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Effects of Mycorrhization on the Micropropagated Banana during the Latest Stages of Development under Commercial Fertilization

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THE effects of arbuscular mycorrhizal (AM) fungi on the micropropagated banana during the latest stages of development and with fertilizer regimes similar to those practiced in commercial crops were studied. Plants were tested at three different stages of formation: acclimatization, nursery and microplot. Two commercial cultivars of banana (*Musa* sp. cv. Williams and Grand naine) were inoculated during hardening off. Each cultivar was inoculated with one of two AM fungi (*Glomus mosseae* and *G. manihotis*) for evaluation of inoculation with mycorrhizal fungi on plant development, the mycorrhizal dependency under the fixed conditions of fertilizer inputs and mycorrhizal colonization. At nursery phase, the same parameters were evaluated. At microplot phase, fresh weight of roots and aerial parts, numbers of suckers, numbers of leaves, leaf area, N, P and K content, and dependency for mycorrhizal were determinated. By completion of the rooting stage, both cultivars showed a positive response to the both AM fungi used for inoculation and the relative mycorrhizal dependency (RMD) of both cultivars was the highest throughout the trial. Following transplanting, inoculated plants of both cultivars, the majority of experimental variables were significantly different for both cultivars in comparison with the controls and root colonization of banana plants by mycorrhizae tended to differ depending on the cultivar. After nine months in microplot conditions and a standard fertilizer regime, banana plants inoculated with *Glomus manihotis* usually, particularly with cv. 'Grand naine', showed a beneficial effect of symbiosis on plant development. However, data on macronutrients (N, P and K) although noticeably higher, did not differ statistically. At the end of this phase, root colonization by both *Glomus* species was relatively important in both cultivars (greater than 79%).

Keywords: Arbuscular mycorrhizal (AM) fungi, Cultivars of banana, Mycorrhizal dependency (RMD), Plant development, Rooting stage, Nursery phase and microplot phase.

The mycorrhizal symbiosis formed between plant roots and endomycorrhizal fungi is of great importance for many crop species. The well-known arbuscular mycorrhizal fungi is part of the microbial community and have been shown to have both plant growth and health promoting activity. The likelihood of using arbuscular mycorrhiza (AM) in crop production systems is increasingly more realistic and studies have increased considerably in the last few years. In the

context of technologies needed for sustainable agriculture, new developments in the potential use of arbuscular mycorrhizal fungi (AMF) have been presented in the last years which should ensure adequate levels of food production along with a reasonably reduced consumption of chemical fertilizers and pesticides (Lovato *et al.*, 1999). Mycorrhizal hyphae can provide the plant with water and some nutrients (P), protect them against diseases and improve the ability of plants to overcome adverse environmental conditions as well as reduce metal phytotoxicity (Varma & Hock, 1999). Therefore, under tropical and semi tropical conditions AM fungi could be highly beneficial to some perennial crops which require nursery production before transplantation to the field (Feldmann & Idzak, 1992). Among these crops, banana is considered promising due to its economic importance for farmer's production (Delvaux *et al.*, 1990). Mycorrhization in vivo has resulted in large increases in the growth and nutrition of this species (Lin & Chang, 1987; Declerk *et al.*, 1995 and Jaizme-Vega & Azcón, 1995) including in the presence of standard fertilization regimes in commercial nurseries (Declerck *et al.*, 2002), with favorable effects on plant behavior when confronted with various soil-borne pathogens such as *Meloidogyne incognita* (Jaizme-Vega *et al.*, 1997) and Pratylenchus goodeyi (Jaizme-Vega & Pinochet, 1997). These results demonstrate the advantages of applying inoculum of fungal AM during root production and acclimatization of micropropagated banana plants, which gives rise to plants that are well developed and have an increased tolerance to attack by soilborne pathogens. Moreover, recent studies on the response of banana to AM symbiosis were very promising (Jaizme-Vega & Azcon, 1995). It is well-known that inoculation with AM fungi gives better results when performed at the early stages of plant development. Thus, under tropical stress conditions in soils which are strongly P-deficient and where crop production is largely dependent on mycorrhizal status of plants, the management of AM fungi is fundamental (Sieverding, 1991). In such environments where the indigenous inoculum potential is low and/or AM fungi poorly or not efficient, adding AM fungi to soil may be considered as highly beneficial to long-term plant production. Development of AM symbiosis, however, is dependent on host plant and edaphic conditions (Plenchette *et al.*, 1983). Significant growth response of micropropagated banana were obtained when plants were inoculated at the beginning of weaning phase (Declerck *et al.*, 1994), depending on varieties and AM fungi used (Declerck *et al.*, 1995).

