Growth Response of Micropropagated Banana Plants to VAM Inoculation

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ABSTRACT

Micropropagated banana plants (*Musa acuminata* colla cv. AAA Giant Cavendish) were inoculated or not with two VAM fungus species, *Glomus mosseae* and *Glomus geosporum*, in two complementary experiments. Inoculation with either species resulted in significantly higher shoot fresh and dry weights as compared to control plants. Shoot P and K contents were also significantly higher in inoculated plants. Nevertheless, there were differences in infection development between the two VAM fungus species. A greater proportion of the roots were infected with *G. mosseae*. This difference in infection development could be related to the growing conditions, particularly concerning the soil pH, which resulted in higher growth and P and K contents in plants inoculated with *G. mosseae*.

La croissance de vitroplants de bananiers mis en présence de champignons MVA.

RÉSUMÉ

Deux expériences complémentaires ont été menées avec des vitroplants de bananier (*Musa acuminata* colla cv. Giant Cavendish) inoculés ou non avec deux souches de champignons mycorhiziens à vésicules et arbuscules (MVA), *Glomus mosseae* et *Glomus geosporum*. On a enregistré une stimulation de croissance ainsi que des teneurs en P et K plus élevées chez les plantes mycorhizées, qu'elles soient inoculées avec *G. mosseae* ou avec *G. geosporum*. Des différences de développement de l'infection ont cependant été observées entre les deux champignons MVA, une infection plus importante étant obtenue avec *G. mosseae*. Cette différence peut être attribuée aux conditions de croissance et plus particulièrement au pH. Cela s'est traduit par des taux de croissance ainsi que des teneurs en P et K plus importants chez les plantes inoculées avec *G. mosseae*.

Respuesta de plantas micropropagadas de banano a una inoculación con MVA.

RESUMEN

Dos experiencias fueron realizadas con vitroplantas de banano (*Musa acuminata* colla cv. Giant Cavendish) inoculadas y sin inoculación de cepas del hongo micorriza, vesiculas y arbúsculos (MVA) *Glomus mosseae* y *Glomus geosporum*. Se encontró una estimulación del crecimiento asi como contenidos de P y K mas elevados en las plantas que fueron inoculadas con *G. mosseae* o *G. geosporum*. Diferencias en el desarrollo de la infección, fueron sin embargo, observadas entre las dos cepas de MVA. Una infección más importante fue obtenida con *G. mosseae*. Esta diferencia puede ser atribuida a condiciones de crecimiento y particularmente al pH. Esto se tradujo en tasas de crecimiento y en contenidos de P y K más importantes en las plantas inoculadas con *G. mosseae*.

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KEYWORDS
Musa, vitroplants, *Glomus*, growth, infection.

MOTS CLÉS
Musa, vitroplant, *Glomus*, croissance, infection.

PALABRAS CLAVES
Musa, vitroplantas, *Glomus*, crecimiento, infección.

introduction

Bananas are of prime importance for the economy of Martinique. With overall annual production exceeding 200 000 t, this crop represents a major fruit export and essential source of income for this small Caribbean island. In the last decade, there has been a decline in banana crop yields in intensive banana cropping systems (DELVAUX *et al.*, 1990). Root diseases are a major constraint, particularly on recent sandy soils derived from volcanic ash and pumice (DELVAUX and GUYOT, 1989). Control of soil-borne pathogens through pesticide treatments is expensive and quite unsatisfactory since suckers and corms are generally used as planting material. Such material can provide a vector for root parasites in pathogen-free soils. In this context, banana productivity could be improved by introducing *in vitro* vegetative propagation, which produces disease-free and highly productive material.

Over the past few decades, there has been an upsurge of interest in vesicular-arbuscular mycorrhizal fungi (VAMF), since they were shown to stimulate plant growth and provide protection against some soil-borne pathogens (STRULLU, 1991). These properties could have a substantial impact since VAM fungi are known to occur in banana cropping systems (ROHINI *et al.*, 1988). Despite the fact that few studies have been conducted to date, inoculation experiments have resulted in increased banana plant growth and P uptake (KNIGHT, 1988; LIN and FOX, 1992), and increased banana plant tolerance to the nematode *Radopholus similis* (UMESCH *et al.*, 1989).

