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SHORT NOTE

AMF-induced biocontrol against plant parasitic nematodes in *Musa* sp.: a systemic effect

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Abstract Although mycorrhizal colonization provides a bioprotectional effect against a broad range of soil-borne pathogens, including plant parasitic nematodes, the commercial use of arbuscular mycorrhizal fungi (AMF) as biocontrol agents is still in its infancy. One of the main reasons is the poor understanding of the modes of action. Most AMF mode of action studies focused on AMF-bacterial/fungal pathogens. Only few studies so far examined AMF–plant parasitic nematode interactions. Therefore, the aim of the study was to determine whether the AMF *Glomus intraradices* was able to incite systemic resistance in banana plants towards *Radopholus similis* and *Pratylenchus coffeae*, two plant parasitic nematodes using a split-root compartmental set-up. The AMF reduced both nematode species by more than 50%, even when the AMF and the plant parasitic nematodes were spatially separated. The results obtained demonstrate for the first time that AMF have the ability to induce systemic resistance against plant parasitic nematodes in a root system.

Keywords Arbuscular mycorrhizal fungi · ISR (induced systemic resistance) · *Pratylenchus coffeae* · *Radopholus similis* · Split-root

Introduction

During the last decades the potential role of arbuscular mycorrhizal fungi (AMF) in controlling plant diseases and pests received an increasing interest. Mycorrhizal colonization provides a bioprotectional effect against a broad range of soil-borne fungi (Dehne 1982; Singh et al. 2000) and nematodes (Pinochet et al. 1996; Elsen et al. 2003a, b; Hol and Cook 2005). The international concern over the excessive use of pesticides and the general ban on the use of methyl bromide by 2005 have given a particular push to the search and use of microbial inoculants as biological pesticides (Paulitz and Belanger 2001). This has resulted in more than 80 microbial products being sold that claim at least some activity against plant pathogens; however, none of them are registered as biocontrol agents. Moreover, none of these products contain mycorrhizal fungal inoculants. Clearly, the commercial use of AMF as biocontrol agents is still in its infancy. One of the main reasons is that the modes of action still need further unraveling, although a major effort was made over the past years (Whipps 2004).

Most AMF mode-of-action studies focused on AMF–fungal pathogen interactions, while only few studies so far examined AMF–plant parasitic nematode interactions (Elsen et al. 2003a, b; de la Peña et al. 2006). In an attempt to summarize the AMF studies, four groups of potential modes of action were defined: (1) direct competition or inhibition, (2) enhanced or altered plant growth, morphology and nutrition, (3) biochemical changes associated with plant defense mechanisms and induced resistance, and (4) development of an antagonistic microbiota (reviewed by Whipps 2004). During AMF colonization, there is little evidence that classic plant resistance responses occur at high levels. However, these responses are greatly stimulated when a subsequent challenge with a pathogen occurs (St-Arnaud and Vujanovic 2007; Gianinazzi-Pearson et al. 1996), but a good AMF colonization is a prerequisite for this response (Cordier et al. 1998; Slezack et al. 2000). It seems that AMF colonization acts as a priming system,

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immunizing the plant to a pathogen. Work with split-root systems, where AMF and AMF-colonized roots are spatially separated from the bacterial and fungal pathogen attacking the root, has clearly indicated that systemic-induced resistance occurs (Cordier et al. 1998; Pozo et al. 2002; Zhu and Yao 2004; Khaosaad et al. 2007).

In most studies, evidence points to a combination of local and systemic mechanisms (Cordier et al. 1998; Pozo et al. 2002; Zhu and Yao 2004). Decreased bacterial and fungal pathogen development in mycorrhizal and non-mycorrhizal parts of mycorrhizal root systems is associated with plant cell responses and accumulation of phenolics (Cordier et al. 1998; Zhu and Yao 2004). In tomato roots infected with *Phytophthora parasitica*, the locally induced resistance in tomato roots is characterized by the immunization of *Glomus mosseae*-containing cortical cells in mycorrhizal tissue to the fungal pathogen, with cell wall appositions reinforced by callose (Cordier et al. 1998). The biochemical analysis of different plant defense-related enzymes showed a local induction of AMF-related new isoforms of the hydrolytic enzymes chitinase, chitosanase and β-1,3-glucanase, as well as superoxidedismutase (Pozo et al. 2002). Systemically, elicitation of host wall thickenings containing non-esterified pectins and PR-1a protein occurred in the nonmycorrhizal root parts. In addition, callose-rich encasement material was formed around the penetrating *P. parasitica* hyphae (Cordier et al. 1998). Furthermore, systemic alterations of the activity of some of the constitutive isoforms were observed in non-mycorrhizal parts of the mycorrhizal tomato plants. The results on the lytic activity against *P. parasitica* also support the systemic effect (Pozo et al. 2002).

