

β -1,3-Glucanase activities in tomato roots inoculated with arbuscular mycorrhizal fungi and/or *Phytophthora parasitica* and their possible involvement in bioprotection

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Abstract

β -1,3-Glucanases in tomato roots were studied after arbuscular mycorrhizal (AM) symbiosis establishment and/or pathogenic infection by *Phytophthora parasitica* by polyacrylamide gel electrophoresis (PAGE). Two species of AM fungi, *Glomus mosseae* and *Glomus intraradices* were tested, and *Phytophthora* inoculation was performed on both non-mycorrhizal and mycorrhizal tomato pre-colonized for 4 weeks with either of the AM fungal species. The protective effect of both AM fungi on tomato plants against *Phytophthora* was assessed. In control roots two acidic β -1,3-glucanase isoforms were constitutively expressed, and their activity was higher in mycorrhizal roots. Two additional acidic isoforms were detected in extracts from *G. mosseae*-colonized tomato roots, but not in *G. intraradices*-colonized roots. Roots infected by *P. parasitica* displayed stronger activities but the pathogen did not induce the isoforms related to *G. mosseae* colonization. Only one basic glucanase isoform was detected whether the plants were non-inoculated or colonized by any of the fungi when inoculated singly. However, when plants were pre-inoculated with *G. mosseae* and post-infected with *P. parasitica* two additional basic isoforms were clearly revealed. Results are discussed in relation to the possible role of the additional acidic and basic β -1,3-glucanase isoforms in the establishment and development of the AM symbiosis, as well as their putative implication in plant bioprotection. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Arbuscular mycorrhizal (AM) associations are the most common type of plant–microbe symbiosis involved in nutrient cycling in natural ecosystems and in man-made agrosystems [1]. Root colonization by AM fungi induces important physiological and biochemical changes in the host plant, enabling it to better overcome biotic and

abiotic stresses [2]. Among these changes, qualitative and quantitative alterations in protein expression within host tissues have been reported in various AM associations [3,4]. A certain local, weak and transient activation of some plant defence mechanisms occurs as a consequence of root colonization by AM formation, although no generalised defence reactions have been found [2,5]. Current research has focused on the differential expression of defence related genes in arbuscular mycorrhizal plants [6,7], including genes coding for hydrolytic enzymes such as chitinases and β -

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1,3-glucanases. The induction of these enzymes in arbuscular mycorrhizas is receiving increased attention due to their possible implication in the regulation of the symbiosis [6,8–10] as well as in plant bioprotection [2,9–11].

Concerning chitinases, their transient activation as well as the induction of new acidic isoforms in response to arbuscular mycorrhizal fungi has been reported in different plants, including tomato [10,11]. Some research has also been undertaken on β -1,3-glucanases in relation to AM symbiosis, showing transient changes in their activities as well as at the mRNA level [7,8,12,13], but up to now only a few studies have dealt with their isoenzyme patterns in AM plants [14,15].

β -1,3-Glucanases (EC 3.2.1.39) are able to partially degrade fungal cell walls by catalysing the hydrolysis of β -1,3-d-glucosidic linkages in β -d-glucans, which are, together with chitin, the major cell wall components of most fungi. These enzymes are constitutively expressed in different organs and tissues of higher plants, and are regulated by normal developmental processes, ethylene and other plant hormones. β -1,3-Glucanases are also induced in plants after pathogen attack and exposure to various biotic and abiotic elicitors, and they usually act in synergy with chitinases [16,17]. Both chitinases and β -1,3-glucanases show a complex isoenzyme pattern with isoforms differing in their biochemical characteristics, primary structure, antigenicity, enzyme activity, subcellular localisation and antifungal properties [17]. They are considered to play an important role in plant defence responses against fungal pathogens, and recent studies involve them in plant resistance against different *Phytophthora* species [18,19], whose main cell wall component is β -1,3-glucan. Although these enzymes have been extensively studied in tomato leaves, only a few reports deal with glucanase activities in tomato root/fungal interactions [20].

The analysis of hydrolytic isozyme patterns following AM formation in comparison to those of pathogenic interactions could clarify the implication of these enzymes in AM symbiosis establishment and functioning, as well as in plant protection against fungal pathogens. In previous studies, chitinase and chitosanase activities in tomato root interactions with *Glomus mosseae* and/or *Phytophthora parasitica* have been investigated [10,11]. The aim of the present work was to

compare two species of AM fungi (*G. mosseae* and *G. intraradices*) in their ability to induce specific β -1,3-glucanase isoforms and to protect the plant against *P. parasitica* attack.

