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SHORT COMMUNICATION

Effect of the arbuscular mycorrhizal fungus *Glomus intraradices* on the false root-knot nematode *Nacobbus aberrans* in tomato plants

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Abstract Information about the interaction between arbuscular mycorrhizal fungi (AMF) and the false root-knot nematode *Nacobbus aberrans* (Thorne, 1935) Thorne & Allen, 1944 is scarce. The effect of *Glomus intraradices* Schenk & Smith on tomato (*Lycopersicon esculentum* L.) cv. Platense inoculated with nematode juveniles from Lisandro Olmos (Argentina) was studied under greenhouse conditions. Six treatments with five replications were performed. After 80 days, nematode reproduction and percentage of AMF colonization in roots were estimated. Some plant growth parameters were also measured. In general, plants with AMF and AMF plus nematodes grew as well as the control without AMF and without nematodes. Furthermore, *G. intraradices* was beneficial in reducing nematode-induced damage in roots (lower number of galls) as well as in having a suppressive effect on parasite reproduction. This is the first study on the use of *G. intraradices* as a possible strategy in the control of *N. aberrans* in tomato.

Keywords *Glomus intraradices* · *Lycopersicon esculentum* · *Nacobbus aberrans* · Nematode reproduction · Mycorrhiza

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Introduction

Some species of plant-parasitic nematodes are a limiting factor in agricultural production in many parts of the world. Their wide host range, association with other organisms (fungi, bacteria, and viruses) in disease complex presents a very challenging problem damaging world's food supply (Saxena 2004). Chemical treatments are currently the most widely used and the reliable way of controlling these pests. However, the increasing use of toxic agrochemicals is being restricted (Djian-Caporalino et al. 2007). In the last years, biological control has become an efficient, economical, and non-polluting method of nematode control.

The arbuscular mycorrhizal symbiosis is a mutualistic association form between plants and wide variety of fungi from the phylum Glomeromycota. The symbiosis is formed by the majority of plants species and is found in ecosystems around the world (Wang and Qui 2006). Colonization of the root system by arbuscular mycorrhizal fungi (AMF) confers benefits directly to the hosts plant growth and development by increasing nutrient uptake and also improves plant tolerance to stress conditions and to the attack of soil-borne pathogens (Sharma et al. 2004). Regarding plant-parasitic nematodes, there is an interest in AMF–nematode interactions because they may enhance parasite resistance or tolerance of AMF-infected plants and their potential value for pest control (Hol and Cook 2005). The likelihood of using AMF in crop production systems is increasingly more realistic, and studies have increased considerably in the last years (Jaizme-Vega et al. 2002).

The false root-knot nematode, *Nacobbus aberrans* (Thorne, 1935) Thorne & Allen, 1944, is a polyphagous species, sedentary endoparasite of roots. It is native to America (Sher 1970). Due to the severe damage it causes to agriculture, it is considered of quarantine importance

(OEPP/EPPO 1984). Losses may reach between 10% and 20% in sugar beet (*Beta vulgaris* L.) in USA (Inserra et al. 1996) and up to 73% in potato (*Solanum tuberosum* L.) in Peru and Bolivia (Canto-Saenz et al. 1996; Franco et al. 1996). To date, the presence of *N. aberrans* has been cited in Argentina, Bolivia, Chile, Ecuador, Mexico, Peru, and USA (Manzanilla-López et al. 2002).

N. aberrans was first detected in northwestern Argentina in 1977 parasitizing several crops and some weeds (Costilla et al. 1977). At present, it has a wide distribution and can be found infesting field-grown and greenhouse-grown crops (Doucet and Lax 2005). In the province of Córdoba, the parasite causes severe damage to tomato (*Lycopersicon esculentum* L.) and pepper (*Capsicum annum* L.) under greenhouse conditions; plants usually exhibit poor development or wilting symptoms in shoot, or may even die when nematode population densities are high.