Two complementary experiments were conducted under greenhouse conditions. In the first experiment, the growth dynamics of *Glomus mosseae* in banana roots were monitored. We also assessed plant growth and water content as influenced by VAM colonization. The second experiment was aimed at evaluating and comparing the effectiveness of two VAM fungi, collected from different soils and edaphic conditions, on growth and nutrition of micropropagated banana plants.

materials and methods

inocula

The *Glomus mosseae* and *Glomus geosporum* strains were collected from clover (*Trifolium subterraneum* L.) for *G. mosseae*, and from banana (*Musa acuminata* colla, cv. AAA Giant Cavendish) for *G. geosporum*. Single fungus pot cultures were then established with *Trifolium subterraneum* L. After 6 months, spores of both VAM fungi were removed from the pot cultures by wet-sieving and decanting. Spores were successively surface-sterilized with calcium hypochlorite (6%) and a solution of chloramine T (20 g/l) containing streptomycin (200 mg/l). The spores were then stirred in 4 l of tap water. One ml aliquots were plated in petri dishes and counted under a stereomicroscope. A volume of water equivalent to 500 000 spores was then sieved, and the supernatant was mixed with 1 l of heat-sterilized sand and used as the original inoculum material.

plant material and growth conditions

Plant material consisted of micropropagated banana plants (*Musa acuminata* colla cv. AAA Giant Cavendish). The experiments were conducted in two main phases.

first experiment

weaning phase

Banana plants were grown in 0.5 l pots for 4 weeks. Ten g of inoculum containing 5 000 propagules were added to each plant, with the controls receiving the same amount of sterilized sand. Each pot contained 0.5 kg of the following material: 10% attapulgite, 2% potting soil and 88% sand. The lactate-acetate extractable P content of this material was 7.40 µg/g. Pots were placed in a growth chamber set at 27/24°C (day/night), 70% relative humidity and a 12L:12D photoperiod. A 3-step degressive shade was supplied to

limit the stress. Luminosity, expressed in PAR (photosynthetic active ray), varied successively from 87 to 130 and 210 µmoles/m^2/s. Each pot received 100 ml weekly supplements of nutrient solution of following composition: N (NH_4NO_3) 20 mg/l, K (KCl) 30 mg/l, Ca ($CaCl_2.H_2O$) 20 mg/l, Mg ($MgSO_4.7H_2O$) 10 mg/l, Fe (Fe-EDTA) 1 mg/l and micro-elements B (H_3BO_3) 0.5 mg/l, Mn ($MnCl_2.2H_2O$) 0.5 mg/l, Cu ($CuSO_4.5H_2O$) 0.02 mg/l, Zn ($ZnSO_4.7H_2O$) 0.05 mg/l, Mo [$(NH_4)6Mo7O_{24}.2H_2O$] 0.01 mg/l.

greenhouse experiment

Banana plants were transplanted in 2.5 l pots containing 2.5 kg of the same material as described above. The duration of this second phase was 8 weeks. Growing conditions were 25/22°C (day/night), with 50% relative humidity and 12D:12L photoperiod.

A 200 µmol/m^2/s luminosity was provided. The nutrient supply included 125 mg ammonium nitrate and 250 mg patenkali (30% K_2O and 10% MgO), applied every 3 weeks.

second experiment

This second experiment was conducted under the same weaning and greenhouse conditions as described above. The soil composition was modified slightly, and consisted of 20% attapulgite, 2% potting soil and 78% sand. The lactate-acetate extractable P of this material was 14.9 µg/g. The nutrient supply during the greenhouse phase was also modified and included 35 mg ammonium nitrate and 65 mg patenkali, applied every 3 weeks.

experimental design and analytical methods

In the first experiment, 70 banana plants were inoculated with *G. mosseae,* the 70 other plants comprized the control group. Ten plants were harvested 1, 2, 3, 4, 6, 9, and 12 weeks after inoculation. The roots were cleared with 10% KOH, washed under tap water and stained with 0.1% trypan blue - lactophenol. Thirty root pieces of 1 cm length were mounted on microscope slides to score mycorrhizal infection (F%), intensity of infection, i.e. hyphal extension in the root cortex (M%), arbuscule intensity in mycorrhizal roots (a%) and in the whole root system (A%), using the method described by TROUVELOT *et al.* (1986). Shoot fresh and dry weights, and water content were also measured 3, 4, 6, 9 and 12 weeks after inoculation with *G. mosseae.* Fresh weight was determined at harvest while dry weight was assessed after drying for 48 h at 65°C. Relative mycorrhizal dependency (RMD) (PLENCHETTE *et al.*, 1983) as a result of mycorrhizal inoculation was also calculated.

