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Further studies on the influence of mycorrhizae on growth and development of micropropagated avocado plants

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Summary — The acclimatization phase during micropropagation of avocado (*Persea americana* Mill) raises problems concerning the survival and development of plantlets *ex vitro*. Comparison of potting mixes showed that survival of plantlets was highest in a soil – sand substratum and was increased by inoculation with *Glomus* sp in a peat – perlite mix. Mycorrhizal infection by *Glomus* sp during the acclimatization process also improved development of micropropagated avocado plants growing in these 2 substrata. Inoculation with other AM fungi showed that *Glomus deserticola,* and to a lesser extent *Glomus mosseae,* improved plant development in the soil – sand mix. Mycorrhiza formation, therefore, appears to play a key role in favouring *ex vitro* development of micropropagated plants of avocado.

***Persea americana* / arbuscular mycorrhizae / micropropagation**

Résumé — Étude approfondie de l'influence de mycorhizes sur la croissance et le développement de plantes d'avocat micropropagées. *La phase d'acclimatation pendant la micropropagation de l'avocat* (Persea americana *Mill) pose des problèmes de survie et de développement des plantes* ex vitro. *La comparaison de mélanges de rempotage montre que la survie est la meilleure dans un substrat sable : sol et qu'elle est améliorée par l'inoculation avec un* Glomus *sp dans le mélange tourbe:perlite. Une infection mycorhizienne par* Glomus *sp pendant le processus d'acclimatation améliore également le développement des plantes d'avocat micropropagées cultivées sur ces 2 substrats. L'inoculation avec d'autres champignons AM montre que* G deserticola, *et à un moindre degré,* G mosseae *améliorent le développement des plantes dans le mélange sol:sable. La formation de mycorhizes joue un rôle clé dans le développement* ex vitro *de plantes micropropagées d'avocat.*

Persea americana / *mycorhize arbusculaire* / *micropropagation*

INTRODUCTION

The acclimatization phase in avocado micropropagation (*Persea americana* Mill) raises problems concerning survival and development of the plantlets *ex vitro*. In a previous study, growth and nutrition of micropropagated plants of avocado were improved by *post vitro* inoculation with the mycorrhizal fungus *Glomus fasciculatum* (Vidal *et al,* 1992). Inclusion of soil in the potting medium appeared to stimulate mycorrhiza formation and effect. Survival, however, although enhanced by mycorrhizal inoculation, was still low. Consequently, further studies have been carried out to define a suitable potting media for plant development and for the expression of mycorrhizal effectivity, and to evaluate the effect of other arbuscular mycorrhizal (AM) fungi, in order to establish the most appropriate conditions of acclimatization for the development of the micropropagated avocado plants.

MATERIALS AND METHODS

For the micropropagation of avocado plants, juvenil shoots were excised from seedlings of the GvarAm-13 rootstock, previously germinated under *in vitro* conditions following the procedures described by Pliego-Alfaro (1988). Cultures were grown on modified MS medium (Barceló-Muñoz *et al,* 1990) in a growth chamber at 25 °C with a 16-h photoperiod and a photosynthetic photon flux of 45 μmol s^{-1} m^{-2} for 4 wk and then allowed to root for 2 additional wk

on rooting medium as described by Pliego-Alfaro and Murashige (1987).

Two different acclimatization procedures were followed. In Experiment 1, shoots with root primordia were transferred to 500-ml sealed glass flasks containing 200 ml peat–perlite (1/1, v/v) medium. Four plantlets per flask were maintained for 4 wk in the greenhouse (25–18 °C day–night, 70% relative humidity) and at the end of this period plants were individually transferred to 250-ml plastic pots containing the tested potting mix and placed in a misting tunnel (100% relative humidity) for an additional 4-wk period before transfer to normal greenhouse conditions. Three different growing substrata were tested: peat–perlite (1/1, v/v) either sterilized by tindalization and non-sterile, and steam sterilized soil–sand (1/1, v/v). *Glomus* sp was the only mycorrhizal fungus used to test its influence on the different potting media following a factorial design.

In Experiment 2, plantlets were directly transferred from the rooting medium in test tubes into the open pots containing a sterile soil – sand mix, which gave best results in Experiment 1, and placed in the misting tunnel as in Experiment 1. The effect of *G deserticola* and *G mosseae* on plant development was studied.

