reprogramming, which is mediated in large part through the activity of NPR1 (NON-EXPRESSOR OF PATHOGENESIS GENES1). NPR1 does not contain any known DNA-binding domain, but interacts with members of the TGA family of bZIP transcription factors to enhance their binding to cognate *as-1*-like promoter elements. It is postulated that NPR1 mediates its function through interactions with TGA factors. However, the contributions made by different TGA factors during the induction of disease defense responses have yet to be determined. Here we report on the characterization of T-DNA insertion mutants within members of the TGA1 clade (TGA1 and TGA4). Like *npr1* mutants, these mutants display enhanced susceptibility to *Pseudomonas syringae* and *Colletotricum*. However, a closer look at the *tga1*/*tga4* mutants reveals that this susceptibility cannot easily be explained as being *npr1*-like in its characteristics. Microarray analysis indicates that NPR1 and clade I TGA factors regulate largely distinct sets of genes, and that the vast majority of the co-regulated defense-related genes are, surprisingly, upregulated in *tga1xtga4*. Furthermore, *tga1xtga4* mutants display a pattern of SA, JA and certain hormones which is disparate to that of *npr1*. kPCR analysis of *tga1/tga4/npr1* triple mutants indicates that many of the gene expression effects of *tga* mutations may be hypostatic to the *npr1* mutation.

Structure-function analysis of the barley MLA resistance protein

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The barley *Mla* locus is exceptionally polymorphic, encoding more than 30 race-specific resistance alleles against the barley powdery mildew fungus, *Blumeria graminis* f sp *hordei*. The molecularly isolated *Mla* genes encode highly sequence-related intracellular R proteins of the CC-NB-LRR subtype. Sequence alignment of the deduced functional MLA proteins reveals exceedingly high sequence conservation in the CC and the NB-ARC domain, with fully identical sequence in some motifs, e.g. the 'EDVID', the 'Walker A' and the 'MHD' motif. We systematically studied the role of some of these motifs in regulating MLA functionality and activity by mutagenesis. We found that mutations in the 'Walker A' motif of the functional MLA10-YFP fusion protein result in loss-of-function as expected but unaltered intracellular distribution pattern, whereas amino-acid substitutions in the 'MHD' motif lead to AVR-independent cell-death. The cell-death inducing activities of 'MHD' mutatant variants are most likely function through the nucleus. Previously we have shown that MLA protein locate in both nucleus and cytoplasm and demonstrated the critical role of nuclear MLA in disease resistance reponses. We further discuss the possible role of cytosolic MLA in triggering immune responses as well as cytosolic fraction of 'MHD' mutant variants in mediating AVR-independent cell-death.

β-1,3-glucan from *Septoria tritici* elicits defence responses in wheat

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Accumulation of β-1,3-glucanase (PR2) and chitinase (PR3) was studied in a compatible (cv. Sevin-isolate IPO323) and an incompatible (cv. Stakado-isolate IPO323) interaction between wheat and the hemibiotrophic pathogen *Septoria tritici* (teleomorph *Mycosphaerella graminicola*). Gene expression studies of β-1,3-glucanase showed that in Stakado (resistant) there was an initial up-regulation of transcript at 1-3 days after inoculation (dai) followed by a subsequent down-regulation. In Sevin (susceptible), a significant up-regulation was seen only from 9 dai. A similar pattern was seen for chitinase. Furthermore, β-1,3-glucanase activity was higher in Stakado than in Sevin, especially in fluid isolated from the leaf apoplast, where the maximum difference in activity between the two cultivars was seen already at 1-3 dai. Since the pathogen lives as a biotroph/endophyte in the apoplastic spaces, this shows the highly localised accumulation of these defence proteins in the vicinity of the pathogen. Further experiments showed that injection of purified β-1,3-glucan from cell walls of *S. tritici* into Sevin at 1 dai was able to give complete protection against disease. This protection was accompanied by increased gene expression of β-1,3-glucanase and deposition of callose. Collectively, these data indicate that resistance in Stakado is dependant on a fast initial recognition of the pathogen, probably due to the presence of β-1,3-glucan in the fungal cell walls and this recognition results in, among other defence responses, accumulation of β-1,3-glucanase. In Sevin, on the other hand, the pathogen spreads unhindered and only elicits accumulation of PR-proteins when it is recognised after having produced sufficient biomass to enter its necrotrophic phase where reproduction occurs.

Analysis of disease resistance induced by phosphorous acid

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Phosphorous acid (H_3PO_3) is widely used either as a fungicide or as a superior source of plant phosphorus nutrition. Because application of phosphorous acid has suppressive effects on a number of plant diseases, it is speculated to activate the plant immune system. In this study, to clarify the mechanism of disease resistance induced by phosphorous acid, we analyzed the effects of phosphorous acid on *Arabidopsis*. *Arabidopsis* ecotype Columbia (Col-0) plants were treated with phosphorous acid by soil-drenching method and inoculated with the virulent bacterial pathogen *Pst* DC3000. By 3 d post inoculation, phosphorous acid-treated plants contained 5-fold lower bacterial titers than control plants. Gene expression analysis indicated that phosphorous acid induced the expression of acidic *PR-1*, a systemic acquired resistance (SAR) marker gene, and *ICS1*, a salicylic acid (SA) biosynthetic gene. Phosphorous acid could not enhance disease resistance against *Pst* DC3000 in *npr1* mutant, indicating that the SA-NPR1 pathway is required for the induction of resistance. These results suggested that phosphorous acid has an ability to induce SAR in *Arabidopsis*.

Characterization of chitin elicitor receptor complex in rice

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CEBiP, chitin elicitor binding protein in rice, plays an important role as a cell surface receptor for chitin elicitor signaling. However, CEBiP seemed not to have any functional intracellular domains for signaling and it was supposed to require the additional factors for signaling through the membrane into the cytoplasm. Our recent study also showed that CERK1 (Chitin Elicitor Receptor Kinase 1), a receptor-like kinase with extracellular LysM domains, is an essential component for chitin elicitor signaling in *A. thaliana*. In rice genome, ten receptor-like kinase genes with LysM domains, named as *OsLysM-RLKs*, were found. Among these, OsLysM-RLK9 showed the highest homology with CERK1 and thus analyzed for its function in chitin elicitor signaling in rice. Knock-down transformants of *OsLysM-RLK9* showed almost no ROS generation as well as phytoalexin biosynthesis in response to chitin elicitor. These results suggested that OsLysM-RLK9 plays an essential role

for chitin elicitor signaling in rice and may form a receptor complex with CEBiP. Blue native PAGE showed the bands with higher molecular weight than those of the CEBiP and OsLysM-RLK9 monomers, suggesting the presence of a sort of receptor complex in the plasma membrane. Yeast two-hybrid analysis showed the positive interaction between CEBiP and OsLysM-RLK9, indicating the possible complex formation by these two proteins through the interaction of their extracellular LysM domains. Further analysis is underway to clarify the role of kinase activity of OsLysM-RLK9 in the chitin elicitor signaling in rice.

The role of jasmonic acid (JA) in phytoalexin production in rice

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When attacked by pathogenic microorganisms, plants respond with a variety of defense reactions, including the production of phytoalexins. Because the production of phytoalexins is induced in rice leaves by treatment with jasmonic acid (JA) or the JA-inducer $CuCl_2$, JA is believed to be involved in the elicitor-induced accumulation of phytoalexins. The causal relationship between endogenous JA and phytoalexin production, however, remain to be defined. In this study, we used the JA-deficient rice mutant *cpm2* to investigate the possible involvement of JA in phytoalexin production in rice leaves. It was first demonstrated that wound-induced JA production was severely suppressed in *cpm2* leaves. Phytoalexins were determined in $CuCl_2$-treated leaves. Diterpenoid phytoalexins accumulated in *cpm2* leaves, similarly as in wild-type leaves, whereas sakuranetin, a flavonoid phytoalexin, accumulated much less in *cpm2* leaves than in wild-type leaves. The wild-type level of sakuranetin accumulation was restored in *cpm2* leaves by the addition of JA. It was concluded that JA is involved in the production of sakuranetin, but not in the production of diterpenoid phytoalexins. This conclusion was further supported by our investigation of the expressions of phytoalexin biosynthetic genes. We also report on the results obtained with the rice *jar1* mutant, which is defective in JA-Ile conjugation.

Functional analysis of elicitor-responsive Group IIa WRKY genes in rice

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WRKY proteins are a large family of transcription factors that mainly participate in plant biotic stress responses. Among them, Group IIa WRKY proteins are predicted as negative regulators of plant basal defense responses. By microarray analysis, we identified three chitin elicitor-responsive Group IIa WRKY genes (*OsWRKY28*, *71* and *76*) from suspension-cultured rice cells. These three WRKY genes were also induced by a sphingolipid elicitor in suspension-cultured rice cells. Fusions of these three WRKY proteins with green fluorescent protein were detected exclusively in the nuclei of onion epidermal cells. These three WRKY proteins were suggested to be transcriptional repressors by a transient assay using the particle bombardment method. Deletion analysis showed that the repression motifs of these WRKY proteins existed in the C-terminal regions of these proteins. Because the expression levels of *OsWRKY28* and *OsWRKY71* were higher than that of *OsWRKY76*, we further investigated functions of *OsWRKY28* and *OsWRKY71*. To have evidence on the functions of these two WRKY proteins in rice defense responses, transgenic rice plants overexpressing *OsWRKY28* or *OsWRKY71* were generated. We also generated transgenic rice plants in which the expression of *OsWRKY28* or *OsWRKY71* was down-regulated by RNA interference. Now, we are trying to perform pathogen inoculation analysis using these transgenic rice plants.

A discovery pipeline for effectors that inhibit plant enzymes

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During infection, pathogens secrete hundreds of non-annotated proteins that are thought to manipulate the host. Many of these proteins are small and can act as inhibitors that target host enzymes. Preliminary data and recent publications demonstrate that activity-based protein profiling (ABPP) is a powerful technology to identify inhibitors. ABPP is based on fluorescent or biotinylated inhibitors (probes) that label active site residues of enzymes in an activity-dependent manner. Preincubation with inhibitors will prevent labeling and suppress signals in the activity profile. We aim to find inhibitors produced by Arabidopsis-adapted pathogens using ABPP. Candidate effectors are selected from genomes of a variety of Arabidopsis pathogens, including *Pseudomonas syringae*, *Hyaloperonospora parasitica*, *Colletotrichum higginsianum* and *Golovinomyces orontii.* Over 200 secreted candidate inhibitors are produced heterologously and screened for inhibiting Arabidopsis enzymes using ABPP on leaf extracts. Other Arabidopsis enzymes which are potentially targeted by pathogen-derived inhibitors are produced by agroinfiltration and tested separately. New inhibitor-enzyme interactions are further characterized by reverse genetics, pull-down assays and *in vivo* localization.

Structure-activity study of rice chitin receptor, CEBiP, using tobacco BY-2 expression system

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CEBiP (Chitin Elicitor Binding Protein) is a transmembrane receptor for chitin elicitor in rice, of which extracellular domain contains three LysM motifs. Although CEBiP is known to show high affinity for chitin oligosaccharides, detailed analysis of the structure-activity relationship of this protein has not been established. For this purpose, we show here the advantage of the use of heterologous expression system with tobacco BY-2 cell line, which neither responds to chitin elicitor, nor contains CEBiP-like protein in the plasma membrane. After the preparation of membrane fractions from the transgenic BY-2 cells expressing CEBiP, they were treated with GN8-Bio, a biocytin conjugate of *N*-acetylchitooctaose, and cross-linked with glutaraldehyde. The heterologously expressed CEBiP was successfully biotinylated and detected by Western blotting with anti-biotin antibody in the microsomal fractions from the BY-2 cells. The binding characteristics of the CEBiP expressed in BY-2 cells showed a good agreement with the known specificity of rice CEBiP. These results suggest that the expression system combined with the affinity cross-linking assay is useful for the analysis of CEBiP and related chitin receptor candidates. Binding characteristics of a series of deletion mutants of CEBiP lacking each LysM domain are being studied.

Differential gene expression in tomatoes during dual infections with an endophytic and a pathogenic *Verticillium*

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Endophytes are usually bacteria or fungi that colonize plants with little symptom development and can cross protect plants from attack by more virulent pathogens. Despite numerous reports of such relationships, the molecular mechanism surrounding this interaction is still poorly understood. In a recent study, we have shown that infection of tomatoes (cv Craigella) by an endophytic *Verticillium dahliae* isolate Dvd-E6 (E6) can severely restrict colonization by virulent *V. dahliae* race (Vd1), regardless of the order of infection, and that cross protection requires genetic interplay between Dvd-E6 and the plant, resulting in increased resistance to Vd1 (Shittu et al., 2009). The aim of the present study was to further investigate host gene expression during dual infections. Craigella susceptible tomatoes were inoculated with Vd1 or E6 alone, or E6 before, mixed with or after Vd1. After 5 or 10 days, the total and relative amounts of Vd1 and E6 in stems and roots were determined by quantitative PCR and RFLP based assay, respectively. Microarray analysis was performed on the CS/Vd//E6, CS/E6//Vd and CS/E6+Vd whole cell nucleic acid extracts. A functional analysis of the microarray results indicates that changes in gene expression during the various combination interactions can be dramatically different. Association studies indicate that many responding genes can be grouped into three categories, based on relative expression in the various dual interactions and that two of the groups correlate with the level of Dvd-E6 or Vd1 in the plant and one corresponds with level of symptoms. The ongoing future direction is to select some genes of interest for further analysis by overexpressing them and also silencing them using RNAi towards a better understanding of their roles in dual infections.

A chimeric receptor approach reveals a role of WAK1 as a receptor of oligogalacturonides

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The cell wall is the extracellular matrix that plays a fundamental role in filtering and interpreting external signals. Pectin, a main component of the cell wall that is continually modified and remodelled during plant growth and development. The characteristic of pectin that determines maintainance of the wall integrity and cohesion of the cells is due to the polyanionic nature of its backbone, i.e. homogalacturonan, which is capable of binding calcium to form the structures called "egg-box". These structures may be broken and fragmented by microbial hydrolases and release oligogalacturonides (OGs) that have elicitor or regulator activity. In Arabidopsis, OGs induce the expression of defense genes and proteins and protect the plant against fungal diseases. In analogy with the role of hyaluronan fragments in the animal innate immunity, OGs may be regarded as host-associated molecular patterns. However, the perception system of OGs is still unknown. Since the response of Arabidopsis to OGs overlaps that of the microbe-associated molecular patterns flagellin and the elongation factor-Tu, it has been hypothesized that the receptors of OGs are similar to their corresponding receptors FLS2 and EFR. On the other hand, candidate receptors of OGs are some members of the Wall-Associated Kinase (WAK) family. WAKs are receptor-like kinases that display the typical eukaryotic Ser/Thr kinase signature and an extracellular domain containing several epidermal growth factor (EGF)-like repeats. Among them, WAK1 is the most characterized: it binds *in vitro* to non-methylesterified homogalacturonans, to elicitor active OGs and the structurally related alginates. However, the detailed role of single WAK receptors remains largely unknown. In order to define the function of WAK1, we have used an approach based on chimeric LRR-RLKs.

Gene expression and changes in carbohydrate and nitrogen metabolism in bean nodules during nitrate exposure

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Nitrate adversely affects nodule functioning in legumes. Mechanisms that have been proposed to explain the negative effect of nitrate on nitrogen fixation include nitrite toxicity, carbohydrate limitation, alteration in the oxygen diffusion barrier, and feed back inhibition by the assimilated N. To gain a better understanding of nitrate-mediated responses in bean (Phaseolus vulgaris cv. Negro Jamapa), we carried out a comparative macroarray analysis using mRNA derived from the nodules of 19-day old plants treated with KNO_3 (10 and 20 mM) for 72 h. Nitrate treatment resulted in a drastic decline in nitrogen fixation activity with a consequent decrease in the nodule ureide content. Nitrate also caused down regulation of sucrose synthase transcript levels as well as the activity, and a decrease in the soluble sugar content in nodules. Analyses of the transcriptome through cDNA macroarray experiments coupled to semi-quantitative RT-PCR, and the amino acid and organic acid contents as measured by HPLC showed how the nodules respond to different concentrations of nitrate. An array of genes involved in several mechanisms such as carbohydrate and amino acid metabolism, and detoxification and signal transduction were differentially expressed in nitrate exposed nodules. Results evidenced a differential response of nodules towards nitrate exposure in comparison to the surrounding root tissues. Microscopic analysis of nodules revealed nitrate-induced structural changes in the infected cells.

Cytochemical characterization of the interaction between *Gremmeniella abietina* and two resistant hosts, *Pinus contorta* and *P. banksiana*

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Gremmeniella abietina causes a serious fungal disease in pine plantations. *Pinus banksiana* and *P. contorta* show resistance and present short tip blight as a symptom without being killed. Little is known about the molecular and cellular mechanisms involved in the relationship between the European race (EU) of *G. abietina* and these two hosts. The aim of the present study was to examine the production of enzymes by the pathogen and to characterize the phenols that contribute to host resistance. Although polygalacturonases were detected early during the infection process, immunocytochemical labelling showed that pine shoot invasion by the EU race is primarily achieved through the production of peroxidases and glucanases. These two enzymes seem to facilitate laccase activity after the alteration of the lignocellulose complex. In response to infection, *P. contorta* and *P. banksiana* formed anatomical barriers mainly composed of suberin and lignin. In addition, other polyphenols were revealed after the examination of tissues under blue light excitation following cytochemical tests involving in particular dimethylamino-cinnamaldehyde, vanillin and NEU reagents. The increase in condensed tannins, proanthocyanidins, catechins, flavonoids and caffeic acid observed after an attack by the EU race of *G. abietina* strongly suggests that these phenolic compounds play an important role in resistance. These two host species also seemed able to produce enzymes altering chitin and ß-1,3-glucans, two pathogen cell-wall constituents. Thus, these results indicate that all the major defense responses in *P. banksiana* and *P. contorta* are brought together sufficiently early during this host-pathogen interaction to halt scleroderris canker development.

Utilization of carbon substrates, and symbiotic performance of plasmid-cured *Rhizobium* and *Burkholderia* isolated from rice

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Plasmids in *Rhizobium* sp. are relatively large, numerous, and difficult to cure. Except for the symbiotic plasmid, little is known about their functions. The primary objective of our investigation was to obtain plasmid-cured derivatives of *Rhizobium leguminosarum bv. phaseoli* and *Burkholderia cepacia complex* by using a direct selection system and to determine changes in the phenotype of the cured strains. Three strains i.e. one of *Rhizobium* and two of *Burkholderia* were utilized that contained three, four, and five plasmids. Phenotypic effects observed after curing of plasmids indicated that the plasmids were involved in the utilization of adonitol, arabinose, catechol, glycerol, inositol, lactose, malate, rhamnose, and sorbitol and utilization of nitrate. Curing of cryptic plasmids also influenced nodulation and growth of plants on nitrogen-deficient media. The alteration in the ability to utilize various substrates after curing of plasmids suggests that the plasmids may encode genes that contribute significantly to the saprophytic competence of rhizobia in soil.

Functional analysis of MAPK interactome in *Oryzae sativa* for deciphering defense signaling network

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Mitogen-activated protein kinase (MAPK) cascade is a complex and fundamental signaling module in eukaryotic cells. They function downstream of sensors/receptors and regulate cellular responses to external and endogenous stimuli. The function of a kinase is regulated by its interaction with other proteins. Hence, it is crucial to identify the interacting partners of a particular protein to trace its function. Here, we used the systemic functional genomics approaches to map a network of Mitogen Activated Protein Kinase (MAPK) cascades in *Orzyzae sativa* (Rice). About 2000 different combination of genes were screened by High-efficiency Yeast two hybrid system, generating six MKK-MPK (Map kinase kinase - Map kinase) binary interacting pairs and six of their binary interacting substrates i.e. transcription factors. Further, we confirmed the interaction by Bimolecular fluorescence complementation (BiFC) in onion cell. Hence, we find that a specific MAP Kinase gene interacts with four others MKKs forming a protein complex. As expected each interacting MKK gene shows phenotypes related to defense response supporting the hypothesis of potential roles of MPK signaling cascade in defense response. In addition, resistance and susceptible phenotypes obtained from pathogenicity test of each interacting MKKs further supports their role in defense response. For example, *OsMEK2*, one of the interacting MKK shows highly resistance phenotype indicating a possible role in positive regulation of defense response mediated by MAPK signaling pathway. Hence, we anticipate that our small scale MAPK interactome allows the exploration of the nature of protein-protein interaction and provides a basis for future functional characterization of these interacting genes in defense response in rice.

Sequence exchanges and targeted mutagenesis based on structural modeling of Gpa2 and Rx1 provide novel insights in NB-LRR protein functioning

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Gpa2 and Rx1 are potato R proteins sharing a high degree of similarity in amino acid sequence, but which recognize two different pathogens, the nematode Globodera pallida and Potato Virus X respectively. By exchanging the Leucine-Rich Repeat (LRRs) sequences between Gpa2 and Rx1, their recognition specificities could be exchanged. However, a concentration dependent autoactivation could be observed as well. The exchange of smaller regions in the nucleotide-binding (NB), ARC and LRR regions helped identifying distinct areas in both the N-terminal half of the LRR and in the ARC2 region involved in this autoactive response. A structural model of the LRR was created by modeling each segment of the LRR to the most similar structure from a LRR crystal structure database. A structure of the NB-ARC region was modeled after the mammalian Apaf-1 structure. Information from coevolutionary patterns and surface characteristics was used to create a putative docking model for the interaction between the LRR and NB-ARC region. The regions shown to be responsible for the autoactive response of some of the chimeric constructs lay on opposite interacting surfaces of the LRR and NB-ARC. Targeted mutagenesis of specific structural features likely to be involved in the domain interaction were perfomed to test assumptions based on the model in functional assays. The implications of the model for understanding NB-LRR type R protein activation will be discussed. Supported by the EU 6th framework program BIO-EXPLOIT CT-2005-513959.

Nucleo-cytoplasmic distribution of the R protein Rx1 and its domains

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Rx1 is a CC-NB-LRR type resistance protein from potato that confers resistance against Potato Virus X. The Rx1 protein has a modular structure of three domains; the N-terminal coiled-coil domain (CC), the nucleotide-binding-ARC (NB-ARC) domain and the C-terminal Leucine-rich repeat (LRR) domain. Recognition is thought to be mediated through the LRR and the signal transduction through the NB-ARC and CC domains, but the exact mechanism is still unknown. The modularity of Rx1 is such that the domains can function even if they are expressed as separate polypeptides (in trans). Until recently the NB-LRR type of R proteins were expected to be localized in the cytoplasm. The subcellular localization of Rx1 was determined by expressing fluorescently tagged Rx1 constructs. Both N- and C-terminal fusions yielded functional R proteins. Confocal microscopy revealed that Rx1 shows a dual localization; nuclear and cytoplasmic. To investigate if any of the domains of Rx have a specific role in the subcellular distribution, a set of fluorescent fusion constructs was made of the individual domains, or combinations thereof. The CC-domain proved to be an important factor in the nuclear localization, whereas the LRR resided mostly in the cytoplasm. Photobleaching experiments show that the CC domain binds transiently to large complexes in the nucleus. Both nuclear accumulation and diffusion characteristics could be attributed to a stretch of 70 amino acid of the CC domain lacking classical nuclear targeting signals. Furthermore, we demonstrated that the nuclear accumulation of Rx1 depends on ATP binding, the co-chaperones SGT-1 and RAR1, and that Rx1 is activated in the cytoplasm upon manipulation of the nucleo-cytoplasmic distribution of its elicitor.

