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Plant hydrolytic enzymes (chitinases and β-1,3-glucanases) in root reactions to pathogenic and symbiotic microorganisms

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Abstract

Within the last decade, a great deal of attention has been devoted to the role of chitinases and β-1,3-glucanases in plant/microbe interactions. While there is strong evidence that these hydrolases are antifungal proteins, there are also recent indications of roles in both plant morphogenesis and plant/microbe signal perception. This paper reviews recent findings pertinent to root/microbe interactions, and discusses the nature and significance of specific hydrolase isoforms in symbioses with arbuscular mycorrhizal (AM) fungi.

Introduction

Due to growing concern about environmental protection, research on the possibility of adding or manipulating microorganisms already present in agricultural systems in order to enhance plant protection against pathogens have received increasing attention in the last few years. There is considerable evidence for the role of arbuscular mycorrhizal (AM) fungi in the control of root pathogens (Caron, 1989; Dehne, 1982; Hooker et al., 1994; Starnaud et al., 1995). The possible mechanisms involved, including competition for colonization sites, direct antibiosis, nutritional aspects and plant defense reactions, have recently been reviewed (Azcon-Aguilar and Barea, 1996). However, the mechanisms of action are still poorly understood, especially if we consider that the observed effects more likely result from different factors acting together. The colonization of roots by AM fungi induces biochemical changes within host tissues. These include stimulation of the phenylpropanoid pathway (Harrison and Dixon, 1993; Morandi et al., 1984, 1996), change in levels of aliphatic polyamines (El Ghachtouli et al., 1995), synthesis of proteins of unknown function (Dumas-Gaudot et al., 1994b; Samra et al., 1995), activation of defense-related genes (Franken and Gnadinger, 1994; Gianinazzi-Pearson et al., 1992; Harrison and Dixon, 1993) and enhancement of certain hydrolase activities (Dumas-Gaudot et al., 1992b; Spanu et al., 1989).

Low priming of defense-related pathways in roots by AM fungi may provoke more rapid responses to subsequent pathogen attack and so contribute to bioprotective effects through mechanisms similar to those known for preimmunization after preinfection by hypovirulent viruses, bacteria or fungi (Kuc, 1987; Ryals et al., 1994; Tuzun and Kloepper, 1994). Plant hydrolytic enzymes, i.e. chitinases and β-1,3-glucanases, appear good candidates amongst molecules with a potential role in bioprotection. Chitinases [poly (1,4-(N-acetyl-β-D–glucosaminide)) glycanohydrolase, EC 3.2.1.14] catalyze the hydrolysis of chitin, a linear homopolymer of β-1,4-linked N-acetylglucosamine residues. β-1,3-glucanases (EC 3.2.1.39) degrade 1,3 β-D-glucosidic linkages in β-D-glucans, and sometimes even 1,3; 1,6-β-glucans. Since the cell walls of many fungi contain chitin and/or β-D-glucans as major structural components (Bartnicki-Garcia, 1968; Wessels and Siestma, 1981), chitinases

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and β-1,3- glucanases were proposed early as potential antifungal compounds in plant disease control (Boller et al., 1983; Boller, 1987; Mauch et al., 1988). This hypothesis has been reinforced by both in vitro experiments (Arlorio et al., 1992a, 1992b; Boller et al., 1983; Broekaert et al., 1988; Mauch et al., 1988; Roberts and Selitrennikoff, 1988) and using transgenic plants over-expressing either plant or microbial chitinase or glucanase genes (Broglie et al., 1991).

Several reviews on chitinases and one on glucanases (Collinge et al., 1993; Flach et al., 1992; Graham and Sticklen, 1993; Sahai and Manocha, 1993; Simmons, 1994) have recently appeared. The aim of this review is to focus on the implication of plant hydrolytic enzymes in root/microbe interactions with special emphasis on symbioses and the possible role of plant hydrolases in bioprotection.