Most of the research works that evaluated AMF interaction with sedentary endoparasitic nematodes have focused on different root-knot nematode (*Meloidogyne*) species of economical importance. It has been observed that root colonization by *Glomus* spp. increased host plant tolerance to *Meloidogyne* spp. and showed a suppressive effect over nematode reproduction, as it occurred in banana (*Musa* AAA) (Jaizme-Vega et al. 1997; Elsen et al. 2002), tomato (Talavera et al. 2001; Shreenivasa et al. 2007), and olive (*Olea europaea* L.) (Castillo et al. 2006). AMF inoculation, however, is not always beneficial for the growth of plants infested by root-knot nematodes (Atilano et al. 1981) or for reduction of the parasite population (MacGuidwin et al. 1985; Atilano et al. 1981; Cofcewicz et al. 2001).

Information about interaction of *N. aberrans* and AMF is very scarce. The only published report indicates that tomato plants inoculated with *Glomus* sp. exhibited a lower number of galls induced by a Mexican population of the nematode (Gardezi et al. 1995). The objective of this work was to evaluate the possibility of using the mycorrhiza *Glomus intraradices* Schenk & Smith as a protective agent against an isolate of *N. aberrans* on a susceptible tomato cultivar. The effects of the AMF–nematode interaction on tomato growth and pathogen development were also studied.

Materials and methods

Nematode isolate

An *N. aberrans* isolate from the locality of Lisandro Olmos (province of Buenos Aires, Argentina), of known capacity for multiplying on tomato, was employed for the study. Nematodes were maintained on tomato cv. Platense under greenhouse conditions. Plants were uprooted, and roots

with galls were gently washed with running water to remove adhering soil particles. Egg masses were removed and placed in Petri dishes containing distilled water. They were maintained at room temperature to allow hatching, and then mobile second-stage juveniles (J2) were extracted with the aid of a pipette.

AMF inoculum

G. intraradices was selected because it showed beneficial effects when interacting with roots of certain plants in the presence of some species of plant-parasitic nematodes (Smith and Kaplan 1988; Jaizme-Vega and Pinochet 1997; Elsen et al. 2001).

The arbuscular mycorrhizal fungus [La Plata, Spetzgazzini Herbarium (LPS), culture Tierra del Fuego 28] was propagated on *Medicago sativa* L. Plants were grown for 6–12 months in pots containing a mixture of perlite and vermiculite (1:1). Plants were watered from below using a capillary system and fed with nutrient solution (three times a week) (Cabello 1997).

Soil preparation

Sandy loam soil was used for all experiments. Soil physico-chemical characteristics were as follows: pH (H₂O), 5.20; clay, 15%; silt, 35%; sand, 50%; organic matter, 2.58%; nitrogen, 0.22%; and available P, 16.08 mg kg⁻¹. The soil was air-dried, powdered, and sieved through a 2-mm-mesh sieve and was steam-sterilized three times (120°C, 1 h with 24 h between the three treatments). The steamed soil was mixed with autoclaved sand (60 min at a pressure of 2 atm) in a 3:1 ratio.

Plant material and treatments

Tomato seeds (cv. Platense) were surface sterilized in 10% sodium hypochlorite (NaOCl) for 5 min and sown in trays containing sterile soil to promote germination. One-month-old seedlings, which had four leaves, were placed individually in plastic containers (20-cm long×4-cm wide, with a capacity of 125 g of soil) containing a mixture of sterilized soil with sand (3:1). Six treatments were performed: (1) plants free of nematodes or AMF (control), (2) plants inoculated with the AMF *G. intraradices* at transplanting (zero time=T₀), (3) plants inoculated with the nematode *N. aberrans* at T₀, (4) plants inoculated with both *N. aberrans* and *G. intraradices* at T₀, (5) plants inoculated with *N. aberrans* 3 weeks after transplanting, and (6) plants inoculated with *G. intraradices* at T₀ and with *N. aberrans* 3 weeks after transplanting.