In *Medicago truncatula* infected with the bacterial pathogen *Xanthomonas campestris*, the induction of a significant number of defense-associated transcripts suggests that AMF symbiosis might induce a response similar to the induced systemic resistance (ISR) response caused by rhizobacteria (Liu et al. 2007). ISR is phenotypically similar to systemic acquired resistance (SAR), but accumulation of salicylic acid (SA) is required for SAR (Durrant and Dong 2004). More experimental evidence points to ISR as the systemic bioprotectional effect against take-all disease in barley, since there was no systemic effect of AMF colonization on SA accumulation (Khaosaad et al. 2007).

Although AMF has proven to have biocontrol potential against plant parasitic nematodes, only limited efforts have been made so far to unravel the modes of action responsible for this biocontrol of plant parasitic nematodes. Therefore, the objective of the present study was to determine whether the AMF *Glomus intraradices* is able to incite systemic resistance in banana plants towards the migratory endoparasitic nematodes, *Radopholus similis* and *Pratylenchus coffeae*.

Materials and methods

Biological material

Tissue-cultured plantlets of the banana cultivar Grand Naine (*Musa* sp. AAA, ITC 1256) obtained from the International Transit Centre (ITC), Bioversity International, K.U. Leuven, Belgium, were used in split-root experiments. The planting material was proliferated, regenerated and rooted in culture tubes on Murashige and Skoog medium including vitamins, 30 g/l ascorbic acid and 2 g/l gelrite with pH 6.2 (Banerjee and De Langhe 1985).

The arbuscular mycorrhizal fungus, *Glomus intraradices*, previously identified as a highly effective symbiont of *Musa* spp. (Declerck et al. 1995), was used. This AMF was originally isolated from bananas in Guadeloupe, France. It was maintained and multiplied for experimental use in sorghum pot cultures in the greenhouse.

Radopholus similis (Ugandan isolate) and *Pratylenchus coffeae* (Ghanaian isolate), both important migratory endoparasitic nematodes of banana (Gowen et al. 2005), were selected for this study. Both nematode species were initially isolated from banana roots and monoxenically maintained on carrot discs at 25±1°C in the dark (Pinochet et al. 1995). When used for inoculation, the nematodes were extracted from the carrot discs using the maceration-sieving method (Hooper et al. 2005).

Experimental design

In each experiment, tissue-cultured plantlets were planted in rock wool cubes and placed in a tray with water. During this rooting phase, the plants received fertilizer (Substral® 7 ml/l) on a weekly basis. Four weeks after planting, enough roots had developed and the split-root set-up was initiated. Both sides of the split-root set-up were filled with a mixture of sand and potting soil (2:1). For the mycorrhizal treatments 300 g of rhizosphere mycorrhizal inoculum was added as a layer. The inoculum consisted of spores, mycelia, and colonized root fragments of 6-month old sorghum plants. Plants not receiving mycorrhizal inoculum received 300 g rhizosphere soil from 6-month old sorghum plants that were not colonized with AMF. The rock wool cubes containing the rooted banana plantlets were placed on top of the split-root set-up. The plants were kept for 6 weeks to allow good root development in both sides of the split-root set-up and to allow good mycorrhizal colonization in the mycorrhizal treatments. After 6 weeks, the right side of the split-root set-up was inoculated with 1,000 nematodes. After 8 (for *R. similis*) or 10 weeks (for *P. coffeae*), the plants were harvested, and the mycorrhizal colonization and nematode reproduction were determined.

The experiments were carried out in a greenhouse at an ambient temperature of 20–27°C, with a 12-h photoperiod

(170-190 PAR) and a relative humidity of 70–90% and irrigated as needed. Eight plants per treatment were included in each experiment. The four treatments in each experiment were as follows: control (i.e., nematodes in right side of split root), co-inoculated (i.e., AMF and nematodes in right side of split root), split root (i.e., AMF in left side and nematodes in right side of split root), and co-inoculated + AMF (i.e., AMF in left side and AMF and nematodes in right side).