Plant chitinases and β-1,3-glucanases, their role in antifungal defense in roots

General biochemical properties of many plant chitinases and β-1,3-glucanases have been extensively compiled (Bol et al., 1990; Boller, 1993; Graham and Sticklen, 1993; Simmons, 1994). Although induction of chitinases and β-1,3-glucanases might be considered as part of a non-specific plant response to various biotic and abiotic stress stimuli, this does not exclude an active role against microbial pests. Whilst induction of these enzymes in aerial plant parts in response to pathogen attacks has received considerable attention, comparatively very few investigations have been concerned with infected roots (Table 1). Research into an antifungal role of chitinases and β-1,3-glucanases has been conducted by both in vitro analyses on inhibitory growth effects against several fungi and in planta using transformed plants in which chitinase and/or β-1,3-glucanase genes are over-expressed.

Antifungal activities of chitinases and β-1,3-glucanases have been studied in vitro using various bio-assays (reviewed in Boller, 1993). Table 2 summarizes the antifungal activities reported up to now for both enzymes tested either alone or in combination against soil-borne fungi. Antifungal activity of plant chitinases was first shown in the bioassay using purified enzymes added on agar plates with the soil saprophyte *Trichoderma viride* (Mauch et al., 1988; Schlumbaum et al., 1986). Using a similar bioassay, several groups confirmed the inhibitory effect of chitinases on the development of rapidly growing soil saprophytes (Arlorio et al., 1992a, 1992b; Broekaert et al., 1988; Huynh et al., 1992; Roberts and Selitrennikoff, 1988; Verburg and Huynh, 1991). However, except for a few cases (Broglie et al., 1991; Shapira et al., 1989), results were rather disappointing with several phytopathogenic fungi (Huynh et al., 1992; Mauch et al., 1988; Verburg and Huynh, 1991), and ectomycorrhizal or ericoid mycorrhizal fungi were not sensitive to chitinaoes (Arlorio et al., 1992a, 1992b). Using a modification of this bioassay with enzymes applied onto small filter disks in contact with the growing fungi, inhibition with chitinases or β-1,3-glucanases was shown only in few cases. However, combinations of both purified enzymes inhibited growth of most tested soil-borne fungi (Mauch et al., 1988). The more reliable spectrophotometric bioassay in microtitre plates (Broekaert et al., 1990; Ludwig and Boller, 1990), which allows observation of fungal growth in liquid media, confirmed previous results of chitinase and glucanase inhibition as well as the synergistic effect of both enzymes. Using such a bioassay, it was shown that growth inhibition of *Nectria hematococca* was only transient (Ludwig and Boller, 1990), suggesting that the fungus has the ability to adapt to the hydrolytic enzymes. Detailed analyses of the antifungal activities of different hydrolases from tobacco tested individually or in combinations have been realized (Melchers et al., 1993; Sela-Buurlage et al., 1993). Tobacco class I chitinase and class I glucanase were demonstrated as being the most active in inhibiting growth of *Fusarium solani* germlings. Class II chitinase showed only a limited inhibitory activity in combination with class I glucanase. Not only plant chitinases display antifungal activity against root pathogens but also bacterial, fungal and even insect chitinases (Anas et al., 1988; Ordentlich et al., 1988; Shapira et al., 1989 and others reviewed in Sahai and Manocha, 1993).

Light and electron microscope studies have established that growth inhibition by the hydrolases is accompanied by swelling and lysis of hyphal tips, indicating that the antifungal activity is due to a weakening of the apex cell walls (Arlorio et al., 1992a, 1992b; Broekaert et al., 1989; Mauch et al., 1988). The theory of the receptivity of nascent chitin of growing filamentous fungi to plant hydrolases has recently been supported by an autoradiographic study of the foliar pathogen *Cercospora beticola*, where purified basic sugar beet class I chitinase was able to degrade newly synthesized radioactively labelled chitin fibers in the hyphal apex, whereas it had no effect on the chitin