2. Materials and methods

2.1. Plant and fungal material

Tomato seeds (*Lycopersicon esculentum* L. cv Earleymech) were surface-sterilised with a commercial bleach solution (10%, v/v), and germinated under sterile conditions on wet filter paper at 28°C for 3 days. Plants were grown in 750-ml (14 cm height) pots containing a sterile mixture of quartz sand and soil (9:1, v/v). Two species of AM fungi were assayed: *G. mosseae* Nicol and Gerd (BEG 12) and *G. intraradices* Smith and Schenck (BEG 72). Mycorrhizal inoculation was carried out by mixing with the growing substrate 10% of a sand/soil (9:1,v/v) based inoculum enriched in fungal propagules and containing chopped mycorrhizal *Allium porrum* L. roots. At potting, plants corresponding to all treatments received an aliquot of a filtrate (< 20 μ m) of both AM inocula in order to provide the microbial populations accompanying the mycorrhizal inocula but free from AM propagules.

Four weeks after potting, a set of plants from each treatment was inoculated with the root pathogen *P. parasitica* var. *nicotianae* [synonymous with *P. nicotianae* van Breda de Haan var. *parasitica* (Dastur) Waterhouse] isolate 201 (kindly provided by P. Bonnet, INRA, Antibes, F). Such a delayed inoculation time with the pathogen was chosen because bioprotection by mycorrhizal fungi occurs mainly when symbiosis is well established before the pathogen attack [2]. *Phytophthora* was grown on a malt-agar medium at 25°C in darkness for 3 weeks, and the inoculum was prepared by washing the growing mycelia with sterile water (16 ml/plate). The suspension obtained was used to inoculate the corresponding tomato plants by injecting 8 ml per plant close to the root system. Control plants were similarly supplied with 8 ml of sterile water.

2.2. Growth conditions and plant harvest

Tomato plants were grown in a controlled envi-

ronment room (25/18°C day/night temperature, 60% relative humidity, 16 h photoperiod with a photosynthetic photon flux of 400 $\mu\text{mol photons m}^{-2}$ per s). They were watered three times per week with Long Ashton nutrient solution at one-fourth phosphorus strength. Plants were harvested 4 and 6 weeks after potting, carefully washed in running tap water, rinsed in deionized water and weighed. The root systems were evaluated for *Phytophthora* necrotic lesions by an arbitrary scale from 0 (no symptom) to 5 (fully necrotic root). The presence of *P. parasitica* in tomato root extracts was evaluated by using a commercial ELISA kit (Agriscreeen, Adgen Diagnostic Systems, Auchincruive, Ayr, UK) following the supplier's instructions. Root systems were immediately frozen in liquid nitrogen, and stored at -80°C until protein extraction. An aliquot of each root system was kept for determination of mycorrhizal colonization by clearing and staining the roots using trypan blue [21].

External mycelia of *G. mosseae* and *G. intraradices* were collected from 4-week-old mycorrhizal tomato plants. The roots were gently washed with sterilized water and put on ice, and the external mycelium was picked up using forceps under a dissecting microscope. *Phytophthora* mycelium was obtained from the fungal colonies growing on malt-agar medium at 25°C in darkness for 3 weeks.

2.3. Protein extraction and quantification

Frozen roots were ground at 4°C in an ice-chilled mortar with liquid nitrogen and the resulting powder was suspended in 100 mM MacIlvaine (citric acid/ Na_2HPO_4) extracting buffer, pH 6.8 (1:1, w/v). Crude homogenates were centrifuged at $15000 \times g$ for 30 min at 4°C and the supernatant fractions were kept frozen at -20°C . Protein extraction from the fungal mycelia was carried out as for root material, except for the amount of extracting buffer (10:1, w/v) and also that crude homogenates were briefly sonicated before centrifugation. In this case, supernatants were concentrated by lyophilization, resuspended in a minimal amount of the extracting buffer and used as crude enzyme extracts. Protein contents were determined by the method of Bradford [22] using BSA as standard.