However, at present there is no information concerning the effects of such symbiotic fungi on the banana plant during the latest stages of development and with fertilizer regimes similar to those practiced in commercial crops.

Therefore, the sequential effects of early mycorrhization on the growth of micropropagated banana plants were studied from the earliest stages of development until nine months after transplanting to the field in microplots.

Material and Methods

Host plant

Micropropagated material of the two most widespread commercial cultivars of banana *Musa* spp. Colla AAA, cvs. "William" and "Grand naine" was used.

Acclimatization stage

Inoculation with AM fungi

Mycorrhization was done during hardening off. Inoculum comprised a homogeneous mixture of rhizosphere soil, spores and rootlets of the host plant. Each cultivar was inoculated with one of two AM fungi, each with 1500 g inoculum per tray (capacity of tray 24 kg) with the following isolates: *Glomus mosseae* from stock collection, multiplied on sorghum, and giving 88% colonization; *Glomus manihotis* Howeler, Sieverding and Schenck, from stock collection, multiplied on tomato, and giving 90% colonization. At inoculation, plants were 10 cm ± 2 cm, had approximately three developed leaves. Inoculation was done in polyethylene (PE) trays (40 x 60 cm, H x L), each tray containing one cultivar/fungus combination with an additional two control trays with non-inoculated plants, one tray per cultivar. Thus there were a total of six trays each with 35 plants. The substrate comprised a steam-sterilized mixture of sandy soil and amended peat in a proportion of 2:1. This phase lasted six weeks in a glasshouse and under a tunnel of black mesh for acclimatization. Irrigation with distilled water took place according to the needs of the plants.

Nursery phase

At the end of acclimatization stage and before transplanting to individual containers, 10 plants of each treatment/cultivar combination were selected and the effects evaluated of inoculation with mycorrhiza on plant development, the mycorrhizal dependency under the fixed conditions of fertilizer inputs, and the extent of colonization by the AM fungi. Parameters relevant to the growth of the plant in general were evaluated at each stage of the investigation as follows: fresh weight (g) of roots and aerial parts, dry weight (g) of aerial parts, length and diameter (cm) of pseudostem, leaf numbers and area (cm^2). Leaf area was calculated with an area meter Li-COR, inc. Lincoln, Nebraska, USA, model Li-3100. The relative mycorrhizal dependency (RMD), defined by Gerdeman (1975) as the degree of mycorrhization needed by plants to produce the maximum growth or yield depending on fertility of the soil, was calculated according to the formula proposed by Plenchette *et al.* (1983) as the numerical expression of this concept:

$$RMD = \frac{\text{DW of plant with AM} - \text{DW of plant without AM}}{\text{DW of plants without AM (DW : dry weight)}} \times 100$$

Colonization by the mycorrhizae was confirmed by observation with a light microscope. Root samples were bleached with 10% KOH and then stained with 0.05% trypan blue in lactic acid as described by Phillips & Hayman (1970) and modified by Koske & Gemma (1989). Percentage root colonization was determined

on 20 1-cm sections of stained root, mounted on slides and examined with a light microscope as described by Brundett *et al.* (1985). Once the determinations were complete 20 plants of each treatment were transferred to 2 L PE bags containing a substrate comprising equal volumes (1:1) of steam sterilized sandy soil and enriched peat. This phase took 14 weeks in glasshouse conditions at temperatures of 27-32°, and a relative humidity of 70-80%. Fertilization was according to the fertilizer regime of a commercial banana nursery. Plants were fertilized twice weekly (100 cc/plant) on alternate days. One of the fertilizer applications was with $(NO_3)_2Ca$ (3 g/L) and NO_3H (0.4 cc/L), and the other application was with SO_4K_2 (3 g/L) and PO_4H_3 (0.2 cc/L). The days on which fertilizer was not applied alternated with irrigation with running water according to the needs of the crop. Plants received a weekly foliar application of micronutrients consisting of 10g L^{-1} mixture from $MnSO_4$ and Fe_3SO_4.