Determination of mycorrhizal colonization

AMF colonization was determined in both root halves of the split-root set-up. Secondary and tertiary root samples were stained with ink-vinegar (Vierheilig et al. 1998). After clarifying, staining and destaining, 20 1-cm fine root fragments were mounted on slides and observed under the light microscope. The frequency of AMF colonization (F%) was calculated as the percentage of root segments colonized by either hyphae or arbuscules or vesicles. In addition, the intensity of colonization (I%), that is the abundance of hyphae, arbuscules and vesicles in each mycorrhizal root fragment, was estimated (Plenchette and Morel 1996).

Determination of root necrosis and nematode reproduction

Root necrosis and nematode reproduction were evaluated 8 weeks (*R. similis*) or 10 weeks (*P. coffeae*) after inoculation. At the end of each experiment, the percentage of root necrosis was measured by scoring five 10-cm longitudinally sliced functional primary roots (Speijer and De Waele 1997). Nematodes were extracted from the inoculated root half by maceration and sieving (Hooper et al. 2005). The number of juvenile, female and male nematodes was determined using a light microscope.

Statistical analysis

Data on mycorrhizal colonization and nematode reproduction were analyzed by analysis of variance (ANOVA) when the conditions for ANOVA (i.e., normal distribution and homogeneity of variances) were met (Statistica® Release 6, Statsoft, Tulsa, OK, USA). The Tukey HSD test was applied for multiple comparisons of group means. Prior to analysis, AMF and nematode data were arcsin(x/100) and log(x+1) transformed, respectively, to reduce the variance in the data.

Results

The split-root compartmental set-up allowed studying the ability of *Glomus intraradices* to induce systemic resistance in banana against two plant parasitic nematodes, *R. similis* and *P. coffeae*.

In both experiments, a well-established and active AMF colonization was observed as hyphal structures, arbuscules and vesicles were present in the stained roots. Moreover, the frequency of colonization was 100% (data not shown). The intensity, which is an indication of the quality of the colonization, ranged from 13 to 24%. In the experiment with *P. coffeae*, in general higher values for intensity were obtained. AMF colonization levels were not affected by inoculation with the nematodes. For each treatment, AMF were absent in the −AMF part of the split-root set-up.

The colonization with *G. intraradices* resulted in a growth depression, as illustrated by the dry root weight (Table 1). Except for the control treatment, the growth depression was observed in both sides of the split-root set-up regardless the presence and/or absence of either the AMF or the nematodes.

Table 1 Dry root weight of *Musa* cv. Grand Naine in a split-root set-up, colonized with the AMF *G. intraradices* and inoculated with the nematode *R. similis* or with the nematode *P. coffeae*

	Split-root with *R. similis*		Split-root with *P. coffeae*	
	Left side	Right side	Left side	Right side
Control	1.4±0.4 B	1.9±0.2 B	1.4±0.2 B	1.1±0.3 B
Co-inoculated	1.3±0.2 AB	1.1±0.1 AB	0.7±0.1 AB	1.0±0.1 AB
Split-root	1.1±0.1 A	1.0±0.2 A	1.0±0.1 AB	0.8±0.2 AB
Co-inoculated+AMF	0.9±0.1 A	0.7±0.1 A	0.8±0.1 A	0.8±0.1 A
P(treatment)	0.002*		0.03*	
P(nematode)	0.67		0.84	
P(treatment x nematode)	0.12		0.41	

Data represent mean±standard error. Capital letters indicate a main effect of treatment, according to the two-way ANOVA and Tukey's HSD test. *Control* (i.e., nematodes in right side of split root), *co-inoculated* (i.e., AMF and nematodes in right side of split root), *split root* (i.e., AMF in left side and nematodes in right side of split root), and *co-inoculated + AMF* (i.e., AMF in left side and AMF and nematodes in right side).