Allicin from garlic, effective in controlling several plant diseases, is a redox toxin that pushes cells into apoptosis

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Allicin is produced in garlic when the tissues are damaged and the substrate alliin mixes with alliin-lyase. Allicin undergoes thiol-disulphide exchange reactions with free thiol groups in proteins and it was suggested that inactivation of thiol-containing enzymes was the basis of its antimicrobial action. We previously demonstrated the effectiveness of garlic juice containing allicin against a range of plant pathogenic organisms. Here we report on the cellular mechanism of allicin action in *S. cereviseae* by measuring changes in the absolute concentrations of reduced and oxidized glutathione. Using the Nearnst equation we showed a shift from highly reduced (E_{hc}= -240 mV) to an oxidized state (E_{hc}= -165 to -167 mV). Changes in the electrical half-cell potential of the GSSG/GSH couple, quantitatively the most important redox buffer in the cell, correlate with biological status and in the range -175 to -160 mV cells are shunted into apoptosis or necrosis. A fluorescence-linked assay to detect activated caspases showed that cells treated with pure synthetic allicin or garlic juice entered apoptosis. We conclude that allicin kills cells by acting as a redox toxin.

Studies on NRC1, a tomato NB-LRR protein involved in downstream defense signaling

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We identified *NRC1* (NB-LRR required for HR-associated cell death-1) as a gene induced upon recognition of the effector Avr4 from the pathogenic fungus *Cladosporium fulvum* by the resistance (R) protein Cf-4 in tomato (Gabriëls et al. (2007) Plant J. 50:14-28). *NRC1* encodes a nucleotide-binding, leucine-rich repeat (NB-LRR) protein that is required for a full Cf-4/Avr4-triggered hypersensitive response (HR) and resistance to *C. fulvum*. Virus-induced gene silencing (VIGS) of *NRC1* in *Nicotiana benthamiana* showed that NRC1 also mediates the HR triggered by other R proteins. Likewise, it was shown by VIGS in *N. benthamiana*, that *SGT1*, *RAR1* and *MEK2*, but not *EDS1*, suppress the HR caused by a constitutively active mutant form of NRC1. This might suggest that SGT1 and RAR1 are involved in the proper formation and/or stability of the NRC1 signaling complexe(s), and that a mitogen-activated protein kinase pathway acts downstream of NRC1. We propose a mechanism in which NRC1 is a common signaling factor downstream of different R proteins. We aim to identify additional components that are required for NRC1 signaling in a forward genetic screen for loss of HR induced by constitutively active NRC1. Plants containing this mutant *NRC1* allele would probably die of necrosis; therefore, we cloned it behind an inducible promoter and will stably transform this construct into tomato. Subsequent EMS mutagenesis of this transgenic line and screening for surviving M2 mutant offspring grown in the presence of the inducer should allow identification of genes involved in NRC1-mediated HR and associated defense responses. Furthermore, we will generate stable transgenic tomato lines that are silenced for *NRC1* expression and will test these for compromised resistance to different types of pathogens.

LIC1* is involved in cell death regulation during nonhost defense response of *Arabidopsis thaliana

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PEN2 encodes a myrosinase that acts in production of antimicrobial compounds critical for nonhost resistance of *Arabidopsis* against powdery mildews. *PEN2* is also involved in nonhost preinvasion resistance against *Colletotrichum* species, suggesting broad-spectrum antifungal activity of the PEN2-generated metabolites. In this study, we screened *lic1* mutants (lesion induced by nonadapted *Colletotrichum*) to identify novel factors in nonhost resistance. The screening identified three *lic* miutants that exhibit lesion development against inoculation of nonadapted *Colletotrichum*. Genetic analysis revealed that the three mutants belonged to the same locus designated *lic1*. In contrast with the *pen2* mutants, nonadapted *Colletotrichum* did not elevate the efficiency of plant invasion in the *lic1* mutant, suggesting that lesion development in the *lic1* mutant is not due to fungal invasion. Map-based cloning revealed that *LIC1* is allelic to *NSL1* encoding a protein with a MACPF (membrane attack complex and perforin) domain. The *nsl1* mutants, tagged by *Ds* transposon, exhibits a dwarf phenotype with spotted necrotic lesions in the absence of pathogen attack, but the *lic1* mutants did not exhibit the *nsl1* phenotype. The *lic1 pad4* and *lic1 eds16* double mutants reduced lesion formation compared with the *lic1* mutant, indicating the involvement of salicylic acid signaling in the *lic1* phenotype. Surprisingly, the *pen2* mutation also partially suppressed lesion development in the *lic1* mutant, suggesting a link between PEN2-mediated antimicrobial response and LIC1. Roles of the MACPF protein in cell death regulation will be discussed.

Characterization of transcription factors involved in chitin-mediated defense signaling

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Chitin, derived from fungal cell walls, is a polymer of β-(1→4)-N-acetyl-D-glucosamine and is a strong elicitor of plant defense responses. As such, it is a pathogen associated molecular pattern (PAMP), a class of molecules known to induce innate immunity in both plants and animals. Our previous study using the ATH1 Affymetrix microarrays consisting of about 23,000 genes identified more than 1,000 genes including 70 transcription factor (TF) genes that specifically responded to the purified chitin elicitor, chitooctaose. Using the more sensitive quantitative-reverse transcription polymerase chain reaction (Q-RT PCR) method, 118 transcription regulators were shown to respond to chitin treatment. Among the major chitin responsive transcription factors (TFs) were AP2/EREBP, C2H2 zinc finer proteins, MYB, and WRKY proteins. In order to elucidate the gene regulatory network involved in chitin signaling, we cloned each chitin responsive TF into yeast two hybrid vectors, pDEST32 and pDEST22 (Invitrogen), and analyzed protein-protein interactions. Ninety two transcription factors, 7 E3 ligases, and 5 mitogen-activated protein (MAP) kinase genes were used as baits for protein interaction analysis. Ninety six transcription factors were used as prey. From the 9,120 combinations assayed by the yeast two-hybrid screen, we found 97 interactions among candidate transcription factors and MAP kinases. Some of these interactions were confirmed by *in vitro* co-immunoprecipitation and in-gel kinase assays. Several these individual interactions have been identified in previous publications. However, here we present the complex network involved in chitin signaling and identify an AP2/EREBP protein as a key, nodal regulator of chitin defense signaling.

Transcriptional profiling analysis of complex regulatory loop between two two-component regulatory systems required for AvrXa21 in *Xanthomonas oryzae* pv. *oryzae*

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Xanthomonas oryzae pv. *oryzae* (*Xoo*), a causal agent of bacterial blight disease in rice, like other bacteria, uses two-component regulatory systems (TCSs) for modulation of gene expression in response to environmental cues. Previously, we have shown that two TCS, composed of RaxR/H and PhoP/Q, are required for AvrXa21 activity by regulate its expression, and RaxR expression behaves as in response to cell population density. Our recently published result suggested that AvrXa21 molecule is quorum sensing molecule, we, now, have promising progress to identify and characterize AvrXa21 molecule. Based on previous proteomic analysis in our lab, we observed the PhoP proteins is increased in *raxR* knockout strains, as well as, the expression of raxR is increased in *phoP* knockout strain suggesting the negative regulatory loop between these two TCS, though little is known about the mechanism whether this effect is from direct or indirect signal transduction. In this study, we demonstrated the signaling crosstalk between two TCS RaxR/H and PhoP/Q in transcription level. The gel shift mobility assay was used to assess in vitro the direct interaction between the response regulator protein and promoter of raxR and phoP, also GFP-promoter fusion was used to assess in vivo the signaling crosstalk of these two TCSs.

Towards identification of effector proteins in the lettuce downy mildew pathogen *Bremia lactucae*

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Bremia lactucae is an obligate biotrophic pathogen of lettuce (*Lactuca sativa*). Like other downy mildews and *Phytophthora* species it belongs to the oomycetes (kingdom Stramenopiles). During infection Bremia grows intercellular hyphae and forms haustoria in host cells. In lettuce cultivation Bremia is mainly controlled by dominant resistance genes that are rapidly overcome by new isolates. Durable resistance is more desirable than ever, as Bremia is also becoming increasingly resistant to fungicides. The aim of this project is to identify Bremia effector proteins and to study their role in the infection process and in disease susceptibility. We have generated 5' cDNA and random-primed normalised cDNA from both Bremia spores and heavily infected lettuce leaves for 454-sequencing. An initial run yielded 46699 assemblies with an average length of 346 base pairs. To increase coverage and lengthen these assemblies additional 454-sequencing is being performed. Potential effectors will be selected from assembled EST sequences by the presence of predicted signal peptides, RXLR-motifs and other known effector characteristics. Currently we are assessing bio-assays to test for disease-promoting and defence suppressing activities of the potential effectors identified by sequencing. We will report on the preliminary analysis of the Bremia transcriptome. The knowledge gained from this project will be used to screen for lettuce lines that are insensitive to the action of important effector proteins that can then be deployed for resistance breeding.

The ABC-transporter BcatrB from Botrytis cinerea exports camalexin and is a virulence factor on Arabidopsis

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Arabidopsis thaliana produces the phytoalexin camalexin in response to abiotic and biotic stress. We have studied the mechanisms of tolerance to camalexin in the fungus Botrytis cinerea, a necrotrophic pathogen on A. thaliana. Exposure of B. cinerea to camalexin induces the expression of BcatrB, an ABC transporter that functions in efflux of fungitoxic compounds. B. cinerea inoculated on wild-type A. thaliana plants yields smaller lesions than on camalexin-deficient A. thaliana mutants. A B. cinerea strain lacking functional BcatrB is more sensitive to camalexin in vitro and less virulent on wild-type plants but is still fully virulent on camalexin-deficient mutants. Pre-treatment of A. thaliana with UV-C leads to increased camalexin accumulation and substantial resistance to B. cinerea. UV-C-induced resistance does not occur in camalexin-deficient mutants. Our experiments demonstrate that an ABC transporter is a virulence factor by increasing tolerance of the pathogen towards a phytoalexin, combined with complete restoration of virulence on host plants lacking this phytoalexin.

Homologues of the *Cladosporium fulvum* effector proteins are present in Dothideomycete species

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Cladosporium fulvum is a non-obligate biotrophic fungal pathogen of tomato that belongs to the Dothideomycete class of ascomycete fungi. Ten effector proteins have been cloned from *C. fulvum* that are classified as avirulence (Avrs: Avr2, Avr4, Avr4E, Avr9) and extracellular proteins (Ecps: Ecp1, Ecp2, Ecp4, Ecp5, Ecp6, Ecp7), and whose recognition in tomato is mediated by the cognate Cf (for *C. fulvum*) resistance proteins. Although the interaction between *C. fulvum* and tomato is regarded as a model for gene-for-gene-based interactions, so far homologues of the *C. fulvum* effector proteins have not yet been identified in other fungal pathoghens. By mining sequenced fungal genomes and by using PCR-based techniques we have now identified for the first time structural and functional homologues of the *C. fulvum* Avr4, Ecp2 and Ecp6 effectors in other Dothideomycete species, including the devastating pathogen of banana *Mycosphaerella fijiensis and* the wheat blotch pathogen *Mycosphaerella graminicola*. Eight cysteine residues are present in the *C. fulvum* chitin-binding protein Avr4 that can protect fungal cell-wall chitin against tomato chitinases that accumulate during infection. The cysteine residues and their spacing are also conserved in the Avr4 homologues present in *M. fijiensis* and other Dothideomycetes, suggesting a similar biological function. Our current studies aim at providing further insight into whether these genes are (i) true homologues shared in ancestors, (ii) have been acquired by horizontal gene transfer, or (iii) are the result of convergent evolution.

GAPDH, a novel component of OsRac1-mediated innate immunity in rice

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Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a glycolytic enzyme with multifunction in yeast and mammalian. Recently, GAPDH was found to

be involved in reactive oxygen species (ROS)-induced cell death in Arabidopsis. Rac small GTPase functions in ROS production and defense gene activation during immune responses, plays a key role in rice (Oryza sativa) innate immunity as part of a complex of regulatory proteins. In this study, using immunoprecipitation we found a cytosolic GAPDH (GAPC) in the OsRac1 complex. To understand the relationship between GAPC and OsRacl in defense, we isolated all GAPC members (OsGAPCs) from rice and analyzed their function. Yeast two-hybrid assays showed that all five OsGAPCs interacted with the constitutively active form of OsRac1 (CA-OsRac1), and OsGAPC2 showed the strongest interaction with CA-OsRac1. It is known that the cysteine residues, especially the enzymatic active site (Cys^{149}) in mammalian GAPDH, play an essential role in cell death. However, cysteine-to-serine mutation of the active site (C154S) in OsGAPC2 did not affect the interaction with OsRac1, suggesting this site may not be important for the interaction. Results of bimolecular fluorescence complementation (BiFC) assays suggest OsGAPC2 and OsRac1 formed a complex *in vivo*. Interestingly, OsGAPC2 interacted with RACK1A, which is a scaffold protein in the OsRac1 immune complex. Together, our current data indicated that Rac GTPase interacts with GAPC *in vitro* and *in vivo*, probably in an enzymatic independent manner. Our ongoing study on OsGAPC over-expression and knock-down plants will further reveal the exact role of GAPC and the detail molecular mechanisms underlay defense response.

The transcriptional regulator Tri6 plays a multi functional role associated with virulence in *Fusarium graminearum*
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Tri6 which encodes for a C_2H_2 zinc finger protein positively regulates the trichothecene pathway genes in *F. sporotrichioides*. Here we report the phenotypic, exo-proteomic and transcriptomic characterization of *Tri6* mutant in *Fusarium graminearum*. Targeted disruption of *Tri6* failed to *in vitro* synthesize 15-acytyldeoxynivalenol, a derivative of the mycotoxin deoxynivalenol (DON). Further, infection on a *Fusarium* susceptible variety of wheat was restricted to the inoculated site. Exo-proteomic evaluation of Δ*Tri6* and wild-type strains revealed that disruption of the *Tri6* gene is associated with effects on secretion. The secreted proteins that are affected have been previously implicated in pathogen virulence. One such protein *Tri*8, located within the trichothecene cluster, is a member of a lip5 class of secreted lipases, and is associated with virulence in *Candida albicans*. Targeted disruption of *Tri*8 in *F. graminearum* resulted in drastic reduction in the virulence of the pathogen. Finally, whole gene expression profiling by Illumina- Solexa technology confirmed that *Tri6*, in addition to regulating secretion also affects transcription of genes involved in sequestering nutrition for pathogen growth.

Identification of proteins interacting with the tomato NB-LRR NRC1
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Plant disease resistance (R) proteins confer immunnity to pathogens possessing the corresponding avirulence (Avr) proteins. Most R proteins localize intracellularly and only a few (e.g Cfs) are extracellular transmembrane proteins. Most intracellular R proteins belong to the nucleotide-binding and leucine-rich repeat (NB-LRR) class. Activation of NB-LRRs is often associated with induction of the hypersensitive response (HR), a form of programmed cell death. NRC1 (NB-LRR required for HR-associated cell death-1) is an NB-LRR from tomato which participates in the signaling cascade required for Cf-mediated resistance to *Cladosporium fulvum.* Silencing of NRC1 in *Nicotiana benthamiana* results in an impaired HR induced by extracellular and intracellular R proteins (Gabriëls et al. (2007), Plant J 50:14-28). To understand how NRC1 regulates the defense response induced by multiple R proteins we focus on the identification of interacting proteins. A thylakoid lumen protein was identified in a yeast two-hybrid screening. Additional yeast two-hybrid and co-immunoprecipitation assays with *in planta*-expressed proteins, employing different domains of NRC1, are in progress. Silencing and overexpression of the thylakoid lumen protein should reveal whether it is required for NRC1 function. Identification of more interactors will be carried out by affinity-purification of NRC1 expressed *in planta* followed by mass spectrometry analysis. The effect on affinity-tags on NRC1 activity was tested by fusing them to an autoactive NRC1 mutant displaying an elicitor-independent HR. In most cases only a slight inhibition on NRC1 acivity was observed. With this research we intend to elucidate whether NRC1 is a true signaling hub for multiple R proteins, required to induce defense responses.

Towards cloning the first Arabidopsis nonhost resistance gene that confers immunity against the soybean pathogen, *Phytophthora sojae*
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The genetic ability of all members of a species to confer complete protection against pathogens, virulent on other plant species is termed as nonhost resistance. Arabidopsis (*Arabidopsis thaliana*) confers nonhost resistance against all soybean pathogens including Asian soybean rust fungus and the oomycete pathogen, *Phytophthora sojae.* We initiated a genetic screen in order to understand the genetic mechanism of Arabidopsis nonhost resistance against the semi-biotrophic soybean pathogen, *P. sojae*. Resistance mechanisms against biotrophic fungi act at two levels: (i) pre-haustorial and (ii) post-haustorial. Three Arabidopsis genes, *PEN1, 2* and *3* were recently shown to be involved in the expression of Arabidopsis pre-haustorial nonhost resistance against a powdery mildew nohost pathogen. We treated seeds of *pen-1-1* mutant with the chemical mutagen, ethyl methane sulfonate to generate random mutations across the genome. *pen-1-1* lacks the functional *PEN1* gene and is penetrated by *P. sojae.* Over 3,000 M_2 families created and screened for identifying mutants that showed loss of *Phytophthora* resistance. We identified 30 *Phytophthora sojae* susceptible (*pss*) mutants. We applied the bulk- segregant analysis to identify molecular markers linked to the *pss1* mutation. The *Pss1* gene has been mapped to a locus that is tightly linked to molecular markers including the SSLP marker, nga707 of the south arm of Chromosome III. Progress towards cloning *Pss1* will be presented.

Comparative analysis of response of Conifer tissues to necrotrophic capability in *Phlebiopsis gigantea* and *Heterobasidion annosum*
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Root and butt rot caused by *Heterobasidion* species is one of the most destructive diseases of conifers in the northern temperate regions. *Phlebiopsis gigantea* (Fr.) Jül., is currently used as biocontrol agent against *Heterobasidion* infection. A major problem is that although the effectiveness of *P. gigantea* in biocontrol has empirically been shown, the long term biological effect of this fungus on conifer trees has not been proven. Equally, mechanism for its biocontrol is still unknown. The most probable mechanism could be due to its induced resistance as it causes lignified cells in conifer tissues. The aims of the present study are to assess the potential risk for the long term use of *P. gigantea* as biocontrol agent in forestry in terms of developing nectrotrophic capability and other impact on the environment. To investigate this, pine seedlings were inoculated with either *P. gigantea* or *H. annosum*. Samples were taken at defined time intervals and examined for morphological changes. Comparative analyses of molecular responses of the host to the two fungi were also documented with aid of Pyrosequencing and qPCR. Initial result indicated that *P. gigantea* induced necrosis both in the phloem and xylem on *P. sylvestris* like *H. annosum*, but there was significant difference ($p < 0.001$) in the lesion size. However, at prolonged incubation, no further increase in lesion size was observed for trees inoculated with *P. gigantea*. Additional separate studies on induced resistance and transcript profiling by pyrosequencing were conducted. The results will be presented and discussed.

Structure-function analysis of the flagellin receptor Arabidopsis FLS2: Glycosylation, cysteine pairs and FLS2-FLS2 association
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FLS2 is a transmembrane receptor kinase of that activates plant defense responses upon binding of bacterial flagellin or flagellin-based peptides such as flg22. For a small minority of plant LRR-RLK proteins the activating ligands, signaling pathways and/or phenotypic impacts have been identified, but little is known about how the signaling activity of the receptor itself is controlled. Using immunoprecipitation from plants expressing two different tagged versions of FLS2, we find that FLS2 is present in FLS2-FLS2 complexes both before and after plant exposure to flg22. FLS2 proteins with mutations at the proposed flg22 binding site revealed that flg22 binding capability is not required for FLS2-FLS2 association. Conserved Cys pairs

flank the large leucine-rich repeat (LRR) domain in FLS2 and many other LRR receptors. Expression of mutant forms of FLS2 in fls2- plants demonstrates that the Cys pair N-terminal to the LRR is required for normal FLS2 stability and function while the Cys pair C-terminal to the LRR is not. Even after restoration of mutant protein abundance by expression from a CaMV 35S promoter, the N-terminal Cys pair is required FLS2-FLS2 association, for flg22 binding, and for flg22-dependent defense signaling. Interestingly, a truncated form of FLS2 containing only the cytoplasmic kinase domain without the extracellular LRR domain has a dominant-negative effect on wild-type FLS2 function. Candidate activity-enhancing mutations have also been identified. Lastly, we discovered that FLS2 is quite insensitive to disruption of multiple putative N-glycosylation sites while the related receptor EFR can be rendered non-functional by disruption of single glycosylation sites. The results will be presented in the context of current models for FLS2 function.

Analysis of the MarR/SlyA family members of *Erwinia chrysanthemi* 3937 reveals a new global regulator of virulence gene expression

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Pathogenicity of *E. chrysanthemi (Dickeya dadantii*), the causative agent of soft-rot disease in many plants, is a complex process involving several factors whose production is tightly regulated by environmental conditions. Members of the MarR/SlyA family of transcriptional regulators regulate a wide variety of biological processes, including adaptation to different environments and virulence. E. chrysanthemi contains 17 regulators of the MarR/SlyA family including PecS, a previously characterized global regulator of the virulence genes (Hommais et al., 2008). The remaining 16 regulators of this family were analysis in order to identify those involved in pathogenicity of the bacterium. This allowed the identification of a new mutant, *mfbR*, strongly affected in virulence on various plants. Among the MfbR targets are the genes encoding plant cell wall-degrading enzymes (pectinases, cellulase and proteases), flagellar components and the oxidative stress response proteins. The expression of these virulence genes is decreased in the *mfbR* mutant, which is in agreement with its reduced virulence. Most of these controls occur through a direct effect because purified MfbR binds to the promoter regions of the genes encoding pectinases and cellulase. Furthermore, MfbR increases and stabilizes the binding of RNA polymerase at these gene promoters, supporting an involvement of MfbR in a direct activation mechanism. These results suggest a co-ordinated regulation of virulence factors by MfbR at various key steps of infection, early (flagellar synthesis and oxidative stress response) and advanced phases (cell-wall degradation).