Microplot phase

After growth for 3.5 months, plants were transferred to larger containers. Prior to this, and as with the first transplanting, 10 plants per cultivar and treatment were evaluated for the effects of the AM fungi, that is the extent of root colonization by mycorrhiza and mycorrhizal dependency. For this last phase of the trial, PE pots 35 cm diameter and 50 L volume were selected and filled with non-sterilized medium of the same materials and in the same proportions as described for the previous transplanting (1:1), and amended with 1.5 g/L of slow release fertilizer (Agro-Top 13:2:44). Once in position in their new pots (10 per cultivar and treatment), the plants were placed amongst other similarly sized pots previously buried up to the upper edge of pot, in the trial plot. Plants were fertilized weekly (1 L/plant), via the localized irrigation system, with the two combinations of fertilizer treatment described previously for banana plants after the first transplanting. Foliar fertilizers were applied fortnightly. The days, on which fertilizers were not applied, plants were irrigated according to the needs of the plants. Plants remained in position for nine months. The trial was then terminated and the effects of symbiosis on development of the banana plants evaluated. The following experimental variables were studied: fresh weight of roots and aerial parts, numbers of suckers, numbers of leaves, leaf area, N, P and K content, and dependency for mycorrhiza. On completion of the foliar analyses, the samples were transferred to a heater for 24 hr at 70° after which nitrogen, phosphorous and potassium contents were determined. For N determination, a semi-microkjeldahl method was used to determine total N, P was determined colourimetrically and K by spectrophotometry of atomic absorption as described by Jackson (1973). Data were analyzed using Statistical Analysis Software (SAS Institute Inc., Cary, NC). One-way ANOVA was used to detect significant differences among mean effects of amendments observed. Means were compared by Fisher's test of least significant differences (LSD) using the statistical package SPSS version 10.0 (SPSS Inc., Chicago, USA).

Results and Discussion

At acclimatization stage

By completion of the acclimatization stage, both cultivars showed a positive response to the two AM fungi used for inoculation (Tables 1a and 2a). Declerck *et al.*

TABLE 1. Effect of *Glomus mosseae* and *G. manihotis* on the development, colonization and mycorrhizal dependency of micropropagated banana cv. Williams at:a. 6 weeks after inoculation, b. 14 weeks after inoculation and c. 9 months after transplanting to microplots.

	Fresh weight (g)		Dry weight (g)	Pseudostem		No. leaves	Leaf area (cm^2)	Colonization	RMD**
	Root	Aerial parts	Aerial parts	Diameter	Length			(%)	
a) 6 weeks after inoculation (acclimatization phase)									
Control	2.6b*	8.6 b	0.5 b	0.9 b	10.4 b	5.2 b	143 b	—	—
G. mosseae	6.4 a	17.5 a	1.1 a	1.2 a	12.9 a	6.3 a	261 a	26	51
G. manihotis	5.5 a	17.8 a	1.0 a	1.2 a	12.1 a	6.0 a	269 a	37	46
b) 14 weeks after inoculation (nursery phase)									
Control	13.1 b*	38.1 b	2.6 b	1.8 b	15.4 b	7.3 b	494 b	15	—
G. mosseae	29.4 a	66.5 a	4.4 a	2.6 a	23.5 a	8.5 a	777 a	59	40
G. manihotis	26.7 a	63.7 a	4.3 a	2.4 a	22.1 a	8.7 a	805 a	38	38

	Fresh weight (g)		No.		Leaf area	Colonization	RMD	Macronutrient (%)		
	Root	Aerial parts	leaves	No. suckers	(cm^2)	(%)	(%)	N	P	K
c) 9 months after transplanting to microplots										
Contro	15.3 b*	9.2 a	14.0 a	3.7 ab	50192 a	59	—	2.89 a	0.185 a	2.52 a
G. mosseae	6.8 ab	6.9 a	14.3 a	2.2 b	44256 a	71	5	2.99 a	0.180 a	2.80 a
G. manihotis	9.8 a	10.0 a	13.7 a	4.5 a	55774 a	74	8	2.71 a	0.183 a	2.41 a

* Means of 10 replicates. Within each column, differences between numbers followed by the same letter are not statistically different with Fisher's test ($P \leq 0.05$).
** RMD: relative mycorrhizal dependency.

(2002) reported that clear evidence on the dependence of banana plants on mycorrhizal symbiosis and growth of micropropagated bananas was significantly increased with the monoxenic AM fungi inoculum, with the indigenous AM fungi inoculum and with both inoculum sources in combination. In this phase, the relative mycorrhizal dependency (RMD) of both cultivars to *Glomus mosseae* and *Glomus manihotis* were the highest throughout the trial and were 35% and 50% respectively. Declerck *et al.* (1995) observed that some banana varieties depended on mycorrhizae, *Glomus macrocarpum* being more efficient in promoting plant growth than *Glomus mosseae.* Benefits have also been reported by Jaizme-Veja *et al.* (1991) and occurred mostly during plant acclimatization (Declerck *et al.,* 1994 and Yano-Melo *et al.,* 1999). In this first phase the percentage colonization by the two inoculated AM fungi was similar for the two cultivars.