2.4. Electrophoresis and enzymatic assays

All extracts were analysed by 15% (w/v) polyacrylamide gel electrophoresis (PAGE) under native conditions, at pH 8.9 according to Davis [23] and at pH 4.3 as described by Reisfeld et al. [24]. For all treatments 7 and 15 μg of proteins were respectively loaded for analyses in acidic and basic electrophoretic systems. A soluble fraction of purified β -glucans from *Saccharomyces cerevisiae*, was used as substrate for β -1,3-glucanase activity [25]. β -Glucans were incorporated at a final concentration of 0.6 mg ml^{-1} directly in the separation gels for the Davis system, or in a 7.5% (w/v) polyacrylamide overlay gel for the Reisfeld et al. [24] PAGE set-up. In the latter, transfer of proteins to the overlay gel was done by blotting for 14 h at 37°C . After electrophoresis, gels were incubated in 50 mM sodium acetate buffer, pH 5.0, for 3 h at 37°C . β -1,3-Glucanase activities on gels were revealed by staining the gels for 15 min with 0.025% (w/v) Aniline blue fluorochrome in 150 mM K_2HPO_4 , pH 8.6, and visualized under long wave UV (365 nm) light [25]. Gels were photographed (Polaroid film No. 665) and scanned (HP ScanJet 3c). All chemicals for electrophoresis were from Bio-Rad (Prat de Llobregat, Barcelona, Spain). Other compounds were from Sigma (Alcobendas, Madrid, Spain).

2.5. Replication and statistical analysis

Two independent experiments were carried out and five replicated plants per treatment were used at each harvest. Data were subjected to ANOVA, followed by Fisher's Protected Least Significant Difference test where appropriate. Since tendencies were similar in both experiments, results from one of them are reported here. All electrophoreses were repeated at least three times, and results on induction of new glucanase isoforms were confirmed in the root extracts from both experiments.

3. Results

3.1. Plant growth and fungal colonization

The growth response of tomato plants subjected to the different mycorrhizal and *P. parasitica* inoculation treatments is presented in Fig. 1. In the absence of the pathogen, there was no significant

effect on plant growth of any of the two AM fungi tested, at any of the harvest times. Only *G. mosseae* (Gm) induced a slight increase in biomass production, but this was not statistically significant. Two weeks after inoculation with *P. parasitica*, shoot and root growth were significantly reduced in non-mycorrhizal (Nm) and *G. intraradices*-colonized plants (Gi), in comparison to their respective, non-inoculated with *P. parasitica*, controls. However, plants mycorrhizal with *G. mosseae* did not show any inhibition of either root or shoot growth as a consequence of *P. parasitica* inoculation.

Mycorrhizal colonization with both *G. mosseae* and *G. intraradices* was well established 4 weeks after potting, when the pathogen inoculation was carried out. More than 40% of the root length became mycorrhizal with *G. mosseae*. After 6 weeks the percentage of root colonization reached 55–60% for the different experiments. These percentages were about 5–10% higher for *G. intraradices*-colonized plants at both harvests (4 and 6 weeks). The AM colonization level was not significantly affected by the inoculation with the pathogen (Table 1).

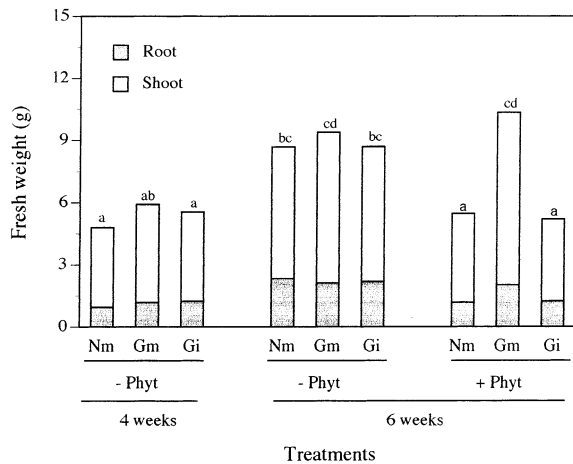


Fig. 1. Effect of mycorrhiza formation with different AM fungi on shoot and root growth of tomato plants post-infected (+Phyt) or not (-Phyt) with the root pathogen *Phytophthora parasitica*, after 4 and 6 weeks of growth. Bars sharing any letter in common do not differ significantly ($P < 0.05$) according to the Fisher's LSD test. Nm, non-mycorrhizal; Gm, *Glomus mosseae*- and Gi, *Glomus intraradices*-inoculated plants.

Table 1

Mycorrhizal colonization, disease index and pathogen amount inside root tissues of 6-week-old tomato plants inoculated (+Phyt) or not (-Phyt) with *P. parasitica*[#]

	Mycorrhizal colonization (%)		Disease index*	<i>Phytophthora</i> biomass**
	- Phyt	+ Phyt	+ Phyt	+ Phyt
Nm	–	–	3	1.94a
Gm	56	58	1	0.50b
Gi	64	63	3	0.98ab

[#] Nm, Non-mycorrhizal plants; Gm, *Glomus mosseae* inoculated; Gi, *Glomus intraradices* inoculated. Data not sharing a common letter differ significantly at $P \leq 0.05$.

* Visual estimation of disease symptoms from 0 (no symptom) to 5 (fully necrotic root).