For nematode inoculation, roots were placed on the substrate and 100 active J2 (initial population=Pi) present in 1.5 ml of water were immediately deposited on the roots,

which were then covered with the substrate. Inoculum of *G. intraradices* was composed of 5 g of a homogeneous mixture of rhizosphere soil, spores (approximately 1,000), and rootlets of the host plant, which were added through the planting hole at the time of transplanting. Five replications per treatment were performed. Temperature in the greenhouse was 24°C (10-h photoperiod). Plants were watered daily and were not fertilized. After 80 days, plants were uprooted and roots were gently washed with water to remove adhering particles.

Assessment of variables and data analysis

At the end of the experiment, the following plant growth parameters were measured: length and dry weight of root and shoot. The percentage of the arbuscular mycorrhiza colonization (%AMC) was assessed according to the technique described by Phillips and Hayman (1970). Briefly, roots were cleared with 10% KOH (15 min at 90°C), then acidified with 1% HCL (1 min, root temperature) and stained in 0.05% trypan blue. Colonization was estimated by the grid-line intersect method (Giovannetti and Mosse 1980) in which 100 segments of 1 cm in length were evaluated under stereomicroscope Leica M420.

Roots of each plant were analyzed using stereoscopic microscope by counting the number of galls present. Root gall index (RGI) was estimated on the basis of the scale proposed for *Meloidogyne* spp. (0=no galls, 1=1–2, 2=3–10, 3=11–30, 4=31–100, and 5=more than 100 galls per root) (Hartman and Sasser 1985). An average of $RGI \leq 2$ was used as a possible indicator of resistance, following Hartman and Sasser (1985). Egg masses were extracted and submerged in 1% sodium hypochlorite solution for 4 min (Hussey and Barker 1973), and eggs were counted. Soil of each container was processed using the centrifugal-flotation technique (Jenkins 1964) for extraction of filiform nematodes present. Final nematode population density (Pf) was estimated based on the number of eggs counted plus the number of nematodes extracted from the soil. Population density was used to calculate the reproduction factor ($RF = Pf/Pi$) (Castillo et al. 1998).

The effect of AMF on nematode population (nematode response), which represents the percentage reduction in nematode numbers on AMF plants, was calculated as follows: the difference between nematode numbers on non-AMF plants and nematode numbers on AMF plants divided by the nematode numbers on non-AMF plants and multiplied by 100 (Hol and Cook 2005).

Plant growth and nematode parameters were analyzed by two-way analysis of variance and contrast test (Tukey, $P \leq 0.05$) to determine the significance of mean differences between treatments. Those parameters that did not exhibit normality were transformed to $\log_{10}(x+1)$ before analysis.

Results

No AMF colonization was observed on the roots of non-mycorrhized plants. The remaining treatments inoculated with *G. intraradices* showed arbuscules, external and intracellular aseptate hyphae (2–6 μm), oval to rectangular intracellular vesicles (25–90 μm), and abundant coils (Fig. 1). Plant growth parameters showed significant differences among treatments, except shoot total length (Table 1). AMF colonization on the root system was significantly higher in plants inoculated with AMF and *N. aberrans* at T_0 (AMF%=45.7) and after 3 weeks (AMF%=47.4) than in nematode-free plants (AMF%=30) (Table 1). Inoculation with *G. intraradices* (AMF at T_0) was associated only with increase in root length compared with the non-inoculated control. On the other hand, inoculation with *N. aberrans* (NEM at T_0) reduced root and shoot dry weight compared with control. Moreover, plants inoculated with nematodes after 3 weeks (NEM after 3 weeks) increased total root length and reduced root dry weight compared with the non-inoculated control. No significant differences were observed among some growth parameters of plants inoculated only with nematodes and of those inoculated with nematodes and AMF (both at transplanting and after 3 weeks), except root total length (at T_0) and root dry weight (after 3 weeks). The comparison of treatments AMF+NEM on the two evaluation dates showed that plants that were inoculated with nematodes after 3 weeks exhibited an increase in biomass for shoot dry weight.

