Effects of in vivo mycorrhization on micropropagated fruit tree rootstocks

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Abstract

In order to establish an endomycorrhizal symbiosis on micropropagated plants and to evaluate its effect on plant growth and survival, three rootstocks belonging to the Prunus genus were used: M51 (P. dulcis), GF677 (P. persica × P. dulcis) and cv Citation (P. domestica × P. persica). The plants were micropropagated and in vitro rooted. Spores of Glomus mosseae (LMSS) and Scutellospora calospora (SCLS) and root fragments of clover or corn mycorrhized by them were used as inocula. Plants were inoculated in: (1) the early acclimatization phase; (2) the transplant phase; (3) in both phases. Two similar experiments were conducted for each plant genotype, differing only in the type of inoculum. Plants were inoculated for each plant genotype, differing only in the type of inoculum. One month after transplanting, plant survival indexes, mycorrhization percentages and growth parameters for each plant were recorded. No significant effects were observed on survival. In M51 no mycorrhizae were found and no significant growth effects were recorded in either experiments. LMSS infected GF677 in the second experiment, but not in the first, when inoculated in the transplant phase, with a better establishment when a second inoculation was done. Citation was not colonized by LMSS in either experiment. SCLS was able to establish mycorrhizal infection of Citation and GF677 only in the first experiment, and only when inoculated in the acclimatization phase. There were significant positive effects of the inoculation phase, the inoculum strain and the plant genotype on growth parameters, regardless of whether symbiosis was established or not. In conclusion, M51 did not seem to be dependent on the establishment of an endomycorrhizal symbiosis, at least with the fungal strains used in our experiments. On the contrary, Citation and GF677 seemed positively influenced by inoculation, even when there was no infection by the mycorrhizas. This fact is probably due to growth promoter components of the inocula — possibly microflora. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Glomus mosseae; Scutellospora calospora; Micropropagated rootstocks; Mycorrhization; Acclimatization

1. Introduction

Most fruit trees and horticultural plants are naturally associated with arbuscular mycorrhizal fungi (AMF). Micropropagation techniques do not permit a spontaneous mycorrhization; besides, the high nutrient contents of the cultural media ensure a good growth of the plants. Nevertheless, many micropropagated species are mycorrhiza-dependent and many problems can arise from the absence of symbiotic fungi during the acclimatization phases in the greenhouse. For micropropagated plants, the role of the endomycorrhization in overcoming transplant stress can be important (Hooker et al., 1994; Subhan et al., 1998). Positive effects on survival and growth were seen after AM inoculation both in micropropagated...
horticultural plants and fruit species, as in *Gerbera* (Wang et al., 1993), avocado (Vidal et al., 1992), apple (Granger et al., 1983; Branzanti et al., 1992), cassava (Azcón-Aguilar et al., 1997), grapevine (Schubert et al., 1988; Lovato et al., 1992), kiwifruit (Schubert et al., 1992), peach and pear (Rapparini et al., 1994, 1996), pineapple (Guillemin et al., 1992; Lovato et al., 1992), plum (Fortuna et al., 1992), *Prunus cerasifera* (Berta et al., 1995), strawberry (Vestberg, 1992) and walnut (Dolcet-Sanjuan et al., 1996). Improved performance of endomycorrhizal plants is usually due to enhanced uptake of nutrients, especially phosphorous. Moreover, AM can enhance resistance to pathogens (Dehne, 1982; Cordier et al., 1996) and protect against nematode parasitism, as reported for banana (Pinochet et al., 1997) and plum (Pinochet et al., 1998).

Three *Prunus* genus rootstocks, M51 (*P. dulcis*), GF677 (*P. persica* × *P. dulcis*) and Citation (*P. domestica* × *P. persica*) were included in this study. Little information is available on the agronomic requirements of M51 and Citation. M51 is under selection at the ISF as almond rootstock. Citation has been introduced in Italy from the USA recently and it is under evaluation as a plum and apricot rootstock (Loreti and Massai, 1995). GF677 is particularly suited to hard environment, with poor fertility, low water stress and high calcareous contents (up to 12%). It is widely used as a peach rootstock and is propagated exclusively by micropropagation (Loreti and Massai, 1995).

Since the main problems for the in vivo establishment of the micropropagated plants are the survival rate and the growth rate, the purpose of this investigation was to evaluate the influence of mycorrhization on plant survival and the growth responses of vitro-plants to inoculation applied in different phases of the acclimatization.

### 2. Materials and methods

M51, GF677 and Citation plants were micropropagated, multiplied and rooted in vitro in appropriate media (Zuccherelli, 1979; Caboni and Damiano, 1994; Liberali and Damiano, 1998), at the following growth conditions: 22°C; photoperiod, 16 h; light intensity, 31.25 µE m⁻² s⁻¹. After the rooting, the plants were transferred to the greenhouse in 80 ml pots containing a sterile mixture of peat and perlite (7:1 by volume).

