Biofertilization of banana (Musa spp. L.) with free-living N\textsubscript{2} fixing bacteria and their effect on mycorrhization and the nematode *Radopholus similis*

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Banana plants (Musa spp. L.) cv ‘Great dwarf’ were amended with free-living N\textsubscript{2} fixing bacteria (FLNFB) and arbuscular mycorrhizal fungi (AMF) and the presence of the burrowing nematode *Radopholus similis* was monitored in the field. Five treatments were applied by inoculating banana roots with four strains of FLNFB, that is C1, C2, C3 and C4 isolated from the rhizoplane of the same banana cultivar, or by keeping them uninoculated. The largest number of nematodes was found in the untreated roots and the lowest in the roots inoculated with C4. The largest percent of mycorrhizal colonization was found when banana roots were inoculated with C1 and the lowest in roots that were not inoculated. The number of *R. similis* decreased with increased colonization with AMF. The largest percentage of necrosis of the banana roots was found when banana roots were not inoculated and the lowest in roots that were inoculated with C4. The necrosis of the roots increased with increased number of *R. similis*, but decreased with increased colonization with AMF. It was found that inoculating the banana cv ‘Great dwarf’ with FLNFB increased the root colonization with AML, and reduced the number of nematodes and root damage.

Key words: Arbuscular mycorrhizal fungi, burrowing or banana-root nematode, free-living N\textsubscript{2} fixing bacteria, plant development.

INTRODUCTION

Plant development is closely related to the interaction with microbiota in the phylosphere and rhizosphere (Green et al., 2006). For instance, 80 and 92% of surveyed plant species and families, respectively, were mycorrhizal with arbuscular mycorrhizal fungi (AMF) being dominant (Wang and Qiu, 2006). Wang and Qui suggested that the origin of arbuscular mycorrhiza coincides with the origin of land plants. In the rhizosphere, AMF and pathogens need to be in equilibrium, so that a normal plant development can occur (Reyes and Valery, 2007). The increase in pathogens populations leads to diseases (Whipps, 2001) thereby hampering plant growth (Siddiqui and Akhtar, 2008). The interaction in the rhizosphere between plant and microorganisms has been studied intensively (Mathesius, 2009) and the importance of certain root exudates in the relationship between plants and microorganisms, beneficial and pathogenic, has been demonstrated (Rojas-Andrade et al., 2003). *Radopholus similis*, the burrowing or banana-root nematode, is one of the most important root parasites of banana plants (Musa spp. L.). Although, effective chemical nematicides exist,
but their high cost and toxicity limit their use. Nematode management for resource-poor farmers relies on the use of hot water-treated suckers (Speijer et al., 1995), tissue culture-derived planting material or mulching (Talwana et al., 2003). None of these methods, however, offers complete control of nematodes (Athman et al., 2006). The roots of the banana, as those of most plants, are colonized by AMF and the intensity of colonization depends on fertilizer use and AMF species (Declerck et al., 2002). The hyphae of AMF reduce the entry points of the nematodes and induce physiological changes that increase plant growth (Andrade et al., 2009). Studies have shown that AMF might reduce the damage done to plants by nematodes (Jaizme-Vega et al., 1997), but the synergistic effect of soil fungi and nematodes might break down the protection of AMF against pathogens (Greipsson and El-Mayas, 2002). It is well known that conventional agricultural practices might have a negative effect on soil fertility due to the use of fertilizers, tillage and pesticides (Gregory et al., 2005). Excessive fertilizer use increases soil salinity, tillage disrupts soil structure accelerating soil organic matter decomposition, while pesticide might negatively affect beneficial organisms (Jorgenson and Kuykendall, 2008). New agricultural practices have been developed to reverse those negative effects (Butler et al., 2007). The introduction of beneficial microorganisms often through inoculation upon planning of crops or biofertilization can improve yields without negatively affecting soil fertility (Avis et al., 2008). Application of organisms that are antagonistic against pests or pathogens is another way to improve crop development (Alabouvette and Steinberg, 2006). Therefore, an alternative way to control *R. similis*, instead of using a nematicide, is to inoculate roots with beneficial symbiotic organisms so that through antagonisms or simple competition the pathogen is prevented to infect the plant (Van der Veken et al., 2008). The objective of this work was to investigate the effect of biofertilizing roots of the banana plant with four free-living N$_2$ fixing bacteria isolated from roots of the similar banana cv 'Great dwarf' and to determine how this affected the colonization of the roots with AMF and the presence of *R. similis*.

MATERIALS AND METHODS

Free-living N$_2$ fixing bacteria

Four free-living N$_2$ fixing bacterial strains C1, C2, C3 and C4 (*Azospirillum* spp.) were isolated from the rhizosphere of banana plants cultures and selected for their high production of indole-3-acetic acid (IAA).