Overall, the presence of *G. intraradices* exerted a protective effect against both *R. similis* and *P. coffeae*, regardless its presence in one or both root halves (Fig. 1). In the experiment with *R. similis*, the final nematode population was significantly reduced by 72% in the co-inoculated + AMF treatment (AMF on both sides) compared with the control treatment (Fig. 1a). Nematode populations in the split-root (AMF on one side and nematodes on other side) and co-inoculated treatment (AMF and nematode on same side) did not differ significantly from the co-inoculated + AMF treatment. However, the *R. similis* population in the co-inoculated treatment did not differ significantly from the control treatment either. In the experiment with *P. coffeae*, similar results were obtained (Fig. 1b). The final nematode population in the co-inoculated + AMF treatment was significantly reduced by 84% than the nematode population in the control treatment. Both the split-root and the co-inoculated treatment did not differ significantly in nematode population from either the control or the co-inoculated + AMF treatment.

No clear differences in nematode population composition were observed in either of the experiments (data not shown). Furthermore, the root necrosis did not differ significantly among the different treatments, ranging from 6 tot 12% in the presence of *R. similis* and ranging from 5 to 12% in the presence of *P. coffeae*.

Discussion

In general, *G. intraradices* reduced both *R. similis* and *P. coffeae* populations in *Musa* cv. Grand Naine between 72 and 84% depending on the treatment. This confirms previous work on banana in similar conditions (Elsen et al. 2003a, b) and more general observations on a wide variety of crops (reviewed by Borowicz 2001), but contradicts that migratory nematodes, such as *R. similis* and *P. coffeae*, are the only group of nematodes whose numbers are greater in AMF-colonized plants (Hol and Cook 2005). Thus, the presented data confirm once again the bioprotective potential of AMF and their potential to increase resistance against migratory endoparasitic nematodes.

The presence or absence of AMF did not influence the nematode damage caused to the root system, while the presence of AMF resulted in a growth depression, as illustrated by the dry root weight. Borowicz (2001) concluded after a meta-analysis of published research papers that AMF tend to exacerbate the harmful effects of nematodes on plant growth and development in general. In addition, several studies reported a growth depression of mycorrhizal plants at high P levels (Sena et al. 2004; Graham et al. 1996). Graham et al. (1996) stated that *Glomus* spp. that were aggressive colonizers of roots at low P-supply, like *G. intraradices*, were also aggressive colonizers at high-P supply, resulting in greater belowground C costs and growth depression. In our case, it can be assumed that the P-content of the substrate used was rather high, since potting soil was included in the mixture. This could explain the growth depression observed in AMF colonized plants.

Many hypotheses have been proposed on the mechanism of the AMF-induced resistance against plant pathogens (Whipps 2004). By using the split-root experimental set-up, we were able to get a better insight. Based on our results, direct competition or inhibition seems not the responsible