All the tomato plants inoculated with *N. aberrans* exhibited roots with galls induced by the nematode (Fig. 1). Both parameters associated with nematode reproduction exhibited significant differences among treatments (Table 1). The two treatments related to *N. aberrans* and AMF inoculation (at T_0 and after 3 weeks) presented the lowest values of RGI and RF, whereas the highest values corresponded to the respective control treatments (NEM at T_0 and NEM after 3 weeks). A positive value of nematode response is an indicator of the decrease in the number of nematodes in mycorrhized plants. A 58.3% reduction occurred when nematode and AMF inoculations were performed at T_0 , and a 23.7% reduction occurred when nematodes were inoculated in pre-mycorrhized plants (compared with respective control: NEM after 3 weeks). Comparison of *N. aberrans* reproduction between the latter treatment performed (AMF at T_0 +NEM after 3 weeks) and control NEM at T_0 showed that nematode reproduction rate was reduced by 63.2%.

Discussion

Chemical nematicides are prohibited due to their potentially detrimental effects on the environment and human health.

Fig. 1 Arbuscular mycorrhizal colonization of *G. intraradices* in tomato cv. Platense infested by *N. aberrans*. **a** Arbuscule. **b** Coil. **c** Vesicles. **d** Tomato roots with galls induced by the nematode. Scale bars: **a** 10 μ m; **b**, **c** 50 μ m; **d** 1 cm

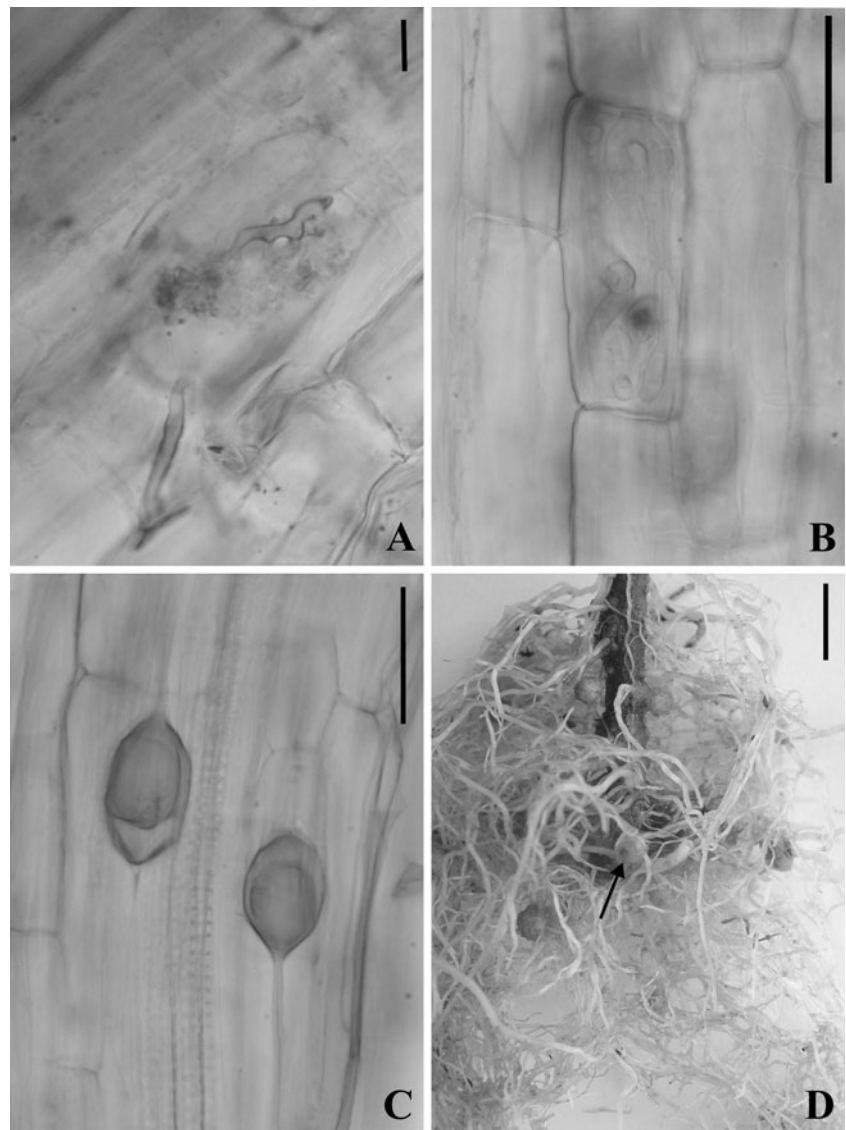


Table 1 Growth parameters of tomato plants cv. Platense, mycorrhizal root colonization of *G. intraradices* (AMF), RGI, and RF of *N. aberrans* (NEM)

