

Arbuscular mycorrhizal inoculation enhances plant growth and changes root system morphology in micropropagated *Annona cherimola* Mill

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Summary — The effect of arbuscular mycorrhizal formation on plant development and root system morphogenesis in micropropagated *Annona cherimola* plants was investigated. A juvenile rootstock was used and already rooted in vitro plantlets were inoculated with arbuscular mycorrhizal propagules at the beginning of their acclimatization phase. Mycorrhization improved plant development (root, stem and leaves), but decreased the specific root length. Arbuscular mycorrhizal formation did not change the degree of branching nor the lateral root frequency for the adventitious roots, but significantly increased the intensity of branching of the first-order laterals. These results corroborate the role of arbuscular mycorrhizal symbiosis inducing beneficial changes in root system morphology and in the general developmental pattern in micropropagated plants.

***Annona cherimola* / arbuscular mycorrhiza / micropropagation / root system morphology**

Résumé — L'inoculation de mycorhizes arbusculaires augmente la croissance de la plante et change la morphologie du système racinaire d'*Annona cherimola* Mill micropropagés. L'effet de la formation de mycorhizes arbusculaires sur le développement de la plante et la morphogénèse du système racinaire d'*Annona cherimola* a été étudié. Un porte-greffe juvénile a été utilisé et des plantes déjà enracinées in vitro ont été inoculées avec des propagules de mycorhizes arbusculaires au début de la période d'acclimatation. La mycorrhization améliore le développement de la plante (racines, tiges, feuilles) mais diminue la longueur spécifique des racines. La formation de mycorhizes arbusculaires ne change pas pour les racines adventives, ni le degré d'embranchement, ni la fréquence des racines latérales, mais augmente sensiblement l'intensité des branchements des racines de premier ordre. Ces résultats corroborent le rôle de la symbiose mycorrhizienne dans l'induction de changements bénéfiques au niveau de la morphologie du système racinaire et en général du développement de plants micropropagés.

***Annona cherimola* / mycorhize arbusculaire / micropropagation / morphologie du système racinaire**

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INTRODUCTION

Annona cherimola Mill, cherimoya, a tropical fruit tree belonging to the Annonaceae family of the Magnoliales native to South America (Gausen et al, 1982), is a plantation crop well adapted to the subtropical conditions of southern Spain, where it is successfully cropped in the Granada and Málaga provinces (Morton, 1987). The rooting pattern of this fruit tree suggests its mycotrophic habit, a fact that was corroborated in previous studies in which *Annona* showed a strong dependence on arbuscular mycorrhizal (AM) formation for optimal growth and development (Azcón-Aguilar et al, 1994a, b).

The role of AM establishment benefiting the survival and development of most of the plants produced in vitro has been demonstrated (Azcón-Aguilar and Barea, 1996; Lovato et al, 1996). This is particularly important for highly mycotrophic plants, like *A. cherimola*; thus AM inoculation appears as a critical factor for the development of the micropropagated *Annona* plants, at the weaning stages. Apart from the well known improvement in mineral nutrition resulting from AM formation, other effects on plant physiology and morphology can be induced as a consequence of AM formation. In this context, it has recently been recognized that AM colonization affects a range of morphological parameters in developing root systems (Atkinson et al, 1994). This can be of particular interest for micropropagated plants (Azcón-Aguilar and Barea, 1996; Lovato et al, 1996) because of the poorly developed root system they produce in vitro which, at least in part, accounts for the high mortality rates on transfer to ex vitro stages. The most commonly described effect of AM inoculation on root development is an increase in root branching. However, other parameters, such as the length of lateral roots, specific root length, etc, have also been demonstrated to be affected by AM colonization (Schellenbaum et al, 1991; Hooker et al, 1992; Berta et al, 1993, 1995; Atkinson et al, 1994).

Accordingly, a series of experiments was designed with the aim of ascertaining the effects of AM fungi on root system morphology in already rooted in vitro plants of *A. cherimola* inoculated at the beginning of their acclimatization phase.

