Effect of inoculation by arbuscular mycorrhizal fungi on the growth of micropropagated pineapple plants

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ABSTRACT
Several experiments were conducted in the greenhouse and in the field with the objective of evaluating the efficiency of arbuscular mycorrhizal fungi (*Glomus clarum, Gigaspora margarita* and *Acaulospora* sp) on the growth of micropropagated plants of pineapple (*Ananas comosus* (L) Merr), cultivar Pérola. During the process of acclimatization of the micropropagated plantlets in a greenhouse, no differences between arbuscular mycorrhizal fungi (AMF) and the control (without AMF) treatments were observed. In the field, 6 months after transplanting the seedlings, it was found that plants inoculated with AMF were higher compared with plants from the control.
After 12 months, the AMF pre-inoculated pineapple plants were taller, had a greater number of suckers and slips, and accumulated more phosphorus and potassium in the D leaves.

KEYWORDS
Ananas comosus, inoculation, vesicular arbuscular mycorrhizae, vitroplants, growth, field crop.

Effet de l'inoculation de mycorhizes à arbuscules sur la croissance de vitroplants d'ananas.

RÉSUMÉ
Divers essais ont été effectués en serre et en plein champ pour évaluer l'efficacité de l'inoculation de mycorhizes à arbuscules (*Glomus clarum, Gigaspora margarita* et *Acaulospora* sp) sur la croissance de vitroplants d'ananas (*Ananas comosus* (L) Merr), cultivar Pérola. Pendant la période d'acclimatation des vitroplants en serre, aucune différence n'apparaît entre les plants mycorhizés et les plants témoins non inoculés.
En revanche, 6 mois après la transplantation des plants en plein champ, les plants inoculés apparaissent plus développés que les plants témoins.
Après 12 mois, ces plants sont plus grands, ont davantage de rejets, et l'analyse de leur feuille D révèle une accumulation plus forte de phosphore (P) et de potassium (K).

MOTS CLÉS
Ananas comosus, inoculation, mycorhizes à vésicule et arbuscule, vitroplant, croissance, culture de plein champ.

Efecto de la inoculación de micorrizas arbusculares sobre el crecimiento de vitroplantas de piña.

RESUMEN
Se efectuaron varios ensayos en invernaderos y en el campo para evaluar la eficacia de la inoculación de micorrizas arbusculares (*Glomus clarum, Gigaspora margarita y Acaulospora* sp) sobre el crecimiento de vitroplantas de piña (*Ananas comosus* (L) Merr), cultivar Pérola. Duranta el período de aclimatación de las vitroplantas en invernaderos, no aparece ninguna diferencia entre los plantones micorrizados y los plantones pruebas sin inocular. En cambio, 6 meses después de la transplantación de los plantones en el campo, los plantones inoculados aparecen más desarrollados que los plantones pruebas. Doce meses después, estos plantones son más grandes, tienen más renuevos, y el análisis de su hoja D revela una acumulación más fuerte de fósforo (P) y de potasio (K).

PALABRAS CLAVES
Ananas comosus, inoculación, micorrizas arbusculares vesiculares, vitroplantas, crecimento, cultivo extensivo.

introduction

Pineapple is widely cultivated in Brazil. However, the low propagation rate and the high incidence of fusariose limit its spread. The fusariose caused by the fungus *Fusarium moniliforme* Sheld, var *subglutinans*, is considered the most serious pineapple disease in Brazil. It is responsible for losses from 30 to 40% (GOES et al, 1986), in large part of the producing regions.

The in vitro micropropagation technique is presented as an efficient alternative that permits a rapid and high propagation rate of disease-free seedlings in a short period of time and in limited space. The estimated yield with this technique is one million uniform plantlets, developed from a single germ in 2 years (PANNETIER and LANAUD, 1976). Studies with different crops (asparagus, banana, sweet potato, cassava, strawberry and grape) have shown evidence of the interaction between micropropagation and arbuscular mycorrhizal fungi (AMF) inoculation techniques, which offer, during the acclimatization phase, a potentially efficient technique to obtain more vigorous and adaptative seedlings for transplanting (MONTEIRO et al, 1991; LOVATO et al, 1995).