** Expressed in absorbance units from the ELISA test.

The extension of necrotic lesions caused by *P. parasitica* in the root system correlated well with the loss of weight: plants mycorrhizal with *G. mosseae* showed less areas affected by necrosis (average rating 1, very few symptoms) than non-mycorrhizal plants (average rating 3, medium level of symptoms), while plants colonized by *G. intraradices* displayed a similar extension of necrotic lesions than the non-mycorrhizals (average rating 3) (Table 1). Disease indices were further supported by data from *P. parasitica* detection by ELISA, since absorbance values of 1.94, 0.5 and 0.98 were obtained respectively for extracts from non-mycorrhizal, *G. mosseae*- and *G. intraradices*-colonized roots infected with *P. parasitica* (Table 1). Plants, mycorrhizal or not, non-inoculated with *Phytophthora* showed absorbance values around 0.1, which correspond to the background value of the assay.

Protein content of the root extracts from mycorrhizal plants (either *G. mosseae* or *G. intraradices*) was significantly higher than that from non-mycorrhizal plants at both sampling times (Table 2). However, no statistically significant differences in protein concentration were found between plants mycorrhizal with either of both fungi. *P. parasitica* inoculation did not significantly affect protein content.

Table 2
Protein content (mg/g root fresh wt.) in tomato root extracts corresponding to the different mycorrhizal treatments[#]

Phytophthora treatment	Mycorrhizal treatment	Time (weeks)	
		4	6
– Phyt	Nm	0.45a	0.53a
	Gm	0.78b	0.95b
	Gi	0.85b	1.09b
+ Phyt	Nm	–	0.43a
	Gm	–	0.82b
	Gi	–	0.59ab

[#] Nm, non-mycorrhizal plants; Gm, *Glomus mosseae*- and Gi, *Glomus intraradices*-inoculated, post-infected (+Phyt) or not (–Phyt) with the root pathogen *Phytophthora parasitica* var. *nicotianae*. Data in the same column not sharing a common letter differ significantly at $P \leq 0.05$.

3.2. Detection of β -1,3-glucanase isoforms

Separation of acidic and neutral proteins from tomato root extracts using the Davis system enabled detection of two main bands with β -1,3-glucanase activity in control tomato roots, corresponding to constitutively expressed isoforms (Fig. 2, lanes Nm). The activity of the upper isoform appears to increase with plant age. Extracts from mycorrhizal roots with either of the

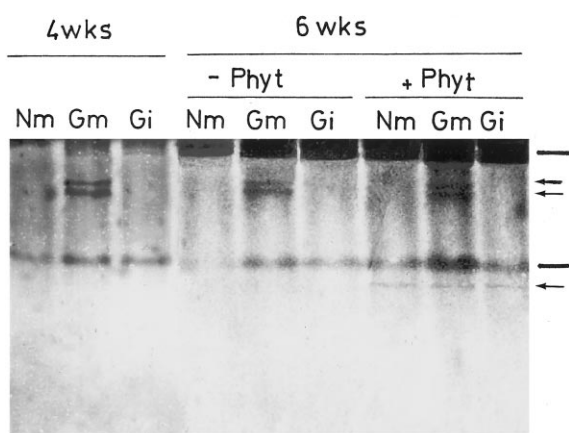


Fig. 2. Acidic β -1,3-glucanase activities after separation of proteins by the Davis system in 15% (w/v) polyacrylamide gels containing soluble β -glucans as substrate. Root extracts from non-mycorrhizal (Nm), *G. mosseae* (Gm), and *G. intraradices*-colonized (Gi) tomato plants after 4 (4 weeks) or 6 weeks of growth (6 weeks) either uninoculated (–Phyt) or inoculated (+Phyt) with *P. parasitica* were studied. Constitutive isoforms are indicated by bars, and additional isoforms by arrows.

AM fungi tested displayed the constitutive isoforms at both sampling times, the one with higher mobility showing enhanced activity (Fig. 2, lanes Gm and Gi). Additionally, two new isoforms were induced in *G. mosseae*-colonized roots (Fig. 2, lanes Gm). A general increase in glucanase activity and a faint band corresponding to an isoform with higher mobility were observed in extracts from *P. parasitica* infected plants, either non-mycorrhizal (Nm) or pre-mycorrhizal (Gm and Gi) (Fig. 2, lanes +Phyt). No β -1,3-glucanase activity was detected in protein extracts from either *G. mosseae* or *G. intraradices* external mycelium (data not shown). *Phytophthora* mycelium displayed β -1,3-glucanase activity, but this activity appeared as a wide lytic zone in the upper part of the gel, and it was not possible to resolve it in differentiated bands, even when different amount of proteins and/or acrylamide percentages were tested (data not shown). This could be due to high affinity between this/these glucanase/s and the glucans embedded in the gel. Affinity during electrophoresis, based on interactions between natural polysaccharides and proteins has been previously described [26].