In both experiments, and after the 4-wk period in the misting tunnel, plants were transferred to normal greenhouse conditions (18–25 °C day–night, 70% relative humidity and natural photoperiod).

In all cases, mycorrhizal inoculum was added to the plants when transferred to the plastic pots. It consisted of 1 g (fresh weight) per plant of clean mycorrhizal onion roots, placed close to the root system. Control plants received filtered leachates of the mycorrhizal inoculum, free from mycorrhizal propagules to compensate for the presence of free-living microorganisms associated with the inoculum.

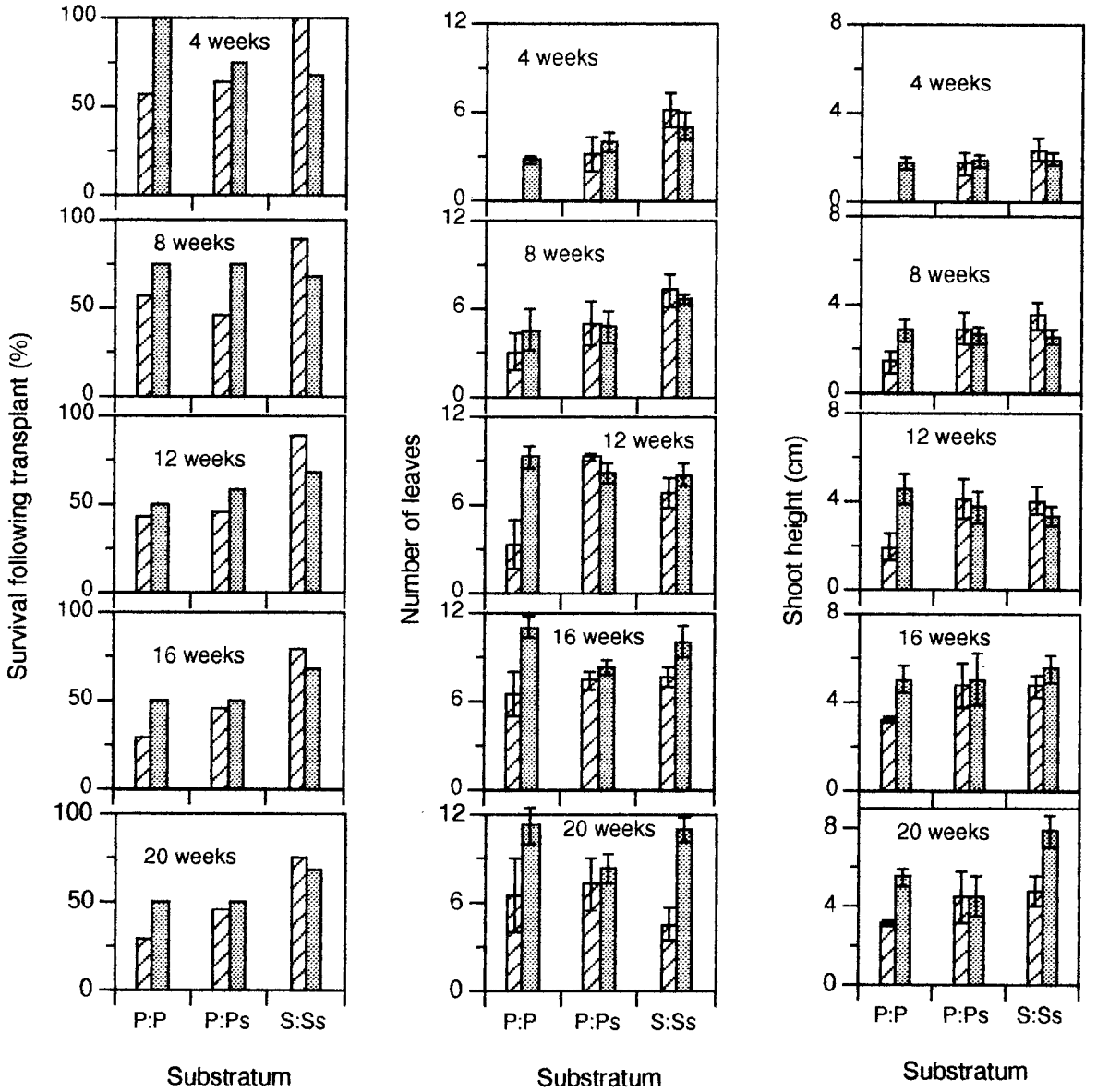

Fig 1. Effect of different potting mixes and of inoculation with *Glomus* sp on survival, number of leaves and shoot height of micropropagated plants of avocado following transplanting (P:P, peat:perlite; P:Ps, sterile peat:perlite; S:Ss, sterile soil: sand; ▨ control uninoculated plants; ▩ mycorrhizal plants). Bars represent SE of means.

