Research article

Effect of mycorrhization on the growth of plantain seedlings in substrate with and without the presence of plant parasitic nematodes

Efecto de la micorrización sobre el crecimiento de plántulas de plátano en sustrato con y sin la presencia de nematodos fitoparásitos

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Rec.: 09.07.11 Acept.: 26.12.11

Abstract

We compared the effect of pre-inoculation with the following arbuscular mycorrhizal fungi (AMF), on the growth of vegetative propagules (suckers) of plantain (*Musa* AAB): 1) *Glomus fistulosum*, 2) *Glomus fasciculatum*, and 3) commercial inoculum (mixture of *G. aggregatum, G. fistulosum, G.manihotis, G. fasciculatum, Kuklosplora colombiana* and *Scutellospora* spp.), compared with a control without AMF, in substrate with and without the presence of *Radopholus similis* and *Helicotylenchus* spp.. Furthermore, we evaluated the effect of AMF on the final amount of plant parasitic nematodes in roots and rhizosphere soil. The results indicated a significant intensification in the mycorrhizal colonization in roots inoculated with commercial inoculum of AMF (40 - 41%) compared with control and other AMFs (<3%). There were over 30% of increase in fresh weight of roots and dry matter (corm and air tissue) variables, in plants inoculated with commercial AMF, even in the presence of plant parasitic nematodes. The final amount of plant parasitic nematodes was not affected by the inoculation of AMF. We conclude that mycorrhizal fungi species' mixture, present within the AMF commercial inoculum, stimulated root growth and nutrient uptake, thereby improving biomass production and offsetting the damage by plant parasitic nematodes.

Key words: *Musa* AAB, mycorrhizal colonization, *Radopholus similis*, root growth.

Resumen

Se comparó el efecto de la pre-inoculación de los hongos micorrícico arbusculares (HMA): *Glomus fistulosum*, *Glomus fasciculatum,* micorriza comercial (*G. aggregatum*, *G. manihotis*, *G. fistulosum, G. fasciculatum, Kuklosplora colombiana* y *Scutellospora* spp.) y un control sin HMA, sobre el crecimiento de propágulos vegetativos de plátano Dominico Hartón (*Musa* AAB) en sustrato con la presencia de *Radopholus similis* y *Helicotylenchus* spp. y sin ella. Adicionalmente se evaluó el efecto de los HMA sobre la cantidad final de los nematodos fitoparásitos en raíces y suelo rizosférico. Los resultados indicaron un incremento significativo en el porcentaje de colonización micorrícica en raíces inoculadas con HMA comercial (40 - 41%), en comparación con el control y los demás HMA (< 3%). Se presentaron incrementos superiores a 30% en las variables de peso fresco de raíces y peso seco (cormo y tejido aéreo) en las plantas inoculadas con HMA comercial, aún en la presencia de nematodos fitoparásitos. La cantidad final de nematodos fitoparásitos no fue afectada por la aplicación de HMA. Se concluye que la micorrización obtenida con las especies de HMA del inóculo comercial favoreció el crecimiento radical y la absorción de nutrientes, mejorando la producción de biomasa y contrarrestando los daños causados por nematodos fitoparásitos.

Palabras clave: Colonización micorrícica, crecimiento radical, *Musa* AAB, *Radopholus similis*.

