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Short communication

Chitinase and β-1,3-glucanase isoforms expressed in pea roots inoculated with arbuscular mycorrhizal or pathogenic fungi

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Abstract

Chitinases were studied in an endomycorrhiza-resistant mutant and wild type pea (*Pisum sativum* L. cv. Frisson) in order to characterize plant hydrolases specific to pathogenic (*Aphanomyces euteiches* and *Chalara elegans*) or mycorrhizal (*Glomus mosseae*) root interactions. Stimulation of constitutive and induction of new chitinase activities was detected by native PAGE for acidic proteins in both pea genotypes inoculated with pathogenic fungi. In contrast, a different additional chitinase isoform was induced in *G. mosseae*-colonized roots. This isoform was also not elicited in chemically-stressed roots, confirming its mycorrhiza-specificity. Investigations of basic chitinase and β-1,3-glucanase activities provided further evidence for differential pea responses during pathogenic and symbiotic interactions.

Endomycorrhizal associations are ubiquitous in the plant kingdom. However, the molecular events governing such symbiosis are poorly understood [Gianinazzi-Pearson, 1995]. Extensive studies of chitinases and β-1,3-glucanases, induced during pathogenic infections, have lead to a suggested role in defence against fungi. Although arbuscular fungi are not considered to induce typical host defense responses, several reports deal with hydrolases in endomycorrhizas [reviewed by Gianinazzi-Pearson, 1995; Sahai and Manocha, 1993]. Specific chitinase isoforms have been reported in tobacco endomycorrhizas [Dumas-Gaudot *et al.*, 1992]. Furthermore, an additional chitinase isoform of plant origin was found in pea genotypes establishing endomycorrhizas [Dumas-Gaudot *et al.*, 1994], and which could be induced as a non-specific response to various fungal infections.

Chitinase activities were studied in roots of *Pisum sativum* L. cv. Frisson or the endomycorrhiza-resistant P2 mutant [Duc *et al.*, 1989] infected with two pathogenic fungi, *Aphanomyces euteiches* Drechs. (Oomycetes) and *Chalara elegans* Nag Rag & Kendrick (Deuteromycetes), selected because of their cell wall composition. Oomycetes are reported to contain cellulose and glucans while Deuteromycetes contain chitin and glucans [Bartnicki-Garcia, 1968]. Comparisons were made with roots inoculated with the arbuscular fungus *Glomus mosseae* (BEG 12) or elicited by chemicals, chitosan [Chang *et al.*, 1992] and acetylsalicylic acid (ASA) [White, 1979], known to mimic pathogenic attack. All root extracts showed 2 constitutive acidic chitinase activities (Fig. 1A, lanes 1–10). In plants infected with *A. euteiches* [see Beghdadi *et al.*, 1992; 10^4 zoospores ml^{-1} inoculum], an avirulent strain SRSF 504 did not interfere with plant growth nor induce root necrosis (data not shown), and only these isoforms were observed (Fig. 1A, lane 4). In contrast, in pea infected by a virulent strain SRSF 502,

