Screening of arbuscular endomycorrhizal fungi for establishment of micropropagated pineapple plants

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Summary — Several arbuscular endomycorrhizal fungi (*Glomus clarum* (LPA16), *Scutellospora pellucida* (LPA20), *Glomus* sp. (LPA21), *Glomus* sp (LPA22) and *Glomus* sp (LPA25)) were tested for plant growth effects and infection development in Queen Tahiti, Smooth Cayenne (clone CY_0) and Spanish varieties of micropropagated pineapple growing in an acid soil under growth chamber tropical conditions. Endomycorrhizal plants of all 3 pineapple varieties grew better than non-mycorrhizal plants. However, increase in plant growth was not related to infection development. Screening of different isolates of arbuscular endomycorrhizal fungi showed some specificity of fungi for promoting growth of the different pineapple varieties. Queen Tahiti and Smooth Cayenne pineapple plants associated with *Glomus* sp (LPA21) grew better than those infected with the other fungi, whilst best growth was obtained for the Spanish variety by inoculating plants with *Glomus* sp (LPA25). The root/shoot ratio was modified by endomycorrhizal inoculation, infected pineapple plants showing a greater increase in shoot production in comparison to root production.

micropropagated pineapple / arbuscular endomycorrhizal fungi / plant growth / infection development

Résumé — Sélection de champignons endomycorhizogènes à arbuscules efficients pour l'établissement d'ananas micropropagés. *Plusieurs champignons endomycorhizogènes à arbuscules (*Glomus clarum *(LPA_{16}),* Scutellispora pellucida *(LPA20),* Glomus *sp (LPA21),* Glomus *sp (LPA22) and* Glomus *sp (LPA25)) ont été testés pour leur développement et leur effet sur la croissance de 3 variétés d'ananas micropropagés (Queen Tahiti, Cayenne lisse (clone CY0) et Spanish) cultivés dans un sol acide et dans une salle climatisée aux conditions tropicales. Pour les 3 variétés d'ananas testées, les vitroplants endomycorhizés présentent une meilleure croissance que les non inoculés. Cependant l'augmentation de croissance n'est pas liée avec l'importance de l'infection. La sélection des différents champignons endomycorhizogènes montrent une certaine spécificité fongique vis à vis des différentes variétés d'ananas. Ainsi les vitroplants des variétés Queen Tahiti et Cayenne lisse, associés avec* Glomus *sp (LPA21), présentent une meilleure croissance que ceux infectés avec les autres champignons tandis que la meilleure croissance pour la variété Spanish a été obtenue avec* Glomus *sp (LPA25). Le rapport du poids de matière fraîche racinaire sur le poids de matière fraîche aérienne est modifié par la présence de champignons endomycorhizogènes; l'effet endomycorhizien sur la croissance de la partie aérienne est supérieur à celui obtenu sur la partie racinaire des vitroplants.*

ananas micropropagés / champignons endomycorhizogènes à arbuscules / croissance de la plante-hôte / infection endomycorhizienne

INTRODUCTION

Micropropagation is a technique of increasing importance for the production of many crops (Sasson, 1992). This includes pineapples which form arbuscular endomycorrhiza (Mourichon, 1981) as do nearly all cultivated plants (Powell and Bagyaraj, 1984; Gianinazzi *et al,* 1990). Endomycorrhization can modify root architecture to give a root system which is better adapted for uptake of mineral nutrient and water (Berta *et al,* 1990), as well as increasing hormone production (Allen, 1985) and resistance to pesticides or root pathogens (Gianinazzi *et al,* 1982; Harley and Smith, 1983). Endomycorrhizal formation is suppressed by practices employed in micropropagation, and it is thus important to consider introducing symbiotic fungi during plant production. However, arbuscular endomycorrhizal fungi differ in their ability to enhance plant

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growth, and to optimise yields it is necessary to screen for the most efficient isolates before developing mass inoculation (Haas and Krikun, 1985). Abbott and Robson (1978) suggested that characteristics of effective isolates are to infect roots rapidly and to efficiently translocate nutrients to plants.

Previous studies on the Smooth Cayenne (clone CY0) variety of pineapple showed that infection with the arbuscular endomycorrhizal fungus *Glomus* sp (LPA21) improved growth under simulated tropical conditions (Guillemin *et al*, 1991). The present paper reports a series of experiments carried out to compare the effect of several arbuscular endomycorrhizal fungi on plant growth and infection development in several varieties of micropropagated pineapple.