Inoculation treatment	Percentage of AMF colonization	Root		Shoot		RGI	RF
		Total length (cm)	Dry weight (g)	Total length (cm)	Dry weight (g)		
Non-inoculated control	–	20.3 \pm 1.4a	0.6 \pm 0.1b	20.2 \pm 2.0a	0.9 \pm 0.1b	–	–
AMF at T_0	30.0 \pm 10.3a	24.9 \pm 2.2b	0.3 \pm 0.2ab	20.4 \pm 1.5a	0.8 \pm 0.1b	–	–
NEM at T_0	–	20.5 \pm 0.9a	0.2 \pm 0.1a	21.0 \pm 3.2a	0.3 \pm 0.1a	2.6b	42.8b
AMF at T_0 +NEM at T_0	45.7 \pm 7.1b	24.1 \pm 3.7ab	0.2 \pm 0.3a	20.1 \pm 1.6a	0.3 \pm 0.1a	1.6a	17.9a
NEM after 3 weeks	–	21.8 \pm 1.9ab	0.5 \pm 0.1ab	19.6 \pm 0.4a	1.0 \pm 0.04b	2.8b	20.7ab
AMF at T_0 +NEM after 3 weeks	47.4 \pm 5.1b	20.8 \pm 2.0ab	0.2 \pm 0.04a	17.6 \pm 1.7a	0.9 \pm 0.2b	2.4ab	15.8a

Data are means of five replicates. Means in the same column followed by the same letter did not differ according to a Tukey test ($P \leq 0.05$) T_0 transplanting time

The use of AMF as biological control of plant-parasitic nematodes is therefore an attractive option to minimize nematode-induced damages (Zhang et al. 2008). The present work is the first evaluation of the effect of *G. intraradices* on tomato plants as a possible tool to control the attack by *N. aberrans*.

The use of *G. intraradices* in tomato plants inoculated with *N. aberrans* juveniles showed significant differences among some growth parameters. In general, plants with AMF and AMF+NEM grew as well as the control without AMF and without nematodes. Similar situations have been reported by Hol and Cook (2005). An overview of AMF–nematode interactions based on data from previous studies revealed that plant responses to inoculation with plant-parasitic nematodes and AMF together differed among nematodes with different feeding types (ectoparasites, sedentary and migratory endoparasites). AMF plants suffered more from ectoparasites than non-AMF plants, while this was opposite for endoparasites (Hol and Cook 2005).

Nematode presence may increase, decrease, or have no effect on root colonization by AMF (Roncadori 1997). The effect would depend on the specific fungal species involved (Waceke et al. 2001). In the present work, lower *G. intraradices* colonization was observed in the absence of *N. aberrans*. Plants inoculated with AMF plus the nematode showed a 52% increase in root colonization (AMF at T₀+NEM at T₀) and a 58% increase (AMF at T₀+NEM after 3 weeks) relative to control (AMF at T₀). Reports of plants parasitized by *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 indicated a slight increase in root colonization by *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe in banana “Grand Naine” (Jaizme-Vega et al. 1997) and *Gigaspora margarita* Becker & Hall on soybean (*Glycine max* (L.) Merr.) (Carling et al. 1989). In the latter case, the nematode also tended to stimulate sporulation by AMF. However, in other situations, AMF colonization has been reduced in the presence of root-knot nematodes, such as tomato infested by *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 and inoculated with *Gigaspora margarita* (Cofcewicz et al. 2001), pyrethrum (*Chrysanthemum cinerariifolium* Vis.) inoculated with *Glomus* sp. and attacked by *Meloidogyne hapla* Chitwood, 1949 (Waceke et al. 2001), or grape (*Vitis vinifera* L.) roots with *Glomus fasciculatum* (Thaxter) Ger. & Trappe emend. Walker and Koske and *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (Atilano et al. 1981). In these cases, the degree to which plant-parasitic nematodes, especially *Meloidogyne* spp., affect mycorrhizae may depend on the effects of nematodes on the plants, rather than on direct effects on fungi (Atilano et al. 1981). The parasite may have obstructed nutrient flow between symbionts, reducing fungus development and efficiency (Cofcewicz et al. 2001).