Mapping avirulence genes in the wheat stem rust fungus, *Puccinia graminis*, by genetics and genomics

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Wheat stem rust caused by *Puccinia graminis* f. sp. *tritici* (Pgt) is one of the most devastating diseases of wheat. Pgt is a dikaryotic, obligate biotrophic fungus. Ug99 (race TTKSK) a new strain of Pgt has spread from northeast Africa to the Arabian Peninsula and recently to central Asia, is a major threat to wheat production worldwide. Resistance to stem rust in wheat is primarily controlled by single dominant genes that follow the classical gene-for-gene model. Over fifty wheat stem rust resistance genes have been characterized. A partial genetic map of Pgt has been constructed and eight avirulence genes mapped (Zambino et al, 2000). Recently, a draft genome sequence of one of the parents (strain CRL 75-36-700-3, race SCCL) from this mapping population was completed. Avirulence genes *Avr*T6 and *Avr*T9a have been mapped the to scaffolds Sc40/Sc110 and Sc3, respectively. The genomic region containing *Avr*T6 is highly polymorphic and preliminary results indicate that this represents divergent lineages rather than recent rapid evolution. Resequencing data of the two parents strains and the F1 of the mapping population, is being used to develop SNP markers to refine the genetic map location of *Avr*T6 and *Avr*T9a. In addition, RNA expression data (microarray and RNA sequencing) and resequencing of additional Pgt strains is being used to confirm genetic mapping data of the avirulence genes.

The *Xanthomonas* effector AvrBsT suppresses the AvrBs1 HR in pepper

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The Gram-negative bacterium *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) causes bacterial spot disease in pepper and tomato plants. Pathogenicity depends on a functional type III secretion system (T3SS) and translocation of more than 20 effector proteins into the plant cell. In case of recognition of a given effector protein by a plant that carries the corresponding resistance (R) gene bacterial growth is arrested and often a hypersensitive response (HR), a rapid local plant cell death, is induced. The effector AvrBs1, e.g. in *Xcv* strain 85-10, elicits a rapid HR in *Capsicum annuum* (pepper) carrying the Bs1 resistance gene. However, the presence of AvrBsT in the plant cell leads to a strong reduction of the AvrBs1-specific HR. AvrBsT is a member of the YopJ/AvrRxv family that is highly conserved in plant and animal pathogenic bacteria and has predicted acetyl transferase activity. A pepper serine/threonine kinase was identified as interaction partner of AvrBsT. Virus-induced gene silencing showed that this kinase is involved in the specific induction of the HR by AvrBs1.

The biotrophic transcriptome of *Colletotrichum higginsianum*, the crucifer anthracnose fungus

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The hemibiotrophic ascomycete *Colletotrichum higginsianum* causes anthracnose disease on *Arabidopsis thaliana*, providing a model pathosystem in which both partners can be genetically manipulated. Following initial penetration by appressoria, biotrophic intracellular hyphae develop inside living host epidermal cells. To survey fungal gene expression during biotrophy, we generated a stage-specific cDNA library from hyphae isolated from infected leaf tissue by fluorescence-activated cell sorting. The high purity of the isolated hyphae (94%) eliminated contamination of the library by sequences derived from host cells or other fungal cell types. Low abundance cDNAs were enriched by 'cold-colony picking'. Sequencing generated 4029 expressed sequence tags (ESTs), which assembled into 1460 unigenes. 47% of the unigenes had no homology to proteins of known function. Homologues of genes related to protein biosynthesis, redox homeostasis and nutrition (biosynthesis of amino acids, vitamins and polyamines, amino acid and sugar uptake transporters) were highly represented in the library. *In silico* prediction of signal peptides and transmembrane domains identified 44 unigenes encoding putative soluble secreted proteins of unknown function that represent candidate fungal effectors. Expression analysis by macroarray and semi-quantitative RT-PCR showed that many of the secreted proteins are plant-induced and highly stage-specific. Overall, our findings suggest that the biotrophic hyphae of *Colletotrichum* may perform similar biological activities as the haustoria of obligate biotrophs, i.e. fungal nutrition and the delivery of effectors for host manipulation.

Reactive oxygen species (ROS) activates defense signaling through protein kinase cascades in rice

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The production of reactive oxygen species (ROS) upon the recognition of pathogen challenge is important for induction of defense responses in plants. However, the molecular mechanism of signal transduction system downstream of ROS production is still unclear. We have demonstrated that OsPti1a negatively regulates a series of defense responses including cell death induction, *PR* genes expression, and accumulation of phytoalexins in rice (Takahashi et al., 2007). Here, we identified a protein kinase, Pik1 (OsPti1a interacting kinase), as an interacter of OsPti1a. Pik1 belongs to an AGC kinase family and exhibits high similarity with Arabidopsis Oxi1/AGC2-1. The expression of *Pik1* was tightly regulated temporally and spatially by ROS stimuli. Furthermore, Pik1 was rapidly phosphorylated and activated by ROS, indicating that Pik1 functions downstream of ROS in rice. MAPK cascade which is a key component of the defense signaling is one of the candidates targeted by ROS. We identified OsMKK4-OsMPK3/6 cascade that mediates defense signals triggered by both elicitor and ROS, and this cascade is involved in induction of defense-related genes, hypersensitive cell death, and phytoalexin production in rice. The mechanism and the biological significance of the defense mediated by these protein kinase cascades downstream of ROS will be discussed.

Characterization of *NON-RCY1-RESISTANCE* (*NRR*) locus required for *RCY1*-conferred resistance to *Cucumber mosaic virus* in Arabidopsis

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Arabidopsis thaliana ecotype Col-0 over-expressing *RCY1*, a CC-NB-LRR class resistance gene, with HA tag (Col::RCY1-HA) shows extreme resistance (ER) to *Cucumber mosaic virus* yellow strain (CMV-Y) but not to other pathogens (Sekine et al., 2008). When ecotype Di-17 was transformed with the *RCY1-HA* construct, about 30% of transformants (Di-17::RCY1-HA) i) accumulated RCY1-HA protein at high level similar to Col::RCY1-HA, ii) exhibited a severe stunted phenotype and iii) were partially compromised in development of ER to CMV(Y). Genetic analysis of F2 progeny between Col::RCY1-HA and Di-17::RCY1-HA indicated that the stunted phenotype was inherited as a single recessive trait which was conferred by recessive *NON-RCY1-RESISTANCE* (*NRR*) gene. The *NRR* gene was mapped between CAPS markers *AG* and *ATMYB3R* on chromosome IV using 914 individual F2 progeny. The compromised development of ER to CMV(Y) also co-segregated with the stunted phenotype. Thus, *NRR* appears to play an important role for complete resistance to CMV(Y) by *RCY1* in Arabidopsis.

An ABC protein responsible for symbiotic nitrogen fixation in *Lotus japonicus*

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Legume plants can establish symbiosis with rhizobia in forming a nitrogen-fixing apparatus that is called nodules. In the nodule formation process, many membrane transport events are involved, but the current understanding of those transport systems and involved transporter is still very limited. As an approach to clarify these transport systems, especially necessary for the nodule formation process, and for the maintenance of its function we chose ATP-binding cassette (ABC) proteins to characterize in detail. ABC proteins constitute one of the largest transporter families in plants and play divergent physiological roles by transporting broad range of substances across membranes. In this study, we have identified an ABC protein gene that is strongly up-regulated during nodulation in the roots of *Lotus japonicus*. The gene designated as *LjABCB1* is coding for 1254 amino acids, which belongs to a full-size ABC protein. The involvement of this gene in nodules is under investigation.

Differential protein expression and peroxidase isozyme pattern in Japanese birch No. 8 plantlets infected with *Inonotus obliquus* IO-U1 strain

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In the previous report (Rahman *et al*., 2008), host-pathogen interactions were investigated on a Japanese birch (*Betula platyphylla* var. *japonica*) Tohoku plantlets after infection with a canker-rot fungus, *Inonotus obliquus* IO-U1 strain. Based on the results obtained, phenolics deposition and necrophylactic peridarm formation are considered to occur as infection-induced responses in Japanese birch Tohoku plantlet infected with *I. obliquus* IO-U1. In the present study, proteome analysis and peroxidase isozyme analysis were performed to clarify the interactions between Japanese birch and *I. obliquus*. Three-month-old sterile plantlets of Japanese birch No. 8 clone were prepared using axillary bud cultured in an MS-based medium. Intact (control 1), injured (control 2) and infected plantlets were prepared for the experiments. Two days after infection, protein sample was extracted from plantlets, and the protein preparation used for analyses. In proteome analysis, 169 protein spots were detected as infection-specific proteins on 2-DE gels. Some mass spectra from these protein spots were subsequently measured using MALDI-TOF-MS, and the following three proteins were identified: glutathione *S*-transferase, heat shock 60 kDa protein, and heat shock 70 kDa protein. On the other hand, peroxidase isozyme pattern was not different among Control 1, Control 2, and infected plantlets in peroxidase isozyme analysis by IEF.

Forced changes in nucleo-cytoplasmic partitioning of the NB-LRR Rx dictate initiation of defence signalling

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In plants, resistance proteins are immune receptors that upon pathogen recognition trigger a signal transduction cascade leading to a rapid defence response. The nucleotide-binding and leucine-rich repeat (NB-LRR) proteins form the major class of immune receptors. The potato NB-LRR Rx confers resistance to potato virus X (PVX) by recognition of the viral coat protein. Rx localises to the cytoplasm, but it was recently shown to also accumulate in the nucleus, despite the absence of a discernible nuclear localisation signal (NLS). In the cytoplasm Rx associates with a RanGTPase-activating protein 2 (RanGAP2), which is required for resistance to PVX (Tameling and Baulcombe, 2007, Plant Cell; Sacco et al., 2007, Plant J). RanGAPs are highly conserved in eukaryotes and are required for the regulation of nucleo-cytoplasmic trafficking of macromolecules (e.g. proteins) through the nuclear pores. Co-expression studies in *Nicotiana benthamiana* revealed that overexpression of RanGAP2 (in the cytoplasm) attenuates the nuclear accumulation of Rx, and largely potentiates the weak autoactive phenotype of a truncated Rx protein. The inverse was observed when the WPP-domain of RanGAP2, which mediates interaction with Rx, was forced to accumulate in the nucleus by fusion with an NLS. Hence, co-expressed Rx hyper accumulated in the nucleus, which coincided with an abolishment of the autoactivity of several Rx mutants. Our data suggest that the ratio between the Rx pool in the cytoplasm and in the nucleoplasm dictates the initiation of defence signalling, possibly via influencing the compartimentalisation of a transcriptional regulator. Further studies will focus on whether such dynamic changes in Rx localisation indeed also occur upon PVX infection and are actually responsible for mediating PVX resistance.

The aldehyde dehydrogenase gene and phosphinorthiricin N-acetyltransferase gene located in pathogenicity island is involved in virulence of *Pseudomonas cichorii*

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Pseudomonas cichorii SPC9018 (SPC9018) causes necrotic lesion and rot on leaves of eggplant and lettuce, respectively. The nucleotide sequences of pathogenicity island (PAI) including the *hrp* genes of SPC9018 and its genetic structure are homologous to those of S-PAI of *P. viridiflava* BS group strains. The consensus *hrp*-box (GGAACC-N_{15-16}-CCANNCA) was identified at putative promoters of *hrpA, hrpF, hrpW, avrE, avrF* and *hrpJ*. RT-PCR analysis showed that expression of *hrp* genes was dependently on HrpL. Interestingly, pathogenicity of SPC9018 on eggplant but not on lettuce plants are *hrp*-dependent (Hojo et al. 2008). In PAI of SPC9018, aldehyde dehydrogenase gene (*aldh*) and phosphinorthiricin N-acetyltransferase gene are located adjacent to *hrpL*. RT-PCR analysis showed that expression of those genes is independent of HrpL. The *aldh*-deficient mutant (Δaldh) and phosphinorthiricin N-acetyltransferase gene-deficient mutant (Δpna) of SPC9018 lost their virulence on eggplant, but retained their virulence on lettuce. Complementing the Δaldh and Δpna with *aldh* and phosphinorthiricin N-acetyltransferase gene originating from SPC9018, respectively, allowed the mutants to cause the disease on eggplant. Furthermore, *aldh* and phosphinorthiricin N-acetyltransferase gene from S-PAI of *P. viridiflava* strain Pv9504 (BS group) also allowed the mutants to complement their virulence on eggplant. These results suggest requirement of *aldh* and phosphinorthiricin N-acetyltransferase gene located in PAI for virulence of SPC9018 on eggplant and functional conservation of those genes between *P. cichorii* and *P. viridiflava*.

Fungal cell surface PAMPs trigger plant basal resistance through activation of mitogen-activated protein kinase pathway

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We have previously reported that *cossd1* mutant, possible altered cell wall composition, of *Colletotrichum orbiculare* induced basal resistance that is mediated by MEK2-SIPK/WIPK pathway in *Nicotiana benthamiana*. Here we evaluated SIPK and WIPK activity against *cossd1* mutant infection by immunocomplex kinase assay. SIPK and WIPK activities in the leaves inoculated with *cossd1* mutant were higher than those with the wild-type. In *SIPK*/*WIPK*-silenced plants, the activation of SIPK and WIPK was not

detected, indicating that *cossd1* mutant induces basal resistance through activation of SIPK and WIPK. To assess whether cell wall structure of *cossd1* mutant induces MAPKs activity, we attempted to infiltrate heat inactivated conidia into plant leaf. When conidial suspension of *cossd1* mutant was infiltrated, both MAPKs were activated higher than those with the wild-type. In addition, this induced kinase activity was similar to the level in leaf with conidia treated with protease. To evaluate the possibility that the wild-type conidia may produce diffusible suppressors on plant surface, we assessed activity using incubated conidial supernatant of the wild-type. However, incubated conidial supernatant did not suppress both MAPKs activities induced by conidia, suggesting that the wild-type conidia did not secret diffusible suppressors. Taken together, these results indicate that the activation level of specific mitogen-activated protein kinase pathway triggered by fungal cell surface determines the effective level of plant basal resistance.

The region in flagellin of *Pectobacterium carotovorum* subsp. *carotovorum* responsible for induction of tobacco cell death

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The flagellin, a building block of flagella, of several plant pathogenic bacteria has been shown to act as pathogen-associated molecular patterns (PAMPs). The flagellin purified from *Pectobacterium carotovorum* subsp. *carotovorum* (Pcc) induced plant cell death in tobacco BY-2 suspension cultured cells, while that from *Dickeya dadantii* (Dd), another genera of soft-rot bacteria, did not. From sequence analysis of *fliC* that encodes flagellin, Pcc flagellin was found to contain the region homologus to flg22 that was first described in *Pseudomonas aeruginosa* to induce plant defense responses. A synthesized peptide of $flg22_{Pcc}$ caused loss of cell viability but not cell death on the tobacco cells. Thus, the presence of another region responsible for induction of cell death was suggested in Pcc flagellin. Analyses using deletion series of Pcc flagellin and chimerical constructs of Pcc and Dd flagellins revealed that the N-terminal peptide of 51-100th amino acids of Pcc flagellin, excluding $flg22_{Pcc}$, can induce tobacco cell death. The site-directed mutants in 11 amino acids that are different from the same region of Dd falgellin were tested for inducibility of cell death of tobacco cells. We identified one of the single amino acid replacements which resulted in the loss of this inducibility.

Identification of a transcription factor regulating hyphal differentiation and growth in *Epichloë festucae*, a mutualistic symbiont of temperate grasses

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The fungal endophyte, *Epichloë festucae*, forms a symbiotic association with perennial ryegrass, *Lolium perenne*. In wild-type associations, *E. festucae* grows systemically in the intercellular spaces of the leaves as infrequently branched hyphae parallel to the leaf axis. *Agrobacterium tumefaciens* mediated T-DNA mutagenesis was used to identify genes of *E. festucae* responsible for maintaining the mutualistic association. The screen identified one mutant, Ag413, which causes severe stunting of the grass host, a host interaction phenotype very similar to that observed for a *noxA* deletion mutant (Tanaka et al., 2006). Sequence analysis of the genomic DNA flanking the T-DNA insertion in Ag413 showed that the T-DNA was inserted into a gene, designated *proA,* encoding a $Zn(II)_2Cys_6$ transcription factor. ProA has homology to Pro1/NosA, positive regulators of sexual development in other ascomycetes. Deletion analysis confirmed that stunting of the host plant is caused by disruption of *proA.* Homologues of *proA* were also deleted in the plant pathogens, *Magnaporthe oryzae* and *Fusarium oxysporum,* but no defect in host pathogenicity was observed.

Proteomic analysis of tomato leaves treated with salicylic acid

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Plants have developed sophisticated mechanisms to cope with biotic and abiotic stresses. Some of these mechanisms are constitutive and others are inducible, such as the accumulation of pathogenesis-related (PR) defence proteins. This induction is mediated by signal molecules that differ on the plant-pathogen interaction. Salicylic acid (SA) plays an important role in the activation of the plant defence response (Heck et al., 2003). Application of exogenous SA is sufficient to induce resistance to many normally virulent pathogens (Verberne et al., 2000) and SA treatment stimulates the expression of a set of defence proteins and the activation of systemic acquired resistance (SAR) in many plants (Murphy et al., 2000; Rajjou et al., 2006). Proteomics is becoming an increasingly important tool because proteins are directly related to function. Recently, advances in proteomics studies have been driven by the development of fluorescence 2-D difference in gel electrophoresis (2-DIGE), based on labelling of different protein samples with fluorescent cyanine dyes which have distinct excitation and emission spectra. Control and treated samples can therefore be run in the same 2-D gel, allowing the control of non-biological variation in differential protein expression experiments. In this work, our aim was to identify proteins that are differentially expressed upon a SA treatment in tomato leaves, by using 2-DIGE technology. Among the 2000 spots detected within the gels, 114 resulted differentially expressed in SA-treated tomato leaves. The proteins were identified by mass spectrometry and mainly classified into two groups: stress and defence proteins and energy and metabolism proteins. The proteome changes found in this study confirm the importance of SA in defence and shed new light in understanding plant pathogenic response.

Pip3, a novel Arabidopsis component targeted by PopP2 that is required for the RRS1-R-mediated resistance

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Ralstonia solanacearum is the causal agent of bacterial wilt. In *Arabidopsis thaliana*, the RRS1-R (TIR-NBS-LRR-WRKY) resistance protein confers a broad spectrum resistance to *Ralstonia*. RRS1-R and its matching type III effector protein, PopP2, physically interact within the plant nucleus. In order to identify other plant components that would participate in the establishment of a PopP2/RRS1-R Resistasome complex, the screening of a Y2H cDNA library was performed using PopP2 as a bait. Several PopP2-interacting partners (called Pips) were identified. Among them, Pip3, a protein of unknown function, is targeted the plant nucleus where it accumulates into subnuclear compartments whose nature remains to determine. Physical interaction between Pip3 and PopP2 was confirmed by co-immunoprecipitation and FLIM experiments. Transient expression assays revealed a stabilization effect of Pip3 by PopP2. Pip3 protein accumulation is indeed increased within plant nucleus upon co-expression with PopP2. A loss-of function approach was developed with the identification of a *pip3* KO mutant and the generation of Pip3 RNAi transgenic lines. Pip3 gene extinction within an *RRS1-R* resistant background leads to a reduction of bacterial multiplication levels in response to both virulent and avirulent strains of *Ralstonia*. Gain-of-function approach could bring new clues about Pip3 function since transgenic Pip3 overexpressor lines show altered root hair development. Taken together, these data suggest that Pip3 is a plant component whose manipulation by PopP2 may be surveyed by RRS1-R. Hypotheses concerning the involvement of Pip3 in the RRS1-R-mediated disease resistance will be presented.

Xanthomonas* T3S effector XopN suppresses PAMP-triggered immunity and physically interacts with a tomato atypical receptor-like kinase and TFT1 *in planta

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XopN is a T3S effector from *Xanthomonas campestris* pathovar *vesicatoria* (Xcv) that is translocated into tomato leaf cells during infection. Xcv ΔxopN mutants are impaired in growth and have reduced ability to elicit disease symptoms in susceptible tomatoes. We present that XopN action *in planta* reduced Pathogen-associated molecular pattern (PAMP)-induced gene expression and callose deposition indicating that XopN is a suppressor of pathogen-triggered immune (PTI) responses during Xcv infection. XopN is predicted to have irregular, alpha-helical repeats suggesting that it may

participate in protein-protein interactions *in planta*. Consistent with this prediction, XopN interacts with the cytosolic domain of a Tomato Atypical Receptor-Like Kinase 1 (TARK1) and four Tomato Fourteen-Three-Three isoforms (TFT1, TFT3, TFT5, and TFT6) in yeast. XopN/TARK1 and XopN/TFT1 physical interactions were confirmed *in planta* by BiFC and pull-down analysis. Site-directed mutagenesis of XopN and TARK1 revealed two signature LXXLL motifs required for XopN/TARK1 binding. Analysis of a XopN LXXLL mutant in Xcv-tomato interactions revealed that this motif contributes to XopN-dependent Xcv growth in tomato but not to XopN-dependent suppression of PTI. Tomato leaves from lines silenced for *TARK1* mRNA expression sustained a higher titer of Xcv ΔxopN compared to leaves expressing *TARK1* mRNA, indicating that reduced *TARK1* expression suppressed the ΔxopN mutant phenotype *in planta*. These data provide the basis for a model in which XopN binds to TARK1 to interfere with TARK1-dependent signaling responses associated with Xcv infection in tomato. In addition, these data reveal that XopN is an important virulence factor that suppresses PTI during Xcv-tomato interactions.

The thermosensitivity of the tobamovirus pepper mild mottle virus strain I (PMMoV-I) is conferred by the helicase domain

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In Capsicum spp, resistance against tobamoviruses is conferred by an allelic serie of genes termed L1-L4 upon its increased effectiveness. The resistance is characterized by the activation of an hypersensitive reaction. Based upon its relationship towards the L3 resistance gene, the Spanish (S) and Italian (I) strains of PMMoV has been identified as P1,2, and P1,2,3 pathotypes, respectively; i.e. avirulent and virulent strains. Our previous data have shown that the resistance conferred by the C. chinense L3 gene is inactive at temperatures above 32ºC. At this temperature the accumulation level of the I strain is reduced with respect to that from the S one. We have established previously that the 126K protein is responsible for the observed differences. By further constructing hybrid viruses within this region, we have determined that the helicase domain within the 126K protein is the responsible for the thermosensitivity of the I strain. It remains open the possibility that this particular feature of the helicase domain within the I strain is responsible for the limited prevalence of this pathotype in Nature with respect to the P1,2 pathotype, in spite of its enhanced pathogenicity towards the Capsicum spp resistance genes.