MATERIALS AND METHODS

Three micropropagated pineapple varieties (Queen Tahiti, Smooth Cayenne (clone CY0) and Spanish) were each inoculated with 1 of 5 arbuscular endomycorrhizal fungi : *Glomus clarum* (LPA16), *Scutellispora pellucida* (LPA20), *Glomus* sp (LPA21), *Glomus* sp (LPA22) and *Glomus* sp (LPA25).

Experiments were carried out in an acid soil (pH 5.0) under simulated tropical conditions (300 µE. s^{-1} m^{-2}, 28–25 °C, 12 h day and 70–90% relative humidity). Pineapple microplants were inoculated during a *post vitro* acclimatization period with root fragments of *Tephrosia ehlenbergiana* infected by one of the fungal isolates (Guillemin *et al*, 1991). One g of infected roots was used to inoculate 10 microplants. After 1 month, pineapple plants were transplanted individually to pots containing 400 g of soil: gravel mix (1:1, v:v). Each pot was watered daily with distilled water and weekly with 2 x 20 ml Hoagland No 2 solution (Hoagland and Arnon, 1950) without phosphate. After 3 months, growth was evaluated *via* several parameters : leaf area (cm^2), shoot and root fresh mass (g) and shoot dry mass (g).

Infection development was estimated by the method of Trouvelot *et al* (1986) after clearing and staining with trypan blue (Philipps and Hayman, 1970). Roots of the Spanish variety were stained also for succinate dehydrogenase (SDH) (Smith and Gianinazzi-Pearson, 1990) and alkaline phosphatase (ALP) (Tisserant *et al*, 1992) activity in order to evaluate living (SDH) and functional (ALP) fungal infections.

Each treatment comprised 5 replicates and all data was analysed by ANOVA and Newman–Keuls test.

RESULTS

Plant growth

Endomycorrhizal plants of all 3 pineapple varieties grew better than non-mycorrhizal plants. However, there were differences among the combinations of plant varieties and fungal isolates.

Optimum growth of Queen Tahiti (table I) and Smooth Cayenne varieties (table II) was obtained with *Glomus* sp (LPA21); leaf area and shoot fresh and dry mass were significantly higher than those of non-mycorrhizal plants. Both varieties infected with *G clarum* (LPA16) also had a significantly higher leaf area.

Spanish variety responded to inoculation with all 5 fungi, with significant effects on all plant growth parameters (table III). Largest increases in shoot growth were obtained with *Glomus* sp

Table I. Leaf area, shoot and root fresh mass and shoot dry mass of uninoculated and endomycorrhizal (*Glomus clarum*(LPA16), *Scutellispora pellucida* (LPA20), *Glomus* sp (LAP21), *Glomus* sp (LPA22) and *Glomus* sp (LPA25)) plants of the Queen Tahiti pineapple variety.

Fungal strains	*Leaf area (cm^2)*	*Shoot fresh mass (g)*	*Root fresh mass (g)*	*Shoot dry mass (g)*
Control	249.2^{c}	21.03^{b}	1.90^{a}	2.27^{b}
Glomus clarum (LPA16)	444.3^{ab}	36.05^{ab}	2.42^{a}	4.26^{ab}
Scutellospora pellucida (LPA20)	363.8^{abc}	30.05^{ab}	1.98^{a}	3.61^{b}
Glomus sp (LPA21)	514.4^{a}	41.57^{a}	3.21^{a}	4.57^{a}
Glomus sp (LPA22)	445.5^{ab}	35.05^{ab}	2.56^{a}	4.15^{ab}
Glomus sp (LPA25)	298.0^{bc}	24.75^{ab}	2.42^{a}	2.85^{b}

Values in a column followed by different letters are significantly different ($P < 0.05$).

Table II. Leaf area, shoot and root fresh mass and shoot dry mass of uninoculated and endomycorrhizal (*Glomus clarum* (LPA16), *Scutellospora pellucida* (LPA20), *Glomus* sp (LPA21), *Glomus* sp (LPA22) and *Glomus* sp (LPA25)) plants of the Smooth Cayenne pineapple variety.

Fungal strains	*Leaf area (cm²)*	*Shoot fresh mass (g)*	*Root fresh mass (g)*	*Shoot dry mass (g)*
Control	413.7^{b}	32.79^{b}	3.46^{ab}	2.95^{a}
Glomus clarum (LPA_{16})	607.4^{a}	48.82^{a}	3.98^{ab}	4.22^{a}
Scutellispora pellucida (LPA_{20})	473.0^{ab}	38.39^{b}	2.94^{b}	3.36^{a}
Glomus sp (LPA_{21})	640.1^{a}	51.07^{a}	4.18^{a}	4.26^{a}
Glomus sp (LPA_{22})	515.9^{ab}	41.13^{ab}	2.80^{b}	3.68^{a}
Glomus sp (LPA_{25})	494.7^{ab}	34.78^{b}	3.18^{ab}	3.98^{a}

Values in a column followed by different letters are significantly different ($P < 0.05$).