In the second experiment, 8 banana plants were inoculated with *G. mosseae*, 8 with *G. geosporum*, with 8 plants in the control group. Plants were harvested 3 months after inoculation. Roots were stained to assess mycorrhizal colonization (TROUVELOT *et al.*, 1986). Shoot fresh and dry weights and water content were determined as described in the first experiment. Chemical analyses were performed on shoot dry matter: P was determined colorimetrically, K by photometry, Ca and Mg by atomic absorption and N by the Kjeldhal method.

results and discussion

first experiment

Infection development followed a sigmoïd curve (Figure 1) characterized by three phases: a lag phase (I), a period of rapid development (II) and a plateau phase (III).

One week after inoculation, 18% of the root system was infected (F%) as observed by the entry points between the roots and the fungus. Arbuscule (A% and a%) development and hyphal extension in the cortex (M%) were inexistant or very low at this early stage of infection (I). The lag phase was followed by exponential development (II) due to the spread of infection. Running hyphae also

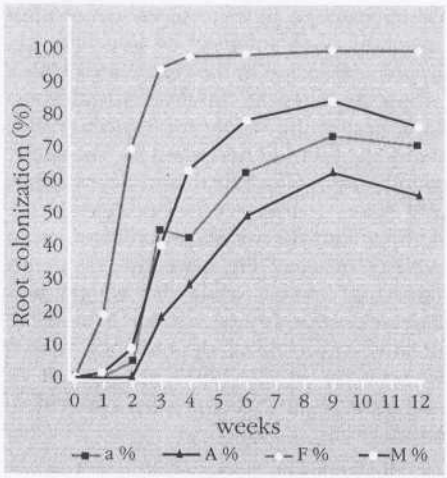

Figure 1
Growth dynamics of Glomus mosseae *in banana roots. F%, frequency of colonization of roots; M%, intensity of mycorrhizal infection in the root cortex; a%, arbuscule intensity in the mycorrhizal roots and A%, arbuscule intensity in the total root system (*TROUVELOT et al., *1986).*

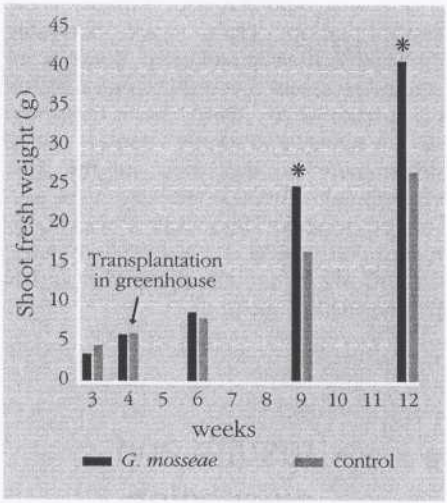

Figure 2
Effect of Glomus mosseae *inoculation on shoot fresh weight in micropropagated banana plants. (*) indicates significant differences at p<0.05 (Newman - Keuls test).*

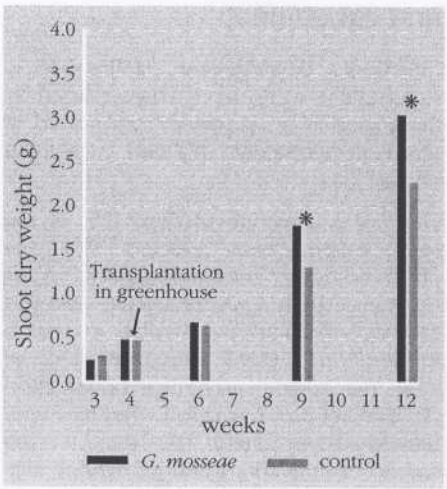

Figure 3
Effect of Glomus mosseae *inoculation on shoot dry weight in micropropagated banana plants. (*) indicates significant differences at p<0.05 (Newman - Keuls test).*

developed new entry points. The cortex was heavily colonized and arbuscules increased in number. Four weeks after inoculation, the entire root system was colonized (F%) and a plateau phase occurred (III). Cortex infection (M%) and arbuscule development (A% and a%) reached a plateau after 6 weeks and remained almost unchanged to the end of the experiment.