Table 1. Soil-borne pathogenic fungi inducing chitinases and/or β-1,3-glucanase activities in root systems of different plants

Plants	Pathogenic fungi	References
Eucalyptus	*Phytophthora cinnamomi*	Albrecht et al. (1994a,c)
Pea	*Aphanomyces euteiches*	Dassi et al. (1996)
	Chalara elegans	
Potato	*Phytophora infestans*	Matton and Brisson (1989)
Tobacco	*Chalara elegans*	Tahiri-Alaoui et al. (1990)
		Dumas-Gaudot et al. (1992a)
Tomato	*Fusarium oxysporum* f.sp.	Benhamou et al. (1989)
	radicis-lycopersici	Benhamou et al. (1990)
Canola	*Rhizoctonia solani*	Benhamou et al. (1992)

layer in the mature cell walls (Collinge et al., 1993; Nielsen et al., 1994).

Direct evidence of hydrolase attack of fungal cell walls in planta has come from immunocytochemical investigations of *F. oxysporum* f. sp. *radicis-lycopersici*-infected tomato roots and 35S chitinase gene-transformed canola roots infected by *Rhizoctonia solani* (Benhamou et al., 1990, 1993; Benhamou, 1993). Chitinase and β-1,3-glucanase accumulated over fungal cell walls, thus supporting the view of their antifungal activities. However, chitinase appeared to be preferentially associated with altered fungal cell structures, suggesting a differential action of the hydrolases.

Many chitinase and β-1,3-glucanase cDNAs or genes have been cloned, and several genetically engineered plants have been constructed with the aim of enhancing resistance to pathogenic fungi. Table 3 lists the plants which have been transformed by over-expressing hydrolase genes and tested for their resistance against soil-borne fungal pathogens. Several reports deal with successful transformation of plants constitutively expressing high levels of class I chitinase accompanied by an increased resistance to root fungal pathogens (Broglie et al., 1991; Jach et al., 1992; Samac et al., 1990; Samac and Shah, 1991; Vierheilig et al., 1993; Yoshikawa et al., 1993). A progressive breakdown of labelled chitin of *R. solani* was further demonstrated at the ultrastructural level in roots of transformed tobacco and canola plants over-expressing a bean chitinase (Benhamou, 1993; Benhamou et al., 1993). Transgenic tobacco expressing high levels of either tobacco class I basic chitinase, tobacco class III acidic chitinase or cucumber class III acidic chitinase all showed increased resistance to *R. solani* (Lawton et al., 1993). Furthermore, *Arabidopsis thaliana* plants transformed to down regulate a class I chitinase gene, using an RNA antisense strategy, were recently demonstrated to be more susceptible to *Botrytis cinerea* (Samac and Shah, 1994). However, as underlined by Stintzi et al. (1993), targeting of hydrolytic enzymes to the right plant cell compartments has to be taken into account with regards to the cellular development of the fungus. Furthermore, since chitinases and β-1,3-glucanases very often act synergistically against fungi containing chitin-glucans in their cell walls, increased resistance could be expected in transformed plants simultaneously constitutively expressing high levels of both enzymes or even other plant defense antifungal proteins. Recently, Jach et al. (1995) showed that transgenic tobacco plants transformed for both barley chitinase and β-1,3-glucanase genes were more resistant to *R. solani* than plants expressing those genes individually, providing additional arguments in favor of combinatorial expression of antimicrobial genes as an effective approach to engineering enhanced crop protection against fungal disease.

