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SHORT NOTE

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Effects of *Glomus intraradices* on the reproduction of the burrowing nematode (*Radopholus similis*) in dixenic culture

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Abstract The effects of *Glomus intraradices* on the reproduction of the burrowing nematode *Radopholus similis* were studied under dixenic culture conditions. The life cycles of both the arbuscular mycorrhizal fungus (AMF) and the nematode were completed in presence of each other and a transformed carrot root as host. The AMF suppressed the *R. similis* population by almost 50% and thus increased protection of the root against the nematode. This reduction was significant for both females and males within roots. There was no correlation between nematode population density and either AMF internal root colonization, external hyphal development or spore production. These results demonstrate that the dixenic system, although artificial, is a valuable tool for studying AMF–nematode interactions, complementing the classical experimental approaches.

Keywords Arbuscular mycorrhizal fungi · *Daucus carota* · Dixenic culture · *Radopholus similis* · Ri T-DNA transformed roots

Introduction

Arbuscular mycorrhizal fungi (AMF) have been shown to reduce damage by soil-borne plant pathogens (reviewed by Azcón-Aguilar and Barea 1996). Among these root pathogens, considerable information has been accumulated on the effects of AMF on nematode reproduction and damage (Smith 1987; Pinochet et al. 1996) and possible mechanisms (Azcón-Aguilar and Barea 1996).

Most research so far on AMF and nematodes has been carried out under greenhouse conditions. This experimental system, however, may yield misleading results under some circumstances due to the presence of undesirable contaminants (Benhamou et al. 1994). The inability to grow these obligate biotrophic fungi in axenic culture led to the development of monoxenic culture systems using excised roots as host partner (Bécard and Fortin 1988; Declerck et al. 1998). Such systems have been used to study interactions with various rhizosphere microorganisms (Benhamou et al. 1994; Filion et al. 1999). However, no research has been dedicated to the AMF–nematode interaction using this culture system. Recently, it was demonstrated that *Radopholus similis* (Cobb) Thorne, a major root-burrowing nematode infecting various economically important crops (Orton Willams and Siddiqi 1973), can infect and reproduce on Ri T-DNA transformed carrot roots (Elsen et al. 2000). This presents an opportunity to study the nematode–AMF interaction under dixenic conditions, i.e. in a system free of organisms other than the two symbiosis partners and the pathogen.

In the present study, mycorrhizal Ri T-DNA transformed carrot roots were inoculated with *R. similis*. The objective of our work was to determine the impact of the AMF on the reproduction of this nematode in a dixenic culture system.

Materials and methods

Biological materials

The AMF strain used was *Glomus intraradices* Schenk and Smith (MUCL 41833). Monoxenic cultures were established in association with transformed carrot (*Daucus carota* L.) roots on modified Strullu-Romand (MSR) medium (see Declerck et al. 1998) but solidified with 4 g l^{-1} Gel Gro. After 5 months culture, several thousand spores were obtained in each Petri dish. Spores were then isolated (see Doner and Bécard 1991) and transferred to sterile water.

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Table 1 Nematode population densities in mycorrhizal (+AMF) and non-mycorrhizal (–AMF) transformed *Daucus carota* roots and MSR medium 10 weeks after inoculation with 25 females of *Radopholus similis*. All nematode population densities were log(*x*+1) transformed prior to analysis (F Females, J juveniles, M males, n.s. not significant according to the Tukey test at $P \leq 0.05$, Rr reproduction ratio: total nematodes recovered from roots and medium relative to the 25 nematodes inoculated)

AMF	Roots				Medium				Total in roots and medium	Rr
	J	F	M	Total	J	F	M	Total		
+AMF	1,662	609	117	2,389	1,238	776	305	2,319	4,707	188
–AMF	2,950	1,739	384	5,072	1,439	2,372	589	4,400	9,472	379
	n.s.	**	*	*	n.s.	*	n.s.	n.s.	**	**

* and ** significantly different according to the Tukey test at $P \leq 0.05$ and $P \leq 0.01$, respectively

An *R. similis* population from Uganda (originally isolated from *Musa* AAA) was maintained monoxenically on alfalfa (*Medicago sativa* L.) callus, grown on modified White's medium (Elsen et al. 1999). Nematodes were extracted from the callus using a modified Baermann funnel (Hooper 1990).

Experimental design

Aliquots of approximately 100 spores of *G. intraradices* were collected from the sterile water with a micropipette and transferred to a 70-mm transformed carrot root on MSR medium. The non-mycorrhizal treatment consisted of transformed carrot roots on the same medium but without the spore inoculum. Petri dishes were incubated horizontally but inverted at 27±0.5°C in the dark for 3 weeks to allow the association to establish. All Petri dishes were then inoculated with 25 females of *R. similis*. Mature females were collected individually with a sterile micropipette and placed in a drop of sterile water on the MSR medium near the root tips. Petri dishes were then incubated horizontally but inverted at 27±0.5°C in the dark for 10 weeks. Eight replicates were established for each treatment.

Assessment of variables

For each replicate, spore production and external hyphal length were estimated under a binocular microscope after 3 and 13 weeks. Spores were counted individually (Declerck et al. 1996) and hyphal length measured using the gridline intersect method (Newman 1966). Root length was also measured using the gridline intersect method after 13 weeks.