Inoculation with *G. mosseae* resulted in a significant increase in banana shoot fresh and dry weights as compared to the uninoculated controls (Figures 2 and 3). Marked differences appeared after 6 weeks, which became significant 9 weeks after inoculation. The increase in dry weight amounted to 0.39 g/week for the mycorrhizal plants and 0.27 g/week for the controls. The RMD for banana plants at 12 weeks was 24.67%. The water contents in control and inoculated banana plants were significantly different (Figure 4). Water content remained almost constant in mycorrhizal plants while it decreased regularly in uninoculated controls. The higher water content observed in the mycorrhizal plants, as compared to the controls, after transplantation in the greenhouse could be explained by the lower resistance to water transport in mycorrhizal plants (SAFIR *et al.*, 1972). These authors further argued that this effect was probably due to improved P nutrition. This is supported by several other experiments showing that mycorrhizal plants could exhibit lower resistance to water flow in phosphate deficient situations (NELSEN and SAFIR, 1982). We suggest that the lower water content in the mycorrhizal plants, as compared to the controls, 3 weeks after inoculation (Figure 4) could be due to stress following mycorrhizal establishment.

second experiment

Both VAM fungi induced marked root infection (F%) (Table 1). Root cortex infection (M%) and arbuscule development (A% and a%) were significantly higher in plants inoculated with *G. mosseae*. These differences in infection development could be related to the growing

conditions. In particular, soil pH seems to have an important role since different VAM fungi are known to proliferate under specific soil pH conditions (STRULLU, 1991). The two symbionts were isolated from soils of pH 6.1 and 7.2 for *G. geosporum* and *G. mosseae* respectively, whereas pH 7.6 was used in our experiments. This pH level may have been unsuitable for effective development of *G. geosporum*. It could have been responsible for the lower root cortex infection (M%) and arbuscule development (A% and a%) with this fungus as compared to *G. mosseae*. Since arbuscules are considered to be the main site of nutrient transfer from the fungus to the plant (SMITH and GIANINNAZZI-PEARSON, 1988) the greater arbuscule development observed with *G. mosseae* could explain the higher effectiveness of this fungus to induce plant growth and increase shoot nutrient content. A 70% increase in growth was obtained with this fungus, with 100% root colonization and 79% arbuscule development. In comparison, growth was increased by 50% with *G. geosporum*, 100% of the roots were colonized but arbuscule development was only 7%. Inoculation with both VAM fungi resulted in significantly higher shoot fresh and dry weights as compared to uninoculated plants (Table 2). Water content was also higher in bananas inoculated with *G. mosseae* and *G. geosporum* (Table 2).

Macronutrient contents (N, P, K, Ca and Mg) also differed in inoculated and control plants (Table 3). Shoot P and K contents were significantly higher, while N, Ca and Mg contents were significantly lower in inoculated plants. The higher P content could be related to various mechanisms, including larger soil volume, which reduces the distance of ion diffusion to the plant roots, faster movement of P into mycorrhizal roots, and rhizosphere modifications (BOLAN, 1991). VAM fungi might also increase the uptake of other nutrients that move to the root surface, primarily by diffusion (ABBOTT and ROBSON, 1984). This could explain the higher K ion content in roots of mycorrhizal banana plants. Nevertheless, no direct mycorrhizal effect on K uptake has been demonstrated to date (HARLEY and SMITH, 1983). Considering the importance of

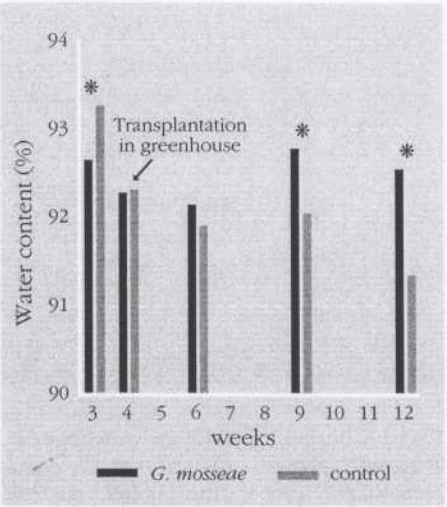

Figure 4
Effect of Glomus mosseae *inoculation on water content in micropropagated banana plants.*
(*) indicates significant differences at p<0.05 (Newman - Keuls test).