Plant chitinases and β-1,3-glucanases in root symbioses

Nodules

Plant roots are colonized not only by pathogens but also by beneficial symbiotic microorganisms. Among these are rhizobia which develop symbiosis with legumes. This interaction normally culminates in the formation of the symbiotic organ, the nodule, which functions in nitrogen fixation (Govers et al., 1987; Nap and Bisseling, 1990). The early stages of infection by rhizobia have been sometimes considered to resem-

Table 2. Soil-borne fungi identified as being sensitive or insensitive to chitinase

Species	Chitinase source	Chitinase effective[a]	Effective with β-1,3-glucanase[a]	References
Sclerotinia sclerotiorum	Insect	Y	nd	Anas et al. (1988)[1]
Sclerotium rolfsii	*Serratia marcescens*	Y	nd	Shapira et al. (1989)[1]
R. solani	*S. marcescens*	Y	nd	Shapira et al. (1989)[1]
R. solani	Bean	Y	nd	Broglie et al. (1991)[2]
S. rolfsii	*S. marcescens*	Y	nd	Ordentlich et al. (1988)[1]
Trichoderma hamatum	Wheat	Y	nd	Broekaert et al. (1988)[2] Broekaert et al. (1990)[3]
T. hamatum	Tobacco	Y	nd	Broekaert et al. (1988)[2]
T. hamatum	Thorn apple	Y	nd	Broekaert et al. (1988)[2]
T. reesi	Wheat	Y	nd	Roberts and
Phycomyces blakeeslanus	Barley	Y	nd	Selintrennikoff (1988)[2]
	Maize	Y	nd	
T. reesi	Arabidopsis	Y	nd	Verburg and Huynh (1991)[2]
T. reesi	Maize seed	Y	nd	Huynh et al. (1992)[2]
T. viride	Bean	Y	Y	Schlumbaum et al. (1986)[2]
T. viride	Bean	Y	Y	Mauch et al. (1988)[2]
T. reesi	Barley seed	Y	Y	Leach et al. (1991)[3]
Fusarium sporotrichioides				
T. longibrachiatum	Pea	Y	Y	Arlorio et al. (1992)[1] a,b
R. solani				
F. solani	Tobacco			
	CH I/GLU I	Y	Y	Sela-Buurlage et al.
	CH I/GLU I	Y	N	(1993)[3]
	CH II PR-3a/ GLU I	N	Y	
	CH II PR-3b/ GLU I	N	Y	
F. solani				
f.sp. *phaseoli*	Pea	N	Y	Mauch et al. (1988)[2]
f.sp. *pisi*	Pea	N	Y	
Hymenoscyphus ericae	Pea	N	N	Arlorio et al. (1992)[1] a,b
Heleboma crustuliniforme	Pea	N	N	
T. reesi	*S. marcescens*	N	nd	Roberts and
P. blakeeslanus	*Streptomyces griseus*	N	nd	Selintrennikoff (1988)[2]
S. sclerotiorum	Arabidopsis	N	nd	Verburg and Huynh (1991)[2]
	Maize seed	N	nd	Huynh et al. (1992)[2]
Nectria hematococca	Pea	tr	tr	Ludwig and Boller (1990)[3]
Thielaviopsis basicola	Pea	nd	Y	Mauch et al. (1988)[1]

[a] Antifungal activities of purified chitinases were tested in vitro by the punched agar plate (Mirelman et al., 1975)[1] or the modified agar plate method in which purified enzymes are applied on small filter papers laid down on agar (Mauch et al., 1988)[2], or by bioassays in microtitre plates (Granade et al., 1985; Ludwig and Boller, 1990)[3].
Chitinase was effective (Y) or not (N); nd= not determined; tr = transient antifungal activity.

