

EXPRESSION PROFILES OF POTATO PROTEINASE INHIBITORS POTENTIALLY USEFUL FOR THE CONTROL OF PHYTOPATHOGENIC FUNGI

D. Turrà

Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli Federico II, 80055 Portici (NA), Italy

Proteinase inhibitors (PIs) are small ubiquitous proteins showing a variety of biological functions in plants, such as stabilization of proteins, modulation of apoptosis and defence against pathogens. They are particularly abundant in storage organs, such as seeds and tubers, and can accumulate in other plant tissues following a variety of different stimuli. Besides patatin, the major protein constituents of potato (*Solanum tuberosum*) tubers are low molecular weight PIs, such as protease inhibitors I (PI-1), II (Pin2) and Kunitz-type (PKPIs). PI-1 and PKPI inhibitors are coded in potato plants by relatively small gene families and synthesized as pre-pro-proteins (signal peptide-propeptide-mature proteins). A minimum of 21 Kunitz inhibitor genes (subdivided, on the basis of their sequence homology, into group A, B, and C) has been found to be present in the potato genome, while contrasting data have been reported for the number of genes belonging to the PI-1 family. In addition, PKPI and PI-1 accumulate in different plant tissues in response to biotic or abiotic stresses. However, little information is today available about the regulation (e.g. after nematode infection) of individual PI-1 genes or PKPI groups. In the present work, a PI mixture, obtained from potato (cv. Desireé) sprouts and showing a strong inhibitory activity against *Botrytis cinerea* fungal proteases, spore germination, hyphal elongation and necrotic activity, was purified and sequenced. Five novel full length PI-1 cDNAs (*PPI3B2*, *PPI3A2*, *PPI3A4*, *PPI2C4* and *PPI2C1A*) and two PKPI genes (*PKI1* and *PKI2*) were isolated, sequenced and characterized. Southern analysis, performed to confirm the presence of *PKI1* and *PPI3B2* genes and to determine the number of PI-1 genes in the genome, indicated that the *PKI1* gene is present as a single copy in the highly polymorphic family of the Kunitz inhibitors of the cultivar Desireé. On the other hand, the hybridizing bands obtained with the *PPI3B2* probe (at least nine) indicate that this gene could be part of a small multigene family (~ 10 members). Alignment of cloned and database retrieved PI inhibitor genes was used to design family, group or gene specific primers to individually analyze target transcripts by semiquantitative RT-PCR. Expression profiles of the three PKPI groups (A, B and C), Pin2 and PR1 (pathogenesis related 1) gene families, and the PI-1 family genes *PPI3A2*, *PPI3B2*, *PPI2C4* were studied in various tissues of Desireé, LB7/4/c-I-7 and P40 potato genotypes, after wounding and infection by *Globodera rostochiensis*. PI, but not PR1 genes, were found to be involved in the plant response triggered by the nematode *G. rostochiensis*, indicating an activation of the defense pathway via jasmonic acid. Moreover, individual PI-1 genes and PKPI groups were expressed in a tissue and genotype specific manner after biotic and abiotic stresses. The differences in the PI expression patterns concerned the intensity, type of inhibitors and rapidity of induction.

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Corresponding author: D. Turrà
Fax: +39.081.2539372
E-mail: davturra@unina.it