Bacteria produce IAA under stress conditions promoting plant growth and shoot water content and thus increase plant drought tolerance (Marulanda et al., 2009). The isolated bacterial strains were kept in nFB culture medium and cultivated in nutrient broth (pH 7.0) at 200 rpm and at 30°C before inoculation.

Plantlets culture and inoculation

Nine hundred banana plantlets were cultivated in the greenhouse in pots containing 5 kg soil from a banana field. The soil contained 320 g kg$^{-1}$ sand, 410 g kg$^{-1}$ loam and 270 g kg$^{-1}$ clay. The plantlets were fertilized with urea (10 g pot$^{-1}$) 60 days after sowing. The plantlets were divided in five groups. Hundred and eighty plants were inoculated with 5 ml of each of the four bacteria (1 $\times$ 10$^8$ cells ml$^{-1}$) eight days after application of urea. The remaining 180 plantlets were not inoculated and served as control.

Field experiment

The plantlets were transferred to the ‘Santa Lucrecia’ farm (14° 40’ 21.56” N; 92° 10’ 27.19” W), a banana plantation in Suchiate, (Chiapas, México), 18 days after bacterial inoculation. Five 1000 m$^2$ plots (180 plants plot$^{-1}$) were outlined (one for each of the four bacterial strains and one for control). Plots were arranged in a complete randomized block design. Each plant was fertilized monthly with 20 g N, 72 g K, 11 g Ca, 4 g Mg and 2 g S. Eight days after the fertilizer was applied, the roots of each plant were inoculated with 30 ml of the previously mentioned bacterial inoculums, i.e. four different strains at 1 $\times$ 10$^8$ cells ml$^{-1}$.

Sampling and assays

After 100, 128, 156, 184 and 240 days, 10 banana plants were selected at random and the rhizosphere collected (30 cm$^3$ soil + roots). The diazotrophic bacteria in the rhizoplane were determined by cultivating them on nFB medium. Nematodes in the roots were extracted with a Baermann funnel as described by Hooper et al. (2005) and counted under a microscope. Nematodes were identified as described by Hunt et al. (2005). The mycorrhizal colonization was determined as described by Giovannetti and Mosse (1980) on previous stained roots as described by Phillips and Hayman (1970). The damage done by the nematodes to the roots was visually determined in three categories: 1) undamaged roots, 2) roots with < 50% damage and 3) necrotic roots with > 50% damage.

Different phenological stages of the banana plants were defined as follows (Soto, 1991). The infante stage was defined as the time between the bud outset and the first formal leaf formation and took between 50 and 60 days, while the reproductive phase covered the time between floral differentiation stage until the fruits were collected.

Statistical analysis

The number of root nematodes, AMF colonization and root necrosis was subjected to one-way analysis of variance (Tukey, p < 0.05) to test for significant differences between the treatments, i.e. different strains, with InfoStat Professional version 2008.

RESULTS

The number of free-living N$_2$ fixing bacteria on the roots...
of inoculated banana plants was $>10^8$ g\(^{-1}\) roots, while in the non-inoculated plants it was between $10^5$ and $10^6$ g\(^{-1}\) roots. The number of nematodes found on the roots remained low until day 128 and started to increase thereafter (Figure 1a). At day 240, the number of nematodes found in the roots was significantly different between the treatments (P<0.05). The largest amount was found in the untreated roots (3618 nematodes 100 g\(^{-1}\)) and the lowest in the roots inoculated with C4 (969 nematodes 100 g\(^{-1}\)). The colonization of the banana roots with AMF increased with a maximum found between 128 and 156 days, and then decreased (Figure 1b). The colonization of the banana roots with AMF was significantly different between the treatments (P<0.05). The largest percent colonization was found when banana roots were inoculated with C1 (32%) and the lowest in roots that were not inoculated (16%). The number of *R. similis* decreased significantly and exponentially with increased colonization of the roots with AMF (P<0.05) (Figure 2a).

The necrosis of the banana roots decreased until day 184 in all treatments, except in the roots that were not inoculated which showed an increase between day 100 and 128 (Figure 1c). After day 184, the number of roots that showed necrosis increased in all treatments. The necrosis of the banana roots was significantly different between the treatments (P<0.05). The largest percentage of necrosis of the banana roots was found when they were not inoculated (26%) and the lowest in roots that were inoculated with C4 (11%). The necrosis of the roots increased significantly with an increased number of *R. similis*, but decreased significantly with increased colonization of the roots with AMF (P<0.05) (Figure 2b and c).