Table 3. Antifungal potential of chitinase and β-1,3-glucanase in roots of transgenic plants

Transgenic plants	Chitinase and/or glucanase genes	Promoter/reporter genes[a]	Pathogenic fungi	Effect[b]	Mycorrhizal fungi[b]	References
Tobacco	Bean chitinase	*CaMV35S/Ch5 B*	*Rhizoctonia solani*	Y	nd	Broglie et al. (1991)
Rape	Bean chitinase	*CaMV3SS/Ch5 B*	*Rhizoctonia solani*	Y	nd	Broglie et al. (1991)
Tobacco	*Serratia marcescens*	*CaMV35S/Ch A*	*Rhizoctonia solani*	Y	nd	Jach et al. (1992)
Arabidopsis thaliana	*Arabidopsis* chitinase	*CaMV35S/Ch* class III/GUS	*Rhizoctonia solani*	G	nd	Samac et al. (1990, 1991)
Tomato	*Arabidopsis* chitinase	*CaMV35S/Ch* class III/GUS	*Phytophthora infestans*	G	nd	Samac et al. (1990, 1991)
Tobacco	Tobacco chitinase	*CaMV35S*/Tob	*Rhizoctonia solani*	Y	N	Vierheilig et al. (1993, 1995)
		CaMV35S/TobΔH		Y	N	
		CaMV35S/TobΔT		N	N	
Tobacco	Bean chitinase			nd	N	Tahiri-Alaoui et al. (unpubl.)
	Aphanocladium chitinase			nd	N	
Tobacco	Soybean β-1,3-glucanase	*CaMV35S/β-1,3-Glu* class I	*Phytophthora parasitica*	Y	nd	Yoshikawaka et al. (1993)
Tobacco	Barley chitinase	*CaMV35S/Ch*	*Rhizoctonia solani*	YY	nd	Jach et al. (1995)
	Barley β-1,3-glucanase	classII/*β-1,3-Glu* class II/Type I				
	Barley RIP*	RIP				

[a] Transgenic plants for chitinase or β-1,3-glucanase (or RIP* = Type I ribosome-inactivating protein) genes are construct using the following promoters: CaMV35S = promoter region of cauliflower mosaic virus 35S transcript; GUS = β-glucuronidase reporter gene; Tob = tobacco chitinase A; TobΔH = tobacco chitinase A deleted for the chitin-binding domain; TobΔT = tobacco chitinase A deleted for the C-terminal extension.
[b] Transgenic plants are proved to be : Y = resistant; YY = highly resistant; N= not resistant; G= localized induction of GUS enzyme activity in infected-transformed plants; nd= not determined.

ble a pathogen attack (Vance, 1983; Vasse et al., 1993). Although there have been some reports dealing with investigations of plant defense molecules and defense-related genes elicited in the rhizobial symbiosis (Grosskopf et al., 1993; Parniske et al., 1990; Werner et al., 1985), only a few studies have focussed on the induction of hydrolases in such symbioses. Staehelin et al. (1992) reported that pathogenesis-related proteins, including chitinases, were induced in soybean nodules infected with *Bradyrhizobium japonicum*. Compared to the low constitutive chitinase activity in roots, elevated levels were found in the uninfected cortex of both effective and ineffective nodules, while activity was present in the entire body of hypersensitively reacting nodules. It has been proposed that in root nodules chitinases may protect the infected zone from external pathogens or the root system from pathogenic rhizobia. In addition, Nod factors have been shown to be substrates for plant chitinases (Schultze et al., 1993), with different degrees of stability against chitinase degradation in vivo and in vitro (Staehelin et al., 1994a, 1994b). The finding that lipooligosaccharide Nod factors are cleaved by chitinases raises the question of whether their stability could be one of the determinants of host specificity. Furthermore, a role of chitinases in controlling plant morphogenesis and cell division has been suggested (De Jong et al., 1992, 1993; Staehelin et al., 1994a, 1994b).

Mycorrhizas

Several studies have centered on elicitation of plant defense responses during ectomycorrhizal (reviewed in Martin and Tagu, 1995; Martin et al., 1995) and

and endomycorrhizal symbioses (Bonfante-Fasolo and Perotto 1992; Gianinazzi et al., 1995; Gianinazzi-Pearson et al., 1995, 1996; Volpin et al., 1994). Increased chitinase activities have been reported (Albrecht et al., 1994a,b,c; Sauter and Hager, 1989) in ectomycorrhizal symbiosis although no chitinase synthesis was found in *Picea abies* roots colonized by *Pisolithus tinctorius* (Wiemken and Ineichen, 1992). Increases in chitinase activity occur rapidly in root tissues before the onset of visible morphological changes. However, comparisons of chitinase isoforms in ectomycorrhizas established with symbiotic fungal strains differing in their aggressiveness to root infections with pathogenic fungi revealed only quantitative differences between the two types of root infections (Albrecht et al., 1994a,c).