At the end of the experiment, Petri dishes were examined for nematode reproduction. Nematodes were extracted from chopped root pieces and medium separately using the maceration-sieving technique. Numbers of juveniles, females and males were determined by counting three 2-ml aliquots from a 200-ml homogenized suspension.

Internal root colonization was estimated in the Petri dishes containing the AMF. Roots were stained as described by Koske and Gemma (1989) and the frequency and intensity of colonization determined as described by Declerck et al. (1996).

Statistical analysis

Before statistical analysis, nematode populations were log(*x*+1) transformed. Single-factor ANOVA was performed and the means were separated by the Tukey test ($P \leq 0.05$) using the Statistica package (Anonymous 1997).

Results

The first secondary spores were formed 1 week after AMF association with the carrot roots. At the time of nematode inoculation, i.e. 3 weeks after mycorrhizal association, 338±244 spores per Petri dish were obtained and the agar surface was covered with a dense hyphal network (513±261 cm). At the end of the experiment, spore production was approximately 3,000 within the range 700–10,000. Mycelium length reached 1,002± 464 cm per Petri dish. The frequency of root colonization was 77.5±17.5% with an intensity of 35.8±12.2%.

In both the mycorrhizal and non-mycorrhizal treatments, the transformed carrot roots developed many lateral roots, both on the agar surface and penetrating the agar. There was no significant difference in development between the mycorrhizal and non-mycorrhizal roots. Root lengths in the mycorrhizal and non-mycorrhizal treatments were 175.9±57.4 cm and 187.1±36.6 cm, respectively.

The numbers of nematodes extracted from carrot roots and MSR medium are given in Table 1. Penetration of roots and reproduction were observed in both the non-mycorrhizal and mycorrhizal treatments. All developmental stages were recovered from both roots and medium. The reproduction ratio (total nematodes recovered from roots and medium relative to the 25 nematodes inoculated) was 188 in the mycorrhizal and 379 in the non-mycorrhizal treatment. In the presence of the AMF, the *R. similis* population was reduced by almost 50% compared with the control. However, this reduction was not significant for all developmental stages. The number of females was reduced in both roots and medium, whereas the number of males was only reduced in the roots. There was no change in the number of juveniles either in roots or medium. Overall, the impact of the AMF on nematode population density was more pronounced in the roots than in the medium. However, no correlation was found between nematode reproduction within roots and population density in the medium and the mycorrhizal parameters, i.e. internal root colonization, external hyphal density or spore production.

Discussion

The results demonstrate that *G. intraradices* grown under dixenic conditions can provide transformed carrot roots with increased protection against *R. similis*. This protection showed itself as a significantly smaller nema-

tode population within the mycorrhizal roots, and to a lesser extent outside the roots, than in the non-mycorrhizal treatment. Both the AMF and the nematode completed their life cycles in presence of each other when associated with a transformed carrot root as host. For *G. intraradices*, a dense hyphal network with numerous spores and a high mycorrhizal root colonization were found at the end of the experiment. For *R. similis*, infection and reproduction within the roots were observed in both the non-mycorrhizal and mycorrhizal treatments. A normal proportion of the population reached the adult stage. The total *R. similis* population was significantly suppressed in the presence of AMF. Indeed, in the non-mycorrhizal roots, reproduction was very high, as already observed by Elsen et al. (2000), while in mycorrhizal roots reproduction was significantly reduced.

The exact mechanism causing reduction of nematode populations observed in our experiment remains uncertain. Factors such as improved nutrient status, microbial changes in the rhizosphere, competition for penetration sites and nutrients, biochemical changes in the plant and anatomical changes in the roots have been proposed as possible mechanisms (Linderman 1994). The dixenic system avoids the influence of parameters such as competition for nutrients in the substrate and microbial changes in the rhizosphere. The absence of correlation between nematode population density (within the root and in the medium) and internal AMF root colonization, external hyphal development or spore production hinder firm conclusions about interactions between the two organisms. The suppressing effect of AMF was, however, more pronounced in the roots than in the medium, suggesting competition within the roots. Biochemical and/or anatomical changes in the mycorrhizal root may be involved in the protection of the root against nematodes. Such an hypothesis was proposed by Benhamou et al. (1994) in connection with a pathogenic fungus. They demonstrated that mycorrhizal colonization of transformed carrot roots can improve protection against *Fusarium oxysporum* f. sp. *chrysanthemi* and that this protection is associated, to a certain extent, with the accumulation of newly formed plant products at the site of infection. This observation was corroborated by the results of Simoneau et al. (1994), who observed an accumulation of symbiosis-related proteins in transformed tomato roots. Some of these proteins were thought to be involved in plant defense (Harrison and Dixon 1993). A systematic histological and cytological study together with biochemical analyses is required to further test this hypothesis as one possible mechanism for horizontal resistance against *R. similis*.

In conclusion, the results reported here confirm the suppressive effect of AMF on nematode reproduction. The *G. intraradices* isolate used appears effective in suppressing the *R. similis* population in roots. Although the dixenic system used is artificial, it may represent a valuable tool for studying the AMF–nematode interaction, complementing classical experimental approaches.

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