MATERIALS AND METHODS

Cultures of *Annona cherimola* Mill cv Fino de Jete were initiated from nodal segments of semi-hardwood *Annona* cuttings following the procedures described by Pliego-Alfaro and Murashige (1987). Shoots were incubated in a basic medium consisting of MS (Murashige and Skoog, 1962) salts, added with i-inositol (100 mg/L), thiamine (100 mg/L), pyridoxine (50 mg/L), nicotinic acid (50 mg/L), glycine (200 mg/L), benzilaminopurine (0.15 mg/L), sucrose (30 g/L) and TC agar (8 g/L). Shoot cuttings, 2.5–3.0 cm long, were removed from the proliferative clumps and prepared for rooting.

The rooting procedure was essentially that described by Azcón-Aguilar et al (1994a). In summary, the shoot cuttings were placed in test tubes (25 x 150 mm) containing the basic medium, added with 1 g/L activated charcoal. They were incubated in light for 3 days, being then subcultured for root induction in a medium similar to the basic one but supplemented with indolebutyric acid (100 mg/L) and citric acid (200 mg/L), with the sucrose content reduced to 15 g/L. Cultures were then incubated for 7 days in darkness, and for a further 3 days in light. Finally, shoots were subcultured in a root initiation/elongation medium which differed from the basic one in that the amount of macro-elements was halved, the content of sucrose rose to 20 g/L and the indolebutyric acid was not added. Cultures were maintained for approximately 2 weeks in light.

All media were adjusted to pH 5.7 prior to autoclaving and the environmental conditions during the rooting phase were 26 ± 1 °C, and 16 h light exposure to 2 000 lux illumination (Sylvania regular spectrum Gro-Lux Lamps, Canada) during the light periods.

At the end of the root initiation/elongation phase, most plantlets had some roots of about 1 cm long. At this stage they were inoculated with AM propagules and brought into acclimatization.

Previous studies (Azcón-Aguilar et al, 1994a) recommended AM inoculation immediately after the acclimatization phase; however, because the aim of this experiment was to study the effect of AM symbiosis on root system morphology, inoculation was practised at the beginning of the acclimatization period. Plantlets were individually transferred to open pots (100 mL) containing a mixture of sterile soil and sand (1:1, v/v) and placed in a misting tunnel (100% relative humidity) for 4 weeks. They were then transferred to another tunnel without mist for another 2 weeks and, at the end of this period, plants were transplanted to bigger pots (250 mL) and then to normal greenhouse conditions (25/19 °C day/night temperatures, 16/8 h photoperiod, 75/90% relative humidity). Hoagland nutrient solution (Hoagland and Arnon, 1983) at 25% strength was used to feed the plants at the rate needed to maintain a suitable soil water content. The AM inoculum consisted of thoroughly mixed rhizosphere sam-

ples of stock cultures, containing spores, hyphae and mycorrhizal root fragments of the AM fungus *Glomus intraradices*. Twenty plantlets per treatment were grown.

Two different experiments were carried out. The first one was a short-term experiment (10 weeks long) designed to establish protocols and experimental conditions. At harvest plant height, the number of leaves, shoot and root weight and root length of adventitious and first-order laterals were recorded.

In the second experiment, and after 8 weeks of growth in the greenhouse, plants were transferred to pots (1 000 mL) containing a peat:sterile soil:sand (2:1:1, v/v) mixture. At harvest, after a 20 week growth period, similar growth parameters as for the first experiment were determined, but a more detailed study of the root system morphology was performed. For root morphology measurements, roots were separated into branching orders and the length of the roots in each order measured using video images with a Digital Image Analysis (version 1.10 A, Delta T Devices Ltd, Burwell, Cambridge, UK).

In the second experiment the following morphometric parameters were determined: number of adventitious roots, number of roots of each order, length of the roots present in each order, total root length, degree of branching of the root system, lateral root frequency and specific root length (Berta et al, 1995). The following formula was applied:

Degree of branching = total root number / total root length

Lateral root frequency = number of roots of order n / length of roots of order $n-1$

Specific root length = total root length / total root weight

Data were analyzed by the analysis of variance and Tukey's test.

RESULTS AND DISCUSSION

Mycorrhizal inoculation substantially increased shoot and root biomass and leaf area (figs 1 and 3), corroborating the strong dependence of *A cherimola* on mycorrhiza for optimal growth (Azcón-Aguilar et al, 1994a, b).

Data from the first experiment showed that AM inoculation increased total root length and, particularly, that of first-order laterals (fig 1). A significant effect of AM inoculation on root development was shown by plotting root length data against those of shoot weight (fig 2). These data show different regression lines for AM and non-AM plants, which suggests a specific effect of AM infection on the production of first-order lateral roots.