Several reports have been published on chitinases and glucanases in AM symbioses since the first investigations by Spanu et al. (1989) and Dumas et al. (1989). Endochitinase and β-1,3-glucanase gene expression was analyzed in bean roots inoculated with *Glomus intraradices* to see whether it could be correlated to observed inhibition of fungal growth under high phosphorus conditions (Lambais and Mehdy, 1993). As previously shown in leek roots (Spanu et al., 1989), higher expression of chitinase was detected in the early stages of colonization under both high and low levels of soil phosphorus. However, expression of chitinase was diminished, but at later stages of mycorrhiza development, to a greater extent under low than under high phosphorus conditions. Suppression under both conditions was related to differential reduction in the level of mRNAs encoding two endochitinase isoforms. Repression of β-1,3-glucanase activity as well as in the levels of two β-1,3-glucanase mRNAs encoding distinct isoforms was also observed at certain stages of mycorrhiza development at both phosphorus levels. From these data, it was suggested that there is a general suppression of plant defense responses during AM development, so favouring fungal growth. However, recent analyses of mRNA accumulation, complemented by in situ hybridization studies, for several defense-related genes including chitinases and glucanases indicate that the intensity of the responses seems more related to the presence of young arbuscules, which are predominant earlier in the development of the symbiosis (Blee and Anderson, 1996). Chitinase activity was also found to increase prior to colonization of alfalfa roots inoculated by the mycorrhizal fungus *G. intraradix*, and again this was interpreted as a host defense response which was subsequently suppressed (Volpin et al., 1994). Vierheilig et al. (1994), investigating chitinase and β-1,3-glucanase activities in various host and non-host plants inoculated with *G. mosseae*, did not find such an early burst in chitinase activity in tomato roots, but they did observe an overall decrease in enzyme activities at a later stage of mycorrhiza formation. Chitinase was also repressed in non-host plants when some sort of mycorrhizal structure was observed in roots, while in two non-host rape cultivars, not forming similar mycorrhizal structures, only late increases in chitinase activity were found. Higher β-1,3-glucanase activities were detected in all *G. mosseae*-inoculated non-host plants, but only at an early stage of the interaction. It was concluded from these observations that AM fungi are indeed recognized by both host and non-host plants, as judged by various plant reactions (ethylene as well as chitinase and β-1,3- glucanase activities), but that none of these seem to influence the outcome of the interaction of the plants with *G. mosseae* nor to be involved in the inability of non-host plants to form arbuscular mycorrhizas.

Generally, all the reported results indicate a transient increase of chitinase and sometimes β-1,3-glucanase activities, interpreted as an early defense response from the plant to the invading fungus. Although a role of plant chitinases in degrading collapsed fungal structures has been suggested (Lambais and Mehdy, 1993), the enzymes do not appear to come into direct contact with intracellular structures of AM fungi nor to bind to external hyphae, unless cell wall proteins and soluble carbohydrates are removed by heat treatment (Spanu et al., 1989). Furthermore, mycorrhiza development and fungal enzyme activity are unaffected in transgenic plants over-expressing chitinase genes from various origins, even though such plants show increased resistance to root pathogens (Table 3; Gianinazzi-Pearson, 1995; Tahiri-Alaoui et al., unpublished results; Vierheilig et al., 1993, 1995). Targeting of tobacco chitinase to intercellular spaces abolished the enhanced resistance to *R. solani*, but had no effect on mycorrhizal colonization by *G. mosseae* (Vierheilig et al., 1993). Considering that the cell walls of AM fungi contain chitin and for some of them β-1,3-glucans (Gianinazzi-Pearson et al., 1994), either these wall components must be inaccessible to the plant enzymes or the fungi somehow inactivate the latter, or there should be other unsuspected roles and functions for hydrolases.