Introduction

Banana and plantain are the fourth most important feeding crops, after rice, wheat and maize (Arias *et al.*, 2004). FAO statistics (FAO, 2009) indicate that Colombia has a plantain cultivated area close to 379,863 ha which positions Colombia as the first plantain exporter of the world, this is the reason why this musacea is a product of economical and social importance for the country. The main difficulties in plantain production in Colombia are referred to the low investment done in crop tasks such as farm arrangement, renovation, fertilization and drainage; these, plus the use of non-certified material to plant, evidences the development of phytosanitary problems like moko (*Ralstonia solanaceraum* Smith), black sigatoka (*Mycosphaerella fijiensis* Morelet), banana weevil (*Cosmopolites sordidus* Germar) and plant parasitic nematodes. According to Araya (2003) after black sigatoka, plant parasitic nematodes are remarkably important since they can reduce crop yield in 80%. Plant parasitic nematodes affecting banana and plantain crops are, in order of importance, migratory endoparasitic *Radopholus similis* (Cobb) Thorne, and *Pratylenchus goodey* (Sher and Allen), ectoparasite *Helicotylenchus multicinctus* (Cobb) Golden, and different species of the sedentary parasite *Meloidogyne* spp., being *Meloidogyne incognita* (Kofoid and White) Chitwood, the specie of highest importance (Gowen *et al.*, 2005; Araya, 2003; Brooks, 2004; Carlier *et al.*, 2003).

The damage to the root system done by these nematodes is translated in low water and nutrient absorption which results in plantain bunch weight loss, longer vegetative cycles and overturn of plants, especially during bunch filling (Sarah *et al.*, 1996; Quénéhervé *et al.*, 1991; Gowen *et al.,* 2005). In tropical conditions, *R. similis* is the most frequent and abundant nematode in any plant developmental stage and, its infection relies on nematodes coming from the corm that move from the mother plant to the new buds and also from nematodes coming from soil (Araya, 2003).

Management of these plant parasites is based on dry nematicides additions together with good agricultural practices (Marin *et al.*, 2000). However, chemical products are highly toxic and its inadequate and indiscriminate use can negatively affect human and animal health, and the environment. Additionally, in Colombia there are not available commercial crops with genetic resistance to nematodes, and the small scale farmers rely only on the removal of affected tissue in the corm without using other different management practices. The above mentioned reasons show the importance of implementing management strategies that can be easily used in an integrated management program.

The use of endomycorrhizal fungi, which established a symbiotic mutualistic association with most of the higher plants, is efficient incrementing plantain and banana suckers growth (Jaizme-Vega *et al.*, 2002; Elsen *et al.,* 2003) and the tolerance mechanisms against plant parasitic nematodes, attributed to a better nutrition and compensation of root damage (Hol and Cook, 2005; Jaizme-Vega and Rodriguez-Romero, 2004; Jaizme-Vega *et al.*, 1997). Therefore, the aim of this study was to evaluate the effect of pre-inoculation with three arbuscular mycorrhizal fungi (AMF) in plantain suckers growth (Dominico Harton) on a substrate with and without plant parasite nematodes, under mesh house conditions. Additionally, the effect of AMF treatments over plant parasitic nematodes population was evaluated in rhyzospheric soil and roots.

Materials y methods

Experimental substrate and experimental location

Substrate was a mix of soil and river sand in a 2:1 proportion. Soil was collected in the experimental station of the International Center for Tropical Agriculture (CIAT) in Santander de Quilichao (Colombia), this soil is

characterized for its low phosphorous content and, it was sterilized with water vapor for two hours per day for two days. The chemical composition of this substrate is on Table 1. After sterilization, substrate was packed in 5 kg. polyethylene bags that were placed under a mesh house located at the Corpoica Research Center located in Palmira (3° 31' N, 76° 19' O, 1001 m.a.s.l. 24°C), Valle del Cauca, Colombia.

Table 1. Chemical composition of the substrate used in this study .

pH	M.O	P	S	B	K	Ca	CIC
	(g/kg)	mg/kg			Cmol/kg		
5.1	36.78	13.7	44.4	0.3	0.2	4	13

AMF inoculum

AMF inoculum sources were added to each bag by hand in the following way: (1) *Glomus fasciculatum* (Thaxt.) Gerd. and Trappe emend. C. Walker and Koske; (2) *Glomus fistulosum* Skou and Jakobsen, both species were obtained from the AMF isolation bank in CIAT; and (3) commercial inoculum consist on a mix of *G. fasciculatum, G. fistulosum, G. aggregatum* Schenck and G.S. Smith, *G. manihotis* Howeler, Sieverding and Schenk, *Kuklosplora colombiana* Oehl and Sieverding and *Scutellospora* spp. Walker and Sanders, species. The inoculum comprises substrate, fragments of colonized roots, spores, sporocarps and hyphae fragments. The amount of inoculum added was established based on the AMF spore number contained on 20 g of commercial inoculum –recommended dose for nursery crops- which was determined by extraction and quantification by the floating method in sugar. In each bag with substrate were added 283 ± 43 spores of AMF. Control treatment consisted in sterile substrate alone.