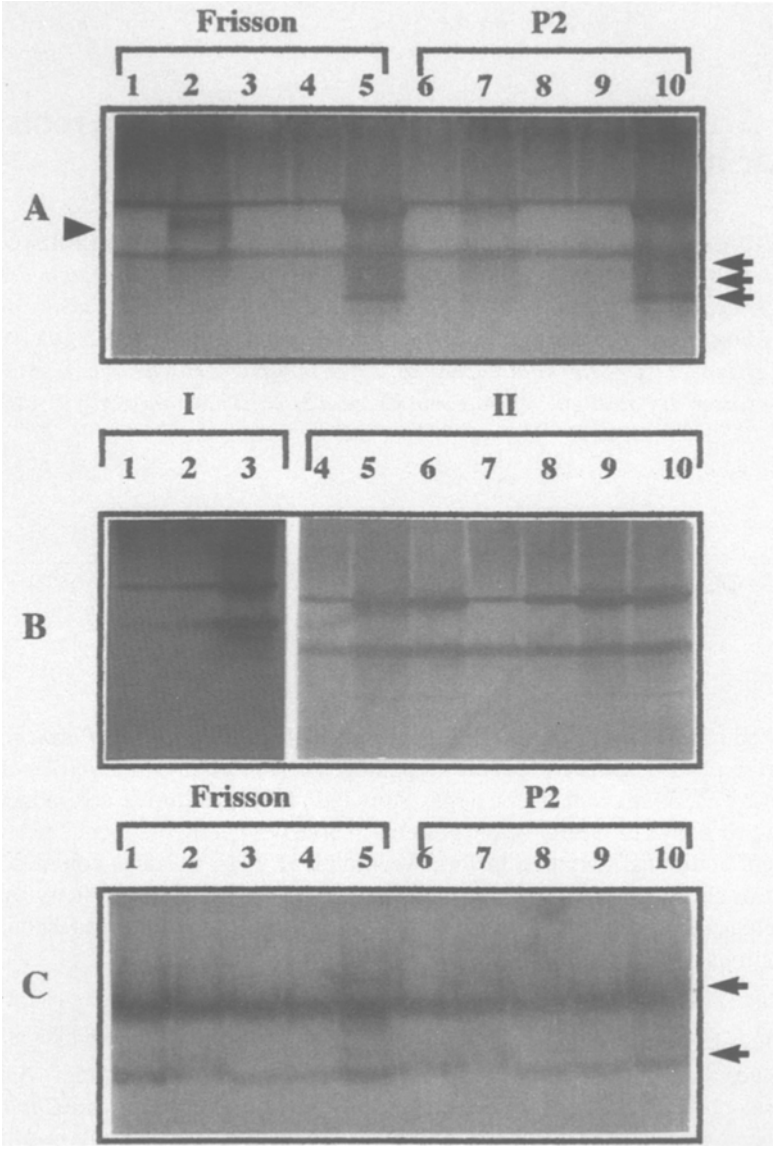

Fig. 1. Acidic (panels A, B; see Dumas-Gaudot *et al.*, 1994) or basic (C; see Trudel and Asselin, 1989) chitinase activities after 15% (w/v) native PAGE. In A, root extracts from uninoculated wild type *P. sativum* (lanes 1, 3) and mycorrhiza-resistant P2 mutant (6, 8), inoculated with *G. mosseae* (2, 7), or avirulent SRSF 504 (4, 9) and virulent SRSF 502 (5, 10) *A. euteiches*. In BI, extracts from uninfected main roots (lane 1), *C. elegans*-inoculated secondary (2) and main (3) roots from wild type pea. In BII, extracts from wild type (lanes 4–6) or P2 mutant (7–10), chemically-stressed for 2 days. Roots were treated with sterile water (4, 8), 4 mM ASA pH 6.5 (5, 9) or 4 mg ml^{-1} chitosan pH 6.5 (6, 10). In lane 7, the extract from non-treated roots was immediately frozen at sampling of the P2 mutant. In C, the same extracts as in A, analyzed for basic proteins. All samples contained 20 mg fresh weight material. Each gel separation was repeated at least twice. Plant growth conditions were according to Dumas-Gaudot *et al.*, 1994, and roots harvested after infection 7, 10 and 21 days for *C. elegans, A. euteiches* and *G. mosseae* inoculation respectively. Additional pathogenesis-related chitinase activities are indicated by arrows, and the mycorrhiza-induced chitinase isoform by an arrowhead.

roots were completely necrotized, and constitutive isoforms were strongly increased. Furthermore, 3 new isoforms with higher mobility were observed (Fig. 1A, lane 5). Similar results were obtained with *C. elegans*-infected roots (strain 84.1, Institut du Tabac, Bergerac, France; 10^6 ml^{-1} phialoconidia suspension), showing necroses on the main and secondary roots already developed at the time of inoculation (Fig. 1B, lane

Table 1. Diagram of changes in acidic and basic chitinase and β-1,3-glucanase isoforms after infection of wild type *P. sativum* roots with *G. mosseae*, *A. euteiches*, *C. elegans*, or after chemical treatment. The same changes were observed in the P2 mutant, except for the mycorrhiza-induced acidic chitinase isoform which was not detected. nd = not determined. Acidic and basic β-1,3-glucanase activities were revealed after blotting (respectively for 2 and 4 h) a 7.5% polyacrylamide overlay gel containing alkali-soluble baker's yeast β-1,3-glucan as substrate. Incubation and staining procedures were as described by Grenier and Asselin (1993)

	control	*G. mosseae*	*A. euteiches* SRSF 502	*C. elegans*	ASA	chitosan
acidic chitinases						
basic chitinases				nd	nd	nd
acidic β-1,3-glucanases				nd	nd	nd
basic β-1,3-glucanases				nd	nd	nd

3). In symptomless secondary roots which grew later, only the constitutive isoforms were detected (Fig. 1B, lane 2). The same patterns as in necrotized roots were observed in wild type pea elicited by chitosan and ASA (Fig. 1B, lanes 5, 6), and in the P2 mutant similarly treated with chemicals (Fig. 1B, lanes 9, 10) or infected by the *A. euteiches* virulent strain (Fig. 1A, lane 10). As previously reported [Dumas-Gaudot *et al.*, 1994], one additional acidic isoform was detected in wild pea roots fully colonized by *G. mosseae* (Fig. 1A, lane 2), but not in the mycorrhiza-resistant mutant (Fig. 1A, lane 7). Extracts were also analyzed for basic chitinase activities. Two isoforms were constitutively expressed in all samples (Fig. 1C, lanes 1–10). Furthermore, 2 additional bands with weak activities were detected in wild type roots inoculated with the *A. euteiches* virulent strain (Fig. 1C, lane 5), while in the similarly inoculated P2 mutant only the isoform with the faster mobility was observed (Fig. 1C, lane 10). In *G. mosseae*-inoculated roots, the 2 constitutive bands were observed without differential responses in either pea genotype (Fig. 1C, lanes 1, 2, 6, 7).