Table III. Leaf area, shoot and root fresh mass and shoot dry mass of uninoculated and endomycorrhizal (*Glomus clarum* (LPA16), *Scutellospora pellucida* (LPA20), *Glomus* sp (LPA21), *Glomus* sp (LPA22) and *Glomus* sp (LPA25)) plants of the Spanish pineapple variety.

Fungal strains	*Leaf area (cm²)*	*Shoot fresh mass (g)*	*Root fresh mass (g)*	*Shoot dry mass (g)*
Uninoculated	311.4^{d}	21.70^{d}	2.27^{d}	2.05^{d}
Glomus clarum (LPA16)	537.1^{b}	37.06^{ab}	3.69^{a}	3.64^{ab}
Scutellispora pellucida (LPA20)	519.7^{b}	36.84^{ab}	3.59^{a}	3.81^{ab}
Glomus sp (LPA21)	539.1^{b}	38.46^{ab}	3.77^{a}	3.71^{ab}
Glomus sp (LPA22)	411.7^{c}	27.60^{c}	2.81^{b}	2.75^{c}
Glomus sp (LPA25)	620.7^{a}	43.37^{a}	3.50^{a}	4.27^{a}

Values in a column followed by different letters are significantly different ($P < 0.05$).

(LPA25), whilst effects on root growth were similar for *G clarum* (LPA16), *Scutellispora pellucida* (LPA20), *Glomus* sp (LPA21) and *Glomus* sp (LPA25). The *Glomus* sp (LPA22) was generally less effective in improving plant growth.

Root/shoot ratio

Root/shoot ratios were modified by inoculation with arbuscular endomycorrhizal fungi (fig 1) and were generally lower than in non-mycorrhizal plants, with the exception of Queen Tahiti infected with *Glomus* sp (LPA25) (fig 1A) which was also the least effective isolate for growth of this pineapple variety. The endomycorrhizal effect on leaf area was negatively correlated ($P = 0.05$) with the endomycorrhizal effect on root/shoot ratio for Spanish variety but not for Queen Tahiti and Smooth Cayenne varieties (fig 2).

Infection development

Levels of infection observed with trypan blue staining differed between isolates (tables IV, V) but were not related to growth increases in any of the inoculated plants. Endomycorrhizal infection intensity (M%) for the Spanish variety by SDH staining (table V) was significantly lower with the fungal isolate LPA22. This isolate also gave a lower arbuscular frequency (A%), evaluated by both SDH and ALP staining, than the other isolates (table V).

