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Genotype-dependent expression of specific members of potato protease inhibitor gene families in different tissues and in response to wounding and nematode infection

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Summary

Protease inhibitors (PIs) are small ubiquitous proteins with a variety of biological functions in plants, including protein stabilization, modulation of apoptosis and defense against pathogens. Kunitz-like inhibitors (PKPIs) and proteinase inhibitors 1 (*PI*-1) are abundant in storage organs of potato plants and are up-regulated in other tissues in response to biotic and abiotic stress. However, little information is available on genotype-dependent regulation of individual *PKPI* group- and *PI*-1 genes. We isolated, sequenced and characterized four novel full-length *PI*-1 cDNAs (*PPI3A2*, *PPI3A4*, *PPI2C4* and *PPI2C1A*) from *Solanum tuberosum* cv. Desirée. Specific primers were developed for *PI*-1 genes *PPI3A2*, *PPI3B2* and *PPI2C4* and the three *PKPI* homology groups A, B and C. Their expression profiles were studied by semi-quantitative RT-PCR in comparison with transcripts of the *PI*-1, *Pin*2 and *PR*1 gene families in various tissues, after wounding and *Globodera rostochiensis* infection of nematode-resistant genotypes P40 and LB7/4/c-I-7, and susceptible cv. Desirée. Individual *PI*-1 genes and *PKPI* homology groups were expressed in a tissue- and genotype-dependent manner after wounding and nematode infection. The differences in PI expression patterns were related to the intensity, type of inhibitors produced, and the kinetics of induction. Therefore, different genotype–environment combinations produce different sets of PI transcripts. Potato plants reacted to *G. rostochiensis* infection by modulating *PKPI*, *PI*-1 and *Pin*2, but not *PR*1 gene expression, suggesting that the jasmonic acid but not the salicylic acid defense

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signaling pathway is activated. PI expression profiles were not correlated with the resistance status of the potato genotype infected with *G. rostochiensis*.

Introduction

Plant reproductive and storage organs, e.g. seeds or tubers, accumulate massive amounts (1–10% of total protein) of protease inhibitors (PIs) (Ussuf et al., 2001). After patatin, the major constituents of *Solanum tuberosum* (potato) tuber storage proteins are low-molecular-weight PIs, such as proteinase inhibitors 1 (*PI*-1), II (*Pin*2) and Kunitz-type protease inhibitors (PKPIs) (Heibges et al., 2003b). PIs orchestrate many aspects of plant physiology including regulation of endogenous protease activity, protein stabilization, modulation of apoptosis, and protection from animals, insects, nematodes and microorganisms (Ussuf et al., 2001; Cai et al., 2003).

In potato, *PI*-1 and PKPIs are both encoded by multigene families and synthesized as pre-pro-proteins (signal peptide-propeptide-mature protein) (Heibges et al., 2003b; Hermosa et al., 2006). Each *PI*-1 gene codes for one subunit of a hexameric protein complex, each characterized by the consensus sequence WPE [V/L] [V/I] Gxxx [K/E] (Vandenbroek et al., 2004; Hermosa et al., 2006). PI-1 monomers interact with the protease by a substrate-type mechanism, through the formation of an exposed reactive center loop. The reactive center provides a "bait" P_1–P'_1 peptide bond (reactive site), which mimics the substrate of the target protease (Bode and Huber, 1992). Depending on the reactive site P_1–P'_1 amino acid residues, plant *PI*-1 can, in fact, exhibit specificity towards vertebrate and invertebrate serine endopeptidases such as trypsin, chymotrypsin, elastase, cathepsin G, subtilisin and *Streptomyces griseus* endopeptidase (Ogata et al., 1991; Bairoch, 1992; Heitz et al., 1993; Vandenbroek et al., 2004).

PKPIs are a highly polymorphic group of proteins subdivided into three groups A, B, and C based on sequence homology (Heibges et al., 2003b). Similarity of PKPI family members within groups ranges from 90% to 98%. Groups A and B are more similar to each other (75% identity on average) than to group C (A vs. C, 34% identity; B vs. C, 38% identity) (Heibges et al., 2003b). Similar to PI-1, PKPIs exhibit different specificity for enzyme inhibition. Group A inhibitor genes code for cathepsin D inhibitors, group B for trypsin, chymotrypsin, and elastase inhibitors and group C for subtilisin, cysteine protease, invertase and α-amylase inhibitors (Svendsen et al., 1986; Ishikawa et al., 1994; Barrett et al., 2001; Heibges et al., 2003a).

PI-1 and PKPI expression is known to be developmentally and environmentally regulated in the Solanaceae. PI-1 transcripts are constitutively present in potato tubers, in etiolated *Nicotiana tabacum* (tobacco) leaves (Kuo et al., 1984), and in floral buds (Heitz et al., 1993). Their expression is induced during fruit ripening (Lincoln et al., 1987), tobacco mosaic virus (TMV) infection (Geoffrey et al., 1990), methyl jasmonate (JAME) application (Lightner et al., 1993), insect attack or wounding (Ryan, 1978). PKPI transcripts are found in potato tubers (A, B and C groups) (Heibges et al., 2003b), young leaves and flower buds (A group), while environmentally regulated in response to JAME treatment (A group), wounding (A and C groups) (Suh et al., 1991; Ishikawa et al., 1994), infection with *Phytophtora infestans* (B and C groups) (Valueva et al., 1998) and TMV (Park et al., 2000).

Several factors involved in plant signaling processes are required for the activation of defense genes in the Solanaceae. A set of such defense genes, including *PI*-1, *Pin*2, aspartic protease inhibitors and carboxypeptidase inhibitors, is locally and systemically insect- or wound-activated by the 18 amino acid mobile signal systemin, through a lipid-based signaling pathway that involves linoleic acid and jasmonic acid (JA) (Dammann et al., 1997; Li et al., 2002; Ryan and Moura, 2002; Díez-Díaz et al., 2004). Another set of defense genes, among others coding for acidic pathogenesis-related (PR) proteins (PR-1, PR-2, PR-5), is regulated by salicylic acid (SA) during the establishment of the pathogen-triggered localized hypersensitive response (HR) and systemic acquired resistance (SAR) (Shirasu et al., 1997; Yang et al., 1997; Xie et al., 1998; Gu et al., 2002). Several PR genes, including glucanase, chitinase, osmotin and PI genes, have been used to improve plant resistance against insects, fungi or nematodes by recombinant DNA technology (Broglie et al., 1991; Liu et al., 1994; Zhu et al., 1994; Urwin et al., 1998). The constitutive expression of *CpTI*, *Oc*-1 and sporamin PI genes in transgenic plants has been shown to effectively limit nematode growth by reducing nutrients' digestibility and fertility (Urwin et al., 1998; Cai et al., 2003). Plant