Table 1
Mycorrhizal infection in micropropagated banana plants 12 weeks after inoculation with *Glomus mosseae* and *G. geosporum*.
F%, frequency of root colonization; M%, intensity of mycorrhizal infection in the root cortex; a%, arbuscule intensity in mycorrhizal roots and A%, arbuscule intensity in the total root system (TROUVELOT *et al.*, 1986).

Treatment	F%	M%	a%	A%
G. mosseae	100	83 (*)	89 (*)	79 (*)
G. geosporum	100	23	43	7

(*) values are significantly different at p<0.05 (Newman-Keuls test).

Table 2
Effect of VAM inoculation on shoot fresh weight (SFW), shoot dry weight (SDW) and water content (WC) in micropropagated banana plants. Values in the same column followed by identical letters are not significantly different at p>0.05 (Newman-Keuls test).

	SFW (g)	SDW (g)	WC (%)
G. mosseae	37.2 [a]	4.5[a]	87.9[a]
G. geosporum	25.0[b]	3.0[b]	88.0[a]
Control	11.3[c]	1.4[c]	87.1[b]

Table 3
Mineral contents in mycorrhizal and non-mycorrhizal micropropagated banana plants. Values in the same column followed by identical letters are not significantly different at p>0.05 (Newman-Keuls test).

	N (%)	P (mg/100 g)	K (mg/100 g)	Ca (mg/100 g)	Mg (mg/100 g)
G. mosseae	2.3^{c}	1273.7^{b}	7300.7^{a}	1458.7^{b}	1516.2^{b}
G. geosporum	2.7^{b}	954.1^{b}	7356.6^{a}	1227.0^{c}	1130.2^{c}
Control	3.2^{a}	554.2^{c}	5750.4^{b}	1819.1^{a}	1700.1^{a}

P and K during the early growth stages in banana plants (MARTIN-PREVEL and MONTAGUT, 1966), the better growth response obtained in mycorrhizal plants was probably due to increased uptake of these two nutrients. The N shoot content results contrasted with previous reports (BAREA *et al.*, 1988; SMITH *et al.*, 1986), indicating that VAM fungi increase nitrogen content in mycorrhizal plants. The only valid explanation would be a dilution effect due to the high growth rate of mycorrhizal plants as compared to the controls (FURLAN and BERNIER-CARDOU, 1989). These authors further observed that K fertilization decreased Ca and Mg contents in VAM and non-VAM plants. Many researchers (HOAGLAND, 1948) have shown that plant contents of Ca or Mg, or both, tend to decrease as K increases. Since the K content in VAM banana tissues was found to be significantly higher than in non-mycorrhizal banana tissues, this clearly resulted in lower Ca and Mg contents due to antagonism between K and these two nutrients.

conclusion

The results of the present study clarified mycotrophic processes in banana plants. The growth dynamics followed a classical three-step development (MENGE, 1984), as occurs in most other biological populations. The results further indicated the dependence of banana plants on mycorrhizal symbiosis. Plant growth was significantly increased following mycorrhizal inoculation.

The presence of VAM fungi in the roots also significantly increased the P and K contents in mycorrhizal plants. The better growth response was probably due to increased P and K uptake, as these two macronutrients are essential during the early growth stages in banana plants (MARTIN-PREVEL and MONTAGUT, 1966). Nevertheless, the growth increases differed between plants inoculated with *G. mosseae* and *G. geosporum*. Despite the lack of absolute specificity accorded to mycorrhizal symbiosis, physiological processes and environmental conditions are determining factors for symbiotic efficiency (SMITH and GIANINAZZI-PEARSON, 1988). It seems likely that the experimental soil pH affected *G. geosporum* infection development and could explain the lower plant growth response obtained with this fungus.

We conclude that efficient associations between VAM fungi and banana plants are formed under greenhouse conditions. Nevertheless, further work is required before this finding can be applied in field situations. Baseline data are now required on mechanisms involved in interactions between the two symbionts and on environmental conditions that favour efficient host-fungus associations.

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