The majority of plant species develop root symbiosis with AMF, which can significantly improve the uptake of soil phosphate, enabling the more efficient use of applied fertilizer (GIANINAZZI-PEARSON, 1986). In study with micropropagated oil palm (*Elaeis guineensis* Jacq), BLAL et al (1990) verified that AMF inoculation increases the efficiency of the use of both rock phosphate and superphosphate for plants growing in acid soils.

The interaction between AMF and pineapple has scarcely been studied. MOURICHON (1981) made the first observations of the presence of AMF in pineapple roots, identifying the structures as part of the genus *Glomus*. AZIZ et al (1990), in work with the Smooth Cayenne cultivar and *Glomus aggregatum* inocula, verified that the fungus stimulated seedling growth only at very low levels of phosphorus (P). On the other hand, JAIZME VEGA and AZCON (1991), in an experiment with micropropagated plantlets of Smooth Cayenne and three AMF species (*G mosseae*, *G fasciculatum* and *Acalospora* sp), observed the efficiency of these fungi on transplanting survival, enhanced plant biomass production and shoot nutrient content, *G mosseae* being the most effective endophyte for pineapple plants. According to LOVATO et al (1994), micropropagated plants of pineapple were dependent on AMF during the acclimatization phase, showing also a bioprotector effect of mycorrhiza, which is characterized by a decrease of damage caused by *Phytophtora cinnamoni* Rands infection and by the *Pratylenchus brachyurus* Godfrey nematode attack (GUILLEMIN et al, 1994 a and b).

Some horticultural practices have created conditions for the successful application of mycorrhizae. However, with agricultural crops the situation is more complex, more research being necessary to promote the rational use of AMF (GIANINAZZI et al, 1995).

The purpose of this work was to evaluate the effect of AMF in micropropagated plantlets of pineapple, cultivar Pérola, which is one of the most planted cultivar in Brazil, during the acclimatization phase and after its transplanting to the field.

materials and methods

Under greenhouse conditions, micropropagated plantlets of pineapple (*Ananas comosus* (L) Merr), cultivar Pérola, 3 cm height, were acclimatized in polystyrene foam trays with 72 cells, each one with a capacity of 100 ml. The substrate constituted of autoclaved vermicompost from cattle manure. Its composition was pH = 5.9, N = 29.8 g, P = 6.1 g, K = 3.9 g, C = 309.89 g and organic matter 557.81 g per kg of dry matter.

The applied treatments were: control treatment in which plantlets were not inoculated, and three treatments with *Glomus clarum* (NICOLSON and SCHENK), *Gigaspora margarita* (BECKER and HALL) and *Acaulospora* sp inoculated plantlets. The choice of these three species of arbuscular mycorrhizal fungi was made due to the availability of these inocula in EMBRAPA-CNPAB[1] and the proved efficiency of the first two fungi in previous experiments.

In treatments with AMF, 60 spores per plant were used. Each treatment consisted of four replicates, the experimental unit being just one plant. During the first period of acclimatization, treatments

(1) Empresa Brasileira de Pesquisa Agropecuária - Centro Nacional de Pesquisa em Agrobiología (Brazil).

were set up separately to prevent possible cross-contamination.

After 135 days, the plants were transplanted to 5 kg polyethylene bags, using an autoclaved mixture of a red-yellow Podzolic (RYP) soil and vermiculite in the rate of 3:1 (v:v). The RYP soil had the following characteristics: pH = 4.9, P = 1.7 μg/cm^3, K = 117 μg/cm^3, Ca = 0.6 and Al = 1.0 meq per 100 cm^3 of soil. This determination was made according to the methodology of Empresa Brasileira de Pesquisa Agropecuária (1979). To this mixture, 1.67 g of Patos de Minas rock phosphate per kg of substrate was added. For this second period, the experimental design was completely randomized.

After 141 days in plastic bags, when the plants were between 30 and 40 cm in height, they were transplanted to the field. To evaluate mycorrhizal colonization, root samples were collected, clarified and strained according to PHILLIPS and HAYMAN (1970). The number of AMF spores in the soil samples from the experimental area was evaluated using the sifting and decantation method of GERDEMANN and NICOLSON (1963).