Basic glucanase isoforms were detected when proteins were separated at pH 4.3 using the Reisfeld system. Only one basic constitutive isoform could be detected in all root extracts (Fig. 3). No time-related differences were found (results not shown), nor when comparing non-mycorrhizal plants (Fig. 3, –Phyt, lane Nm) with those colonized with either *G. mosseae* or *G. intraradices* (Fig. 3, –Phyt, lanes Gm and Gi). However, significant differences in response to *P. parasitica* infection were found (Fig. 3, +Phyt). *G. mosseae*-colonized plants clearly reacted to the presence of the pathogen through the induction of two new isoforms (Fig. 3, +Phyt, lane Gm), while these isoforms were not detected in non-mycorrhizal nor in *G. intraradices*-colonized plants infected by the pathogen (Fig. 3, +Phyt, lanes Nm and Gi), and, as indicated above, they were also absent in plants colonized by *G. mosseae* alone (Fig. 3, –Phyt, lane Gm).

4. Discussion

Tomato growth was not significantly improved by inoculation with any of the AM fungi tested in

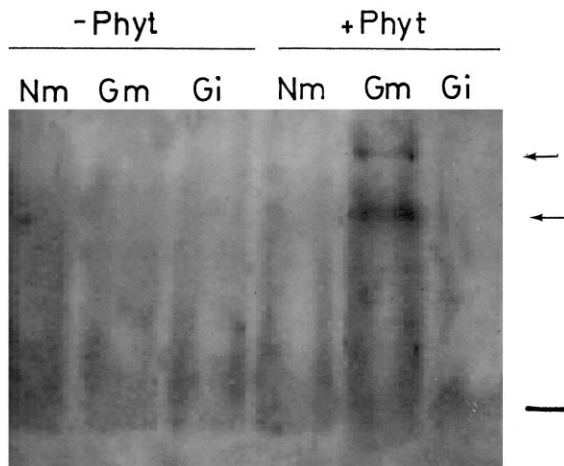


Fig. 3. Basic β -1,3-glucanase activities after separation of proteins by the Reisfeld system in 15% (w/v) polyacrylamide gels and blotting to a 7.5% polyacrylamide overlay gel containing soluble β -glucans as substrate. Root extracts from non-mycorrhizal (Nm), *G. mosseae* (Gm), and *G. intraradices*-colonized (Gi) tomato plants either uninoculated (–Phyt) or inoculated (+Phyt) with *P. parasitica* were studied. Results correspond to extracts from plants harvested after 6 weeks of growth (2 weeks after pathogen inoculation). Constitutive isoforms are indicated by bars, and additional isoforms by arrows.

the present study, as was expected because the substrate used, based mainly in sand, does not impose important limitations to nutrient diffusion towards the root. This is important when trying to avoid the indirect effect due to a better nutritional status of mycorrhizal plants on the susceptibility to pathogens. However, the inoculation of tomato roots with *P. parasitica* resulted in a visible reduction of shoot and root weights and the appearance of necrotic areas in the root system. Colonization by the AM fungus *G. mosseae* decreased the negative effect of *P. parasitica* infection, in relation to both plant weight reduction and root necrosis. These results agree with previous reports [11,27], confirming *G. mosseae* as a protective agent of tomato plants against *P. parasitica* under different experimental conditions. A similar reduction in disease symptoms was not detected in *G. intraradices*-colonized plants. Thus, it can be argued that the ability of AM symbiosis to enhance resistance or tolerance in roots against soil-borne pathogens is not similar for different AM fungi and needs to be ascertained for each particular combination of AM fungus, host plant genotype, pathogen and environmental conditions [2].

Hydrolytic enzymes have been proposed to play a role in plant development, morphogenesis and plant/microbe signalling, in addition to their evidenced antifungal character [16]. Southern blot analysis indicates that the tomato β -glucanase family is relatively small, with a few genes encoding either acidic or basic glucanases [17]. We have detected one basic and two acidic isoforms constitutively expressed in tomato roots. Two newly induced acidic β -1,3-glucanase isoforms have been detected in the present work in *G. mosseae*-colonized roots, but these new isoforms were not detected in *G. intraradices*-colonized plants. Dumas-Gaudot et al. [14] reported the induction of a β -1,3-glucanase isoform during AM symbiosis in pea plants, whose activity depended on the AM fungus involved, but no similar response was found in leek or onion. In the present work *P. parasitica* infected root extracts displayed a faint new acidic isoform, different from any of those induced by *G. mosseae*. Thus, the induction of the new β -1,3-glucanase isoforms in *G. mosseae*-colonized roots seems to be a specific response in certain plant/AMF interactions: specificity concerning the AM fungus, as shown in the present work, and the plant, as evidenced by Dumas-Gaudot et al. [14]. This specificity, besides the absence of β -1,3-glucanase activity detected in the extraradical mycelium of *G. mosseae*, strongly supports a plant origin for the activity.