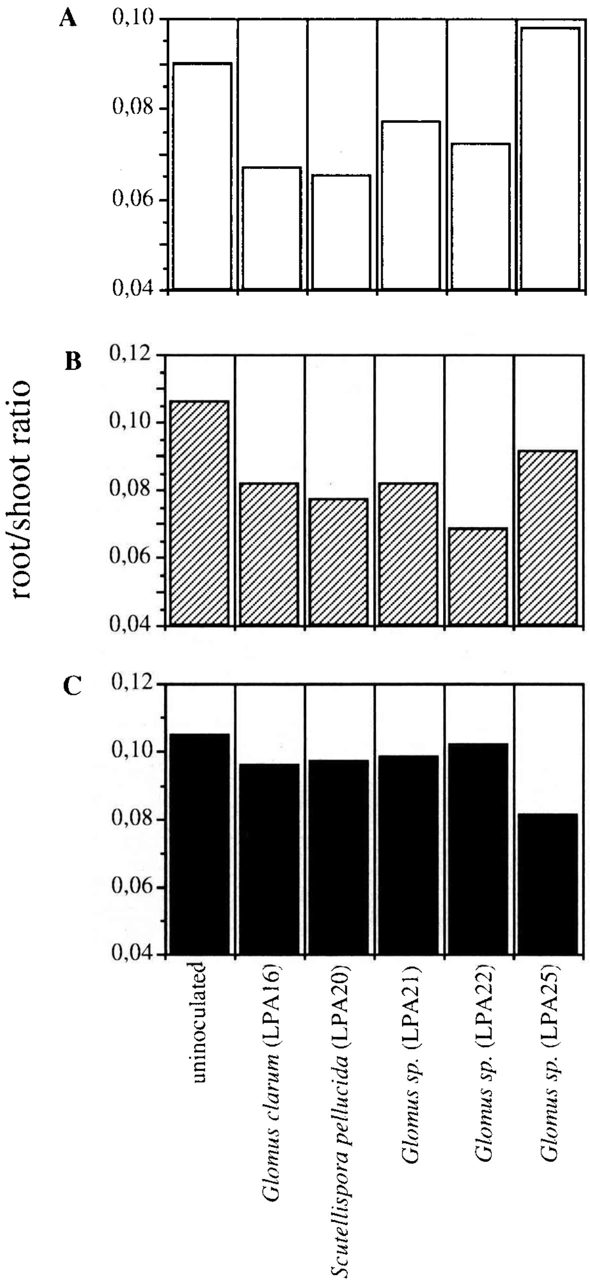

Fig 1. Root/shoot ratio of uninoculated and endomycorrhizal (*Glomus clarum* (LPA16), *Scutellispora pellucida* (LPA20), *Glomus* sp (LPA21), *Glomus* sp (LPA22) and *Glomus* sp (LPA25) plants of Queen Tahiti **(A)**, Smooth Cayenne **(B)** and Spanish **(C)** pineapple varieties.

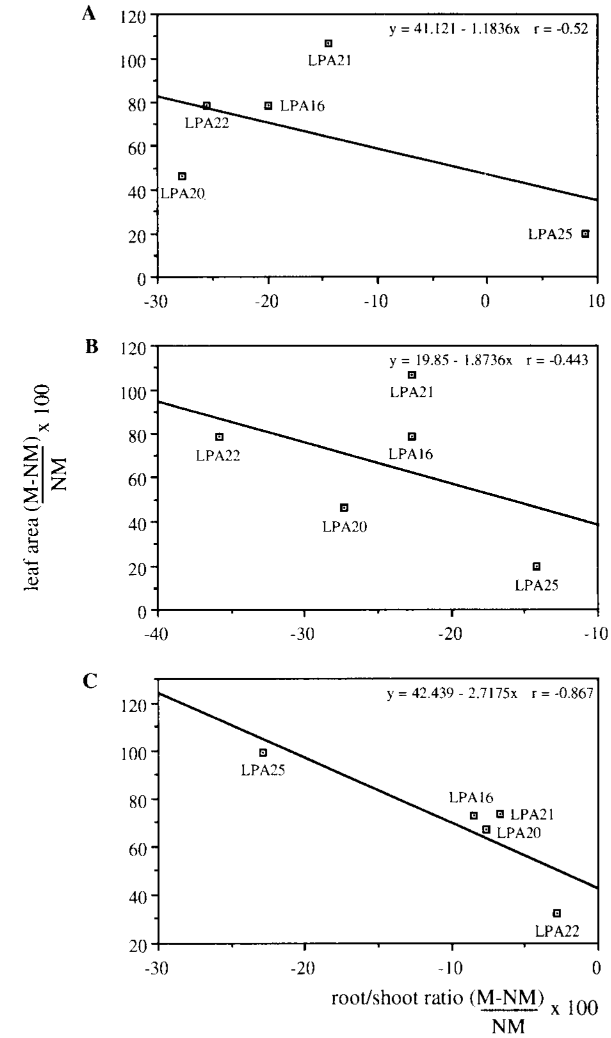

Fig 2. Negative correlations between endomycorrhizal effects on leaf area and on root/shoot ratio of Queen Tahiti **(A)**, Smooth Cayenne **(B)** and Spanish **(C)** pineapple varieties.

DISCUSSION AND CONCLUSION

Screening of different arbuscular endomycorrhizal fungi for their growth-promoting effects on 3 pineapple varieties indicated some specificity of isolates. Of the fungal isolates tested, *Glomus* sp (LPA21) appeared to be most effective for growth of the Queen Tahiti and Smooth Cayenne varieties of pineapple, and *Glomus* sp (LPA25) for the Spanish variety. These observed differences in effectivity underline the necessity to select arbuscular endomycorrhizal fungal isolates. However, these tests were carried out under conditions without competition from other fungi. If such results are to be translated into field predictions, it is necessary to know whether these efficient fungi are also able to establish and persist in the presence of competition by indigenous arbuscular endomycorrhizal fungi (Powell, 1982). Furthermore, it would be of interest to test whether inoculating with a mixture of fungal isolates is more efficient in promoting the growth of different pineapple varieties than inoculation with individual fungal isolates. Sieverding (1989), however, has suggested that it would be more interesting to find one isolate that is effective with a wide range of plant species, since interactions can occur between different isolates in mixtures.

