INSECT AND FUNGAL ENZYME INHIBITORS IN STUDY OF PLANT VARIABILITY AND EVOLUTION*

INHIBITORY HMYZÍCH A HOUBOVÝCH ENZYMŮ PŘI STUDIU VARIABILITY A EVOLUCE ROSTLIN

A. Konarev[1], D. Vaughan[2] and P. Shewry[3]

[1]*All-Russian Institute for Plant Protection, St. Petersburg, Russia*
[2]*National Institute of Agrobiological Resources, Tsukuba, Japan*
[3]*IACR-LARS, University of Bristol, UK*

ABSTRACT: Amylase and proteinase inhibitors were found to be highly polymorphic in seeds and vegetative tissues of wild and cultivated species of wheat, rice, other cereals, *Vigna* sp., kidney bean, potato, sunflower and other *Compositae*. The spectra of various inhibitors determined by isoelectric focusing are specific for species, genomes or varieties and reflect evolutionary relationships between species in various plant groups. Potato varieties differed in their capability to accumulate inhibitors in leaves in response to damage and in the spectra of induced inhibitors. Inhibitors can be effectively used in studies of plant diversity, evolution and plant-parasite co-evolution in combination with other protein and DNA markers.

Keywords: trypsin; chymotrypsin; subtilisin; insect α-amylase; cysteine proteinase; inhibitor; fungi; wheat; rice; cereals; potato; vigna; sunflower; safflower; *Compositae*; evolution; diversity

ABSTRAKT: Inhibitory amylázy a proteinázy v semenech a vegetativních pletivech planých i pěstovaných druhů pšenice, rýže, jiných obilnin, druhů *Vigna* sp., fazolu, slunečnice a jiných hvězdnicovitých jsou vysoce polymorfní. Spektra různých inhibitorů, stanovená izoelektrickým fokusováním, jsou specifická pro druhy, genomy nebo odrůdy a odrážejí evoluční vztahy mezi druhy v různých botanických uskupeních. Odrůdy bramboru se lišily ve schopnosti akumulovat inhibitory v listech při reakci na poškození a ve spektrech indukovaných inhibitorů. Inhibitory mohou být účinně využívány v kombinaci s jinými proteiny a markery DNA ke studiu diverzity rostlin, jejich evoluce a koevoluce s parazity.

Klíčová slova: trypsin; chymotrypsin; subtilizín; hmyzí α-amyláza; cysteinová proteináza; inhibitor; houby; pšenice; rýže; obilniny; brambor; *Vigna*; slunečnice; saflor; hvězdnicovité; evoluce; diverzita

INTRODUCTION

The seeds and vegetative parts of higher plants contain various proteinaceous inhibitors of insect, fungal, mammalian and endogenous proteinases. The inhibitors may be involved in plant defense systems against harmful organisms and may also play regulatory roles during plant development (Shewry and Lucas, 1997). Furthermore, plant inhibitors are of interest in relation to problems of host/parasite co-evolution (Konarev, 1996), as markers in studies of plant diversity and evolution (Konarev, 1982, 1996) and as potential drugs with antiviral and other properties. Genes encoding potent and stable inhibitors can be transferred to other plants to improve their pest or fungal resistance (Ryan, 1990). The biochemical properties of hydrolase inhibitors are particularly well studied in the families *Fabaceae*, *Poaceae* and *Solanaceae*. Some 12 inhibitor families can be recognised based on their amino acid sequences and target proteinases (Shewry, 1999). However, the evolutionary variability of inhibitors in these taxa was not investigated in detail. The inhibitors of sunflower and other species of the *Compositae* remained unstudied until recently. We have, therefore, (i) determined the polymorphism, distribution, variability, genetic control and biochemical properties of α-amylase and proteinase inhibitors in lines, varieties

*This work was carried out according to Programme of Fundamental Investigations on Plant Protection of Russian Academy of Agricultural Sciences and supported under JIRCAS fellowship (Japan), a Joint Project (683072.P810) and "Ex-Agreement Visit from the Former Soviet Union" grants from Royal Society of London for the first author.

and wild accessions of wheat, *Aegilops,* rice, potato, cowpea, kidney bean, sunflower and other *Compositae* species and (ii) searched for novel inhibitors. Special attention has been given to inhibitors of insect α-amylases and cysteine proteinases, trypsin, which is a typical digestive enzyme of insects, mammals and fungi, and subtilisin, a proteinase of microorganisms.