Additional investigations on β-1,3-glucanases revealed differences only in basic activities in pathogen-infected roots. Two isoforms already present in all extracts were detected. However, the faster migrating band showed a stronger activity in virulent *A. euteiches*-inoculated roots (Table 1). The same results were obtained in P2 roots (data not shown). These results are in agreement with the known functions of β-1,3-glucanases during pathogenesis [Simmons, 1994]. No specific modifications were observed in mycorrhizal roots. However, we must be cautious before rejecting implication of β-1,3-glucanases in endomycorrhizal symbioses, especially when considering that such enzymes may be acting on different substrates [Coté *et al.*, 1991], and act synergically with chitinases *in vitro* [Mauch *et al.*, 1988].

In conclusion, using pea genotypes differing in their ability to form mycorrhizas inoculated with arbuscular/pathogenic fungi, or chemically elicited, we clearly demonstrated the specificity of one mycorrhiza-induced acidic chitinase isoform in pea. Besides the major putative function of chitinases as defense

proteins with antimicrobial activities, some potential roles for either the liberation of signal molecules or organogenesis and differentiation have been reported [Sahai and Manocha, 1993]. Such hypotheses need to be tested, especially considering that infection with arbuscular mycorrhizal fungi modifies root morphology and architecture [Berta *et al.*, 1990]. The additional chitinase isoform constitutes so far the first example of a protein of known function which is specifically induced in endomycorrhizas. A purification protocol is presently underway in order to further characterize the mycorrhiza-induced pea chitinase isoform. Furthermore, a cDNA library from *G. mosseae*-inoculated roots is being screened with homologous [Vad *et al.*, 1991] and heterologous chitinase probes.

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References

Bartnicki-Garcia S (1968) Cell wall chemistry, morphogenesis, and taxonomy of fungi. Ann Rev Microbiol 22: 87–108

Beghdadi A, Richard C and Dostaler D (1992) L'*Aphanomyces euteiches* des luzernières du Québec: isolement, morphologie et variabilité de la croissance et du pouvoir pathogène. Can J Bot 70: 1903–1911

Berta G, Fusconi A, Trotta A and Scannerini S (1990) Morphogeneric modifications induced by the mycorrhizal fungus *Glomus* strain E3 in the root system of *Allium porrum* L. New Phytol 114: 207–215

Chang MM, Hadwiger LA and Horovitz D (1992) Molecular characterization of a pea β-1,3-glucanase induced by *Fusarium solani* and chitosan challenge. Plant Mol Biol 20: 609–618

Coté F, Cutt JR and Asselin A (1991) Pathogenesis-related acidic β-1,3-glucanase genes of tobacco are regulated by both stress and developmental signals. Electrophoresis 4: 173–181

Duc G, Trouvelot A, Gianinazzi-Pearson V and Gianinazzi S (1989) First report on non-mycorrhizal plant mutants (Myc-) obtained in pea (*Pisum sativum* L.) and faba bean (*Vicia faba* L.). Plant Sci 60: 215–222

Dumas-Gaudot E, Asselin A, Gianinazzi-Pearson V, Gollotte A and Gianinazzi S (1994) Chitinase isoforms in roots of various pea genotypes infected with arbuscular mycorrhizal fungi. Plant Sci 99: 27–37

Dumas-Gaudot E, Furlan V, Grenier J and Asselin A (1992) New acidic chitinase isoforms induced in tobacco roots by vesicular-arbuscular mycorrhizal fungi. Mycorrhiza 1: 133–136

Gianinazzi-Pearson V (1995) Morphofunctional compatibility in interactions between roots and arbuscular endomycorrhizal fungi: molecular mechanisms, genes and gene expression. In: Kohmoto K, Singh RP and Singh US (eds) Pathogenesis and host-parasite specificity in plant diseases. Vol. II (pp. 251–263) Pergamon Press, Elsevier Science, Oxford

Grenier J and Asselin A (1993) Detection of β-1,3-glucanase activity in gels containing alkali-soluble yeast glucan. Anal Biochem 212: 301–302

Mauch F, Mauch-Mani B and Boller T (1988) Antifungal hydrolases in pea tissue. II. Inhibition of fungal growth by combination of chitinase and β-1,3-glucanase. Plant Physiol 88: 936–942

Sahai AS and Manocha MS (1993) Chitinases of fungi and plants: their involvement in morphogenesis and host-parasite interaction. FEMS Microbiology Reviews 11: 317–338

Simmons CR (1994) The physiology and molecular biology of plant 1,3-β-D-glucanases and 1,3;1,4-β-D-glucanases. Critical Reviews in Plant Sciences 13: 325–387

Trudel J and Asselin A (1989) Detection of chitinase activity after polyacrylamide gel electrophoresis. Anal Biochem 178: 362–366

Vad K, Mikkelsen JD and Collinge DB (1991) Induction, purification and characterization of chitinase isolated from pea leaves inoculated with *Ascochyta pisi*. Planta 184: 24–29

White RF (1979) Acetylsalicylic acid (aspirin) induces resistance to tobacco mosaic virus in tobacco. Virology 99: 410–412