Table IV. Percent endomycorrhizal infection (M%: intensity of infection and A%: arbuscule frequency) observed with trypan blue staining in roots of uninoculated and endomycorrhizal (*Glomus clarum* (LPA16), *Scutellospora pellucida* (LPA20), *Glomus* sp (LPA21), *Glomus* sp (LPA22) and *Glomus* sp (LPA25)) plants of Queen Tahiti and Smooth Cayenne pineapple variety.

Fungal strains	*Queen Tahiti*		*Smooth Cayenne*	
	M%	*A%*	*M%*	*A%*
Uninoculated	0^{d}	0^{d}	0^{c}	0^{d}
Glomus clarum (LPA16)	74^{c}	36^{c}	87^{a}	69^{a}
Scutellospora pellucida (LPA20)	82^{b}	54^{b}	71^{b}	40^{c}
Glomus sp (LPA21)	91^{a}	69^{a}	79^{ab}	58^{b}
Glomus sp (LPA22)	92^{a}	71^{a}	83^{ab}	68^{a}
Glomus sp (LPA25)	87^{ab}	53^{b}	89^{a}	57^{b}

Values in a column followed by different letters are significantly different ($P < 0.05$).

Table V. Percent endomycorrhizal infection (M%: intensity of infection and A%: arbuscule frequency) observed with trypan blue, succinate dehydrogenase (SDH) and alkaline phosphatase (APL) staining in roots of uninoculated and endomycorrhizal (*Glomus clarum* (LPA16), *Scutellospora pellucida* (LPA20), *Glomus* sp (LPA21), *Glomus* sp (LPA22) and *Glomus* sp (LPA25)) plants of Spanish pineapple variety.

Fungal strain	*Trypan blue*		*SDH*		*ALP*	
	M%	*A%*	*M%*	*A%*	*M%*	*A%*
Control	0^{c}	0^{c}	0d	0^{c}	0^{b}	0^{d}
Glomus clarum (LPA16)	74^{ab}	36^{a}	51^{a}	22^{a}	36^{a}	17^{a}
Scutellospora pellucida (LPA20)	70^{b}	35^{a}	52^{a}	19^{a}	32^{a}	15^{b}
Glomus sp (LPA21)	85^{ab}	40^{a}	54^{a}	22^{a}	35^{a}	14^{b}
Glomus sp (LPA22)	92^{a}	33^{a}	36^{c}	13^{b}	30^{a}	10^{c}
Glomus sp (LPA25)	69^{b}	35^{a}	43^{b}	20^{a}	33^{a}	14^{b}

Values in a column followed by different letters are significantly different ($P < 0.05$).

The root/shoot ratio of pineapples (a C_4/CAM plant) was decreased by endomycorrhizal infection, so that endomycorrhizal plants showed a greater increase in shoot production than root production. Such modifications in biomass distribution have often been observed in C_3 plants (Harley and Smith, 1983; Gianinazzi and Gianinazzi-Pearson, 1986; Schubert and Hayman, 1986). The root/shoot ratio was negatively correlated with the endomycorrhizal effect on growth of the 3 varieties of pineapple, but this correlation was less important for the Queen Tahiti and Smooth Cayenne varieties (figs 2A,B). Although endomycorrhizal growth effects were greatest in these 2 varieties when infected with *Glomus* sp (LPA_{21}), this arbuscular endomycorrhizal fungus induced a proportionally greater root production in comparison to the other fungi tested.

Levels of infection observed with trypan blue staining were not related to increased plant growth in any of the 3 varieties. However, the lower SDH and ALP activities present in roots of the Spanish variety may partly explain the lower growth responses in these plants. However, Vierheilig and Ocampo (1989) have suggested that SDH activity does not indicate the efficiency of an arbuscular endomycorrhizal fungus in promoting plant growth. The efficiency of a fungus for the enhancement of plant growth could perhaps be better assessed by ALP activity in endomycorrhizal roots (Tisserant *et al*, 1992). For a better understanding of the physiological basis of variations in the effectivity of arbuscular endomycorrhizal fungi, other parameters should also be analysed, such as external hypha production (network around the roots) (Abbott and Robson, 1985), longevity of hyphae in soil (Sylvia, 1988), efficiency of uptake and transport of phosphorus to the host roots (Sylvia and Burks, 1988; Jakobsen *et al*, 1992), and interactions between external hyphae and mycophagous invertebrates (Rabatin and Stinner, 1991).

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