MATERIAL AND METHODS

Seed material was obtained from the Vavilov Institute of Plant Industry (St. Petersburg). Most of the study was carried out using simple and sensitive methods to detect amylase (Konarev, 1982; Konarev and Fomicheva, 1991) and proteinase (Konarev, 1986; Konarev *et al*., 1999b, 2000a) inhibitors among other plant proteins, separated by isoelectric focusing (IEF), electrophoresis or thin layer gel-filtration. These methods were also effective in monitoring stages of purification of novel inhibitors by gel-filtration, affinity chromatography and reversed-phase HPLC.

RESULTS AND DISCUSSION

Cereals. In wheat (*Triticum* L.) and *Aegilops* L. the spectra of insect amylase and proteinase inhibitors determined by IEF are specific for species, genomes or varieties and reflect evolutionary relationships between diploids and polyploids (Konarev, 1982, 1996). The polymorphism of inhibitors of chymotrypsin and subtilisin (C/SI) is comparable with that of prolamins and can be used in wheat variety identification.

In rice, inhibitors of trypsin, subtilisin and insect α-amylases were found in accessions of *Oryzae sativa* and other diploid species with the A genome but not in diploid species with the B and C genomes or tetraploid species with the BBCC and CCDD genomes. They therefore reflect divergence between rice species with A and other genomes.

Fabaceae. Data for four serine and cysteine proteinase inhibitor systems allowed evolutionary links between *Vigna* species to be estimated and identified diploid forms that may be related to the donors of the genomes of tetraploids. Data on 74 inhibitor band positions were in good agreement with data on morphology and DNA analysis and, in addition provided new information on the evolutionary relationships between species of the subgenus *Ceratotropis* (Konarev *et al*., 1999c, 2000b).

Potato. *Solanum tuberosus* varieties differed in the spectra of proteinase inhibitors present in tubers, in their ability to accumulate inhibitors in leaves in response to damage and in the spectra of induced inhibitors. Ability to accumulate inhibitors was found only among varieties resistant to Colorado beetle (Konarev and Fasulati, 2000).

Compositae. Multiple molecular forms of proteinaceous inhibitors of digestive proteinases of animals and extracellular proteinases of phytopathogenic fungi were identified in seeds of wild and cultivated *Helianthus* and other *Compositae* species. *H. annuus* seeds contain at least two types of trypsin inhibitors (TI) and bifunctional trypsin/subtilisin inhibitors (T/SI). The main TI, characteristic of the majority of *Helianthus* and some *Tithonia* species, is unique among plant proteinase inhibitors in its small size (1500) and cyclic structure (Konarev *et al*., 1999a, 2000a; Luckett *et al*., 1999). The TIs and T/SIs vary widely between *H. annuus* lines and wild *Helianthus* species in their presence or absence, composition, hydrophobicity and pI. Analysis of F_2 hybrids indicated that three loci encoding T/SI components were linked (Konarev *et al*., 1999a, 2000a). Similar components were found in annual diploid species with the B genome but not in perennials with the A genome. The T/SI present in seeds and vegetative organs were active against extracellular proteinases of the white rot fungus *Sclerotinia sclerotiorum*, an important pathogen of sunflower, indicating a possible protective role (Konarev *et al*., 1999b). T/SI, C/SI and T/C/SI with mass near 7500 are widely distributed in other *Compositae* species, being present in species of the subfamilies *Carduoideae* (genera *Carthamus*, *Centaurea*, *Cirsium*), *Cichorioideae* (*Lactuca*, *Taraxacum*) and *Asteroideae* (genera *Cosmos*, *Dahlia, Bidens*) etc.