Differential host-responses to several AM fungi have already been described. Lambais and Mehdy [8] found both differential regulation and expression of defence-related genes coding for chitinases and β -1,3-glucanases in soybean colonized by two strains of *G. intraradices* differing in infectivity. The different ability of diverse AM fungi to modify defence-related enzyme expression in various host plants, besides time-related variations in these activities, and the different approaches used, makes it difficult to draw conclusions about the regulation of these enzymes in AM symbiosis.

Arbuscular mycorrhizal fungi contain β -1,3-glucans, chitin and/or chitosan and glycoproteins [28]. All these molecules are known to be potent elicitors of plant defence reactions [29]. When colonization by AM fungi is successful, some fungal strategies of self camouflage may occur such as important wall modifications during the colonization process, or repression of the induced plant defence mechanisms [28]. In fact, the host plant

influences wall morphology and composition of AM fungi as they develop within the root tissues. Lemoine et al. [30] described a progressive disappearance in wall β -1,3-glucans during root colonization. This was confirmed for *G. mosseae* developing in tomato roots. Extraradical hyphal walls contained a large quantity of β -1,3-glucans, but they were less abundant in intercellular hyphae and became undetectable in arbuscules [28]. In this context, it can be hypothesized that the new acidic glucanase isoforms could help the plant to control the AM fungus development. However, it is difficult to understand the induction of new glucanase isoforms by *G. mosseae* and not by *G. intraradices*. It remains to be clarified if this can be related to the different colonization dynamics of both fungi, or to differences in their wall composition and/or structure.

Important differences have also been found among non-mycorrhizal, *G. mosseae*- and *G. intraradices*-colonized plants concerning responses to infection by the pathogen *P. parasitica*. While one basic isoform was detected in controls or mycorrhizal roots, two new basic isoforms were present in extracts from roots post-infected with the pathogen only when they were previously colonized by *G. mosseae*.

β -1,3-Glucans are a normal component of hyphal walls of the oomycete *P. parasitica*. High levels of glucanase activity have been correlated with plant resistance to fungal pathogens, including oomycetes [31]. Transgenic tobacco overexpressing β -1,3-glucanase was resistant to *P. parasitica* var. *nicotianae* [32]. Recently, Cordier et al. [33] have reported modifications in *P. parasitica* wall during infection of mycorrhizal tomato root systems, since β -1,3-glucans were not detected in *Phytophthora* hyphal walls when the development of the pathogen was blocked by papilla formation in the mycorrhizal tissues. This reaction was not observed in non-mycorrhizal tomato plants, suggesting some sort of break down action by the mycorrhizal plant. Therefore the role of β -1,3-glucanases could be crucial. Since different isoforms can have different subcellular localisation and can be differentially regulated, the study of the role of specific isoforms is crucial to understanding the features in pathogen/host plant interactions.

In the present study, changes in acidic and basic β -1,3-glucanase isoform patterns were detected in response to mycorrhizal colonization and patho-

gen infection. However, available information does not allow to correlate conclusively the acidic or basic character of the isoenzymes with their in vivo functions. The induction in *G. mosseae*-colonized roots of new acidic isoforms was independent of the presence of the pathogen. Vierheilig et al. [34], using transgenic plants, reported that constitutive expression of an acidic glucanase delayed root colonization by *G. mosseae*, while it was not affected by the expression of a basic one. These data suggest a role for specific acidic isoforms in controlling *G. mosseae* development inside the roots. On the other hand, the induction of two basic isoforms only occurred in plants infected with *P. parasitica* and previously colonized by *G. mosseae*, the plants that better overcome the pathogen infection. Consequently, basic isoforms appear to be good candidates to be involved in tomato plant protection by *G. mosseae* against the fungal pathogen *P. parasitica*. This hypothesis is also supported by studies showing that the antifungal activity of plant protein extracts against different *Phytophthora* species lies in basic glucanase isoforms [18,19,35]. Work is in progress trying to gain further insights into the precise role of the different induced β -1,3-glucanase isoforms in mycorrhiza and pathogen interactions.