Rice blast genome resequencing and association genetics reveals three novel avirulence genes, AVR-Pia, AVR-Pii and AVR-Pik/km/kp, whose products are recognized inside rice cells

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Rice blast caused by the ascomycete fungus *Magnaporthe oryzae* is the most devastating fungal disease of rice, a major food staple of billions of people. With the objective of isolating novel avirulence (*AVR*) and effector genes from *M. oryzae*, we carried out a large-scale DNA polymorphism study of secreted protein genes predicted from the published genome sequence of isolate 70-15. Nucleotide diversity of 1,036 *M. oryzae* genes among 46 isolates of a worldwide collection was extremely low (theta = 8.2×10^{-5}), suggestive of recent dispersal of the pathogen. However, no association between DNA polymorphism and *AVR* was identified, which prompted us to carry out genome resequencing of Ina168, an *M. oryzae* isolate known to contain nine *AVR* genes. Remarkably, a total of 1.68-Mb regions, comprising 316 candidate effector genes, were present in Ina168 but absent in the assembled sequence of isolate 70-15. Association analyses of these 316 genes identified three novel *AVR* genes, *AVR-Pia*, *AVR-Pii* and *AVR-Pik/km/kp* corresponding to five previously known *AVR* genes. *M. oryzae* genetic complementation validated these *AVR*s. Transient transformation of rice protoplasts and stable transformation of rice plants showed that these *AVR* products are recognizedinside rice cells possessing the cognate *R*-genes. *AVR-Pia* and *AVR-Pii* have evolved by gene gain/loss processes, whereas *AVR-Pik/km/kp* has evolved by gain/loss as well as nonsynonymous nucleotide substitutions presumably driven by arms-race with rice *R*-gene evolution. Candidate effector genes, such as the isolated AVRs, show higher association with transposable elements and seem to be evolving predominantly by gain/loss processes.

A universal PCR assay for detecting fungal pathogens of apple

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Apple is an important economic fruit crop. It is susceptible to various diseases caused by fungi, bacteria and insects amongst which the fungal diseases are of major importance. Early detection of the pathogens during asymptotic stage can be useful for timely adoption of treatments to prevent establishment of the disease and the associated yield loss. We will describe a PCR based strategy for timely detection of fungal pathogens of apple. Using the sequence information available at NCBI, a pair of universal primers has been synthesized which can amplify DNA fragments from almost all apple associated fungal pathogens. A restriction enzyme is identified by insilico analysis of the universal primer amplifiable sequences of various fungal pathogens of apple that can generate length polymorphisms in the PCR amplicons that are resolved by agarose gel electrophoresis. The method has been validated in wet lab experiments and is found to be capable of detecting and distinguishing all the tested apple pathogens that were isolated from Indian orchards. The present paper also describes our strategy of using the robustness and sensitivity of quantitative real-time PCR (RT PCR) to sensitively detect, identify and quantitate the biomass of these apple pathogens.

Jasmonate signaling is required for susceptibility to the root infecting fungal pathogen *Fusarium oxysporum*

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The root infecting fungus *Fusarium oxysporum* is the causal agent of vascular wilt in over 100 different plant species, including important crops such as cotton, banana and tomatoes. *Arabidopsis thaliana* is also susceptible to *F. oxysporum* infection and provides an excellent model system to dissect mechanisms of plant resistance. While defense responses mediated by jasmonic acid (JA) are often necessary for resistance against necrotrophic pathogens, we found that the JA-perception mutant *coi1*, but not JA-biosynthesis mutants, was highly resistant to *F. oxysporum* and to lesion induction from fungal culture filtrates. The *coi1*-mediated resistance was independent of JA-dependent defense gene expression but correlated with reduced expression of senescence-associated genes and reduced fungal colonisation at later stages of infection when leaf necrosis was highly developed in wild-type plants. Our results suggest that *coi1*-mediated resistance acts through the inhibition of *F. oxysporum*-incited lesion development and plant senescence. To further investigate the basis of *coi1*-mediated resistance, we have studied JA-signalling components that act downstream of COI1. This has included the analysis of repressors of JA signaling known as JAZ proteins, where we have observed differential gene expression patterns and roles for JAZ proteins in resistance to *F. oxysporum*. In an un-biased approach to identify genes controlling this interaction we are also undertaking a global-wide analysis of Arabidopsis genes by screening collections of homozygous insertion mutants with a high-throughput quantitative infection assay. From these analyses, we aim to develop a detailed map of host genetic mechanisms determining vascular wilt disease.

Effector candidates from cereal powdery mildew and rust fungi share localization motif

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Powdery mildew and rust fungi are obligate fungal pathogens that depend on having haustoria in host cells. Haustoria, which are separated from the host cytoplasm by a plant cell-derived extrahaustorial membrane, secrete effector proteins to the plant cell in order to suppress defence and stimulate the plant to re-direct nutrient to the pathogen. We have generated a cDNA library from barley epidermal strips with a high density of mature powdery mildew haustoria and made 10,000 EST reads. Two-thirds of the ESTs represented approximately 3,000 fungal genes. We listed these genes according to the number of ESTs obtained and found that the top of the list was dominated by genes encoding small (90-130 amino acids) proteins with N-terminal signal peptides (SP). These proteins are novel and generally unrelated, but they all share a short amino acid motif in the N-terminal of the mature protein. In total, we found 120 proteins with these characteristics being represented in our library. We consider these proteins effector candidates secreted to the plant cell, since a proteome analysis of isolated haustoria only identified four of them among 204 fungal proteins. Subsequent analysis of publically available

fungal genome and EST data revealed that wheat stem rust and wheat leaf rust fungi also encode large numbers of proteins with these characteristics, including the same motif. Transient plant expression of constructs encoding SP-GFP fusion proteins, with and without the motif, strongly suggests that the motif controls plant cell localization of these effector candidates.

The gene *BvGLP-1* encoding for a germin-like protein regulates the *Hs1^{pro-1}*-mediated nematode resistance by its oxalate oxidase activity in sugar beet and *Arabidopsis thaliana*

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Germins and germin-like proteins (GLPs) constitute highly diverse family of plant proteins and are involved in many developmental stages and stress-related processes. *Hs1^{pro-1}* locus confers resistance to the beet cyst nematode *Heterodera schachtii* in sugar beet (*Beta vulgaris*). To understanding the molecular mechanism governing the *Hs1^{pro-1}*-mediated nematode resistance, transcript profiling was conducted on both of sugar beet with the cDNA-AFLP-analysis and Arabidopsis with the ATH1 GeneChip hybridization experiments. This approach has identified the gene *BvGLP-1* from sugar beet. The gene encodes for an oxalate oxidase-like germin protein that is highly up-regulated in the resistant, but not in the susceptible sugar beet in response to nematode infection. Also, a homolog of *BvGLP-1* gene, *GLP3*, was identified from the ATH1 Genechip, which represents the highest upregulated gene in the *Hs1^{pro-1}* transgenic Arabidopsis genome. These data strongly suggest an active role of *BvGLP-1* in the *Hs1^{pro-1}* mediated resistance. For functional analysis, we transferred *BvGLP-1* into sugar beet roots und Arabidopsis plants and challenged the transgenic roots and plants with the beet cyst nematode. Our data demonstrate that expression of *BvGLP-1* in nematode feeding cells of both sugar beet roots and Arabidopsis plants was sufficient to initiate nematode resistance while knockout of the homolog gene of *BvGLP-1* in Arabidopsis significantly increased in plant susceptibility to the nematode infection. Moreover, we demonstrate that BvGLP-1 exhibits oxalate oxidase activity in plant cells and regulate the expression of the pathogenesis-related proteins. These data suggest that *BvGLP-1* play a central role in regulating plant nematode resistance. A possible signal transduction pathway was discussed.

Arabidopsis actin depolymerizing factor AtADF4 mediates defense signal transduction triggered by the *Pseudomonas syringae* effector AvrPphB

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The actin cytoskeleton has been implicated in plant defenses against pathogenic fungi and oomycetes with limited, indirect evidence. To date, there are no reports linking actin with resistance against phytopathogenic bacteria. The dynamic behavior of actin filaments is regulated by a diverse array of actin-binding proteins, among which is the Actin Depolymerizing Factor (ADF) family of proteins. Here, we demonstrate that actin dynamics play a role in the activation of gene-for-gene resistance in *Arabidopsis thaliana* following inoculation with the phytopathogenic bacterium *Pseudomonas syringae* pv. tomato. Using a reverse genetic approach we explored the roles of *Arabidopsis* ADFs in plant defenses. AtADF4 was identified as being specifically required for resistance triggered by the effector AvrPphB, but not AvrRpt2 or AvrB. Recombinant AtADF4 bound to monomeric actin (G-actin) with a marked preference for the ADP-loaded form, and inhibited the rate of nucleotide exchange on G-actin, indicating that AtADF4 is a *bonafide* actin depolymerizing factor. Exogenous application of the actin disrupting agent cytochalasin D partially rescued the *Atadf4* mutant in the AvrPphB-mediated hypersensitive response, demonstrating that AtADF4 mediates defense signaling through modification of the actin cytoskeleton. Unlike the mechanism by which the actin cytoskeleton confers resistance against fungi and oomycetes, AtADF4 is not involved in penetration resistance. Collectively, this study identifies AtADF4 as a novel component of the plant defense signaling pathway, and provides strong evidence for actin dynamics as a primary component that orchestrates plant defenses against *P. syringae*.

Allelic tobamovirus resistance genes from *Capsicum* plants code for single, independently evolved CC-NBS-LRR proteins with different spectrum for virus recognition

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Unlike bacterial and fungal pathogens, it seems difficult for viruses to acquire new effectors because of their limited genome size. Through co-evolution, plant-virus interactions have generated a series of virus strains and host resistance genes that provide good models to study molecular interactions in pathogen perception by plants. Capsicum plants have allelic genes, L^1, L^2, L^3 and L^4, which provide increased tobamovirus resistance: L^1 confers resistance to the P_0 pathotype of tobamoviruses; L^2 confers resistance to P_0 and P_1; L^3 confers resistance to P_0, P_1 and $P_{1,2}$; L^4 confers resistance to P_0, P_1, $P_{1,2}$ and $P_{1,2,3}$. From five different Capsicum species, we cloned seven allelic *L* genes, each encoding a single CC-NBS-LRR type resistance protein. Functional analysis of the genes revealed that the hierarchical interactions between tobamoviruses and *L* genes are conditioned by the interaction between different tobamovirus coat proteins and allelic *L* gene products that have different recognition spectra: i.e., L^1 showed limited, L^2 and L^3 showed broader, and L^4 showed the broadest recognition spectra. Analysis of chimeras between L^3 and a functionally uncharacterized paralog, and those between L^1 and the other *L* genes revealed: 1) both CC-NBS and LRR domains have roles in the determination of the recognition spectrum; and 2) amino acid changes in different LRR units are responsible for broadening the recognition spectrum. The results suggested that *L* genes have evolved independently from each other during the diversification of the genus *Capsicum*. Different *Capsicum* species are likely to have been under selective pressure from different pathotypes of tobamoviruses as well as environmental conditions.

Fluorescent proteins for the co-localization of symbionts and endophytes in legumes

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Bacterial endophytes can promote plant growth and yield, suppress pathogens, solubilize nutrients or contribute to nitrogen uptake in plants. The use of fluorescent reporters is nowadays a key tool for studying microbe-plant interactions. In a previous work we assessed the presence of different endophytes in addition to rhizobia inside root nodules of wild legume plants and showed that rhizobia share nodules with a variety of different co-infecting taxa (Muresu et al., 2008 FEMS Microbiol. Ecol. 63:383). In the present report we explore the plant-endophyte relationships attempting the co-localization of endophytes *(Pseudomonas* sp.*, Enterobacter agglomerans)* and rhizobia *(Rhizobium leguminosarum* bv. *trifolii)* by the introduction of different fluorescent proteins as bacterial markers for the different kinds of bacteria. This would help their localization throughout the plant and in particular the distinction of rhizobia from other endophytes co-infecting root nodules. Green fluorescent protein (GFP) and red fluorescent protein (RFP) markers were integrated into the bacterial chromosome. For the marked constructs, a suicide plasmid, carrying the gfp gene in a transposable element was mobilized into the endophytic species. To obtain rfp-tagged bacteria, a replacement of gfp with rfp was made starting from the pRL765gfp plasmid. The use of dual fluorescence markers will allow to co-localize different bacterial taxa within plant tissues and will enable to plan different innovative applications in the field of symbiosis, biocontrol and other endophytic plant-microbe interactions.

A target for the *Fusarium* mycotoxin zearalenone in plants: Inhibition of HSP90 ATPase

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The *Fusarium* mycotoxin zearalenone (ZON) is well known for its strong estrogenic activity in animals. Plants do not have an estrogen receptor, and it

is an open question whether the *Fusarium* metabolite ZON has a biological role in plant-pathogen interaction. We have identified a prominent target for zearalenone: Hsp90. Zearalenone and more strongly beta-zearalenol (bZOL) inhibit ATPase activity of purified yeast Hsp90 (ScHsp82p) in vitro. Hsp90 is necessary for the stability of many client proteins such as signal transduction components, and has been shown to be necessary for plant defense. Microarray experiments of ZON treated *Arabidopsis* plants showed marked changes in gene expression. Many genes encoding proteins with a role in cell wall remodeling and peroxidases were repressed, and small heat shock proteins and AtHsp90-1 were upregulated. Also many candidate detoxification genes were induced. ZON was found to be rapidly converted into ZON-glucoside and ZON-sulfate in plants. Both conjugates are no longer inhibitors of Hsp90 ATPase *in vitro*. The finding that ZON and its biosynthetic precursor bZOL are Hsp90 inhibitors also raises the question about the mechanism of self resistance in the toxin producing fungus. We have engineered yeast strains with increased ZON sensitivity (deletion of several ABC transporters) and with deletions of the endogenous yeast *HSP90* genes (*hsp82 hsc82*), that express as sole source of Hsp90 either the yeast or *F. graminearum* gene. The strain expressing the *Fusarium* cDNA showed increased ZON resistance, suggesting that amino-acid differences in of FgHsp90 might be responsible for target insensitivity. (Supported by the Austrian Science Fund FWF F37).

Characterization of the *Pseudomonas syringae* type III effector HopA1

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Pseudomonas syringae is a host specific plant bacterial pathogen that requires a type III secretion system to translocate effector proteins into plants for pathogenicity. The type III effector protein HopA1 (formerly HopPsyA) was first characterized in *P. syringae* pv. *syringae* 61 and is encoded by a gene located in the Hrp pathogenicity island. Another strain, *P. syringae* pv. *tomato* DC3000, also contains a *hopA1* allele in a different region of the chromosome. When expressed in tobacco (*Nicotiana tabacum*), HopA1 elicits a hypersensitive response (HR), consistent with HopA1 being recognized by a resistance protein inducing effector-triggered immunity. We have determined that the C-terminal two thirds of HopA1 (98-375 aa) is required for the elicitation of an HR on tobacco when the DNA is transiently delivered into plant cells using *Agrobacterium*-mediated transformation, suggesting that this is the HopA1 portion that is recognized by the plant immune system. Several *hopA1* alleles from other *P. syringae* strains have been cloned and they fall into two groups. Group 1 members are similar to $HopA1_{61}$ and when transiently expressed in tobacco and *N. benthamiana* elicit an HR and when expressed in yeast induce cell death. Group 2 members, which are similar to $HopA1_{DC3000}$, only elicit an HR in tobacco and are not lethal to yeast. Bioinformatic analyses show that all HopA1s share similarity with the *Photorhabdus luminescens* insecticidal toxin Mcf2. Several invariant residues between Mcf2 and HopA1 have been identified and we have made site-directed mutations in many of these residues in both $HopA1_{61}$ and $HopA1_{DC3000}$. We are currently determining the effects that these substitutions have on the ability of HopA1 to elicit an HR and kill yeast as well as HopA1's ability to contribute to virulence.

A novel class of scaffold proteins regulates rhizobial and fungal infections by interfering with essential signaling components

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Plant-specific remorin proteins have been suggested to be involved in signaling processes and to form oligomeric structures. However, functions have not been assigned probably due to lethality of transgenic plants or functional redundancy within this multigene family. We recently identified a member of the remorin family that controls infection of rhizobia during root nodule symbiosis proving for the first time that these proteins are essential for at least certain types of plant-microbe interactions. This protein that localizes to subdomains of the plasma membrane and more specifically accumulates on nodular infection threads and the symbiosome membrane interacts with at least three receptor-like kinases (RLKs). Phenotypical analysis of knock-down plants and induction of the gene by isolated bacterial Nod Factors (NFs) also imply roles of the protein during pre-infection and early infection stages. To decipher the domain structure we characterised putative motifs required for RLK binding, plasma-membrane association and oligomerisation of the protein. We show that this remorin is spatially induced in distinct patches during NF perception and initial bacterial infection. We also identified another member of this protein family that is strongly induced during mycorrhizal infections and controls fungal infection. Results from transcriptional induction and protein localization patterns in combination with the identification of new interaction partners of these remorin proteins reveal essential functions of remorins during both types of symbiotic interactions. We will present data confirming that these remorins are novel scaffolding proteins with key positions within the symbiotic signaling cascade.

Suppression of lipoxygenase, allene oxide synthase, allene oxide cyclase and 12-oxophytodienoic acid reductase in pea altered susceptibility to *Mycosphaerella pinodes*

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The establishment of the plant-fungal pathogen interaction eventually causing diseases often involves the production by pathogens of virulence factors, which counter or attenuate host disease resistance. In *Mycosphaerella pinodes*-causing leaf spot (blight) of pea, two structurally related glycopeptides named Supprescin A and B (referred to suppressors) are produced in the pycnospore germination fluid. The nature of Supprescin B, *O*-glycosyl and other substitutions consisting of peptide moiety are important determinants for suppressing the host defense. Interestingly, the suppressor induces *12-oxophytodienoic acid reductase* (*OPR*) in pea, which is likely involved in the jasmonic acid (JA) synthesis (Matsui et al. 2004). In this study potential pea genes including *lipoxygenase* (*LOX*), *allene oxide synthase* (*AOS*), *allene oxide cyclase* (*AOS*) and *OPR* were invesigated to explore the role of JA-mediated cellular process in the achievement of susceptibility, using an Apple latent spherical virus (ALSV)-based VIGS technology (Yaegashi et al. 2007). In *LOX*-, *AOS*-, *AOC*- or *OPR*-silenced pea, lesion formation induced by *M. pinoses* were remarkably reduced. No difference in lesion formation was found among each silenced plants, indicating that LOX, AOS, AOC and OPR equally contribute to normal disease sympton development in pea. In fact in wild type plants exogenous JA promoted lesion formation by *M. pinodes*. Similarly, silencing of *LOX*, *AOS*, *AOC* and *OPR* reduced sensitivity to a phytotoxin corronatine, which is believed to function through JA-dependent process. Collectivelly, these results indicate that JA synthesis and/or JA-mediated cellular process are required for suppressing SA-dependent defenses during a compatible interaction between pea and *M. pinodes*.

Towards the demonstration of the transcriptional regulatory activity of RRS1 proteins during bacterial wilt disease in *Arabidopsis thaliana*

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The RRS1-R resistance gene confers broad spectrum resistance of *Arabidopsis thaliana* ecotype Nd-1 to *Ralstonia solanacearum*, the causal agent of bacteria wilt disease. An allelic form of the gene, RRS1-S, is present in the Col-0 susceptible ecotype. They encode TIR-NBS-LRR proteins with C-terminal domain characteristic of WRKY plant transcription factors (Deslandes et al, 2002). An interaction was detected within plant nuclei, between RRS1 and the bacterial Avr protein Pop2, a *Ralstonia solanacearum* type III effector targeted to the plant nucleus (Deslandes et al, 2003). We postulate that the transcriptional output of the plant genome could be altered due to the interaction between Pop2 and RRS1 proteins, which probably affects RRS1 activity. The objective of this work is to demonstrate the transcriptional activity of the RRS1 proteins and study their activity in response to bacteria. To this end (i) Expression pattern of RRS1 genes is being analysed in *Arabidopsis* transgenic plants. (ii)Transcriptomic approaches have been developed. (iii) In vitro and in vivo binding to W box are being tested. Data currently obtained will be presented.

Allelic diversity of genes coding eukaryotic translation initiation factors in Stone Fruit trees (*Prunus* species)

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Recessive resistance against viruses corresponds to a passive mechanism due to the absence or to the inapropriate nature of a host factor specifically required by the virus to complete its cycle. The dominant allele can then conceptually be envisionned as encoding a susceptibility factor needed by the

virus to be able to infect the host plant. Indeed, one of the major findings in the last few years was the identification of natural recessive resistance genes against several important potyviruses in vegetable crops, all of them encoding the translation initiation factor eIF4E or one of its isoforms. Interestingly, we showed recently that a knock-out mutant for *eIF(iso)4E* or for *eIF(iso)4G* in *Arabidopsis thaliana* is resistant to *Plum pox virus* (PPV) (Decroocq et al., 2006; Nicaise et al., 2007). These results suggest that many if not all Potyviruses probably use identical or closely related host factors in widely different plants to complete their life cycle. As a consequence, the identification of a plant susceptibility factor in any given potyvirus-plant pathosystem is very likely to be transferable to stone-fruit trees since PPV, the causal agent of sharka disease, belongs to the potyviruses. We postulate that most of the *Prunus* species bear a susceptibility allele of *eIF4E* and *eIF4G* and that some rare variants might occur in the natural *Prunus* population and germplasm collection. The purpose of this study is therefore the detection of polymorphism in the *Prunus eIF4E* and *eIF4G* homologues and isoforms and to correlate natural polymorphisms with a PPV resistance phenotypic trait.

The VirB-protein-induced small heat-shock protein HspL is a molecular chaperone promoting efficient VirB/D4 T4SS-mediated DNA transfer in *Agrobacterium tumefaciens*

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Agrobacterium tumefaciens is a Gram-negative plant pathogenic bacterium which causes crown gall disease by transferring and integrating its T-DNA into the host genome. We previously employed proteomics approach to discover a chromosomally encoded small heat-shock protein HspL, which was induced by the virulence (*vir*) gene inducer acetosyringone (AS) (Lai et al., 2006). Expression analysis in various *vir* mutants revealed that AS-induced HspL protein accumulation is triggered by the expression of certain *virB* genes (*virB6*, *virB8*, and *virB11*) encoding components of the type IV secretion system (T4SS). Biochemical fractionation analysis suggested that HspL associated with the membranes and co-fractionated with VirB protein complexes extracted with mild detergent. The protein-protein interaction studies using the yeast two-hybrid system, co-purification in *E. coli* and *A. tumefaciens* indicated that HspL interacted directly with VirB8, a key assembly factor of the T4SS. All analyzed VirB proteins accumulated at lower levels in the *hspL* deletion mutant early after AS-induction suggesting that HspL may directly impact protein stability of individual VirB proteins or of the T4SS complex. The tumorigenesis efficiency and the VirB/D4-mediated conjugal transfer of an IncQ plasmid RSF1010 derivative between *A. tumefaciens* strains were reduced in the absence of HspL. By protein aggregation prevention assay, we also demonstrated that HspL possesses a chaperone activity. The data presented here demonstrate that VirB-induced small heat-shock protein HspL interacts with VirB8 and suggest that it may function as a VirB chaperone to stabilize VirB proteins and/or the T4SS complex in promoting efficient DNA transfer and tumorigenesis.