Induction of specific hydrolase isoforms in AM symbiosis. What is their meaning?

Approaches to detect hydrolase activities directly after protein separation in PAGE according to Trudel and Asselin (1989) have shown that chitinase activities are higher in roots of leek, onion and pea colonized by several *Glomus* species as compared to non-mycorrhizal plants (Dumas-Gaudot et al., 1992b). Furthermore, several additional chitinase isoforms were found to be active in mycorrhizal roots together with the constitutive chitinases present in control roots (Dumas-Gaudot et al., 1992b). Further investigations on a mycorrhiza-induced chitinase isoform in several pea genotypes and a mycorrhiza-resistant myc$^-$ pea mutant (Duc et al., 1989) have led to its characterization as an acidic chitinase isoform, with an apparent molecular mass of approximately 27 KDa (Dumas-Gaudot et al., 1994a). Evidence that this isoform is of plant origin comes from the fact that it was induced in pea roots colonized with different *Glomus* species (Dumas-Gaudot et al., 1994a) and was not present in extracts of extraradicular hyphae, germinated spores or mycelium of the mycorrhizal fungus (Dumas-Gaudot et al., 1994a; Slezack et al., unpublished results).

Although there is strong evidence for their host origin, a doubt still exists that the novel isoforms could originate from the mycorrhizal fungi either as usual components playing a role in fungal morphogenesis and autolysis, or as host-inducible enzymes (Sahai and Manocha, 1993). Considering that arbuscules are typically formed by mycorrhizal fungi in the parenchyma cortical host cells, the chitinases may have a specialized function in hyphal extension and branching, as has been reported for non-mycorrhizal fungi (Cabib et al., 1992; Kuranda and Robbins, 1991; Rast et al., 1991).

Comparisons of different host plants inoculated with one AM fungus have shown that novel chitinase isoforms vary depending on the plant species, lending further support to the host origin of mycorrhiza-induced chitinase isoforms (Gianinazzi et al., 1995). By comparing pea, tobacco and tomato roots infected by AM or pathogenic fungi it has also been demonstrated that different chitinase isoforms are activated by the two types of fungi (Dassi et al., 1996; Dumas-Gaudot et al., 1992a; Pozo et al., 1996), demonstrating that there is elicitation by AM fungi of specific root chitinase isoforms which differ from both constitutive and pathogen-induced ones. In tomato roots which were first inoculated by the AM fungus *G. mosseae* and two weeks later by the pathogenic fungus *Phytophthora nicotianae* var. *parasitica*, all additional chitinase isoforms (mycorrhiza and pathogen elicited) were detected. Interestingly, chitinase isoforms from mycorrhizal tomato roots also differed from pathogen-elicited enzymes in that they displayed a greater renaturing capacity after root extracts had been submitted to denaturing SDS-PAGE.

As already mentioned, only a few reports deal with β-1,3-glucanase elicitation in AM roots (Blee and Anderson, 1996; Lambais and Mehdy, 1993; Vierheilig et al., 1994). In earlier investigations, we found no evidence for increased β-1,3-glucanase activities or induction of novel basic or acidic isoforms in AM roots (Dumas et al., 1989; Dumas-Gaudot et al., 1992b), although pea root inoculations by pathogenic fungi led to a typical host response with the induction of new basic β-1,3-glucanase isoforms (Dassi et al., 1996). Recently, however, elicitation of one acidic β-1,3-glucanase isoform has been detected in tomato roots inoculated with the AM fungus *G. mosseae* (Pozo et al., unpubl. results). Although the plant or fungal origin as well as the biochemical characteristics of this additional β-1,3-glucanase isoform have to be determined, it is the first time that a new β-1,3-glucanase isoform has been reported in an AM symbiosis. This may be relevant to the recent finding that β-1,3-glucan polymers, present in the inner walls of the spores and external hyphae walls of fungi belonging to the Glomineae became undetectable in arbuscules developing within the host root (Lemoine et al., 1995).