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References

- [1] J.L. Harley, S.E. Smith, Mycorrhizal Symbiosis, Academic Press, London, 1983.
- [2] C. Azcón-Aguilar, J.M. Barea, Arbuscular mycorrhizas and biological control of soil-borne plant pathogens—An overview of the mechanisms involved, Mycorrhiza 6 (1996) 457–464.
- [3] E. Dumas-Gaudot, P. Guillaume, A. Tahiri-Alaoui, V. Gianinazzi-Pearson, S. Gianinazzi, Changes in polypeptide patterns in tobacco roots colonized by two *Glomus* species, Mycorrhiza 4 (1994) 215–221.
- [4] K. Benabdellah, C. Azcón-Aguilar, N. Ferrol, Soluble and membrane symbiosis-related polypeptides associated with the development of arbuscular mycorrhizas in tomato (*Lycopersicon esculentum*), New Phytol. 140 (1998) 135–143.

- [5] V. Gianinazzi-Pearson, E. Dumas-Gaudot, A. Golotte, A. Tahiri-Alaoui, S. Gianinazzi, Cellular and molecular defence-related root responses to invasion by arbuscular mycorrhizal fungi, *New Phytol.* 133 (1996) 45–57.
- [6] M.R. Lambais, M.C. Mehdy, Differential expression of defense-related genes in arbuscular mycorrhiza, *Can. J. Bot.* 73 (1995) 533–540.
- [7] K.A. Blee, A.J. Anderson, Defense-related transcript accumulation in *Phaseolus vulgaris* L. colonized by the arbuscular mycorrhizal fungus *Glomus intraradices* Schenck and Smith, *Plant Physiol.* 110 (1996) 675–688.
- [8] M.R. Lambais, M.C. Mehdy, Soybean roots infected by *Glomus intraradices* strains differing in infectivity exhibit differential chitinase and β -1,3-glucanase expression, *New Phytol.* 134 (1996) 531–538.
- [9] E. Dumas-Gaudot, S. Sleczak, B. Dassi, M.J. Pozo, V. Gianinazzi-Pearson, S. Gianinazzi, Plant hydrolytic enzymes (chitinases and β -1,3-glucanases) in root reactions to pathogenic and symbiotic microorganisms, *Plant Soil* 185 (1996) 211–221.
- [10] M.J. Pozo, C. Azcón-Aguilar, E. Dumas-Gaudot, J.M. Barea, Chitinase and chitinase activities in tomato roots during interactions with arbuscular mycorrhizal fungi or *Phytophthora parasitica*, *J. Exp. Bot.* 49 (1998) 1729–1739.
- [11] M.J. Pozo, E. Dumas-Gaudot, S. Sleczak, C. Cordier, A. Asselin, S. Gianinazzi, V. Gianinazzi-Pearson, C. Azcón Aguilar, J.M. Barea, Induction of new chitinase isoforms in tomato roots during interactions with *Glomus mosseae* and/or *Phytophthora nicotianae* var *parasitica*, *Agronomie* 16 (1996) 689–697.
- [12] M.R. Lambais, M.C. Mehdy, Suppression of endochitinase, β -1,3 endoglucanase, and chalcone isomerase expression in bean vesicular-arbuscular mycorrhizal roots under different soil phosphate conditions, *Mol. Plant-Microbe Interact.* 6 (1993) 75–83.
- [13] H. Vierheilig, M. Alt, U. Mohr, T. Boller, A. Wiemken, Ethylene biosynthesis and activities of chitinases and β -1,3 glucanase in the roots of host and non-host plants of vesicular-arbuscular mycorrhizal fungi after inoculation with *Glomus mosseae*, *J. Plant Physiol.* 143 (1994) 337–343.
- [14] E. Dumas-Gaudot, J. Grenier, V. Furlan, A. Asselin, Chitinase, chitinase and β -1,3 glucanase activities in *Allium* and *Pisum* roots colonized by *Glomus* species, *Plant Sci.* 84 (1992) 17–24.
- [15] B. Dassi, E. Dumas-Gaudot, A. Asselin, C. Richard, S. Gianinazzi, Chitinase and β -1,3-glucanase isoforms expressed in pea roots inoculated with arbuscular mycorrhizal or pathogenic fungi, *Eur. J. Plant Pathol.* 102 (1996) 105–108.
- [16] F. Mauch, B. Mauch-Mani, T. Boller, Antifungal hydrolases in pea tissue. II. Inhibition of fungal growth by combinations of chitinase and β -1,3-glucanase, *Plant Physiol.* 88 (1988) 936–942.
- [17] C.R. Simmons, The physiology and molecular biology of plant 1,3- β -d-glucanases and 1,3;1,4- β -d-glucanases, *Crit. Rev. Plant Sci.* 13 (1994) 325–387.