Complex interactions among multiple hormone signaling sectors in plant inducible defense

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The major signaling mechanisms for the pattern-triggered immunity (PTI) and the effector-triggered immunity (ETI) are not known due to involvement of poorly characterized signaling pathway(s), functional redundancy among signaling pathways, or both. To test the possibility of functional redundancy we constructed an Arabidopsis quadruple mutant *dde2*/*ein2*/*pad4*/*sid2*. The level of PTI was tested using microbe-associated molecular pattern (MAMP) –induced resistance against *Pseudomonas syringae* pv. *tomato* DC3000 (*Pto* DC3000). In the quadruple mutant, ~80% and ~45% of flg22- and elf18-induced resistance, respectively, was lost. In the quadruple mutant, ~80%, ~20%, and ~50% of ETI triggered by the effectors AvrRpt2, AvrRpm1, and AvrPphB, respectively, was lost. Furthermore, the quadruple mutant was more susceptible to a necrotrophic fungal pathogen, *Alternaria brassicicola*, than *dde2* or *pad3* single mutants. These results show that the signaling network defined by the four genes is mostly responsible for flg22-PTI and AvrRpt2-ETI and that the signaling network is shared among PTI, ETI, and resistance against a necrotroph. To precisely estimate the effects of single wild-type genes and their interactions, we measured flg22- and elf18-induced resistance, AvrRpt2- and AvrRpm1-ETI, and resistance against *A. brassicicola* in all the possible combinations of the four gene mutations and fitted a mixed general linear model to the obtained data. This analysis demonstrated that each of the four genes can positively contribute to resistance against both biotrophic and necrotrophic pathogens and that functional redundancy among the four genes is the basis of robust AvrRpt2-ETI.

LysM domain-containing proteins in *Medicago truncatula* and recognition of GlcNAc-containing compounds

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Chito- and LipoChito-oligosaccharides (COs and LCOs) are important signaling molecules for pathogenic and symbiotic interactions, respectively. Their recognition involves putative receptors which are membrane-bound proteins with extracellular domains containing Lysin motifs (LysM domains). How, in legumes, these structurally-related LysM-containing proteins might discriminate two structurally-related signals, enabling the plant to distinguish between friends and foes, is a fascinating question. Our main goal is to understand the molecular basis of Nod factor recognition in *Medicago truncatula* by studying the binding of LCOs to NFP and LYK3, their putative receptors, in comparison with LYM1 and LYM2, which are possible orthologs of the rice chitin-binding protein CEBiP (Kaku et al. 2006). Different strategies to generate recombinant versions of the extracellular LysM domains have been set up, including the production of recombinant proteins in *E. coli*. Surface Plasmon Resonance (Biacore technology) and radioligand binding assays have been performed to search for a physical interaction of the recombinant proteins with LCOs. The interaction data will be presented and discussed in comparison to molecular modeling studies of individual LysM domains. Moreover, data will be presented on the spatial and sub-cellular localisation of LYM2 and discussed with respect to the potential biological role of this protein. This work was funded in part by the French Agence National de la Recherche (contract ANR-05-BLAN-0243-01 "NodBindsLysM" and contract ANR-08-BLAN-0208-01"Sympasignal").

Phosphate solubilizing microorganisms associated with Phaseolus vulgaris

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Many soil microorganisms are able to transform insoluble forms of phosphorus to a accessible soluable form, contributing to plant nutrition as a plant growth-promoting microorganisms (PGPM). Arbuscular mucorrhiza (AM) is most broadly wide-spread form vegetable microbial interaction. The mushrooms of the type Glomeromycota and majority overland plants participate in its formation. In base of the relations between macro and micro symbiont lies exchange a product metabolism, as a result which mushroom gets the carbohydrates, but plant get phosphorus and other elements. (AM) fungi of the unique phylum Glomeromycetes are obligate biotrophs that live symbiotical within plant roots. This ancient symbiosis confers benefits directly. We have isolated (AM) from root haires of legume plants and studies their symbiosis with plants Phaseolus vulgaris. All the experimental conditions were controlled in laboratory (Green-House) and experimental territory of the institute of Genetics. Were are screening new symbiotical mutants to beans, studied their genetic and morphological analysises, is revealled stage of the development nitrogen fixation and endomycorrhizal symbiosis.

PAMP perception mediates recruitment of the *Arabidopsis* PEN3 ABC transporter to sites of pathogen detection and activation of PEN3 defense function

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The *Arabidopsis* PEN3 ABC transporter is required for full penetration resistance to the barley powdery mildew *Blumeria graminis* f. sp. *hordei* (*Bgh*) and also participates in resistance to the nectrotrophic fungus *Plectosphaerella*

cucumerina and the oomycete *Phytophthora infestans* (Stein et al., 2006). PEN3 resides in the plasma membrane and is recruited to sites of attempted penetration by invading fungal appressoria (Stein et al., 2006). We report that recruitment of the PEN3 transporter to sites of cell wall reinforcement, known as papillae, is triggered by perception of PAMPs such as flagellin and chitin and requires the corresponding pattern recognition receptors (PRRs). Recruitment of PEN3, but not the PEN1 syntaxin, to sites of papilla deposition requires intact actin filaments, but is not affected by inhibitors of microtubule polymerization or vesicle trafficking. Additionally, PAMP-induced recruitment of PEN3 to sites of papilla deposition is unaltered in the presence of inhibitors of PRR endocytosis and downstream signaling such as wortmannin and K252A or in the absence of the BAK1 kinase that interacts with FLS2 and is required for full responsiveness to flagellin. PEN3 was previously found to be phosphorylated in response to PAMP perception (Benschop et al., 2007; Nuhse et al., 2004; Nuhse et al., 2007). We generated alanine substitution variants at sites of PAMP-induced PEN3 phosphorylation and found that phosphorylation of PEN3 is required for its defense function, but does not affect recruitment of the transporter to sites of papilla deposition. Current work is aimed toward understanding the mechanisms through which PRRs initiate recruitment of PEN3 and other defense proteins to sites of papilla deposition and elucidating the significance of PEN3 phosphorylation for its defense function.

Unraveling the role of the plant DNA repair machinery in the T-DNA integration process

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During *Agrobacterium*-mediated plant transformation (AGMT), the molecular machinery within the plant cell that carries out the T-DNA integration is now receiving in-depth scientific because of its importance. Studies in yeast have identified the involvement of the Non-Homologous End Joining (NHEJ) pathway in T-DNA integration. In plants, the requirement of *Histone H2A*, *Histone H3, KU80, VIP2* and chromatin associated proteins during T-DNA integration has been established. *KU80* gene is the only one from the NHEJ pathway that has been well studied. Using virus-induced gene silencing (VIGS) in *Nicotiana benthamiana* and Arabidopsis mutants, we characterized other components of NHEJ pathway for their role during AGMT. Here we present data for the role of one of the key genes of the NHEJ pathway that encodes X-Ray Cross Complementing protein 4 (XRCC4) in T-DNA integration. VIGS of the *XRCC4* gene in *N. benthamiana* showed an increase in stable transformation. Since an exon knockout of *XRCC4* is not available in *A. thaliana*, over-expression lines were generated and shown to be highly deficient in transformation. We also show for the first time an interaction of the *Agrobacterium tumefaciens* VirE2 protein with XRCC4 both *in vitro* and *in planta*. Studies on RNAi lines of *XRCC4* and mutants of other NHEJ components will be presented.

Molecular mechanism behind cross-talk between salicylic acid- and jasmonic acid-dependent defense pathways in Arabidopsis

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The signaling molecule salicylic acid (SA) plays an important role in plant defense against biotrophic pathogens, while the plant hormone jasmonic acid (JA) is implicated in protection against attack by necrotrophic pathogens and insects. The SA and JA signaling pathway can cross-communicate to fine-tune the plant's defense reaction in response to the type of invader that is encountered. In Arabidopsis thaliana, SA was observed to repress expression of JA-responsive genes, among which PDF1.2 and VSP2 (Spoel et al., 2003: Plant Cell 15:760-770). Here, we aim to unravel how SA can exert its antagonistic effect on JA-responsive gene expression and where in the JA signaling pathway SA exerts its antagonistic action. CORONATINE INSENSITIVE 1 (COI1) is an essential component in the JA signaling pathway. We found that plants mutated in COI1 were still able to show SA-mediated suppression of JA-responsive genes, suggesting that SA targets the JA signaling pathway downstream of COI1. Promoter analysis of JA-induced genes that are suppressed by SA revealed an overrepresentation of the GCC box in their promoters. In addition, using plants that carry the GUS reporter gene under control of the TATA box and four copies of the GCC box, we found that the GCC box is a sufficient element for SA-induced suppression of JA-induced gene expression. AP2/ERF-domain proteins form a family of transcription factors that are able to bind the GCC box. The AP2/ERF transcription factors ORA59 and ERF1 function as important activators of the JA responsive gene PDF1.2. We speculate that SA can repress JA-responsive genes via interference with the function of ORA59 and/or ERF1.

Transcriptional regulators of rhizobacteria-induced priming for defense

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Arabidopsis develops broad-spectrum disease resistance in response to root-colonization by nonpathogenic Pseudomonas fluorescens WCS417r bacteria. This induced systemicresistance (ISR) is based on priming of the innate immune system, which results in an augmented response to subsequent pathogen attack. To study a putative role of transcription factors (TFs) in WCS417r-mediated priming, we performed a qPCR-based genome-wide screen for WCS417r-responsive TF genes. This screen demonstrated that WCS417r induced TF genes that have been related to the regulation of jasmonate (JA)-dependent defenses, such as ERF1 and MYC2. Interestingly, promoter analysis of WCS417r-primed genes also pointed to a regulating role of MYC2. The concerning promoter regions were enriched for the cis-element CACATG, which serves as a docking site for MYC2. Moreover, ISR was abolished in mutants that were disrupted in MYC2, demonstrating a necessity of MYC2 in ISR. These data suggest that JA-related TFs, amongst which MYC2, accumulate upon WCS417r-treatment and amplify pathogen-triggered defense-gene expression. Remarkably, WCS417r also renders Arabidopsis more resistant to the biotrophic oomycete Hyaloperonospora arabidopsis, though JA-regulated defenses are not effective against this pathogen. Microscopical analysis indicated that WCS417r-treatment leads to a higher incidence of impenetrable callose-rich papillae at H. arabidopsis infection sites. Previously, priming of this response by beta-aminobutyric acid (BABA) was shown to be abolished in impaired in BABA-induced sterility 2 (ibs2) and ibs3 mutants. A similar assay demonstrated that IBS2 and IBS3 are also compulsory for ISR against H. arabidopsidis, indicating partial overlap in the signaling pathways underlying both priming phenomena.

Priming Arabidopsis innate immunity

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Specific environmental stimuli can prime the plant's innate immune system to express an augmented defence reaction upon subsequent pathogen attack. Application of the xenobiotic compound beta-aminobutyric acid (BABA) mimics various forms of biologically induced priming. For instance, BABA primes salicylic acid (SA)-inducible defenses causing augmented activation of the *PR-1* gene after subsequent pathogen attack. This BABA-induced priming of *PR-1* is associated with enhanced expression of 22 WRKY genes, of which the majority requires the defence regulatory protein NPR1. Promoter analysis identified a putative *cis*-element that is strongly over-represented in BABA-inducible, NPR1-dependent *WRKY* genes. BABA also triggers enhanced acetylation of histone H3 at the WRKY-binding W-box region of the *PR-1* promoter. This priming-related chromatin remodeling functions independently of NPR1 and does not target promoters of the jasmonic acid (JA)-inducible *PDF1.2* gene or the constitutively expressed *GAPDH* gene. Hence, BABA-induced priming of the *PR-1* gene is associated with NPR1-dependent induction of *WRKY* genes and NPR1-independent chromatin remodeling of the *PR-1* promoter. Apart from priming of SA-dependent defenses, BABA can also prime SA-independent defense mechanisms, such as deposition of callose-rich papillae upon infection by fungi or oomycetes. This priming of cell wall defense requires intact abscisic acid (ABA) signaling. A mutagenesis screen in the background of SA non-accumulating NahG identified in the *impaired in BABA-induced immunity 1* (*ibi1*) mutant. Subsequent characterization of this mutant revealed involvement of light-dependent ROS signaling in ABA-dependent priming of cell wall defense.

The *Cladosporium fulvum* effector proteins Avr4E and Avr9 are genuine virulence factors

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Cladosporium fulvum (syn. *Passalora fulva*) is a biotrophic fungal pathogen that causes tomato leaf mold. To establish a successful infection, *C. fulvum* secretes effectors during colonization of the host, ten of which have been characterized. For the majority of these characterized effectors, cognate *C.*

fulvum (Cf) resistance loci that mediate resistance (so-called effector-triggered immunity) have been identified in tomato. In absence of Cf-mediated recognition, the *C. fulvum* effectors are thought to play a role in pathogen virulence and previous studies have indeed shown that three of these effectors, Avr2, Avr4 and Ecp6, contribute significantly to *C. fulvum* virulence. We now demonstrate that two additional effector proteins, Avr4E and Avr9, are similarly required for full pathogen virulence. Heterologous expression of *Avr4E* or *Avr9* in tomato results in enhanced colonization of *C. fulvum* strains that lack these effectors. Moreover, Avr4E-producing tomato displays enhanced susceptibility to the vascular wilt pathogen *Verticillium dahliae*. Our results show that Avr4E and Avr9 are potent effectors and strengthen the hypothesis that all *C. fulvum effector* proteins are genuine virulence factors.

Quantitative analysis of the Arabidopsis defense proteome and phospho-proteome

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The Arabidopsis-*Pseudomonas syringae* pv. *tomato* (*Pto*) interaction is an intensively studied model system for plant immunity. Mutant hunts and gene expression profiling have driven the field to a new plateau of understanding. However, many gaps remain in our understanding of how resistance (R)-proteins work. Signaling partners of R-proteins that are lethal if mutated or are genetically redundant will not be recovered in mutant screens. Microarray studies cannot detect the primary signaling events that ultimately give rise to transcriptional changes. To complement these approaches, we use sensitive proteomics methods using LC-MS/MS to identify proteins whose properties are regulated by immune signaling. We make use of the conditional avrRpm1 effector expression system to induce immune signaling through the R-protein RPM1 in adult plants. After the first (phospho-)proteome analysis from 3 replicates and 2 time-points, we identified approximately 4600 proteins and 1900 phospho-peptides (corresponding to 900 modified proteins), of which 330 and 220, respectively, were reproducibly changed in level. Our study confirms several changes observed at transcript level, and identifies regulation of proteins known to be involved in immune signaling like TRX5, CML24, PEN1 and PEN3. Novel exciting findings include changes in (phosphorylation-)levels of proteasome-related proteins, calcium/calmodulin binding proteins, kinases, phosphatases and transcription factors. Besides functional analysis of these proteins, we continue proteomics based analysis of the Arabidopsis immune system by parsing signaling pathways using mutant backgrounds, and we are building an interactome by identification of protein complexes after immuno-precipitations with various proteins around the RPM1 module.

Non-host resistance against *Phytophthora capsici* is mediated by both R gene and basal defense mechanisms

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Non-host resistance is a form of plant immunity in which all accessions or cultivars of a plant species are resistant to all strains of a pathogen species. *Phytophthora capsici* is a soil-borne oomycete that causes Phytophthora blight disease in many solanaceous and cucurbit plants worldwide. Interest on *P. capsici* has increased considerably in the last two years with the sequencing of its genome. Yet, molecular interactions between *P. capsici* and its host have been poorly studied, and more remarkably, single gene resistance against *P. capsici* has not been reported to date. In this work, we have tested a number of *Nicotiana* species for their ability to recognize the PcAvr3a protein. We found that several *Nicotiana* species tested responded to PcAvr3a with an HR, suggesting recognition by an endogenous R gene. Infection assays demonstrated that recognition of PcAvr3a correlates with resistant to *P. capsici*, suggesting an avirulence function for PcAvr3a. A strategy to silence P. capsici genes by RNAi is currently in progress to further study the role of PcAvr3a and other candidate genes involved in resistance/pathogenesis. In addition, to identify genes important for disease resistance to *P. capsici*, we used VIGS of candidate defense genes in *Nicotiana* spp. Susceptibility to *P. capsici* was enhanced when the multigene family of a NB-LRR R gene, *I2/R3a*, was silenced in *N. edwardsonii*. Silencing of two other components of the resistance signaling pathway in plants, *Eds1* and *Sgt1*, also enhanced susceptibility to *P. capsici* in *Nicotiana*. Furthermore, salicylic acid was found to be important for defense against *P. capsici* by using *Arabidopsis* mutant lines, suggesting that non-host resistance to this pathogen may involve different pathways and mechanisms of resistance.

Identification of *Nicotiana benthamiana* genes involved in PAMP-triggered immunity

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Plants have evolved elaborate mechanisms to defend themselves against disease-causing microbes they encounter in the environment. The first line of defense is triggered by the recognition of structurally conserved pathogen or microbial-associated molecular patterns (PAMPs or MAMPs) by pattern recognition receptors (PRRs), which leads to PAMP-triggered immunity (PTI). Important features of PTI include callose deposition at the cell wall, generation of reactive oxygen species (ROS), activation of mitogen-activated protein kinase (MAPK) cascades, changes in intracellular calcium concentration and the activation of defense related genes. In order to identify genes involved in PTI, we used a virus-induced gene silencing (VIGS) approach to silence over 3,000 genes in *N. benthamiana*, which is increasingly being used as a model system to study plant-pathogen interactions. A cell death-based assay performed on silenced plants was used to select for genes that may play a role in PTI. Seven candidate genes were obtained from the screen, and several methods were used to examine their possible role in PTI and to place them in known PAMP-PRR signaling pathways (see also poster by S. Chakravarthy). Results from the screen and possible functions of the candidate genes in different aspects of PTI will be presented.

Localization and cross-complementation studies with *Medicago truncatula* DMI1 (Does not Make Infections 1) and its homologs in *Lotus japonicus*

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Medicago truncatula DMI1 (Does not Make Infections 1) and its homologs in *L. japonicus,* CASTOR and POLLUX, play a crucial role in early symbiotic signaling leading to establishment of legume nodulation and arbuscular mycorrhization. Although, these cation channels have been localized to the nuclear envelope, the precise localization at nuclear membrane level was not known. Transmission electron microscopy on MtDMI1::GFP expressing roots using high pressure freezing and cryo-substitution technique demonstrates that DMI1 preferentially localizes on inner nuclear membrane over the outer nuclear membrane. A DMI1 paralog, MtCASTOR was cloned and characterized for its role in root endo-symbioses using RNA interference and complementation studies. We show that MtCASTOR localizes to nuclear envelope like its homologs, but does not seem to play a major role in legume nodulation and arbuscular mycorrhization. Pairwise split-ubiquitin assay and bimolecular fluorescence complementation (BiFC) assay suggest that DMI1 and MtCASTOR form homo-multimers. Cross-species complementation analyses suggest that MtDMI1 is able to fully complement *castor, pollux* mutants as well as *castor/pollux* double mutants of *L. japonicus*. Whereas LjCASTOR or LjPOLLUX alone or together when expressed under the influence of strong promoters or native promoters are unable to restore *dmi1* mutation in *M. truncatula*. Interestingly, gain of function mutations on key residues in the pore region of LjPOLLUX enable to fully complement *dmi1* in *M. truncatula* and a *castor/pollux* double mutant in *L. japonicus*. Thus, we demonstrate that not the expression levels but key residues in the pore region govern the functionality of these ion channels.

Symbiotic methylobacteria influence strawberry flavour

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Strawberry flavour is composed of more than 350 different chemical substances. Amongst them, two furanoid compounds 2,5-dimethyl-4-hydroxy-

2H-furanone (DMHF) and 2,5-dimethyl-4-methoxy-*2H*-furanone (DMMF) play an important role. In literature different biosynthetic pathways for these compounds are discussed. A symbiotic interaction of methylotrophic bacteria with callus cultures of strawberry has been supposed to lead to an increased biosynthesis of DMHF and DMMF in plant cells. In order to prove the influence of methylotrophic bacteria on the biosynthesis of furanoid compounds in strawberries and to enhance the sensory properties of the produced fruits, two selected strains, *Methylobacterium extorquens* DSM 21961 and *Methylobacterium mesophilicium* DSM 21962, were applied on strawberry plants in greenhouse and in field studies. The abundance of the applied bacteria on leaves was monitored over a period of several weeks using a specific quantification system based on a real time PCR. On the other hand, a *gfp*-tagged strain of Methylobacterium extorquens was used to investigate colonization patterns of the bacterial cells on plant leafs in detail under a confocal laser scanning microscope. The produced fruits were analyzed regarding their concentrations of DMHF and DMMF by GC-MS. Additionally the overall flavour of the fruits was evaluated by a highly trained sensory test panel. The results of the current investigations indicate an enduring colonization of strawberry plants by methylobacteria and an influence on strawberry flavour. Fruits of treated and untreated plants significantly differed in content of DMHF and DMMF and could be distinguished by the sensory test panel. Based on these results further experiments in greenhouse and in larger scale on agricultural areas are planned.

NSP1 and NSP2 proteins: Two GRAS proteins allowing coordination of epidermal and cortical programs during symbiosis in *Medicago truncatula*

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Legumes establish symbiosis with bacteria, called Rhizobia. During this interaction, a new organ, the nodule is formed. Plant perception of the bacterial signalling molecule Nod factor is sufficient to initiate nodulation in the root cortex. It is unlikely that Nod factor can freely diffuse into cortical cells. This implies that Nod factor perception at the epidermis activates a developmental process in the cortex via a diffusible factor that links epidermal and cortical programs. Perception of Nod factor leads to the activation of calcium oscillations which are likely decoded by CCaMK, whose function requires two GRAS transcription factors, NSP1 and NSP2. Moreover, activation of a cytokinin receptor (CRE1/LHK1) alone is sufficient for nodule formation and also requires NSP1 and NSP2. This implies a dual role for these proteins functioning downstream of CCaMK in the epidermis and downstream of CRE1/LHK1 in the cortex. To explore *NSP1* and *NSP2* roles in these two programs several approaches are being developed. First, we are studying their expression profiles and characterizing specific promoters of epidermal and cortical root cells to explore their function in both tissues. Recently, *ENOD11*, *ERN1* and *NIN* have been identified as NSP1 targets in response to Nod factor treatment (Hirsch et al., 2009). We are now looking if those targets and additional targets are bound after cytokinin treatment. We are also looking at possible interactions between NSP1 and NSP2 and the cytokinin pathway. Finally, a suppressor screen of *nsp2* is in progress to identify new components of these developmental processes. These analyses should reveal how NSP1 and NSP2 could participate to the coordination of epidermal and cortical programs leading to nodule formation.