Conclusions and perspectives

It is evident from in vitro experiments and from transgenic plants over-expressing genes coding for hydrolases that plant chitinases and/or β-1,3-glucanases have an antifungal role against pathogenic soil-borne fungi. Although there are some cases of unaltered resistance in transgenic plants showing high chitinase gene expression, the prospects for using genetically engineering plants with improved resistance still appear promising. However, there are also some limitations for their use. For example, since the targeting of the expressed genes has to be evaluated with respect to the cellular growth of the invading fungi, it would be difficult to simultaneously improve resistance to different fungi growing into distinct host cellular compartments. In addition, the fact that some fungi have the capacity of adapting to high levels of chitinase and β-1,3-glucanase (Ludwig and Boller, 1990) constitutes

an other restraint, unless means for suddenly increasing their concentration in the vicinity of the advancing hyphae will be discovered.

The roots of most plant species are natural hosts not only to pathogenic fungi but also to beneficial mycorrhizal fungal symbionts. Over-expression of antifungal proteins in transgenic plants does not limit the development and functioning of AM fungi. A direct antifungal role of such enzymes as evoked for plant/pathogen interactions therefore seems unlikely. Moreover, mycorrhiza-induced chitinase isoforms appear to be a general phenomenon in AM symbioses, further suggesting that such enzymes should have other, so far unknown, functions. Although their role in AM symbioses still has to be elucidated, it is interesting to speculate that they may somehow be involved in plant/microbe signalling, as has recently been proposed for rhizobial symbiosis (Staehelin et al., 1994a, 1994b). Investigations to test this hypothesis are presently being carried out. However, in order to understand the role of mycorrhiza-induced chitinase isoforms in AM associations, several questions need to be answered: Do they compete for the same substrates as other isoforms (chitin or altered forms of chitin)? Do they share similar cellular sites of accumulation ? Are they intracellular or extracellular ? Are they only locally induced in colonized root parts or are they induced also far from infection points ? Can they be induced by elicitors released from mycorrhizal fungi ?

In relation to biocontrol of root pathogens in AM plants, several points dealing with the potential contribution of plant hydrolytic enzymes need thorough examination. The possibility that triggering of mechanisms involved in defence reactions by pre-colonization of roots by AM fungi helps the plant to quickly respond to subsequent pathogen attack still has to be considered. Although we have shown that AM fungi induce chitinase isoforms which differ from those induced by a pathogenic fungus, the mycorrhiza-induced chitinases still appear good candidates for releasing oligosaccharide elicitors from the chitinous AM fungal cell walls, and these may in turn stimulate the general defense responses of plants (Hahn et al., 1989; Lamb et al., 1989). This hypothesis would take into account the fact that bioprotection in AM plants is effective when roots have been pre-colonized by AM fungi (Cordier et al., 1996; Linderman, 1994). Furthermore, although until recently no typical endogenous substrates for chitinases have been identified in plants, Benhamou and Asselin (1989) have reported the presence of abundant N-acetylglucosamine residues, in the form of glycolipids, in secondary walls of plants. Collinge et al. (1993) underlined these data in relation to the fact that rhizobia secrete lipo-oligosaccharide Nod factors. Recently, chitinases have been proved to be able to hydrolyse some of the Nod factors (Staehelin et al., 1994a,b). In the present status of our knowledge on mycorrhiza-induced chitinase isoforms, it is necessary to determine firstly if they have any antifungal activities against soil-borne pathogens or even against other microorganisms, and secondly if they are able to release elicitors from AM fungi.

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