After AMF inoculation, it was sown in each bag one plantain sucker from Dominico Harton (*Musa* AAB Simmonds) of 10 cm height and 3 cm wide approximately, obtained by induction of lateral buds budding in corms under high temperature (50 °C approximately). Soil with suckers was kept with a moisture close to field capacity level to avoid any water stress.

Plant parasitic nematodes inoculum

Plantain ((*Musa* AAB Simmonds) roots that were infected with nematodes were collected in farms from Montenegro and Armenia, Quindio (Colombia). Plant parasitic nematodes were isolated by the method of Niblack and Hussey (1987) and identified based on morphologic and morphometric characteristics (Mai and Mullin, 1996) as *R. similis* and *Helicotylenchus* spp., they were concentrated, counted on a stereoscope in 1 ml aliquots and adjusted to a concentration (individuals/ml of water) of 167 *R. similis* and 24 *Helicotylenchus* spp. Nematode inoculation was done 2 months after sowing the suckers, by doing three holes in the soil (1 cm diameter x 2 cm depth) separated 1,5 cm from the pseudostem base (Dosselaere *et al.*, 2003; Zum Felde *et al.*, 2006). In each hole 2 ml of suspension solution with nematodes were added to a total of 1000 *R. similis* and 144 *Helicotylenchus* spp. per plant. The control treatment was done using the same volume of sterile water.

Treatments were done in a complete random experimental design to compare the effect of AMF: *G. fistulosum*, *G. fasciculatum*, commercial mycorrhiza (mix *G. aggregatum*, *G. manihotis G. fistulosum, G. fasciculatum, Kuklosplora colombiana* and *Scutellospora* spp.) and a control without AMF; in the growth of suckers on a substrate with or without nematodes (Table 2). Each treatment was replicated four times using four plants as experimental unit.

Plants were removed ninety days after inoculation and the infestation results on growth variables were, root fresh weight (g) and dry weight (corm and aerial tissue) (g).

The final amount of soil nematodes was determined on a 100 cm^3 rhyzospheric soil subsample from each plant using the centrifugation and flotation in sugar method described by Niblack and Hussey (1987). To determine the nematode number in roots, they were cut in 2-3 cm length fragments, homogenized and a 10 g subsample was grinded on a blending machine for 10 seconds intervals at low and high speed, then nematodes were isolated by the centrifugation and floating using the sugar method (Niblack and Hussey, 1987). The number of nematodes

was evaluated on three aliquots of 5 ml from a sample of 25 ml, using a counting camera and a stereoscope. Identification of *R. similis* and *Helicotylenchus* spp. was done by the method described previously. Finally, nematode number was expressed in 100 cm^3 of soil and 1g of roots.

AMF mycorrhizal colonization in roots was determined in 1 cm root segments, cleared in a water bath (90 °C) with KOH (2.5%) for an hour; then they were submerged in HCl (2%) at room temperature for an hour, later they were dyed with trypan blue under water bath (90 °C) for an hour. Samples were mounted on microscope slides and evaluated on a microscope (40X) using the notation scale of Trouvelot (Trouvelot *et al.,* 1986) (Figure 1). Results were analyzed with Mycocalc software (Clapp y Zhao, 2001) to calculate the percentage of mycorrhizal colonization (MC).

Before the analysis was done, the number of plant parasitic nematodes was transformed (log_{10} (x + 1)) to adjust the data to a normal distribution. Treatment differences were evaluated with variance analysis, using the GLM (general lineal model) procedure with the AMF inoculation treatments as source of variation. Data mean were separated by the Tukey's comparison test (P < 0.05), likewise, Pearson multiple correlations among the evaluated variables and the number of plant parasitic nematodes in soil and roots and mycorrizhal colonization were done. All the analysis was done using SAS version 9.1 software (SAS Institute Inc, 2004).