Hexanoic acid-induced resistance is effective against *Pseudomonas syringae* in tomato plants

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In addition of basal resistance, plants are able to develop an enhanced state of resistance so called indicer resistance (IR). This state of resistance can be triggered upon appropiate stimulation. We have demostrated that tomato plants treated with hexanoic acid displayed enhanced resistance against *Pseudomonas syringae* (Pst) DC3000 strain. After 72hpi hexanoic acid treatment reduced the disease rate and the colony forming units in infected plants. To establish the implication of SA-, J-, ET- and ABA- signaling pathways in the hexanoic acid-induced resistance, the expression of *PR1*, *LoxD*, *ACCOx* and *ASR1* marker genes was analyzed by RT-quantitative PCR. A significant increase of JA marker genes was observed at early stage of infection (up to 48hpi). Hormone analysis showed that hexanoic acid induced a faster and stronger accumulation of OPDA, a JA precursor upon infection. These results addressed the question about the involvement of JA pathway in the hexanoic acid-induced resistance against Pst. The present data contribute to the knowledge of induced resistance in tomato and supports the use of hexanoic acid as a crop protection strategy based on natural compounds.

Functional characterization of Meloidogyne incognita nematode effectors

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The Southern root-knot nematode Meloidogyne incognita is one the most damaging plant pathogen worldwide. Its potential host range encompasses more than 2,000 plant species. This obligate root parasite establishes an intimate relationship with its host plants, inducing the redifferentiation of root cells into specialized feeding cells. The successful development of feeding cells is essential for the growth and reproduction of the nematode. The induction and maintenance of the giant cells result from the injection of nematode effectors into the plant cells. Global expression analysis of the host response showed that the successful establishment of the nematode is associated with the suppression of plant defence responses (Jammes et al., 2005). The importance of plant defence suppression during plant-pathogen interactions has been highlighted in recent studies on bacteria, fungi and oomycetes. Successful pathogens seem to have evolved specialized strategies to manipulate the response of host plants. In contrast to bacteria, little is known about the identity and function of the effectors of nematodes. There is increasing evidence that a substantial portion of the effector proteins secreted by plant parasitic nematodes acts inside the host cell, for example in the cytoplasm or in the nucleus (Huang et al., 2006; Elling et al., 2007). Several effectors from M. incognita have been identified by proteomic and transcriptomic approaches that could regulate defence or host cell development via protein-protein interactions. Among the characterized nematode effectors, some have orthologs in animal parasites. In this project we are analyzing the role of selected nematode effectors in the manipulation of signaling pathways leading to plant susceptibility and giant cell induction.

Molecular factors affecting fumonisin biosynthesis in *Fusarium verticillioides*: A study on *FUM1* putative promoter

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Fumonisins are mycotoxins -produced mainly by *Gibberella moniliformis* (anamorph *Fusarium verticillioides*)- that contaminate maize and maize-based products and cause great concern for human and animal health. Several biosynthetic (*FUM*) genes are known; among them, *FUM1* encodes a key polyketide synthase. To study the genetic and environmental factors linked to fumonisin production, we amplified the putative *FUM1* promoter (pFUM1; about 1000 bp upstream of the start codogen) from a toxigenic *F. verticillioides* (Fv) strain. pFUM1 was used to generate transgenic Fv strains expressing the N-terminus of FUM1 translationally fused to GFP under the control of pFUM1. Currently, pFUM1-driven transcriptional activity is being tested in fumonisin-inducing and non-inducing conditions, to estimate the degree of correlation between the fluorescence observed and transgene transcription (quantified by RT-qPCR); and between the latter and transcription of the endogenous *FUM1*. If correlations turn out to be acceptable, these transgenic strains could be used as indicators of fumonisin production at the single-cell level during plant infection and tissue colonization. Moreover, bioinformatic analyses on the putative promoter sequences of all known FUM genes suggest the presence of a statistically over-represented, 6-bp sequence; this motif could cis-act on the regulation of *FUM1* transcription, since it is repeated twice in pFUM1. At present, we are generating a synthetic pFUM1 version where this motif is mutated, and preparing promoter-reporter constructs as above. The analysis (by fluorescence microscopy and RT-qPCR) of the corresponding Fv transformants will allow an immediate comparison with the activity of the Wt promoter, and thus help us to validate the bioinformatic predictions.

ENHANCED DISEASE SUSCEPTIBILITY1-dependent systemic acquired resistance signaling

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Immunity in plants is based in part on recognition of pathogen effector molecules by corresponding plant RESISTANCE (R) gene products. This type of response is called effector triggered immunity (ETI) and is accompanied by the induction of a long-lasting broad spectrum defense, systemic acquired resistance (SAR), in remaining healthy tissues of the plant. ENHANCED DISEASE SUSCEPTIBILITY1 (EDS1) is a lipase-like protein that, together with its homologs and interaction partners PAD4 and SAG101, is required for basal resistance and ETI in response to certain R proteins (TIR type). Although ETI in response to CC-type R proteins is completely intact in *eds1* mutant plants, SAR is abolished. We show that EDS1 is required for SAR signal perception/amplification in the systemic tissue, but also for SAR signal generation/transmission from tissue undergoing ETI downstream from activation of the CC-type R protein RPM1. Thus, petiole exudates from infected wild type (wt) leaves induced expression of the defense gene *PR1* in naive wt plants, but not in the *eds1* mutant. Conversely, petiole exudates from infected *eds1* leaves did not induce *PR1* expression in naive wt plants. Since *eds1* plants do not send an SAR signal in spite of the execution of a fully active ETI response, we set out to map the SAR signal signature in wt compared to *eds1* tissue conditionally expressing the bacterial effector AvrRpm1 that activates RPM1. We have investigated protein and small molecule profiles in apoplast extracts from these tissues, and potential new *EDS1*-dependent SAR signaling components (and their role in pathogen defense in plants) will be discussed.

Enhancement of the wheat defence response to fungal pathogens by modifying the pectin component of the cell wall

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In several plant-pathogen interactions, the plant cell wall represents the main barrier to penetration and/or colonization of the host tissue. Because of this, its reinforcement should increase plant resistance. One way to reach this goal and test this hypothesis is the modification of the pectin component of the cell wall. Pectin is secreted in a highly methylesterified form and is demethylesterified *in muro* by pectin methylesterase (PME). The activity of PME is regulated by specific protein inhibitors (PMEIs). Since highly methylesterified pectin can be less susceptible to hydrolysis by enzymes such as fungal endo-polygalacturonases (endo-PGs), the inhibition of endogenous PME by PMEI might increase pectin resistance to degradation by fungal PGs. In order to verify this possibility in wheat, a number of wheat lines expressing the pectin methylesterase inhibitors AcPMEI (from *Actinidia chinensis*) has been produced. This inhibitor is active on endogenous PME and transgenic lines showed a reduced PME activity. No obvious phenotypic differences between the transgenic lines and the wild type plants have been observed, however, the degree of methylation is higher in the transgenic compared to the wild type plants. Moreover, transgenic tissue is more resistant to digestion by fungal PGs and transgenic plants showed a significant reduction of symptoms following the infection with the fungal pathogen *Bipolaris sorokiniana*. Infection experiments with the floral pathogen *Fusarium graminearum* are also in progress.

Combining metabolomics and genomics – Elucidating the function of the flavin monooxygenase BS3 in plants and protozoa

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The pepper Bs3 resistance gene mediates recognition of *Xanthomonas* strains that express the matching effector protein AvrBs3. The transcription-factor-like AvrBs3 protein binds and transcriptionally activates the Bs3 gene. Bs3 encodes a structurally atypical flavin monooxygenase (FMO) that upon expression triggers cell death. Expression of Bs3 in yeast causes growth arrest whereas *Escherichia coli* seems to be unaffected by this particular type of FMO. In this project tools originating from the genomics and metabolomics trade are combined to elucidate the biochemical basis of BS3 function. In the last years our group has established a reliable LC-MS platform for the analysis of various developmental and stress-induced changes as well as for the biochemical phenotyping of mutants in *Arabidopsis thaliana* and crop plants. The UPLC-ESI-QTOF-mass spectrometer combination was used to find possible substrates, products or product intermediates of the Bs3-protein in plants, yeast and prokaryotes. Preliminary results suggest that the Bs3 protein is involved in the primary metabolism. Most recent results on the metabolite-based analysis of Bs3 will be presented.

Endocytosis plays an important role in *Cf-4*-mediated resistance of tomato to *Cladosporium fulvum*

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The *Cf-4/Avr4*-gene-for-gene pair of tomato and the pathogenic fungus *Cladosporium fulvum*, respectively, is responsible for an incompatible interaction between host and pathogen. The Cf-4 protein is a trans-membrane, receptor-like protein. Upon perception of the *C. fulvum* effector Avr4, its short intracellular domain which lacks a clear signaling signature, is thought to participate in the initiation of a signal transduction cascade eventually leading to resistance. Interestingly, the cytoplasmic C-terminus of Cf-4 contains a putative YxxΦ motif (YpaW), which is a sorting signal that binds to clathrin-associated proteins and therefore might serve as an endocytosis signal. By site-directed mutagenesis we show that the integrity of this motif is required for full functionality of the Cf-4 protein. The clathrin-associated proteins are so-called adaptor-protein (AP) complexes that mediate the recruitment of clathrin to membranes, thereby initiating endocytosis, and bind to the sorting signal. This binding occurs through the medium subunit of the AP complex, referred to as μ-adaptin. Indeed, yeast two-hybrid studies revealed that the C-terminus of Cf-4 interacts with tomato μ-adaptins. Furthermore, silencing of genes encoding these μ-adaptins results in loss of full *Cf-4*-mediated HR and resistance to *C. fulvum*. These results suggest that endocytosis plays an important role in *Cf-4*-mediated resistance. Current investigations aim to further clarify the role of the putative endocytosis signal in Cf-4 function. Furthermore, we want to identify all tomato μ-adaptins and test them for interaction with Cf-4 in yeast two-hybrid screens. Interestingly, the Cf-2 and Cf-5 proteins appear to lack this sorting signal in their cytoplasmic part and therefore these will be included as controls as they are expected not to interact with the adaptins.

OsRap2.6 is a candidate of downstream effector of OsRac1 in rice innate immunity

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Plants use two innate immune systems to respond to pathogen infection. OsRac1 is localized in the plasma membrane and is involved in various defense related functions. OsRac1 interacts with RACK1 and plays an important role in plant defense (Nakashima et al. 2008). In a yeast two hybrid screening, we identified OsRap2.6 as one of the interactors of RACK1. OsRap2.6 is a transcription factor with an ERF domain which has sequence homology with Arabidopsis 2.6 (AtRap2.6). The ERF domain binds specifically to the GCC box. Modified Yellow Fluorescence Protein (YFP-fusion protein) showed that OsRap2.6 is located in the nucleus and the cytosol in rice protoplasts. OsRap2.6 interacted with Constitutively Active (CA) and Dominant Negative (DN) form of OsRac1. The next challenge is to examine how OsRap2.6 interacts with OsRac1 which is located in the plasma membrane. It's important to find out the actual role of OsRap2.6 in defense response and where OsRap2.6 is located in the OsRac1 defense pathway. The key issue will be to find out whether OsRap2.6 binds with RACK1A in the OsRac1 immune complex. This and other results will be presented.

A gain-of-function mutation reveals a novel role of the phosphate transporter gene *SUP3* in plant innate immunity

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A detailed understanding of the defense gene networks is critical in advancing our knowledge on the mechanisms of plant defense. However, it remains

challenging to identify defense regulatory genes and elucidate their functions. Taking advantage of the unique defense-dependent dwarfism conferred by the Arabidopsis mutant *acd6-1*, we developed a genetic screen to identify *acd6-1 suppressor* (*sup*) mutants, which potentially harbor mutations in novel defense genes. Among the genes identified was *SUP3*, encoding a phosphate transporter widely conserved in plants. The *sup3-1* mutant was found to suppress constitutive defense and small size in *acd6-1*. In addition, *sup3-1* was compromised in basal defense against virulent *Pseudomonas syringae* strains. We also found that *sup3-1*-conferred susceptibility could be rescued by exogenous salicylic acid (SA) treatment, suggesting SUP3 acting upstream in SA signaling. Consistent with the role of SUP3 in regulating SA-mediated defense, genetic analysis indicated that *sup3-1* acted additively with several known SA regulators, ALD1, EDS5, and SID2, to affect *acd6-1*-conferred phenotypes. The *sup3-1* mutant is disrupted in the fifth exon of the *SUP3* gene, leading to the accumulation of a shorter transcript. The *sup3-1* mutant is dominant, possibly due to the action of the truncated protein. Transgenic expression of the DNA fragment containing the *SUP3-1* region in the wild type recapitulated *sup3-1*-conferred susceptibility to *Pseudomonas* infection. In addition, introducing extra copies of the full length *SUP3* genomic fragment into the wild type also resulted in enhanced disease susceptibility. These data suggest that SUP3 is a novel negative regulator of basal defense, acting independently of ALD1, EDS5, and SID2 in the SA-mediated defense pathway.

Characterization of the putative type six secretion systems in *Pseudomonas syringae* pv. *tomato* DC3000

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Gram-negative bacteria possess at least six distinct secretion systems to export proteins to the environment or deliver effectors into host cells in response to different stimuli. In search of the completed genome sequence of *Pseudomonas syringae* pv. *tomato* (*Pst*) strain DC3000, two operons which contain genes homologous to those of the recently identified type VI secretion systems were identified. The biological functions of these gene clusters in the plant pathogens, including *Pst* DC3000, are still unknown. Therefore, in this study, mutants deleted of *icmF* homologs are generated and their effects on growth, motility, virulence and secretion of possible substrates are investigated.

Membrane associated stigmasterol plays an important role in plant innate immunity

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Plant nonhost resistance, a form of innate immunity, is the most common form of disease resistance exhibited by plants against the majority of potential pathogens in nature. We used virus-induced gene silencing in *Nicotiana benthamiana* to identify genes involved in nonhost resistance. Eleven genes were identified to be involved in type I and/or type II nonhost resistances by individually silencing ~4,000 genes from a normalized NbcDNA library. One of them encodes squalene synthase (SQS), a key enzyme catalyzing the first enzymatic step in sterol biosynthesis. Silencing *SQS* gene in *N. benthamiana* caused plant cell membrane leakage resulting in more nutrient accumulation in the apoplast. The Arabidopsis *SQS1* RNAi lines were not only susceptible to nonhost pathogens, *Pseudomonas syringae* pv. *tabaci* and *P. syringae* pv. *syringae*, but also more susceptible to virulent pathogens, *P. syringae* pv. *tomato* DC3000 and *P. syringae* pv. *maculicola*, when compared with the wild-type Arabidopsis. We also discovered that a mutation in Arabidopsis *SMT2*, a gene encoding sterol methyltransferase (downstream enzyme in phytosterol biosynthesis), compromised nonhost resistance. Metabolite analysis indicated that, compared to the wild-type Arabidopsis, *SQS* RNAi lines and a *smt2* mutant produced less stigmasterol. Strikingly, the gene *AtCYP710A1* converting sitosterol to stigmasterol was dramatically induced and stigmasterol was significantly increased in wild-type plants upon inoculation with nonhost pathogens. Arabidopsis *cyp710a1* mutant and overexpressors of *AtCYP710A1* are currently being characterized and the results will be presented. Our data suggest that cell membrane components especially membrane associated stigmasterol plays an important role in plant innate immunity against bacterial infections.

Development of targeted gene knock-out system for *Grosmannia clavigera* using homologous recombination

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The ecological and economic sustainability of forests are increasingly threatened by native and alien (invasive) insect pests that can carry diverse fungal pathogens, as illustrated by the current mountain pine beetle (MPB) epidemic in western Canada. The MPB-vectored fungi (e.g. *Grosmannia clavigera*) rapidly colonize the tree phloem and sapwood of pine host trees, and kill the tree by blocking water transport to the crown. As well, the pathogenic fungi stain the tree sapwood, reducing the industrial value of the wood and wood fibres. The wood discoloration is caused by fungal melanin that is synthesized through the dihydroxynaphthalene (DHN) pathway. We built full deletion cassettes for two genes of the pathway, polyketide synthase (PKS, 6.5kb) and scytalone dehydratase (SD, 0.6kb), by first extracting their sequences from our the *G. clavigera* draft genome sequence recently developed in our program. The cassettes included 1.5 to 3 kb of flanking DNA sequence from each open reading frame (ORFs), and the hygromycin resistance gene. We assembled the cassettes in a yeast recombination system and used *Agrobacterium*-mediated transformation (AMT) to transform *G. clavigera*. To increase the homologous AMT recombination efficiency, we also generated split-marker fragments. We will discuss the efficiency and stability of the different transformation methodologies.

The Arabidopsis CBP60g and h proteins define a critical node in salicylic acid signaling

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We have studied two closely-related members of the Arabidopsis calmodulin-binding protein 60 (CBP60) gene family, designated g and h. CBP60g is a bona fide calmodulin (CaM)-binding protein, but CBP60h lacks a CaM binding site and does not bind CaM. Plants with *cbp60g* mutations have a defect in MAMP-induced salicylic acid (SA) production at nine hours after challenge, and show enhanced disease susceptibility (eds) to *Pseudomonas syringae* pv. *maculicola* strain *Psm* ES4326. However, SA levels 24 hours after *Psm* ES4326 infection are normal. Site-directed mutagenesis experiments showed that CaM binding is required for the functions of CBP60g in SA production and limitation of *Psm* ES4326 growth. Plants with *cbp60h* mutations have a defect in SA production following *Psm* ES4326 infection, and are also eds to *Psm* ES4326. However, SA levels at nine hours after a MAMP challenge are normal. Double *cbp60g,h* mutations cause a severe defect in SA production during a MAMP response, infection by *P. syringae* pv. *tomato* strain *Pst* DC3000 *avrRpt2*, or infection by *Psm* ES4326. They are also severely eds at a level greater than the SA synthesis mutant *sid2*, and comparable to highly pleiotropic *pad4* mutants. Thus, the two genes define a critical and partially redundant function in SA signaling, with CBP60g playing a more important role during a MAMP response, and CBP60h playing a more important role later during pathogen infection. Expression profiling revealed that the *cbp60g,h* double mutation affects all the genes affected by *sid2*, as well as a subset of *SID2*-indpendent genes affected by *pad4*. This analysis places the CBP60g,h node downstream of the PAD4/EDS1 node and upstream of SA synthesis in the plant defense signaling network.

Promoter analysis of 22 new genes affecting RPP4-mediated resistance against downy mildew suggests the existence of a 'lockdown' system

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The battle between pathogen and plants occurs at three fronts—PID (pre-invasion defense) conferred by physical and chemical barriers, PTI (pathogen-associated molecular patterns triggered immunity) initiated upon the recognition of pathogen derived molecules (flagellin, EF-Tu, chitin) and ETI (effector-triggered immunity) mediated by resistance (R) proteins. Recent studies on ETI suggested that R-mediated resistance may involve gene regulation. However R protein associated transcriptional control of defense is yet to be understood. Previously, our expression and phenotype profiling of T-DNA insertion mutant populations identified 22 new components affecting RPP4 mediated resistance against downy mildew. Study of the promoters of these genes after K-means clustering suggests the enrichment of several cis-elements Surprisingly, these elements are also enriched in the promoters of the transcription factors (TFs) that may bind these sites. Additionally, Pearson correlation analysis of the expression profiles of these TFs in leaves indicates that they also have similar expression pattern when challenged by pathogen. Collectively, these data implies that RPP4 rapidly induces defense through a 'lockdown' system in which self-activation and cross activation of TFs help amplify the signal. Besides the explosive pattern, mathematical simulations of

simple 'lockdown' system suggest the possible existence of a bi-stable or even multi-stable situation in some parameter spaces, which help explain the elaborate spatial and temporal regulation of ETI.

Determining the virulence function of the HopAF1 (HopPtoJ) family of *P. syringae* type III effectors

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Many plant pathogens encode the type III secretion system for translocating effector proteins into the host during infection. Strains of *Pseudomonas syringae*, our plant bacteria model system, which are not capable of delivering the type III effectors are nonpathogenic. Therefore, the functions of type III effectors are essential for disease. However, the biochemical functions of type III effectors during disease manifestation are largely unknown. Our lab is interested in HopAF1 (previously known as HopPtoJ), a confirmed type III effector that is widely distributed in at least nine strains of *P. syringae*. Previously, no function in virulence has been associated with HopAF1, and no disease resistance gene that recognizes it has been defined in any plant species. Based on secondary structure prediction, we suggest that HopAF1 has deamidase activity. Deamidation is required for the virulence activity of bacterial toxin Cytotoxic Necrotizing Factor 1 from pathogenic strains of *E. coli*. HopAF1 also contains the consensus sequence for acylation at the N-terminus. Preliminary data gathered via confocal microscopy of HopAF1 expressed *in planta* supports the prediction of localization at the plasma membrane. Additionally, using yeast two-hybrid screens we have identified plant fibrillins and methylthioadenosine nucleosidases as possible host cellular targets. We propose that HopAF1 family members use deamidation to regulate plant host cell factors to enhance virulence. We will continue to use microscopy, biochemistry, and pathology assays to determine the role of HopAF1 in plant disease. Our research will improve the understanding of molecular mechanisms by which type III effectors cause disease.

Virulence of *Fusarium sulphureum* MRC514 with altered trichothecene profile on potato cultivars: A role for toxin inactivation by glucoside formation in plant resistance

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Fusarium sambucinum causes dry rot of potatoes and produces type A trichothecenes. Despite the high toxicity and large amounts of toxins produced in infected tissue, trichothecene production is generally believed to be irrelevant for virulence on potato, based on the report that a *tri5* gene disruption mutant strain showed unchanged virulence. We have reinvestigated this question using a different diacetoxyscirpenol (DAS) producing strain, MRC514 *F. sulphureum* (= synonym for *sambucinum*). Visual symptoms and ergosterol measurements clearly showed that inactivation of *TRI5* in this strain reduced virulence on 22 tested potato cultivars, which showed a wide variability in the ability to control pathogen spread after wounding of whole tubers. We have also used metabolic engineering to alter the trichothecene profile. DAS production was changed into deoxynivalenol (DON) production by disruption of *TRI13* and introduction of a *F. graminearum TRI1* gene. The resulting *tri13*::*nptII* Fg*Tri1–HygB* DON-producing strain was almost as virulent as wild-type. We have also tried to engineer DON-glucoside formation in this strain by introduction of an *Arabidopsis* glucosyltransferase UGT73C5 gene using a nourseothricin marker. Using LC-MS/MS techniques we observed that potatoes have the ability to detoxify DON into DON-3-glucoside. The molar percentage of inactivated toxin (D3G/(D3G+DON) varied significantly between different cultivars. We propose that quantitative differences in the ability to detoxify the "new" virulence factor DON contributes to differences in the resistance level against the engineered strain.