Nursery phase

Following transplanting, the positive effect of the AM fungi on plant development was maintained for 3.5 months after mycorrhization. For inoculated plants of both cultivars, the majority of experimental variables were significantly different for both cultivars in comparison with the controls Tables 1b and 2b. The development of RMD was similar for both cultivars completing this phase of the trial with averages of 40% for both AM fungi on Williams, and 30% and 20% respectively for *Glomus mosseae* and *Glomus manihotis* on Grand naine (Tables 1b and 2b).

Root colonization of banana plants by mycorrhizal inoculation tended to differ depending on the cultivar and *Glomus* strains. Thus, roots of "Williams" inoculated with *G. mosseae* had twice the mycorrhiza infection in comparison with the beginning of the study, similar results being maintained on roots colonized by *Glomus manihotis*. Such difference were reported in literature (Declerck *et al.*, 1994 & 1995) *Glomus mosseae* was shown the more infective on Williams and other cultivars, as compared to *G. macrocarpum* (Declerck *et al.*, 1995). However with plants of cv. Grand naine, no changes in root colonization were observed in comparison with the first transplanting. During the trial, from 14 weeks onwards 15% root colonization by contaminant AM fungi were noted in control plants of both cultivars (Tables 1b and 2b) but without significant effects on plant development. These endophytes are able to disperse in irrigation water or by uncontrolled contamination in the nursery containing the plants.

These data confirm those already published on the benefits of early mycorrhization of plants in the first phases of development of this crop (Declerck *et al.*, 1995 and Jaizme-Vega *et al.*, 1997).

Microplot phase

The results of the second phase of the trial in which the effects of the AM fungi on mycorrhiza-treated plants in the *in vivo* phase for three months and transplanted to non-sterile medium, showed that after nine months in microplot conditions and a standard fertilizer regime, banana plants inoculated with *Glomus manihotis* usually, particularly with cv. Grand naine, showed a beneficial effect

TABLE 2. Effect of *Glomus mosseae* and *G. manihotis* on the development, colonization and mycorrhizal dependency of micropropagated banana cv. Grande naine at: a. 6 weeks after inoculation, b. 14 weeks after inoculation and c. 9 months after transplanting to microplots.

	Fresh weight (g)		Dry weight (g)	Pseudostem		No.	Leaf area	Colonization	
	Root	Aerial parts	Aerial parts	Diameter	Length	leaves	(cm^2)	(%)	RMD**
a) 6 weeks after inoculation (acclimatization phase)									
Control	3.1b*	8.2b	0.50b	0.97b	8.7b	5.5a	155b	-	-
G. mosseae	5.5a	12.9a	0.80a	1.15a	8.7b	6.3a	223a	27	38
G. manihotis	6.0a	11.9a	0.75a	1.18a	9.8a	6.5a	216a	24	34
b) 14 weeks after inoculation (nursery phase)									
Control	22.8b*	40.5b	2.8b	2.0b	14.7b	7.7a	514b	14	-
G. mosseae	33.9a	57.5a	3.9a	2.5a	16.3a	8.5a	722a	26	29
G. manihotis	36.4a	50.7a	3.5ab	2.4a	15.9ab	8.0a	662a	30	29

	Fresh weight (g)			No.	Leaf area	Colonization	RMD	Macronutrient (%)		
	Root	Aerial parts	No. leaves	suckers	(cm^2)	(%)	(%)	N	P	K
c) 9 months after transplanting to microplots										
Contro	7.1a*	7.8a	14.9b	2.4a	41845b	59	-	2.84a	0.176a	2.65a
G. mosseae	7.7a	11.5ab	19.1ab	3.6a	57733ab	72	31	3.03a	0.189a	3.00a
G. manihotis	9.5a	13.6a	23.2a	4.0a	61660a	83	42	3.00a	0.184a	3.03a

*Means of 10 replicates. Within each column, differences between numbers followed by the same letter are not statistically different with Fisher's test ($P \leq 0.05$).
** RMD: relative mycorrhizal dependency

of symbiosis on plant development, with RMDs of approximately 40%. These values are considered to be relatively high for the conditions of the trial (Tables 1c and 2c) moreover there was an increase in the other experimental variables. Declerck *et al.* (1994) has shown that micropropagated bananas inoculated with mycorrhizal fungi show significant increases in nutrient content and plant growth compared to uninoculated controls. However, data on macronutrients (N, P and K) although noticeably higher, did not differ statistically (Tables 1c and 2c). This lack of response in nutrient content of aerial parts can be interpreted as typical for a mycorrhiza-treated plant receiving soluble fertilizer. Plants of cultivar "Williams" showed a smaller response to the AM fungi after the microplot phase, plants inoculated with *Glomus mosseae* showing a development and nutritional state equal or slightly less than control plants. This part of the trial used nonsterilized substrate which, together with other conditions in the trial, explained the data.