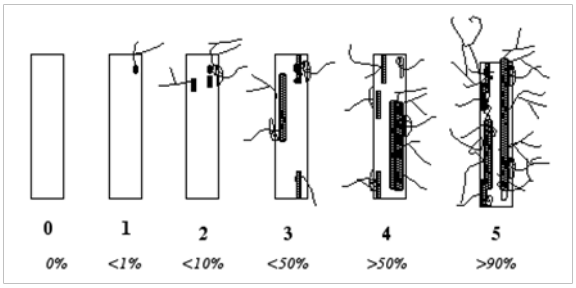

Figure 1. Evaluation scale for mycorrhizal colonization percentage in roots.
Source: Trouvelot *et al.* (1986).

Results and discussion

In the absence of nematodes it was observed a significant increment in the root production with commercial AMF, which was two times higher than the one obtained with *G. fistulosum* (Table 2). Inoculated plants with commercial AMF incremented their root fresh weight average in 60% and 40% in comparison with inoculated control plants and plants inoculated with *G. fasciculatum*, respectively, although these differences were not significant. Dry weight of corm and aerial part was not different (P > 0.05) between treatments; however, it was observed that plants inoculated with AMF had 30% more biomass than the control without AMF.

The percentage of mycorrhizal colonization was significant (P < 0.05) between treatments, this was 41% in commercial AMF inoculated plants, whereas the control without AMF, and

Table 2. Efecto de la aplicación de hongos formadores de micorriza arbuscular sobre las variables de crecimiento y la colonización micorrícica en raíces, evaluada 150 días después de la inoculación de HMA.

Treatment	Without plant parasitic nematodes			With plant parasitic nematodes		
	Root fresh weight (g)	Dry weight (g)	% Mycorrhizal colonization	Root fresh weight (g) (g)	Dry weight (g)	% Mycorrhizal colonization
G. fistulosum	8.9 b$^{\varphi}$	3.7	3 a	9.0	3.0 b	0 a
G. fasciculatum	13.5 ab	4.4	1 a	13.9	4.2 ab	0 a
AMF mix	21.9 a	5.5	41 b	17.6	5.5 a	40 b
Control without AMF	15.4 ab	4.2	0 a	11.5	3.3 b	0 a
Treatment effect[1]	*	ns[1]	**	ns	*	**

$^{\varphi}$ Average in each column followed by the same letter are not statistically different according to Tukey´s test (P ≤ 0.05).

[1]ns: statistically not significant interaction; *: significant at P ≤ 0.05; **: highly significant at P ≤ 0.001.

the ones inoculated with *G. fistulosum* and *G. fasciculatum* showed less than 3% inoculation (Table 2). Biomass and root increment was positively correlated with the percentage of mycorrhizal colonization (Table 3), which demonstrates the beneficial effect of mycorrhizal in plantain suckers of Dominico Harton. Similar results were found in the plant growth variables when soil was inoculated with plant parasitic nematodes. In this case, a significant increment of 30% in dry weight of plants inoculated with commercial AMF was observed when compared to other treatments The highest fresh root biomass was obtained in suckers grown with the commercial inoculum, however the differences were not significant (P > 0.05). The increment on fresh root weight and plant dry weight of plants inoculated with commercial AMF and plant parasitic nematodes were higher than the control without nematodes. Mycorrhization increments growth and favors morphological changes in roots which helps plants to tolerate better nematode damage, since roots in plants with mycorrizhae are highly branched counteracting the negative impacts of nematodes (Hol and Cook, 2005).