Twelve and 13 replicate plants per treatment were used in Experiment 1 and 2 respectively. Survival of plants, number of leaves and shoot height were determined at different times following transplanting, and standard errors were calculated.

RESULTS

Experiment 1

Survival of the plantlets *ex vitro* (fig 1) was higher in the soil–sand mix and in *Glomus* sp-inoculated plants growing in the peat–perlite mix. *Glomus* sp did not produce consistent effects on the development of plants growing in the sterile peat–perlite substratum. However, mycorrhizal inoculation significantly improved shoot height and number of leaves of plants growing in the nonsterile peat–perlite and sterile soil–sand mixes. These effects were evident after 12 wk growth. Since a higher rate of survival of the plants was obtained in the soil–sand mix, this substratum was selected for the second experiment.

Experiment 2

The acclimatization conditions used in this experiment gave increased survival rates of plantlets, reaching values of 85% after 16 wk growth in all treatments (fig 2). Mycorrhizal inoculation had no effect on the survival of the plants, but improved plant development. *Glomus deserticola* and to a lesser extent *Glomus mosseae* increased shoot height, leaf number and vigour of the plantlets (fig 2).

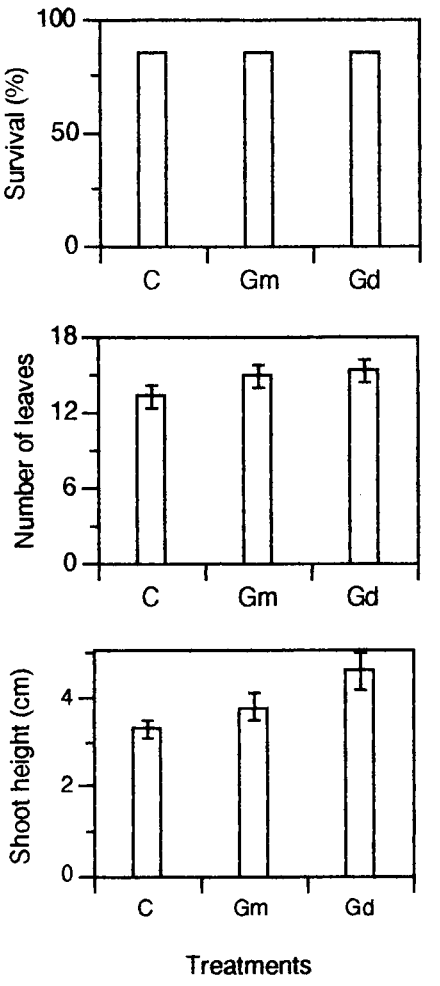

Fig 2. Effect of the inoculation with different arbuscular mycorrhizal fungi on survival and development of micropropagated avocado plants after 16 wk growth following transplanting (C, uninoculated control; Gm, *Glomus mosseae;* Gd, *Glomus deserticola).* Bars represent SE of means.

DISCUSSION AND CONCLUSION

The present results confirm that mycorrhizal inoculation by different AM fungi is effective in promoting plant growth and development of micropropagated avocado (Vidal *et al,* 1992). They also show that it is important to define the potting mix and endomycorrhizal fungus to be used if maximum benefits are to be gained after transplanting *ex vitro.* Although plants were not analysed for mineral content, it is probable that this effect is, at least partially, due to the well-known mycorrhizal activity in increasing mineral uptake by plants. However, mycorrhizal inoculation has also been reported to increase the production of lateral roots in mycorrhizal, micropropagated grapevine plants as compared to uninoculated ones (Schellenbaum *et al,* 1991). Thus, a morphogenetic effect of the AM fungi on the root system of micropropagated plants of avocado cannot be excluded. Rooting of micropropagated avocado is a problem and therefore possible effects of endomycorrhizal formation on root morphogenesis requires further research.

In conclusion, mycorrhizal formation appears to play an important role in assuring satisfactory *ex vitro* development of micropropagated plants of avocado.

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