Thus, in many plant taxa the spectra of various inhibitors can be specific for lines, varieties, single biotypes, species, groups of species, genomes and genera. Spectra of inhibitors reflect evolutionary links between species within a subgenus or genus or between diploid and polyploid species. The use of inhibitors as markers of plant resistance to harmful organisms can be based on their biological activity or on their physiological or genetic linkage with resistance factors. Methods of detection of amylase and proteinase inhibitors are applicable to representatives of many plant families and can be used to search for novel inhibitor types. Inhibitors can be effectively used in studies of plant diversity, evolution and plant-parasite co-evolution in combination with other protein and DNA markers.

REFERENCES

Konarev A. V. (1982): Component composition and genetic control of insect α-amylase inhibitors from wheat and *Aegilops* grain. Dokl. VASKhNIL, *6* : 42–44

Konarev A. V. (1986): Analysis of protease inhibitors from wheat grain by gelatine replicas method. Biochemistry (Moscow), *51*: 195–201.

Konarev A. V. (1996): Interaction of insect digestive enzymes with plant protein inhibitors and host parasite co-evolution. Euphytica, *92*: 89–94.

Konarev A. V., Anisimova I. N., Gavrilova V. A., Shewry P. R. (1999a): Polymorphism of inhibitors of hydrolytic enzymes present in cereal and sunflower seeds. In: Mugnozza G. T. S., Porceddu E., M. A. Pagnotta M. A. (Eds): Genetics and Breeding for Crop Quality and Resistance. Kluwer Acad. Publ.: 135–144.

Konarev A. V., Anisimova I. N., Gavrilova V. A., Rozhkova V. T., Fido R., Tatham A. S., Shewry P. R. (2000a): Novel proteinase inhibitors in seeds of sunflower (*Helianthus annuus* L.): Polymorphism, inheritance and properties. Theor. Appl. Genet., *100*: 82–88 .

Konarev Al. V., Fasulati S. R. (2000). Variability of potato varieties in ability to accumulate proteinase inhibitors. Trudy VIZR (in press).

Konarev Al. V., Fomicheva Yu. V. (1991): Cross analysis of the interaction of alpha-amylase and proteinase components of insects with protein inhibitors from wheat endosperm. Biochemistry (Moscow), *56*: 628–638.

Konarev A. V., Kochetkov V. V., Bailey J. A., Shewry P. R. (1999b): The detection of inhibitors of the *Sclerotinia sclerotiorum* (Lib) de Bary extracellular proteinases in sunflower. J. Phytopathol., *147*: 105–108.

Konarev A. V., Tomooka N., Ishimoto M., Vaughan D. A. (1999c): Variability of the inhibitors of serine, cysteine proteinases and insect alpha-amylases in Vigna and Phaseolus. In: Mugnozza G. T. S., Porceddu E., Pagnotta M. A. (Eds): Genetics and Breeding for Crop Quality and Resistance. Kluwer Acad. Publ.:173–181.

Konarev A. V., Tomooka N., Vaughan D. A. (2000b): Proteinase inhibitor polymorphism in the genus *Vigna* Savi subgenus *Ceratotropis* and its biosystematic implications. Euphytica (accepted, in press).

Luckett S., Garcia R. S., Barker J. J., Konarev A. V., Shewry P. R., Clarke A. R., Brady R. L. (1999): High-resolution structure of a potent, cyclic proteinase inhibitor from sunflower seeds. J. Mol. Biol., *290*: 525–33

Ryan C. A. (1990): Protease inhibitors in plants: gens for improving defenses against insects and pathogens. Annu. Rev. Phytopathol., *28*: 425–449.

Shewry P. R. (1999): Enzyme inhibitors of seeds: types and properties. In: Shewry P. R., Casey R. (Eds): Seed Proteins. Kluwer Acad. Publ.: 587–615.

Shewry P. R., Lucas J. A. (1997): Plant proteins that confer resistance to pests and pathogens. Adv. Bot. Res., *26*: 135–192.

Received for publication on June 22, 2000

Corresponding author:

Dr. Alexander K o n a r e v, All-Russian Institute for Plant Protection, St. Petersburg, Russia, tel: + 7 812 xxxxxxxxx, fax: + 7 812 xxxxxxx, e-mail: al_konarev@hotmail.com