G. intraradices inoculation was highly beneficial for the host in reducing the damage caused by the nematode in roots (lower number of galls). The highest RGI was recorded in the absence of AMF; in its presence, RGI decreased by 38.5% at T₀ and in the treatment performed after 3 weeks, by 14.3%. Reduction in the number of galls due to the effect of AMF agrees with results indicated in the only experiment conducted so far in tomato infected by *N. aberrans* and inoculated with *Glomus* sp. (Gardezi et al. 1995). Although RGI was reduced in root systems colonized by *G. intraradices*, the most important effect was observed in the considerable decrease of parasite multiplication rate (58.2% at T₀, 23.7% in the treatment performed after 3 weeks and up to 63% when the latter treatment is compared with control NEM at T₀). Results agree with previous reports on the suppression of different nematode species by AMF (including *G. intraradices*), such as *Pratylenchus goodeyi* Sher & Allen, 1953 and *M. incognita* in banana (Jaizme-Vega et al. 1997; Jaizme-Vega and Pinochet 1997), *M. incognita* and *M. javanica* in olive (Castillo et al. 2006), *M. incognita* in tomato (Talavera et al. 2002), *Radopholus citrophilus* Huettel, Dickson & Kaplan, 1984 in rough lemon citrus (Smith and Kaplan 1988), and *Radopholus similis* Cobb, 1893 and *Pratylenchus coffeae* (Zimmerman, 1898) Filipjev & Schuurmans Stekhoven, 1941 in carrot root cultures (*Daucus carota* L.) (Elsen et al. 2001, 2003). Mechanisms involved in nematode suppression by AMF are still a matter of speculation (Elsen et al. 2002). Some possible factors include enhanced nutrient status of the plant, biochemical changes in plant tissue, anatomical changes, modified root exudation, and changes induced in root morphology (Hooker et al. 1994).

In the present work, the lowest reproduction of *N. aberrans* was obtained in the treatment involving pre-mycorrhized plants and further attacked by nematode juveniles. Because no tomato cultivars resistant to *N. aberrans* have been obtained so far, this approach involving AMF–nematode interactions might represent an effective nematode management strategy in commercial tomato production, particularly in continuous cropping under greenhouse conditions. Although the study was not repeated, our results demonstrate the possible advantage of a previous application of AMF inoculum in the soil devoted to develop tomato seedbeds. Thus, once the plant is transplanted to the field or greenhouse in soil infested by *N. aberrans*, the roots would already be colonized by AMF. This would confer the plant with certain degree of tolerance to the pathogen attack and, at the same time, would contribute to limit nematode population densities in the soil. However, as sterilized soil was used, it should be taken into account that other native soil microbes might affect the mycorrhizosphere.

N. aberrans populations exhibit a great variation on behavior, either on a single host or on a range of plants

(Manzanilla-López et al. 2002; Lax et al. 2006). Therefore, there are different races within the species (Inserra et al. 1985; Castiblanco et al. 1999; Manzanilla-López et al. 2002). On the other hand, some AMF species have proved to be more efficient, to a greater or lesser degree, than others in suppressing plant-parasitic nematodes (Waceke et al. 2001; Hol and Cook 2005). For this reason, it is important to conduct further studies in this field, incorporating other nematode populations with different degree of pathogenicity; it is also important to evaluate their interaction with other mycorrhizal species and/or isolates as well as with other plant species of importance for agriculture.

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