In the second experiment the AM response can be summarized as follows: in general, inoculation improved the growth of root, stem and leaves (fig 3), and increased the number and length of the roots (fig 4) but, in turn, AM formation induced a decrease in the specific root length (fig 4). It did not change the degree of branching of the root system nor the lateral root frequency for the adventitious roots, but significantly increased the intensity of branching of the first-order laterals (fig 4).

Modifications in the morphology of the root system induced by AM colonization merit some comments. Morphological, modelling and topological methods have been used to investigate effects of AM on root morphology and/or archi-

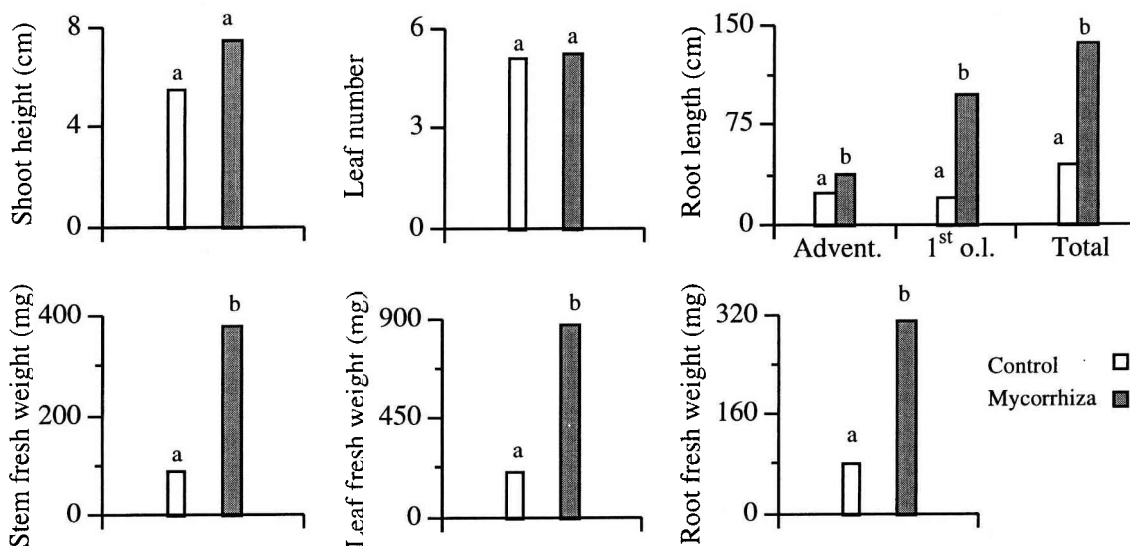


Fig 1. Effect of arbuscular mycorrhizal inoculation on several growth parameters (stem fresh weight, shoot height, leaf fresh weight, leaf number, root fresh weight, root length) of *Annona cherimola* plants micropropagated from a juvenile rootstock. Inoculation was done at the beginning of the acclimatization period and plantlets were 10 weeks old when harvested. For each parameter, mean values (20 replicates) sharing the same letter did not differ significantly ($P < 0.05$) according to Tukey's test. Advent.: adventitious roots; 1st o.l.: first-order laterals.

ture (Atkinson et al, 1994). In agreement with Fitter and Stickland (1991), Schellenbaum et al (1991), Berta et al (1993) and Atkinson et al (1994), the topological method allows architectural approaches which, in turn, make it possible to evaluate the costs and benefits of root architecture related to the root potential for soil resource exploitation. This type of assessment cannot be achieved by applying a morphological develop-

mental analysis. However, the latter is inherently dynamic and it is therefore most useful in describing changes which reflect developmental processes (Atkinson et al, 1994). Because this fits into the aims of the present study, a simple developmental method was used. Such an approach allowed us to detect changes in root morphology induced by the symbiotic interactions. One of the main effects derived from the mycorrhizal status was that the root number in any order lateral was increased as well as the length in all cases. This basically agrees with the findings by Schellenbaum et al (1991), Hooker et al (1992) and Berta et al (1995), in comparing AM versus non-AM control plants. It is difficult to reach generalizable conclusions on the pattern of modifications induced by AM due to the complexity of the factors involved, such as AM fungal species, host plant, soil fertility and the prevailing environmental conditions (Schellenbaum et al, 1991; Atkinson et al, 1994).