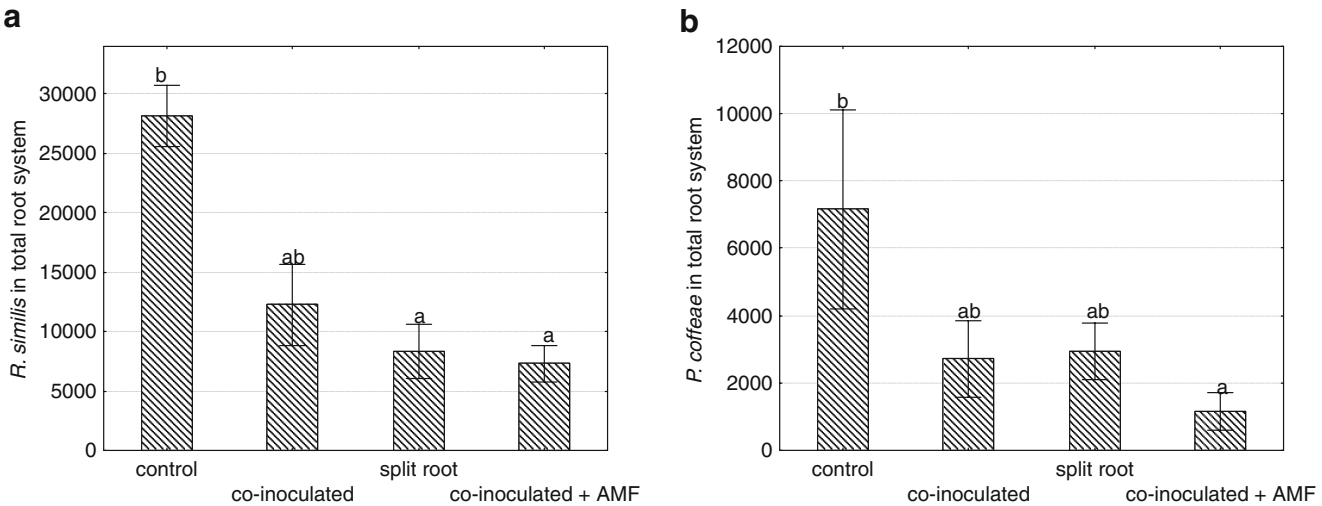

Fig. 1 Total numbers of nematodes in inoculated root half of *Musa*, cv. Grand Naine, 8 weeks after nematode inoculation; **a** *Radopholus similis*, **b** *Pratylenchus coffeae*. Error bars represent standard error. In each graph, *different letters* indicate significant differences ($P \leq 0.05$) according to the Tukey HSD test; *control* (i.e., nematodes in right side of split root), *co-inoculated* (i.e. AMF and nematodes in right side of split root), *split root* (i.e., AMF in left side and nematodes in right side of split root), and *co-inoculated + AMF* (i.e., AMF in left side and AMF and nematodes in right side)

mechanism, since bioprotection also occurs when AMF and nematodes are spatially separated. Moreover, because of the lack of growth promotion by AMF in our system, well-accepted mechanisms as improved plant growth and nutrition do not seem to play a role. The reduced nematode population in the split-root set-up clearly demonstrates that the AMF-induced bioprotection is at least partially systemically induced. For fungal pathogens, this has been shown previously (Cordier et al. 1998; Pozo et al. 2002; Zhu and Yao 2004; Fritz et al. 2006; Khaosaad et al. 2007), but for nematodes, this is the first report. Only one study reported on the use of a split-root experimental set-up to study whether AMF-induced bioprotection occurs through a local or systemic mechanism. De la Peña et al. (2006) suggested that nematode suppression by AMF in *Ammophila arenaria* did not occur through a systemic plant response but through a local mechanism only, which contradicts our results. Knowledge concerning induced systemic resistance (ISR) toward plant parasitic nematodes is scarce (Hasky-Günther et al. 1998; Munif et al. 2001; Siddiqui and Shaukat 2002; Vu et al. 2006). A split-root experimental set-up demonstrated that rhizosperic and endophytic bacteria, like *Bacillus* and *Pseudomonas* spp. induced systemic resistance against cyst (Hasky-Günther et al. 1998) and root-knot nematodes (Munif et al. 2001; Siddiqui and Shaukat 2002). Vu et al. (2006) reported ISR by an endophytic *Fusarium oxysporum* against *R. similis* in banana. Only one study suggested that AMF induce a defense response against root-knot nematodes in the mycorrhizal grapevine roots, which appeared to involve transcriptional control of VCH3 expression throughout the whole root tissue (Li et al. 2006).

ISR has been defined as a physiological state of enhanced defensive capacity by a range of non-pathogenic micro-organisms and biological control agents (Van Loon et al. 1998; Bakker et al. 2007). ISR is phenotypically similar to systemic acquired resistance (SAR) that is triggered by exposing the plant to virulent, avirulent, and non-pathogenic micro-organisms, or artificially to chemicals (Vallad and Goodman 2004). Unlike SAR, ISR does not involve the accumulation of pathogenesis-related proteins or salicylic acid (Pieterse et al. 1996), but instead relies on pathways regulated by jasmonate and ethylene (Knoester et al. 1999; Pieterse et al. 1998).

Similar mechanisms appear to be involved in ISR and autoregulation, i.e., a phenomenon where already existing nodules or arbuscular mycorrhizal roots suppress the further establishment of symbiosis in other root parts (Vierheilig et al. 2000; Catford et al. 2003), since both ISR and autoregulation require high levels of AMF colonization (Vierheilig 2004). The autoregulation of rhizobial and/or mycorrhizal symbiosis seems to involve flavonoids, like formononetin and ononin (Catford et al. 2006). However, this has never been studied for ISR induced by AMF.

In conclusion, the results provided support the involvement of plant-mediated mechanisms other than improved nutrition. For the first time, the presented research demonstrates that AMF have the ability to induce systemic resistance against plant parasitic nematodes in a root system. The pathways involved in the induction and signalling of this ISR still need further investigations.

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