The soil in the field where the plantlets were finally transplanted showed the same characteristics as the RYP soil earlier. The space between plants was 40 cm and each plant was fertilized with 0.8 g of single superphosphate, 2.5 g of Patos de Minas rock phosphate, 0.8 g of potassium chloride and 1 l of dried cattle manure.

In the field, the experimental design was identical to the one used during the acclimatization phase. It should be mentioned that no floral induction was performed. The plants in the field were observed for a period of 18 months of growth.

For statistical analysis, ANOVA 1, from the MSTAT program, was used. Duncan's multiple range test procedure was used to determine whether significant differences occurred between treatments. The statistical analysis of suckers and slip numbers was done with transformed data ($x + 1$).

results and discussion

The substrate used during the first period of acclimatization was not suitable for seedling preparation because it could not provide a good particle aggregation, even in the presence of roots, thus making it difficult to remove the plants from the trays without damage.

In the second period of acclimatization, when soil and vermiculite were used, seedling growth was better. There was no observable effect of AMF inoculation on plant height and vigor. Similar results were reported by AZIZ et al (1990) with Smooth Cayenne seedlings originating from the crown. This lack of response to AMF inoculation suggests that the slow growth of the seedlings created a low rate of P uptake, and hence there was little necessity for mycorrhizal infection. NIEMI and VESTBERG (1992) also reported no difference between the AMF treatment and the control when working with micropropagated strawberry seedlings, and a low infection level of the AMF was observed.

At the field transplanting stage, control plants presented no mycorrhizal colonization. In the evaluation of native AMF population, an average of 50 spores per 50 ml of soil was found, the predominant species being *Glomus macrocarpum* and *Acaulospora* sp.

In the field, it was found that, at 6 and 12 months, mycorrhizal plants were significantly taller than the controls without AMF, with the exception of the *Gigaspora margarita* treatment after 6 months (fig 1). The positive response obtained during this period can be explained by the growth of the plant metabolism, which increases nutrient demand

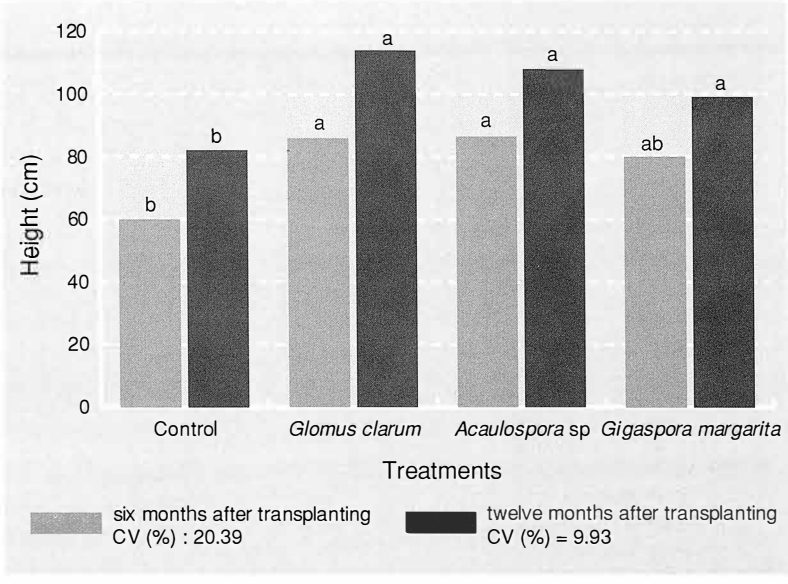


Figure 1
Arbuscular mycorrhizal fungi inoculated pineapple height at 6 and 12 months after transplanting to the field, compared with plants without inoculation (control). For a same color (clear or dark gray), values followed by the same letter are not different according to Duncan's multiple range test ($P \leq 0.05$). The given variation coefficients (CV) refer to the average of all treatments.

Table I
Dry matter and phosphorus and potassium accumulation in "D" leaves of pineapple, 12 months after transplanting to the field [1].