- [18] Y.J. Kim, B.K. Hwang, Isolation of a basic 34 kiloDalton β -1,3-glucanase with inhibitory activity against *Phytophthora capsici* from pepper stems, *Physiol. Mol. Plant Pathol.* 50 (1997) 103–115.
- [19] S.Y. Yi, B.K. Hwang, Purification and antifungal activity of a basic 34 kDa β -1,3-glucanase from soybean hypocotyls inoculated with *Phytophthora sojae* f. sp. *glycines*, *Mol. Cell.* 7 (1997) 408–413.
- [20] N. Benhamou, J. Grenier, A. Asselin, M. Legrand, Immunogold localization of β -1,3-glucanases in two plants infected by vascular wilt fungi, *Plant Cell* 1 (1989) 1209–1221.
- [21] S. Rajapakse, J. Creighton Miller, Methods for studying vesicular-arbuscular mycorrhizal root colonization and related root physical properties, in: J.R. Norris, D.J. Read, A.K. Varma (Eds.), *Methods in Microbiology*, Academic Press, New York, 1992, pp. 301–316.
- [22] M.M. Bradford, A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding, *Anal. Biochem.* 72 (1976) 248–254.
- [23] B.J. Davis, Disc electrophoresis. II. Method and application to human serum proteins, *Ann. New York Acad. Sci.* 121 (1964) 404–427.
- [24] R.A. Reisfeld, V.J. Lewis, D.E. Williams, Disk electrophoresis of basic proteins and peptides on polyacrylamide gels, *Nature* 195 (1962) 281–283.
- [25] J. Grenier, A. Asselin, Detection of β -1,3-glucanase activity in gels containing alkali-soluble yeast glucan, *Anal. Biochem.* 212 (1993) 301–302.
- [26] F. Côté, J. Letarte, J. Grenier, J. Trudel, A. Asselin, Detection of β -1,3-glucanase activity after native polyacrylamide gel electrophoresis: Application to tobacco pathogenesis-related proteins, *Electrophoresis* 10 (1989) 527–529.
- [27] A. Trotta, G.C. Varese, E. Gnani, A. Fusconi, S. Sampo, G. Berta, Interactions between the soilborne root pathogen *Phytophthora nicotianae* var *parasitica* and the arbuscular mycorrhizal fungus *Glomus mosseae* in tomato plants, *Plant Soil* 185 (1996) 199–209.
- [28] A. Golotte, C. Cordier, M.C. Lemoine, V. Gianinazzi-Pearson, Role of fungal wall components in interactions between endomycorrhizal symbionts, in: H.E.A. Schenck, R. Herrmann, K.W. Jeon, N.E. Muller, Schwemmler (Eds.), *Eukaryotism and Symbiosis*, Springer, Berlin, 1997, pp. 412–428.
- [29] M. John, H. Röhrig, J. Schmidt, R. Walden, J. Schell, Cell signalling by oligosaccharides, *Trends Plant Sci.* 2 (1997) 111–115.
- [30] M.C. Lemoine, A. Golotte, V. Gianinazzi-Pearson, S. Gianinazzi, Localization of β (1-3) glucan in walls of the endomycorrhizal fungi *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe and *Acaulospora laevis* (Gerd. & Trappe) during colonization of host roots, *New Phytol.* 129 (1995) 97–105.
- [31] T.L. Graham, M.Y. Graham, Cellular coordination of molecular responses in plant defense, *Mol. Plant-Microbe Interact.* 4 (1991) 415–422.
- [32] M. Yoshikawa, M. Tsuda, Y. Takeuchi, Resistance to fungal diseases in transgenic tobacco plants expressing the phytoalexin elicitor-releasing factor, β -1,3-endoglucanase, from soybean, *Naturwissenschaften* 80 (1993) 417–420.
- [33] C. Cordier, M.J. Pozo, J.M. Barea, S. Gianinazzi, V. Gianinazzi-Pearson, Cell defense responses associated

- with localized and systemic resistance to *Phytophthora* induced in tomato by an arbuscular mycorrhizal fungus, *Mol. Plant-Microbe Interact.* 11 (1998) 1017–1028.
- [34] H. Vierheilig, M. Alt, J. Lange, M. Gut-Rella, A. Wiemken, T. Boller, Colonization of transgenic tobacco constitutively expressing pathogenesis-related proteins by the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae*, *Appl. Environ. Microbiol.* 61 (1995) 3031–3034.
- [35] F. Meins, P. Ahl, Induction of chitinase and β -1,3-glucanase in tobacco plants infected with *Pseudomonas tabacci* and *Phytophthora parasitica* var *nicotianae*, *Plant Sci.* 61 (1989) 155–161.