The absence of a good symbiotic association between *G. fistulosum* or *G. fasciculatum* with plantain plants is not explicable, since other studies (Reyes *et al.*, 1995; Hurtado, 1996; Jaramillo and Rivillas, 2001) reported over 80% colonization of these species in plantain and banana roots. In the same manner, bioprotection effects of *G. fasciculatum* against *R. similis* in banana are cited (Siddiqui and Mahmood, 1995; Umesh *et al.*, 1988). Possibly, the *G. fistulosum* and *G. fasciculatum* strains used in this study require a higher number of spores/ g of inoculum, or they were affected by the soil physical and chemical conditions that affected their adaptation and colonization, compared with other genus or species on the commercial AMF inoculum. This suggests that the inoculation of a strain alone cannot be more effective than a mix of strains. Nowadays, it is accepted the specificity between the host and the inoculated strain in the mycorrhizal association, there are evidences in the response diversity according to different spores from the same strain (Ian Sanders, University of Lausanne, Suiza, 2010 -personal communication). Elsen *et al.* (2003) indicate that the relative mycorrhizal dependency (RMD), defined as the degree of plant de-pendence on the mycorrhizae to get the maxi-mum growth or yield under a soil fertilization level, relies on the variety and the AMF. It has been observed a high variation in RMD between seven varieties of *Musa* spp. (group AAA) inoculated with two AMF species under greenhouse conditions (Declerck et al., 1995). RMD was highly variable according to the inoculated *Glomus* spp. specie (Declerck *et al.*, 1995).

The obtained results confirm the beneficial effect in growth of *Musa* spp.suckers (Declerck *et al.*, 1995; Rizzardi, 1990; Elsen *et al.*, 2002; Jaizme-Vega *et al.,* 2002), as a response to the mycorrhization presented by the commercial AMF inoculums. In this case, the increment was associated with a higher nutrient absorption, because it was found that the plants with mycorrhizae had higher contents of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and cupper

Table 3. Pearson correlation coefficients for the evaluated variables: Dominico Harton plantain seedlings, percentage of mycorrhizal colonization in roots and total number of plant parasitic nematodes in roots and soil.

Variable	Root fresh weight	Dry matter	Mycorrhizal colonization (%)
% Mycorrhizal colonization	0.60*	0.67*	—
Nematodes in roots	-0.17	-0.19	-0.02
Nematodes in soil	-0.09	-0.06	0.02

* Averages in each column are significantly different at P ≤ 0.05.

(Cu) than control plants without mycorrizhae (data not shown), which is in agreement with other studies (Allen, 1996; Smith and Read, 1997; Pinochet *et al.*, 1997; Clark and Zeto, 2000; Ramos *et al.,* 2009).

In this study, the mycorrhizal colonization was not affected by the presence or absence of plant parasitic nematodes, which is demonstrated with the low correlation index shown between both variables (Table 3), and by the colonization percentage obtained with comercial AMF (Table 2) which was 40% and 41% similar, respectively, in soil with and without nematodes. Elsen *et al.* (2002) found that the intensity of the mycorrhizal association was not affected in the presence of *R. similis* and *P. coffeae.* However, plant parasitic nematodes can degrade cells that contribute with the AMF nutrition (Hol and Cook, 2005). This effect is seen with a longer exposure time of the roots to these nematodes, which is required for a higher degradation of root cells.

Related to the number of plant parasitic nematodes in roots and soil, the results demonstrate that the inoculation method was suitable. In general, an average of 5473 *R. similis* vermiforms in the total root average (13 g/plant) were found, which is five times higher than the inoculated amount (1000 *R. similis*). Additionally, it was demonstrated the health of the material used to sow, given that the root system of the suckers sown in soil without nematodes stayed healthy without any nematode in roots or soil. This reveals the importance of using pathogen free material to sow, to reduce the amount of initial inoculum being a critical factor for a crop in the field.

The advantage of using a propagation system by induction of buds and thermotherapy, is the amount of material produced from a small number of healthy plantain corms in a short time, which is accessible to small farmers by a low price (US$0.35/plant) (González, 2011).