The M flax-rust resistance protein expressed in *Pichia pastoris* is purified with ADP bound within its nucleotide binding pocket

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Plant disease resistance (R) proteins play a vital role in defending plants against pathogenic attack. M is a flax R protein that confers resistance against the biotrophic flax rust fungus, *Melampsora lini*, and is a member of the most abundant structural class of R proteins, those that contain a nucleotide binding site (NBS) and a domain of leucine-rich repeats (LRR). Proteins within this class have previously been shown to possess the capacity to bind and hydrolyse ATP (Tameling et al., 2002; Tameling et al., 2006 and Ueda et al., 2006), and together with other research this has led to the formulation of a number of models to explain R protein activation. We can express and purify near full-length and truncated variants of M using a *Pichia pastoris* expression system. Analysis of purified monomeric M using a luciferase-based ATP quantification assay has demonstrated that ADP is associated with our purified M protein. Subsequent examination of the non-functional P-loop mutant, where the highly conserved Lysine is exchanged for Leucine (MK286L), shows a significantly reduced ADP binding capacity. The nucleotide preference and binding efficiency of M was not changed when the TIR domain was removed. Of most interest is that when this system was used to analyse a mutant M protein that causes an autoactive HR *in planta*, recombinant protein of this mutant had an altered nucleotide preference. We anticipate that our methods will allow us to uncover residues in and around the NBS that affect the binding and nucleotide preference of the pocket, and thus give us an insight into the possible active and inactive states of this class of R protein.

The potential role of LysM domain-containing proteins in peptidoglycan perception in *Arabidopsis thaliana*

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Pathogen-associated molecular patterns (PAMPs) trigger immune reactions in plants. A prominent example is represented by peptidoglycans (PGNs) that are main constituents of bacterial cell walls. We have shown that PGNs derived from both, Gram-negative and Gram-positive bacteria elicit defense responses in *Arabidopsis thaliana*, including the activation of MAP kinase cascades, the induction of defense related genes, NO production and medium alkalinization (Gust et. al., 2007). In order to identify a corresponding PGN receptor or binding protein in *Arabidopsis*, we have taken a reverse genetic approach. As LysM domains were originally described as PGN binding motifs in bacterial lysins, and as plant LysM proteins have been implicated in fungal chitin perception (Miya et al., 2007; Kaku et al., 2006), we have selected T-DNA insertion lines for genes encoding LysM domain-containing proteins and receptor-like kinases. Screening for altered responses, we identified an insertion line with higher susceptibility for infections with *Pseudomonas syringae* pv. *tomato* DC3000 and decreased induction of defense-related genes following PGN elicitation. The corresponding protein was recombinantly expressed and showed a strong binding to Gram-positive and Gram-negative PGN. Thus, we provide evidence that a LysM domain-containing protein might be involved in the PGN perception machinery of *Arabidopsis thaliana*.

Activation of RacGTPase-mediated defence signalling by pathogen-associated molecular patterns and guanine nucleotide exchange factor in rice

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Previously, we showed that the small GTPase OsRac1 plays a key role in pathogen-associated molecular patterns (PAMPs)-induced immune responses in rice. Overexpression of the constitutively active form of OsRac1 enhances reactive oxygen species production in rice cells treated by PAMPs, such as rice blast fungus–derived N-acetylchitooligosaccharide and CerebrosideA elicitors. To study the temporal dynamics of OsRac1 activation in vivo, we developed an intramolecular fluorescence resonance energy transfer (FRET) biosensor that composed of OsRac1, the CRIB domain of human PAK1, which binds specifically to the GTP-bound form of OsRac1, and variants of the green fluorescent protein, Venus and SECFP, which function as FRET donor and acceptor chromophores, respectively. Using time-lapse FRET microscopy, we found that OsRac1 was rapidly activated by PAMP treatment in rice protoplasts transiently transformed with the FRET biosensor. To isolate the regulator of OsRac1, using yeast two-hybrid screening, we identified a guanine nucleotide exchange factor (GEF) that possessed a PRONE domain,

which we designated as OsGEF7. In vitro assay showed OsGEF7 possessed GEF activity for OsRac1. Yeast two-hybrid assay also indicated that the OsRac1-OsGEF7 interaction was suppressed by the C-terminal region of OsGEF7. Furthermore, FRET analysis showed that the PRONE domain of OsGEF7 and full-length OsGEF7 with a phospho-mimic mutation in the C-terminal region could activate OsRac1 in transient rice protoplast transformation assay, but not the wild type full length OsGEF7. Therefore, the results suggest that OsRac1 is activated by OsGEF7and phosphorylation of the C-terminal region of OsGEF7 is required to activate OsGEF7.

Molecular and genetic analysis of plant-oomycete interactions in the lettuce-*Bremia lactucae* pathosystem

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Bremia lactucae is an obligate biotrophic oomycete that causes the most serious disease of lettuce throughout the US and worldwide. In order to investigate the molecular basis of pathogenicity and host specificity in this pathosystem, we are characterizing the activities of effector proteins of *B. lactucae* and their plant targets using data mining and functional analyses to identify and characterize candidates. Bioinformatic analysis of cDNA and genomic sequence assemblies has so far identified several candidate genes with RXLR motifs, which are known to be involved in pathogenicity in other oomycetes. Candidate effectors are being functionally characterized using several methods: Y2H assays for interactions with a library of potential plant targets, transient expression assays for HR-eliciting activity on a differential set of resistant lettuce cultivars, and assays for HR-suppressing activity during transient coexpression with known HR-elicitors. We will also use a yeast functional secretion assay to identify secreted proteins without known motifs. *B. lactucae* has a fairly small genome size of ~50-100 Mb and we are currently assembling a high-coverage draft genome using sequences from several libraries, including BAC-end Sanger reads and single, paired-end, and mate-end Illumina reads. We will use sequence-based, multiplexed bulked segregant analysis to relate sequence to phenotypes. These resources will enable efficient discovery of candidate effectors and facilitate development of disease resistant lettuce cultivars.

Functional analyses of bacterial effector proteins using a viral expression system in Arabidopsis

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Bacterial pathogens such as Pseudomonas and Ralstonia cause disease by secreting a repertoire of effector proteins into the host cell using the Type III apparatus. Activities of these proteins are essential for the pathogen's virulence; their recognition induces plant defenses leading to resistance. The enzymatic activities of most secreted proteins and their molecular targets in plant cells are unknown. We produced a library of recombinant Tobacco Rattle Virus (TRV) clones harboring sequences encoding effector proteins and tested their pathogenicity in Arabidopsis. Infection with virions containing GFP or GUS reporter genes was nearly symptomless and the virus spread systemically. The expression of effector-encoding sequences often affected virus movement and severe morphological abnormalities were observed in infected plants. Known bacterial avirulence determinants restricted the systemic spread of recombinant TRV and/or induced necrosis in the veins along the path of virus movement. Such reactions were dependent on cognate R genes and known components of defense pathways. In addition to known avirulence determinants, we identified more than 20 effectors capable of reducing virus movement, affecting growth of infected plants including their light perception, and/or inducing necrosis. Responses to the infection with recombinant TRV clones were polymorphic among different ecotypes of Arabidopsis, enabling mapping of the corresponding plant genes. Because of TRV's simplicity in comparison with other pathogens, this novel virus-based pathogenicity assay is an informative tool for dissecting the functions of effector proteins originating from bacteria, fungi and oomycetes. TRV infects a broad range of hosts; therefore this assay can be readily extended to species beyond Arabidopsis.

Activation tagging in Arabidopsis to identify resistance against *Verticillium* spp.

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Soil-borne vascular wilt diseases caused by *Verticillium* spp. are among the most notorious diseases worldwide, causing severe yield and quality losses in food, feed and industrial crops. Regardless of the economical importance of *Verticillium* wilt disease, little is known about the molecular basis of *Verticillium*-host interactions and genes playing key role in these interactions. We have undertaken a forward genetics approach to identify host genes that play an important role in the interaction of Arabidopsis with *Verticillium*. To this end, an activation tagged Arabidopsis mutant library was screened with *V. dahliae*. From this screening, four mutants with enhanced resistance were identified that also appeared to display enhanced resistance towards *V. albo-atrum*. The enhanced resistance phenotypes have been confirmed by quantifying the fungal biomass *in planta* using real-time PCR. To further study whether the enhanced resistance of the mutants is specific to *Verticillium* spp. or also concerns other pathogens, the mutants were challenged with the fungal pathogens *Botrytis cinerea*, *Plectosphaerella cucumerina* and *Alternaria brassicicola*, and the bacterial pathogen *Pseudomonas syringae*. Interestingly, the mutants displayed differential responses towards these pathogens. Currently, analyses to identify the T-DNA insertion sites are ongoing. This research is supported by the Dutch Technology Foundation STW, applied science division of NWO and the Technology Program of the Ministry of Economic Affairs.

Type III effectors of *Xanthomonas oryza* pv. *oryzae* suppress PAMPs-triggered immunity in rice

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Plant bacterial pathogens equipped with the type III secretion system (TTSS) generally deliver different TTSS effector proteins into plant cells. These TTSS effector proteins modulate the function of crucial host regulatory molecules and allow bacteria to invade plant cells. So far, we have isolated 16 TTSS effectors from *Xanthomonas oryza* pv. *oryzae* (*Xoo*; Furutani et al. MPMI 2008). To identify *Xoo* TTSS effectors that inhibit host immune responses, we generated transgenic rice plants expressing each of 10 *Xoo* effectors. These transgenic plants were inoculated with the TTSS-deficient *hrpX* mutant of *Xoo*. The *hrpX* mutant is not able to grow in the wild-type plant, because infection of the *hrpX* mutant strongly induces PAMPs-triggered immunity (PTI) in host cells. Transgenic plants expressing *Xoo* effectors showed different levels of susceptibility to the *hrpX* mutant, indicating that these effectors have the abilities to inhibit PTI inside plant cells. Especially, transgenic plants expressing four of 10 effectors developed severe disease lesions of the *hrpX* mutant. Thus, it is likely that these four effectors may block the important steps of PTI in rice. Identification of target proteins of these Xoo effectors is in progress.

Comparative investigation of Arabidopsis non-host resistance to *Pseudomonas syringae* pv. *tomato* strain T1 and susceptibility to strain DC3000

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Pseudomonas syringae pv. *tomato* (Pto) DC3000 has long been used as a probe to unravel the Arabidopsis immune system. DC3000 uses a type III secretion system (T3SS) to inject over 30 effectors into Arabidopsis cells to subvert defenses and hijack plant cells in favor of pathogen growth. *P. syringae* pv. *phaseolicola* (*Pph*) isolates are unable to overcome the Arabidopsis immune system and are frequently used to investigate the basis of Arabidopsis non-host resistance. However, the large number of genomic differences between DC3000 and these distantly related isolates make it difficult to apply a comparative genomics approach to determine which genomic differences are at the basis of their different abilities to cause disease in Arabidopsis. In contrast to this problem, *Pto* T1 is a very close relative of DC3000 but is a non-pathogen of Arabidopsis. Comparative genomics of DC3000 and T1 has thus great potential for gaining insight into their different abilities to elicit and/or suppress Arabidopsis immunity. Dramatic differences in T3E repertoire were found between T1 and DC3000. One of these differences is the presence of *avrRpt2* in the T1 genome and its absence from the DC3000 genome. Although AvrRpt2 is well known to elicit effector-triggered immunity (ETI) in many Arabidopsis ecotypes, an *avrRpt2*-disruption mutant of T1 (T1') is still non-pathogenic to Arabidopsis. Additionally, T1' is incapable of causing disease in Arabidopsis defense mutants defective in immunity triggered by pathogen associated molecular patterns (PTI), ETI, and salicylic acid signaling. We present a comparison of

Arabidopsis immune responses triggered by T1, T1', DC3000, and derived T3SS-deficient strains and show how these responses are modulated by individual T1/DC3000-specific effectors.

Functional genomics analysis of ribosomal proteins in host-virus interactions

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Plant viruses are obligate intracellular pathogens whose infections are accompanied by dramatic changes in the mRNA accumulation (i.e. the expression) of host genes. Transcript profiling studies have provided insight into the different functional groups of genes that are induced or suppressed by plant viruses as well as mechanisms that control their expression. One striking outcome of these studies is the large number of genes encoding cellular ribosomal proteins that were induced in response to *Turnip mosaic virus* (TuMV) infection. Subsequent studies showed that other viruses, such as tobamoviruses, are also able to induce the mRNA expression of these genes in both *Arabidopsis thaliana* and *Nicotiana benthamiana*. Ribosomal proteins are structural components of both the small and large subunits of the ribosome, and for most of them, specific functions are unknown. The increased expression of ribosomal proteins suggests an increased capacity for protein synthesis during viral infection or that viruses potentially modify the ribosomal protein content of infected cells. To begin to investigate the roles of ribosomal proteins in viral infection, virus-induced gene silencing (VIGS) was used to knock down their expression in *N. benthamiana* plants. Functional analysis of five ribosomal proteins by VIGS supports the hypothesis that ribosomal proteins are required for virus accumulation. Interestingly, TuMV required all five of the ribosomal proteins tested, but *Tobacco mosaic virus* (TMV) only required four of the five. The observation that TuMV has a different requirement for ribosomal proteins than TMV may be due to the different translation strategies used by these two un-related viruses.

Mutation in *Ralstonia solanacearum* pathogenicity determinant RSc0411 (LptC) causes attenuation of type III secretion system and altered lipopolysaccharide biogenesis

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LptC, a member of the novel protein family DUF1239, is suggested to play an essential role in *E. coli* lipopolysaccharides (LPS) transport into the outer membrane. Here we investigated functions of the *Ralstonia solanacearum* DUF1239 protein, a protein encoded by RSc0411, in pathogenesis and LPS biogenesis. *R. solanacearum lptC* mutant was defective in membrane integrity and various pathogenesis-related properties. Notably, the induction of the type III secretion system was attenuated and production of rough LPS was reduced in *lptC* mutant. Comparative characterization of an LPS-defective *rfaF* mutant indicated that the altered LPS biogenesis of *lptC* mutant did not account for most of the altered phenotypes. The organization of DUF1239-related gene cluster is conserved among the bacteria analyzed, while sequence homology among orthologous genes in the cluster and those involved in LPS biogenesis varied accordingly with phylogenetic relationships. Complementation tests revealed that only DUF1239 members in bacteria phylogenetically related to *R. solanacearum* were functional to rescue the mutant's defects, suggesting certain specificity in the LptC-involved pathogenesis mechanism and LPS biogenesis machineries may have evolved. Collectively these results evidence a novel and crucial role of LptC in early pathogenesis and LPS biogenesis of *R. solanacearum*.

NPR1 is involved in the antagonistic interaction between SAR signaling and ABA-mediated signaling

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Systemic acquired resistance (SAR) is a potent innate immunity system in plants that is effective against a broad range of pathogens. SAR development in dicotyledonous plants is mediated by salicylic acid (SA). Here, using different types of SAR-inducing chemicals, including 1,2-benzisothiazol-3(2H)-one1,1-dioxide (BIT) and benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH), which act upstream and downstream of SA in the SAR signaling pathway, respectively, we show that treatment with abscisic acid (ABA) suppresses the induction of SAR in Arabidopsis. In an analysis using several mutants in combination with these chemicals, treatment with ABA suppressed SAR induction by inhibiting the pathway both upstream and downstream of SA, independently of the jasmonic acid (JA)/ethylene (ET)-mediated signaling pathway. Suppression of SAR induction by the NaCl-activated environmental stress response proved to be ABA-dependent. Conversely, the activation of SAR suppressed the expression of ABA biosynthesis-related and ABA-responsive genes, some of which were NPR1-dependently but others were not, indicating that NPR1 protein or signaling downstream of NPR1 appears to contribute to the cross-talk. These data have revealed that antagonistic cross-talk occurs at multiple steps between the SA-mediated signaling of SAR induction and the ABA-mediated signaling of environmental stress responses.

Identification and characterization of *Hyaloperonospora arabidopsidis* RxL(R)-type effectors function in *Arabidopsis thaliana*

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Hyaloperonospora arabidopsidis (Hpa) is a natural oomycete pathogen of *Arabidopsis* that causes downy mildew disease. Co-evolution between *Hpa* and its host is reflected in the extensive genetic variation in pathogen isolates and responses of different *Arabidopsis* accessions. As an obligate biotroph, *Hpa* deploys a range of effector proteins that contribute to suppression of host immune responses in order to establish a feeding relationship for growth and reproduction. We know little about how oomycete pathogens manipulate their hosts to establish successful colonization. To understand this process better we have isolated ~25 *Hpa* candidate effector proteins expressed during leaf infection that have a conserved RxL(R) motif and are predicted to be translocated to the plant cell cytoplasm. Highly polymorphic RxL(R) candidates that suppress host defences in transient assays were selected and cloned for further functional studies. RxL(R) overexpression *Arabidopsis* transgenic lines have been generated and are now being screened for alteration of host immune responses. Using bacterial delivery of RxL(R) proteins into the plant cytoplasm via the *Pseudomonas syringae* Type III secretion system (TTSS) to a range of Arabidopsis accessions we aim to assess whether pathogen effectors have driven selection of specific plant *R*-genes. We will present our results so far.

The C-terminal domain of *Pseudomonas syringae* effector AvrPto has novel virulence and avirulence activities

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AvrPto is *Pseudomonas syringae* type III effector protein that is delivered into plant cells during the host-*Pseudomonas* interaction. AvrPto is an 18-kD protein with a central core that adopts an a-helix bundle structure, while ~30 amino acids at both the N- and C-terminus are unstructured and flexible. In this study, we show that AvrPto has two distinct virulence determinants that have similar and additive effects: the CD-loop and the C-terminal phosphorylation sites. The CD-loop is responsible for suppressing PAMP-triggered immunity (PTI). Mutation in the CD-loop abolishes the recognition of AvrPto in tomato by the resistance protein Pto; disruption of the phosphorylation of the C-terminus of AvrPto weakly affects Pto recognition. In tobacco, recognition of AvrPto is dependent on phosphorylation of the C-terminus and is not affected by CD-loop. The resistance triggered by phosphorylated C-terminus of AvrPto is termed Rpa. Interestingly, Rpa is present in various *Nicotiana* species, but is not observed in any *Solanum* species tested. Therefore, closely related genera in the Solanaceae recognize the same effector AvrPto by different mechanisms.

A new positive regulator PrhG for the *hrp* regulon in *Ralstonia solanacearum*

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Ralstonia solanacearum is a soil-borne plant pathogen. Genes encoding a type III secretion system are indispensable for pathogenicity and form the *hrp* regulon. The *hrp* regulon is positively regulated by HrpB and negatively

controlled by PhcA. Although a signal cascade PrhA-PrhR/PrhI-PrhJ-HrpG is demonstrated to activate the *hrpB* expression, several factors regulating the *hrpB* expression remain to be elucidated. We randomly mutagenized a *R. solanacearum* OE1-1 reporter strain with Tn5 transposon and screened mutants, which reduced the *hrp* regulon expression. After mapping the transposon insertion sites and measurements of the expression levels of the *hrp* regulon, mutants were classified into three groups. (1) Mutations in the genes responsible for aromatic amino acid biosynthesis (2) Mutations in the genes whose function are unknown and completely lost the *hrp* regulon expression (3) Mutations in *hrpG* and a *hrpG* homologous gene. We focused on the *hrpG* homologous gene, named *prhG*, in this study. The *prhG* deletion mutant reduced the *hrpB* expression as well as the *hrpG* mutant. Although HrpG and PrhG are response regulators of a two-component system and activated the *hrpB* expression, regulation mechanism of both genes was quite different. While PhcA negatively regulates the *hrpG* expression, PhcA was demonstrated to positively regulate the *prhG* expression. The *prhG* expression level increased concomitantly with bacterial cell density. When inoculated tomato plants, the *hrpG* mutant completely lost virulence, while the *prhG* mutant showed weaker pathogenicity than the wild type. Then all together, we hypothesized that for the *hrpB* activation HrpG plays a major role in the early stage of infection and PrhG works at higher cell density after proliferation in the intracellular spaces.

Two novel *Magnaporthe oryzae* effector genes show contrasting evolutionary patterns imposed by positive selection

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Effector genes involved in host-pathogen interaction are known to rapidly co-evolve by arms race, leaving signatures in the genome sequence of the pathogen. To identify such effector genes, we conducted a large-scale DNA polymorphisms study of *M. oryzae*, followed by an association analysis of polymorphisms with avirulence (AVR) phenotypes. This approach revealed two effector genes *RU528* and *pex31* (*AVR-Pik*). *RU528* has a paralog *RU879* in the *M. oryzae* genome showing a large nucleotide divergence, indicative of a long-time maintenance of the paralogs in the population. Evidence for strong positive selection in *RU528* was obtained based on analyses of the two paralogs (Ka/Ks =2.1), within-species orthologs (Ka/Ks = 2.2), as well as among-species orthologs (Ka/Ks = 1.8 ~ 2.0). Rice plants overexpressing *RU528* became more susceptible to *M. oryzae* than control plants indicating that RU528 has a virulence effector activity but no association with avirulence was detected. In contrast, DNA polymorphisms of *pex31* showed association with *AVR-Pik*, *AVR-Pikm* and *AVR-Pikp*. *Pex31* contains five allelic variants A-E differing by only five nonsynonymous substitutions. Genetic complementation in *M. oryzae* confirmed that pex31-D has the activity expected for *AVR-Pik*, *AVR-Pikm* and *AVR-Pikp*. Rice protoplast genetic transformation experiments showed that pex31-D was recognized by both rice *R*-genes *Pik* and *Pik-p*, whereas pex31-E was recognized only by *Pik*, and pex31-C neither by *Pik* nor *Pik-p*. In contrast that *RU528* preserves divergent alleles and paralogs for a longer time, pex31 evolution seems to be recently driven by the recognition specificity of *R*-genes, which were introgressed through rice breeding.

Transgenic potato plants expressing constitutively active StCDPK5 show late blight resistance, but early blight susceptibility

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Reactive oxygen species (ROS) are implicated in plant innate immunity. NADPH oxidase (RBOH; Respiratory Burst Oxidase Homolog) plays a central role in oxidative burst in potato. We have isolated a potato calcium-dependent protein kinase 5 (StCDPK5) that activates StRBOHB by phosphorylation of its N-terminal region. In addition, we confirmed that StCDPK5 phosphorylates the N-terminal regions of StRBOHA to D, and heterologous expression of StCDPK5 and StRBOHs in *Nicotiana benthamiana* resulted in oxidative burst. The transgenic potato plants that carry a constitutively active StCDPK5 driven by a pathogen-inducible promoter of the potato showed high resistance to late blight pathogen *Phytophthora infestans*. We observed infection sites for a virulent isolate of *P. infestans* under a microscope. Cystspores failed to penetrate the epidermal cell and HR-like cell death was observed. Strong 3,3-diaminobenzidine (DAB) staining, which shows H_2O_2 accumulation, was detected in the attacked cells. In contrast, these plants showed high susceptibility to early blight necrotrophic pathogen *Alternaria solani*. At the penetration sites, strong DAB staining was also observed, but internal hyphae increased in the mesophyll cell compared with wild-type potato leaves. Furthermore, we verified increase in biomass of *A. solani* using quantitative-PCR, suggesting that oxidative burst confers high resistance to biotrophic pathogen, but high susceptibility to necrotrophic pathogen.