The absence of differences between inoculated and non-inoculated plants grown in the nonsterilized soil was probably due to the competition between introduced and native AM fungi species either directly or indirectly. The combination of different AM fungi generally results in synergistic effects on growth (Edathil *et al.*, 1996) but in some instances on competition effects as demonstrated by Pearson *et al.* (1994). In our experiment, it is probable that the inoculated fungi had to compete with the indigenous AM fungi population both for colonization of now roots and nutrients. The inoculant had probably a great competitive advantage over the indigenous fungi as it is already present in the root system at transplanting in the trial, but this advantage could be lost as root system developed and competition evolved.

At the end of this phase, root colonization by both *Glomus* species was relatively important in both cultivars (greater than 79%). Large root colonization is generally followed by substantial stimulation of growth. Attention is drawn to the high level of colonization of roots of control plants.

Conclusion

Many works have shown that mycorrhizal inoculation is of most benefit to banana plants when introduced to previously disinfected soil. In general and particularly in the last phase of the trial, it can be confirmed that, at the latest stages of the crop, that biotechnological resource showed promise for the improvement of production.

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تأثير الميكوريزا على كرمات الموز خلال المراحل المتأخرة من النمو تحت ظروف التسميد التجارية

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تم دراسة تأثير فطريات الميكوريزا على كرمات الموز خلال المراحل المتأخرة من النمو مع التسميد بنفس معدلات التسميد التى تتم داخل صوب التربية.

تمت الدراسة على ٣ مراحل من النمو (التأقلم والشتل والنقل للأرض المستديمة) مع استخدام صنفين من كرمات الموز صنف وليامز وصنف جراند ناين حيث تم تلقيحهما خلال مرحلة التأقلم (التقسية) بواحد من نوعين من فطريات الميكوريزا (*Glomus mosseae* and *G. manihotis*) لتقييم تأثير التلقيح على معدل نمو الكرمات ومعدل اعتماد الكرمات على فطريات الميكوريزا فى النمو تحت ظروف التسميد العادية بالإضافة إلى تقدير نسبة استعمار فطريات الميكوريزا لجذور النبات. وعند مرحلة الشتل تم تقدير نفس القياسات. أما فى مرحلة النقل للأرض المستديمة فقد تم تقدير الوزن الرطب للجذور والأجزاء الهوائية (الخضرية) وأنابيب الامتصاص وأعداد الأوراق ومساحة الورقة والمحتوى من النيتروجين والفوسفور والبوتاسيوم بالإضافة إلى معدل الاعتماد على فطريات الميكوريزا وقد أظهرت النتائج أن:

١. بعد تمام تكوين الجذور اظهر كلا من صنفين الموز استجابة موجبة للتلقيح بكل من نوعي فطريات الميكوريزا، وكان معدل الاعتماد على الميكوريزا فى النمو (RMD) عاليا لكل من صنفي الموز خلال تلك المرحلة.
٢. مع استمرار النقل (مرحلة الشتل) أظهرت النباتات الملقحة بفطريات الميكوريزا استجابة عالية لكل القياسات السابقة مقارنة بالنباتات غير الملقحة وكانت اغلبها معنوية. بينما أظهرت نسبة استعمار جذور الموز بفطريات الميكوريزا اختلافا معنويا اعتمادا على صنف الموز المستخدم.
٣. بعد ٩ أشهر (مرحلة النقل للأرض المستديمة) وتحت ظروف التسميد القياسية كانت الاستجابة عالية لصنف جراند ناين (مقارنا بصنف وليامز) للتلقيح بكل من نوعين فطريات الميكوريزا المستخدمة. فى حين زادت نسبة النيتروجين والفوسفور والبوتاسيوم زيادة ملحوظة إلا أنها كانت غير معنوية. على الرغم من أن نسبة استعمار الجذور بفطريات الميكوريزا كان عاليا (٧٩٪).