In the treatments that included nematodes inoculation, the final numbers of these vermiforms in both rhyzospheric soil and roots, was not different between the treatments ($P < 0.05$) (Table 4). Nonetheless, the total number of nematodes was higher in roots of plants inoculated with *G. fistulosum* (424 *R. similis*/g of roots) which explains why those had the lowest values in the growth variables, even lower than the controls without AMF. In rhyzospheric soil, the average number of plant parasitic nematodes was between 3 and 24 vermiforms/100 cm^3 of soil ($P < 0.05$). The fact that the number of nematodes on roots was higher than the one on soil is associated with the endoparasitic habit of *R. similis* and the ectoparasitic habit of *Helicotylenchus* spp. (Gowen *et al.*, 2005).

In both, banana and plantain, AMF can provide protection against plant parasitic nematodes, using diverse action modes like damage reduction or plant tolerance improvement against the disease (Jaizme-Vega and Pinochet, 1997; Jaizme-Vega and Rodriguez-Romero, 2004), nematode population reduction (Bagyaraj *et al.*, 1979; Jaizme-Vega *et al.*, 1997; Elsen *et al.*, 2002; Elsen *et al.*, 2003; Elsen et al., 2008; Oyekanmi et al., 2008; Van der Veken et al.,

Table 4. Effect of AMF application on number of plant parasitic nematodes in rhizospheric soil and suckers roots of Dominico Harton plantain, 90 days after inoculation with 1000 and 144 vermiforms/plant of *R. similis* and *Helicotylenchus* spp., respectively.

Treatments	Nematodes in 1 g of roots		Nematodes in 100 cm^3 of rhizospheric soil	
	R. similis	***Helicotylenchus* spp.**	***R. similis***	***Helicotylenchus* spp.**
G. fistulosum	465	3	8	10
G. fasciculatum	375	6	35	5
AMF mix	420	7	14	23
Control without AMF	424	8	5	4
Treatment effect[1]	ns	ns	ns	ns

[1] ns: statistically not significant.

2010; Becerra-Encinales *et al.*, 2010), and systemic resistance induction (Elsen et al., 2008; Zhang et al., 2008).

The present study did not show reduction on nematode populations in the roots, which is in agreement with the findings of Hol and Cook (2005) who found that the only group with high numbers in AMF colonized plants, is the one with the migratory nematodes *R. similis* and *P. coffeae*. In this case, the protection effect is associated to a direct alteration on plant growth, morphology and nutrition (Zhang et al., 2008; Whipps, 2004) which makes plants more tolerant to damage by nematodes (Hol and Cook, 2005).

Conclusions

- The inoculation of a single AMF specie is not the most effective in contrast to the inoculation of an AMF mix, which was seen in this study with the commercial inoculum.
- The results found in this study indicate that the increment on root and biomass was positively correlated with the percentage of mycorrhizal colonization obtained by pre-inoculation with spores of the species *G. aggregatum*, *G. manihotis, G. fistulosum, G. fasciculatum, K. colombiana* and *Scutellospora* spp., that were part of the commercial AMF inoculum, even with the presence of nematodes.
- This work demonstrate that mycorrhization does not affect development and reproduction arrest of plant parasitic nematodes. The bioprotection effect was more associated with a direct alteration on plant growth, morphology and nutrition, which makes plants more tolerant to nematode damage. However, response type and scale vary with the environment, host genotype, nematode species and populations, and fungi isolates (Hol and Cook, 2005).

In this way, the use of healthy suckers induced by budding and thermotherapy plus AMF addition, is presented as a practice to reduce nematode dispersion in the field and enhance vegetative growth in the nursery, in accordance with an integrated management plan for plantain crops.

Acknowledgments

This work was part of the Project on sanity improvement and competitiveness of plantain crops, by the development of new management strategies for soil and nutrition, financed by the National Founds for Vegetable and Fruits Development and the Ministry of Agriculture and Rural Development. We are grateful to the Phytopatologist Francia Varón and to Silverio González, FEDEPLATANO president, for their contributions to this study and, to the reviewers of this article for their valuable contributions.

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