The AM-induced decrease in the specific root length over time was similar to that found by Berta et al (1995), but the available data from the present study does not permit any further discussion of the reasons accounting for that pattern.

Overall, the results from the reported experiments corroborate the role of AM symbiosis inducing changes in root system morphology and, in general, in the developmental patterns in micropropagated plants. These changes lead to

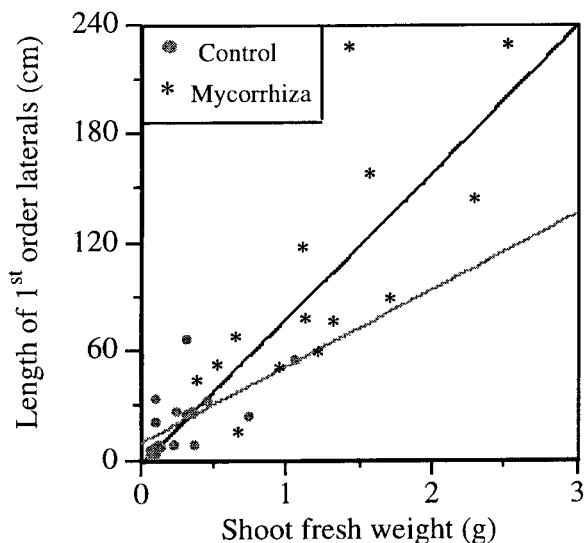


Fig 2. Correlation between length of first-order laterals and shoot weight for control and arbuscular mycorrhizal (*Glomus intraradices*) *Annona cherimola* plants. Inoculation was done at the beginning of the acclimatization period and plantlets were 10 weeks old when harvested.