**DISCUSSION**

**Root colonization with arbuscular mycorrhizal fungi (AMF)**

The colonization of banana roots changed with time. In
cereals, AMF root colonization is related to the phenological stage of the plant (Gavito and Varela, 1993; Majic et al., 2008). Colonization increased during the vegetative phase and decreased during the reproductive phase. Photosynthetic products are transported to the roots where they are used by the microorganisms in the rhizosphere. The microorganisms proliferate and their metabolites and/or released nutrients are taken up by the plants. When the plant matures and the reproductive phase starts, photosynthetic products are used for flowering and seed development. The plant metabolites released in the rhizosphere decreases, microbial growth slows and the AMF loosen their association with cortical cells.

The level of colonization with AMF found in this experiment for banana plants not inoculated were less than those reported. Adriano (2001) found a 58 to 88% colonization with *Glomus mosseae* for the banana cv 'Great dwarf' during the reproductive phase. Jaizme-Vega and Pinochet (1997) found a 47% colonization of roots of the same banana cultivar with *G. mosseae*, 34% with *G. aggregatum* and 33% with *G. intraradices*.

![Figure 2. The correlation a) between the number of nematodes *Radopholus similis* (per 100 g roots) and the percentage of arbuscular mycorrhizal fungi colonization (%), b) the number of nematodes *Radopholus similis* (per 100 g roots) and necrosis (%) of the roots c) necrosis (%) of the roots and the percentage of arbuscular mycorrhizal fungi colonization (%) of the roots of the banana plant (*Musa* spp. L.) cv 'Great dwarf' inoculated with four different free-living N$_2$ fixing bacteria.](image-url)
Population of *Radopholus similis*

Jaizme-Vega et al. (1997) reported that the banana cv ‘Great dwarf’ colonized by the AMF *G. mosseae* were less susceptible to infections with the root-knot nematode *Meloidogyne incognita* favouring plant growth. Contrarily, Jaizme-Vega and Rodriguez-Romero (2004) reported that the same banana plants inoculated with *G. mosseae*, *G. aggregatum* and *G. intraradices* had the same number of *Pratylenchus goodeyi* and *M. incognita* than the untreated plants. However, Elsen et al. (2008) found that AMF have the ability to induce systemic resistance against plant parasitic nematodes *R. similis* and *Pratylenchus coffeae* in the root system. AMF reduced both nematode species by more than 50%, even when the AMF and the plant parasitic nematodes were spatially separated. Similar results were obtained with maize (*Zea mays* L.), olive (*Olea europaea* L.) (Castillo et al., 2006) and kiwi (*Actinidia deliciosa*) (Verdejo et al., 1990). The mycorrhiza had neither effect on the number of pathogenic nematodes nor was the number of AML affected by the pathogens.

**Root damage**

Jaizme-Vega et al. (1997) reported a 36 to 64% reduction in root galling caused by the root-knot nematode *M. incognita* when micropropagated banana plants cv ‘Great dwarf’ were inoculated with *G. mosseae* reaching 58 to 88% of AM root colonization. Jaizme-Vega and Rodriguez-Romero (2004) found that banana plants cv ‘Great dwarf’ inoculated with *G. mosseae*, *G. aggregatum* and *G. intraradices*, and infected with the nematode *P. goodeyi*, had 4, 15 and 13% lesions, respectively, while the plants that were not inoculated had 28%. The colonization with *G. mosseae* was 47%, with *G. aggregatum* 34% and with *G. intraradices* 33%. No differences, however, in nematode populations were found. Elsen et al. (2008) found that the number of lesions in the roots of banana cv ‘Williams’ amended with ±1850 mycorrhizal spores and infected with 5000 eggs and juvenile *M. javanica* one month later, were 29% lower with *G. mosseae*, 25% with *G. macrosporum* and 16% with *G. caledonium* compared to plants not inoculated with mycorrhizae. They suggested an inhibitory effect of the micorrhiza on the infection with nematodes through changes in the nutrition of the plant, biochemical changes in the plant tissue, e.g. an increase in amino acids, peroxidases, quinases, phytoalexins, increases in lignin, stress reduction, changes in the microbial populations in the roots and a more dense root system. Vaast et al. (1998) found a decrease in lesions by *P. coffeae* in the roots of coffee (*Coffea arabica* L.) when inoculated with the mycorrhizae *Acaulospora mellea* or *G. clarum*. Castillo et al. (2006) found similar results when studying the effect of mycorrhiza on the pathogenicity of *M. incognita* and *P. vulnus* on roots of olive tree. Castillo et al. (2006) suggested that the reduction in pathogenicity was not related to a reduction in infection rate, but was related to a higher tolerance towards the nematode.

Our findings conclude that inoculating roots of the banana plant cv ‘Great dwarf’ with free-living N2 fixing bacteria isolated from roots of the similar plant resulted in a higher colonization of the roots with AMF, a lower number of pathogenic nematodes and healthier roots.

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**REFERENCES**