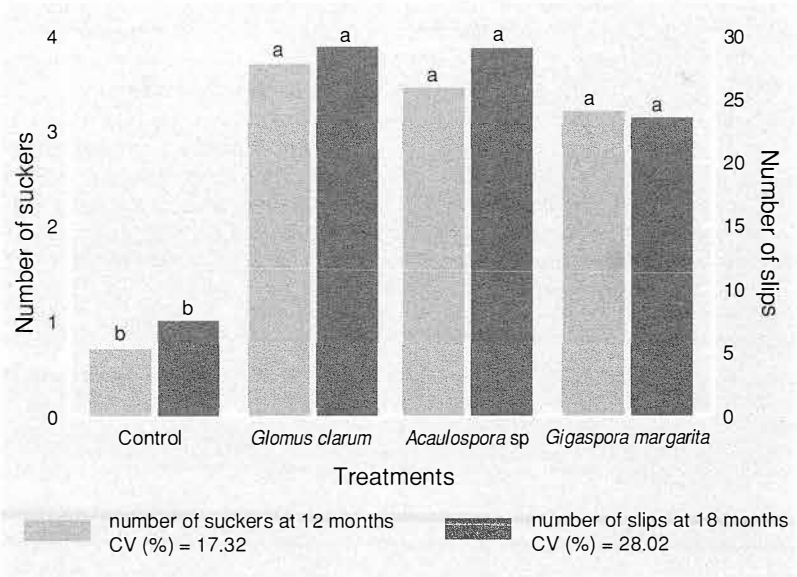
Treatment	*Dry matter (g/leaf)*	*Phosphorus (mg/leaf)*	*Potassium (mg/leaf)*
Control	2.15 b	2.91 b	51.38 b
Glomus clarum	3.15 a	4.11 a	81.20 a
Acaulospora sp	3.19 a	4.39 a	81.50 a
Gigaspora margarita	2.83 a	3.88 a	73.20 a

(1) Mean values of four replicates. a, b : Values followed by the same letter are not different according to Duncan's multiple range test ($P \leq 0.05$).

and stimulates the symbiosis to work effectively. According to SIQUEIRA and FRANCO (1988), the mycorrhizal intensity, as well as its functioning and host answer, depends on both the fungus and the plant, in addition to environment.

After 12 months growth, the mycorrhizal plants showed greater dry matter accumulation in D leaves than the controls (table I). It was also observed that mycorrhizal treatments had a greater P and K content in the D leaves (table I). These data indicate that mycorrhizal plants showed a greater vigor and a better nutritional status when compared to the non-mycorrhizal plants. The better nutritional status of mycorrhizal plants is primarily due to a more efficient P absorption (COOPER, 1984; RIZZARDI, 1990).

Figure 2
Number of suckers and slips in arbuscular mycorrhizal fungi inoculated pineapple at 12 and 18 months, respectively, after transplanting to the field, compared with plants without inoculation (control). For a same color (clear or dark gray), values followed by the same letter are not different according to Duncan's multiple range test (P ≤ 0.05). The given variation coefficients (CV) refer to the average of all treatments.



In a subsequent work in the field, GARCIA (personal communication, 1992) observed no differences in height and shoot weight of micropropagated plantlets of Smooth Cayenne and Pérola, previously inoculated with AMF.

However, P was greater in the AMF inoculated plants. COOPER (1984) reported that the plant response to mycorrhizal infection varies slightly, depending on the efficiency of each endophyte, as well as its ability in adapting to different environmental, nutritional and physical conditions.

It is possible to observe that the number of suckers and slips (fig 2) was greater in the AMF treatments than in the control without inoculation. This result is very promising as pineapple plants propagate principally vegetatively. CABRAL (1984) considered the number of slips a genetic characteristic, influenced by environment and the vegetative growth of the plant. The harvested fruits at 12 months presented a normal aspect although they were of a small size.

The use of the in vitro culture technique of the axillar shoots was satisfactory for the production of healthy and viable pineapple seedlings.

conclusion

Under the conditions of this study, pineapple plants of the cultivar Pérola responded positively, under field conditions, to prior inoculation with the three arbuscular mycorrhizal fungi used. Results also suggest that the increased vigor and growth of the mycorrhizal plants constituted a preponderant factor for an increase in the sucker and slip production compared with the non-inoculated controls.

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