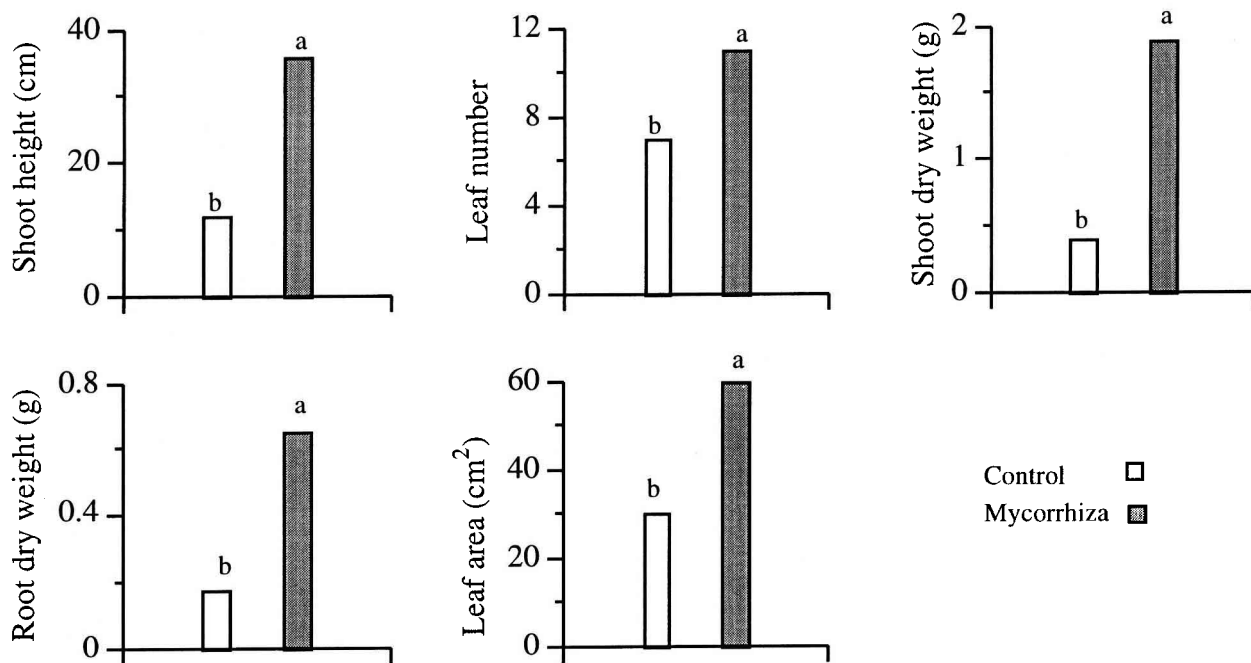


Fig 3. Effect of arbuscular mycorrhizal inoculation on several growth parameters (root dry weight, shoot height, leaf area, leaf number, shoot dry weight) of *Annona cherimola* plants micropropagated from a juvenile rootstock. Inoculation was done at the beginning of the acclimatization period and plantlets were 20 weeks old when harvested. For each parameter, mean values (20 replicates) sharing the same letter did not differ significantly ($P < 0.05$) according to Tukey's test.

plantlets more efficient for nutrient acquisition and better equipped to cope with the stress situations characteristic of the micropropagation processes (Berta et al, 1995). They also support

the cooperation of both biotechnological approaches (micropropagation and mycorrhization) towards a more sustainable horticultural production (Azcón-Aguilar and Barea, 1996; Lovato et al, 1996).

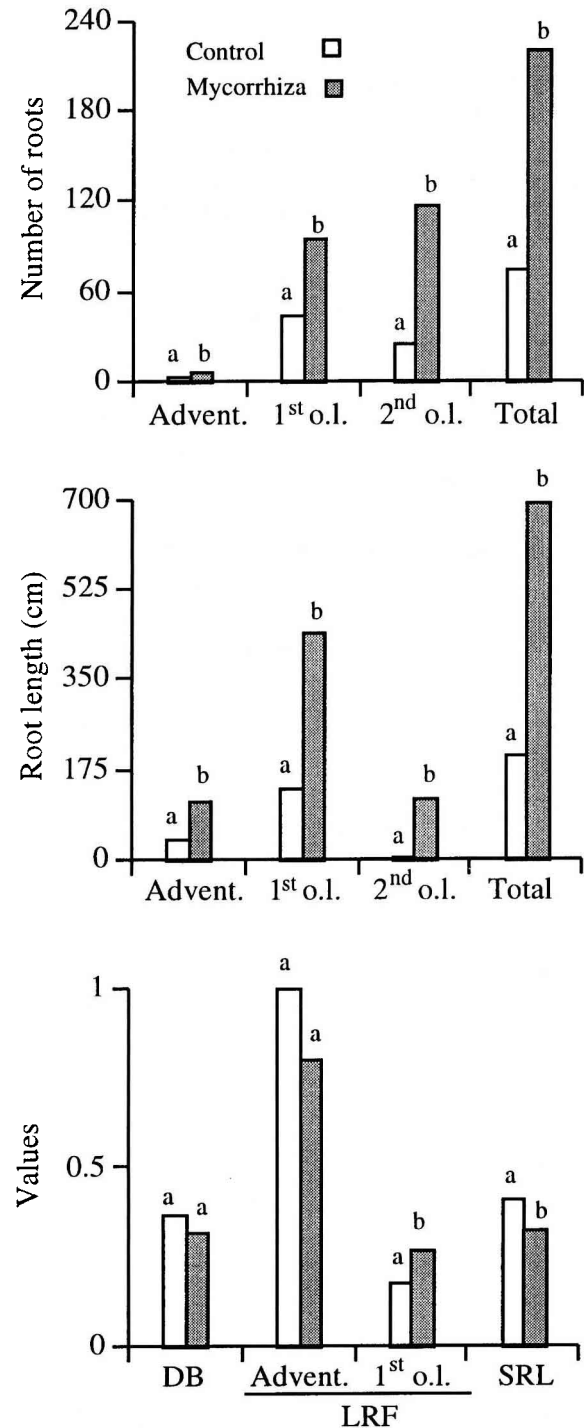


Fig 4. Effect of arbuscular mycorrhizal inoculation on root system morphology of *Annona cherimola* plants micropropagated from a juvenile rootstock. Inoculation was done at the beginning of the acclimatization period and plantlets were 20 weeks old when harvested. For each parameter, mean values (20 replicates) sharing the same letter did not differ significantly ($P < 0.05$) according to Tukey's test. Advent.: adventitious roots; 1st o.l.: first-order laterals; 2nd o.l.: second-order laterals; DB: degree of branching; LRF: lateral root frequency; SRL: specific root length.

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