The role of the transcription factor MYB72 in induced systemic resistance

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Root colonization by selected strains of non-pathogenic rhizobacteria triggers an induced systemic resistance (ISR) in diverse plant species. In *Arabidopsis thaliana*, ISR mediated by *Pseudomonas fluorescens* WCS417r requires an intact response to jasmonate / ethylene as well as the key-regulator NPR1. Previous studies identified the transcription factor MYB72 as a novel regulatory component of early signaling steps of ISR. Its transcriptional activity was locally induced upon WCS417r colonization of the roots and mutants disrupted in *MYB72* were abolished in their ability to generate ISR. Despite the fact that MYB72 is essential for ISR, it is not sufficient for its establishment, as MYB72 overexpressors do not display increased levels of resistance. This result indicates that either MYB72 requires post-translational activation or other interacting partners are co-required. Yeast two-hybrid analysis revealed that MYB72 physically interacts *in vitro* with the ETHYLENE INSENSITIVE3 (EIN3)-LIKE3 transcription factor EIL3. Here, we analyzed the subcellular localization of MYB72 and tested whether MYB72 and EIL3 interact *in planta.*

Quantitative proteomics of flagellin-induced plasma membrane compartmentalization reveals new components of plant immunity signaling

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Plasma membrane compartmentalization is a well-characterized mechanism to spatially and temporally regulate cell-autonomous immune signaling/responses in animal cells, supposedly based on the lipid-driven formation of membrane rafts. To elucidate immediate-early protein dynamics at the plasma membrane in response to the bacterial pathogen-associated molecular pattern (PAMP) flagellin (flg22) we employed quantitative mass spectrometric analysis on detergent-resistant membranes (DRMs) of *Arabidopsis thaliana* suspension cells. This revealed profound changes in DRM protein composition. These comprised the enrichment of proton ATPases and receptor-like kinases, including the flagellin receptor FLS2. We applied reverse genetics and pharmacological interference to address a potential contribution of a subset of these proteins in flg22-triggered responses, which revealed two novel players in elicitor-dependent oxidative burst control. Our data provide evidence for dynamic, ligand-induced changes in the membrane compartmentalization of PAMP signaling components.

Bioinformatic analysis of Phaseolus vulgaris Hsp70 during symbiosis with Rhizobium

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Hsp70 chaperone family is highly conserved across the three domains of life (archaea, bacteria and eukarya). Its memebers have been studied in diverse organisms, and have been classified in constitutive expressed or stress activated proteins. Hsp70s are localized to the cytoplasm, chloroplast, endo-plasmic reticulum or mitochondria. Particularly, the synthesis of Hsp70s localized in organelles is increased in response to diverse physical or chemical stresses, having a relevant function in protein-folding required for cell homeostasis. A mitochondrial Hsp70 of Phaseolus vulgaris was reported some years ago (Vidal et al., 1993). In the symbiosis Phaseolus vulgaris - Rhizobium

the function of this protein has not been fully established. We constructed a contig with Phaseolus vulgaris ESTs to clone a complete non mitochondrial Hsp70, based on high conservation of this proteins. We confirm the expression of Hsp70 in leaves, stem, roots and nodules trough a Western Blot assay. We will present results of the Hsp70 - GFP fusion protein expression and the Hsp70 gene silencing in common bean roots and nodules. Based on presented data, we propose that Hsp70 has an important chaperone role during the symbiosis since Rhizobium infection generates a variety of stresses, one of them, the high translational rate, due to the enhanced metabolism of infected cells. This work was partially supported by CONACYT 3357

The role of *DMR6* in disease susceptibility

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Plants are constantly exposed to micro-organisms including pathogens. They are resistant to the vast majority of potential pathogens due to a multilayered defence system. Plant disease susceptibility, on the other hand, is determined by virulence factors from the pathogen, their targets in the host and the suppression of plant defences. To gain insight into the molecular mechanisms underlying disease susceptibility, we study the interaction between the oomycete pathogen *Hyaloperonospora arabidopsidis* (*Hpa*), causing downy mildew, and its host Arabidopsis. For several *downy mildew resistant* (*dmr*) mutants, generated by EMS, we have cloned the corresponding genes. Of these, *DMR6* encodes for an 2-oxoglutarate iron (II)-dependent oxygenase of unknown biological function. Genetic analysis revealed that *dmr6*-mediated resistance requires the activation of defence responses. The enhanced expression of defence-associated genes in the *dmr6* mutant strongly suggests that *DMR6* acts as a negative regulator. This is supported by the finding that Arabidopsis overexpressing *DMR6* are more susceptible to *Hpa*. The enhanced susceptibility of *DMR6* overexpression in Arabidopsis was also observed when challenged with the bacterium *Pseudomonas syringae* pv. *tomato* DC3000 and the oomycete *Phytophthora capsici*. The combined data suggest that a substrate accumulates in the *dmr6* mutant that activates plant defence, including the expression of *DMR6*. We propose that, in wild-type plants, the intrinsic role of *DMR6* as a negative regulator is to reduce the level of DMR6 substrate to prevent overactivation of defence.

Transcript profiling in the nonhost-interaction of barley against *Magnaporthe* - Comparative analysis including rust and powdery mildew nonhost interactions revealed non-redundant sets of regulated genes

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Rice blast disease caused by *Magnaporthe oryzae* is a major threat in rice agriculture. Resistance of new cultivars is short-lived because of a shift to new pathogen races. A promising approach for breeding of resistant crops might be, therefore, the utilization of mechanisms of the durable nonhost type of resistance. Barley is an alternative host for *M. oryzae* but shows typical nonhost interactions to other species of the genus *Magnaporthe*. Penetration resistance and a hypersensitive response of attacked epidermal cells were identified as crucial defence mechanisms for the arrest of pathogen growth in nonhost interactions. We used pharmacological approaches in combination with barley mutants to sequentially suppress the epidermal resistance aiming at the enforcement of fungal growth into the mesophyll. Transcript profiling of stripped barley epidermal tissue infected with *Magnaporthe* host or nonhost isolates was performed using cDNA macroarrays. Statistical analysis of four biological replicates resulted in 180 candidate genes characteristically regulated in the nonhost situation. Within up-regulated genes a significant over-representation of genes encoding lipid metabolism-related proteins, in particular lipid transfer proteins, was detected. Meta-data analysis of transcriptional responses was done in comparison to two additional pairs of adapted (host) and non-adapted (nonhost) fungal isolates of the *genera Blumeria* (powdery mildew) and *Puccinia* (rust). Significantly, nonhost resistance against the non-adapted pathogens was not associated with a common nonhost-specific regulon. Moreover, a link between nonhost resistance and basal host defence was suggested.

Histidine- and urocanate-specific transporters in plant associated *Pseudomonas*

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The histidine utilization (*hut*) locus of *Pseudomonas fluorescens* SBW25 confers ability to grow on histidine and urocanate (the first intermediate of the histidine degradation pathway) as a sole source of carbon and nitrogen. Previous work has defined the genes involved in histidine catabolism and regulation. Here we report the identification of three transport systems and extend our understanding of histidine utilization to the population level. Deletion of *hutTu* eliminated growth on urocanate, implicating that HutTu is an urocanate transporter. Uptake of histidine is complex: inactivation of *hutTh* and *hutXWV* (ABC type transporter) caused a significant delay of growth on histidine. A total of 230 *Pseudomonas* strains were isolated from sugar beets grown in Oxford (UK) and Auckland (NZ) and their ability to grow on histidine and urocanate was examined. Results revealed considerable variation of phenotypes, for example, strains capable of growing on histidine but not on urocanate (His^+, Uro^-) and vice versa (His^-, Uro^+). Interestingly, His^+, Uro^- strains were common in the Auckland population, whereas His^-, Uro^+ strains were more prevalent in the Oxford population. Introduction of cloned copies of *hutTh* and *hutTu* from SBW25 restored the ability of many naturally His^- and Uro^- strains to utilize histidine and urocanate, respectively. Together, the data indicate that *Pseudomonas* populations are polymorphic with respect to the transporters. Finally, we have taken an experimental evolutionary approach to dissect the origin of transporter diversity in the plant environment.

Characterization of ACD6 complex

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The integral membrane protein ACCELERATED-CELL-DEATH 6 (ACD6), which contains a transmembrane region and cytoplasmic ankyrin repeat motif, is a necessary positive regulator of cell death and defense responses in Arabidopsis. A leucine to phenylalanine substitution in the transmembrane domain of ACD6 causes gain-of-function phenotypes in the *acd6-1* mutant, which shows spontaneous cell death, high levels of the defense signal salicylic acid (SA) and increased disease resistance. Analysis of intragenic *acd6-1* suppressors suggested that ACD6 is a two-domain protein in which the ankyrin repeat domain and predicted transmembrane region communicate. To gain a better understanding of ACD6 function, we used two-dimensional blue native gel electrophoresis to analyze microsomal fraction solubilized with Triton X-100 from transgenic plants expressing functional HA tagged ACD6/ACD6-1. ACD6:HA or ACD6-1:HA proteins migrated at a molecular mass of 600 to 870kD with different abundances. Additionally, the level and the size of ACD6/ACD6-1:HA complexes were increased after treatment with the in both SA agonist benzothiazole (BTH) or *Pseudomononas syringae* infection. Moreover, we have generated transgenic plants expressing HA tagged ACD6 and ACD6-1 with eight intragenic mutations that disrupt ACD6-1 function under the control of ACD6 promoter to examine the effects of those mutations on its ability to form complexes. Further studies are in progress to determine components of the complexes containing ACD6-1 or ACD6 after defense stimulation.

Visualizing the transfer of internally tagged VirE2 from *Agrobacterium* to plant cells

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In plants, *Agrobacterium* VirE2 interacts with plant proteins, such as VIP1, VIP2, importin α-1, importin α-4, and plant expressed VirE2. In plants, VirE2 likely interacts with a VirD2-T-DNA complex and facilitates its nuclear import. Interaction between VirE2 and VIP1 has been confirmed both in yeast and in plants. However, transfer of VirE2 from *Agrobacterium* to plant cells and its interaction with host proteins have not yet been visualized. We engineered VirE2 protein by inserting a cCFP tag after its 10th amino acid residue (VirE2-cCFP), and introduced the engineered virE2-cCFP gene into a *virE* operon deletion *Agrobacterium* strain, ΔE. When expressed from an additional plasmid in ΔE, *Agrobacterium* containing either VirE2-cCFP or wild-type VirE2 can incite tumors equally efficiently, indicating that the tagged VirE2 protein can be transported through the T4SS apparatus and interact with proteins in the plant. To visualize the movement of VirE2-cCFP, we used the ΔE strain expressing VirE2-cCFP to infect plant cells expressing a fusion protein, mCherry-aptamer2-nVenus. Aptamer2 is a short peptide which interacts with VirE2 protein in bimolecular fluorescence complementation in plants. After VirE2-cCFP protein is transferred from *Agrobacterium* to plant cells, VirE2 would interact with plant expressed aptamer2 peptide, resulting in folding between nVenus and cCFP and the generation of yellow fluorescence at the protein interaction site. Preliminary results indicate that VirE2-cCFP delivered from *Agrobacterium* to transgenic *Arabidopsis* plants or tobacco cells expressing mCherry-aptamer2-nVenus

produces yellow fluorescence. This is the first direct visualization of VirE2 transfer into plant cells.

Isolation of aflatoxin B1-degrading fungi among the mycoflora coincidental with toxigenic *Aspergillus flavus*

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Aspergillus flavus is a common opportunistic fungal pathogen of plants. It produces numerous secondary metabolites. Of main concern are aflatoxins, which are potent carcinogens. Pre- and post-harvest infection of various crops by *A. flavus*, and resultant contamination of food products with aflatoxins, is a serious international food safety problem. One approach for reducing this contamination may be use of aflatoxin-catabolizing microorganisms. We discovered a number of such organisms among fungi co-colonizing natural substrates with toxigenic *A. flavus*. These microorganisms included strains of *Epicoccum nigrum*, *Gliocladium roseum*, *Colletotrichum atramentarium*, *Bipolaris sorokiniana*, *Rhizoctonia cerealis*, *Chaetomium* sp. and *Ophiobolus* sp. These fungal isolates, along with toxigenic and nontoxigenic strains of *Aspergillus* (A10+ and P2654, respectively), were screened for aflatoxin B1-catabolizing activity. The fungi were cultivated on casein hydrolyzate-containing liquid Czapeck's media, supplemented with B1 (200-300 ng/ml). After 7-day incubation at 28° C and 200 rpm, levels of B1 in cultural filtrates were quantified by HPLC. All natural isolates, except for those of *R. cerealis* and *Ophiobolus* sp., possessed significant B1-degrading activity. *Chaetomium* sp. had the highest activity, degrading 94% of the aflatoxin. Interestingly, the non-toxigenic strain of *A. flavus*, P2654, possessed no B1-degrading activity. Alternatively, the toxigenic strain, A10+, degraded the B1 by 50%. Our results show there are microorganisms, within the mycoflora that includes *A. flavus*, which could help with B1-decontamination. The results also suggest there is a genetic mechanism in *A. flavus* that regulates both pathways involved in the biosynthesis and catabolism of aflatoxins.

Identification of pathway genes involved in *mlo*-based resistance to tomato powdery mildew

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MLO is the family of heptahelical transmembrane proteins in plants. In barley, Arabidopsis and tomato, loss-of-function mutation(s) in *Mlo* orthologues is/are sufficient to confer full resistance to different powdery mildews, demonstrating that MLO represents a conserved plant host cell protein required in powdery mildew pathogenesis. The *mlo*-based resistance requires correct functioning of other genes like *Ror1* and *Ror2* in barley and *Pen1*, *Pen2* and *Pen3* in Arabidopsis. *Pen1* and *Ror2* had been studied are functionally homologous syntaxins. In our research, we studied the involvement of *Pen* genes in *mlo*-based resistance in tomato and Arabidopsis. The tomato *Pen* homologues were isolated and functionally analyzed by virus-induced gene silencing (VIGS). Silencing of tomato *Pen1* and *Pen3* homologues restored (partially) the penetration of tomato powdery mildew (*Oidium neolycopersici*) in the tomato *mlo*-mutant. While, fungal sporulation was only observed on the plants in which tomato *Pen1* homologue was silenced. In contrast, in Arabidopsis clear sporulation of *O. neolycopersici* was observed on the *Atmlo2/pen3* double mutant, but not on the *Atmlo2/pen1* double mutant through fungal penetration ratio was increased. It is likely that that the *Pen* genes are functionally conserved only for pre-penetration resistance mediated by loss-of-function mutation(s) in *Mlo* orthologues in Arabidopsis and tomato.

Bacterial effector gene *avrRxo1* encodes an ATP binding protein that physically interacts with Rxo1 to trigger broad resistance in both monocot and dicot plant species

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Why few NB-LRR genes function across taxonomic species whereas others exhibit restricted taxonimc functionality is not known. We recently demonstrated that the NB-LRR/effector gene pair *Rxo1/avrRxo1* functions in both monocots and dicots to trigger defense responses. We demonstrated that *avrRxo1* encodes an ATP binding protein that physically interacts with the NBS domain of Rxo1. The ATP binding function and nuclear localization of AvrRxo1 are required for virulence function. Transgenic *Arabidopsis* plants expressing *avrRxo1* suppressed both basal defense responses and *Rps2*-mediated disease resistance. Co-expression of both wild type and mutants of *Rxo1* along with *avrRxo1 in planta* suppressed nuclear localization and the virulence function of AvrRxo1.

Identification and characterization of host targets of *Pseudomonas syringae* effector HopZ1 in soybean

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Many gram-negative bacteria of plants and animals depend on the type III secretion system (T3SS) to secrete and translocate largely distinct sets of effectors (type III secreted effectors or T3SEs) into host cells. In the last few years, substantial progress has been made to understand the functions of T3SEs by studying their interactions with host targets. However, the molecular mechanisms of the majority of T3SEs are largely unknown. HopZ effectors from the plant pathogen *Pseudomonas syringae* are homologs of YopJ acetyltransferase, which are widespread in both plant and animal pathogens. Among the five HopZ members, HopZ1a most resembles the ancestral allelic form. It has been demonstrated that HopZ effectors have undergone allelic diversification in response to selective pressures imposed by the host defense system. HopZ1b is an endogenous T3SE of *P. syringae* pv. glycinea, the causal agent of soybean bacterial blight. HopZ1b promotes bacterial *in planta* multiplication in soybean; however, HopZ1a triggers hypersensitive response. We hypothesize that HopZ1a and HopZ1b interact with distinct sets of plant proteins, leading to their different functions in soybean. In order to test this hypothesis, yeast two-hybrid screenings were carried out using HopZ1a and HopZ1b as the baits to identify HopZ1-interacting proteins (ZINPs) in soybean. These ZINPs were classified into three groups: 1) interacting with HopZ1a, but not HopZ1b; 2) interacting with HopZ1b, but not HopZ1a; 3) interacting with both HopZ1a and HopZ1b. The interactions of ZINPs and HopZ1 alleles were then confirmed by *in vitro* co-immunoprecipitation and *in vivo* split-YFP assays. Two ZINPs are further investigated for the molecular mechanisms underlying functionality and evolution of HopZ effectors.

Temperature modulates plant defense responses through disease resistance proteins

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Temperature influences almost every aspect of plant growth and development as well as its interaction with other organisms. An elevated growth temperature often inhibits plant defense responses and renders plants more susceptible to pathogens. The molecular mechanisms underlying the temperature modulation of plant defenses were not known, and this temperature sensitivity poses a challenge to agriculture especially in the current global climate changes. The plant immune system consists of multiple layers of defense responses to various pathogen attacks, and temperature could regulate multiple components in those defenses. Here we show that two NB-LRR coding disease resistance (*R*) genes involved in recognizing pathogen effectors are responsible for the temperature sensitivity in disease resistance. An elevated temperature reduces the nuclear localization of an Arabidopsis R protein named SNC1 and a tobacco R protein named N, leading to the inhibition of defense responses mediated by these R genes. Further, specific residue alterations in the SNC1 and the N proteins counteract this temperature effect and sustain defense responses at high temperatures. These findings provide a potential means to generate effective plant immune responses within a broader temperature range.

Photoinhibition through targeted D1 degradation or inhibition does not appear to play a major role in HR induce by *Pseudomonas syringae* AvrB in soybean

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The possible role of inhibition of photosynthetic electron transfer in the hypersensitive defense response (HR) has been proposed in several studies

(i.e. Allen et al 1997, Seo et al. 2000). We examined the possible role in HR of the inhibition of photosystem II (PSII) with a focus on the labile nature of the D1 subunit of PSII in soybean. We concluded that photoinhibition through D1 is probably not playing a major role in *Pseudomonas syringae* AvrB-induced HR in soybean based on results of several experiments. One, co-infiltration of the D1 interfering herbicide bentazon with *P. syringae* carrying *avrB* led to a slight enhanced multiplication of the pathogen, not a decrease. Second, western blots of D1 protein levels showed no significant change in D1 quantities within the first 24 hours post inoculation (hpi), although qRT-PCR indicated a 7-fold decrease in gene expression. Third, the western blots showed that the D1 protein appeared to be largely intact during the first 24 hpi. Fourth, microarray gene expression profiles showed that leaves infected with *P. syringae* carrying *avrB* were more similar to glyphosate treated leaves (tissue death through aromatic amino acid starvation) than to tissue treated with the D1 inhibiting herbicide bentazon. Although not absolutely conclusive, together these results suggest that photoinhibition through targeted D1 degradation or interference, is probably not playing a major role in AvrB-induced HR in soybean.

Cellulose is involved in the formation and stability of *Rhizobium gallicum* UPRM 8053 biofilms in an abiotic surface

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Bacterial biofilms are communities embedded in a matrix mostly composed of exopolysaccharides. Other components, such as cellulose, might be part of that matrix. The presence of cellulose in the matrix has been reported in some *Salmonella* strains, several environmental *Pseudomonas* isolates, and in *Agrobacterium tumefaciens*, among others. Several *Rhizobium* and *Bradyrhizobium* strains produce cellulose fibrils but their role in biofilm formation, if any, is not clear. The objective of this work was to determine whether cellulose affects the formation or the stability of biofilms in the plant symbiont *Rhizobium gallicum* UPRM 8053. *R. gallicum* colonies show a strong red color when grown in TY medium with Congo red suggesting the production of cellulose by the bacterium. However, other components and a low pH could also give positive results. To test for cellulose involvement in biofilm formation or stability, two approaches were used: 1) an initial addition of 0.2% cellulase to wells of a polysterene microtiter plate containing a suspension of *R. gallicum*, followed by incubation for 2 days, 2) an addition of 0.2% cellulase to wells containing two-day old *R. gallicum* biofilms followed by incubation for 2 additional days. In both cases, the attached biofilm was evaluated with crystal violet. In independent experiments, the presence of cellulose fibrils was assessed by atomic force microscopy (AFM). Our results show that cellulase prevents biofilm formation and that 2-day-old biofilms are altered by its presence, suggesting that cellulose is involved not only in biofilm formation but also in the stability of preformed biofilms. This is to our knowledge, the first report indicating that cellulose might be a key component in the formation and maintenance of *R. gallicum* biofilms.

Identification of a host specificity factor in the biotrophic maize pathogens *Ustilago maydis* and *Sporisorium reilianum*

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Ustilago maydis and *Sporisorium reilianum* are smut fungi with a narrow host range. *U. maydis* and the *S. reilianum* variety SRM produce smut symptoms only on maize, while the *S. reilianum* sorghum variety SRS produces spores on sorghum. Microscopic analysis showed that after plant penetration *S. reilianum* reaches the inflorescence via growth along the vascular bundles of the respective host plant. SRS hyphae can also ramify in maize plants and reach the inflorescence but do not differentiate into spores. We hypothesized that genes responsible for virulence on maize should be present in the maize pathogens *U. maydis* and SRM but absent in the sorghum pathogen SRS. Using genome comparison and heterologous PCR/Southern analysis we identified three putative candidate genes. One of these genes codes for a secreted protein that is highly conserved between *U. maydis* and SRM. Deletion of this gene in *U. maydis* drastically reduced virulence. Expression of the respective SRM ortholog in SRS led to strains that more efficiently reached the inflorescence as deduced by an increased incidence of phyllody – a typical symptom associated with *S. reilianum* infection. This shows that this effector supports fungal proliferation in maize. Expression of this SRM effector in SRS did not lead to a significant reduction of virulence on sorghum. However, sorghum plants infected with this strain showed an increased appearance of small dark spots above the veins of sorghum leaves. This suggests an increased host defense response at locations colonized by the fungus. The enhanced maize colonization mediated by this effector suggests a contribution to host specificity.

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