Inoculum was obtained from cultures of *Glo- mus mosseae* (LMSS) and *Scutellospora calospora* (SCLS) on clover (first experiment) and corn (second experiment). Sand containing spores and infected root fragments was added as middle layer to the transplant substrate in the ratio 1:8 by volume.

Three inoculation times were used: (1) at the early weaning phase, when plants were transferred from in vitro to in vivo (Iₜ); (2) at the transplant phase, during the transfer from the closed tunnel to the open bench, 1 month after the start of acclimatization (Iₜ); (3) at both stages (I_D). Two experiments were conducted for each *Prunus* genotype.

The number of plants surviving transplantation was recorded after 1 month. Plants were sacrificed and the area of leaves and root systems were digitized by a scanner (Microtek ScanMaker IIHR, by Microtek International, Taiwan), at 200 dpi and 400 dpi, respectively and analyzed with a specific software (DT-SCAN, Delta-T devices, Cambridge, UK) for the determination of the surfaces. Dry weight of shoots, leaves and roots was determined after oven drying at 70°C for 48 h. The following were recorded for each plant: shoot dry weight (SDW, mg), root dry weight (RDW, mg), plant height (mm), leaf area (LA, mm²), root length (RL, mm), mean root diameter (mm).

From these data the following indices were also calculated: shoot/root ratio (S/R), specific root length (SRL, cm/mg), leaf area/root length ratio (LA/RL, mm²/mm).

Mycorrhizal status was evaluated through the microscope on subsamples, according to the method of McGonigle et al. described in Brundrett et al. (1994), after clearing in 10% KOH, acidification with 1% HCl for 24 h, and staining 20 min at 70°C with trypan blue 0.05%, according to Koske and Gemma (1989).

Statistical analyses were performed with NCSS statistical software (Hintze, 1996) with one-way analysis of variance respectively on inoculum type (control, LMSS and SCLS) and time of inoculation; differences were tested by Fisher’s LSD and Kruskal-Wallis Z.

### 3. Results

No significant effects were observed on plant survival (significance evaluated by Fisher’s exact test) (Fig. 1). M51 was never mycorrhized and in the
Fig. 1. Survival percentages (%) at the transplant phase (about 1 month after the inoculation). The micropropagated plants were inoculated during the transfer from the in vitro to the in vivo conditions (early weaning phase).

Table 1
Growth parameters observed in Citation in the first experiment

<table>
<thead>
<tr>
<th>Treatments</th>
<th>IT</th>
<th>Number of plants</th>
<th>Mycorrhizal infection (%)</th>
<th>RL (mm)</th>
<th>LA/RL (mm²/mm)</th>
<th>SRL (cm/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>–</td>
<td>8</td>
<td>0.0</td>
<td>9005.1±765.5 b</td>
<td>23.5±1.9 a</td>
<td>5.1±0.6 b</td>
</tr>
<tr>
<td><em>G. mosseae</em></td>
<td>I_W</td>
<td>4</td>
<td>0.0</td>
<td>6225.9±1002.4 ab</td>
<td>38.8±7.2 ab</td>
<td>4.4±0.8 ab</td>
</tr>
<tr>
<td></td>
<td>I_T</td>
<td>8</td>
<td>0.0</td>
<td>5547.4±906.8 a</td>
<td>31.7±9.0 ab</td>
<td>4.9±0.4 b</td>
</tr>
<tr>
<td></td>
<td>I_D</td>
<td>4</td>
<td>0.0</td>
<td>6180.7±881.5 ab</td>
<td>26.5±2.3 ab</td>
<td>4.3±0.7 ab</td>
</tr>
<tr>
<td><em>S. calospora</em></td>
<td>I_W</td>
<td>3</td>
<td>10.5</td>
<td>3784.3±1305.2 a</td>
<td>47.2±2.8 b</td>
<td>3.1±0.9 a</td>
</tr>
<tr>
<td></td>
<td>I_T</td>
<td>8</td>
<td>0.0</td>
<td>5216.0±539.9 a</td>
<td>36.3±3.7 ab</td>
<td>3.3±0.3 a</td>
</tr>
<tr>
<td></td>
<td>I_D</td>
<td>3</td>
<td>0.0</td>
<td>5310.2±2342.3 a</td>
<td>25.1±0.9 ab</td>
<td>4.2±0.5 ab</td>
</tr>
</tbody>
</table>

*IT*=inoculation time; *I_W*=inoculation at the early weaning phase; *I_T*=inoculation at the transplant phase; *I_D*=double inoculation. RL=root length; LA/RL=leaf area/root length ratio; SRL=specific root length. Different letters indicate significant differences (*p*<0.05).

other species mycorrhiza establishment was poor (Tables 1–3). Citation was never infected by LMSS (Tables 1 and 2). LMSS infected GF677 only in the second experiment, when inoculated in the transplant phase, with a better establishment on double inoculation (Table 3). SCLS established mycorrhizal infection on Citation and GF677 only in the first experiment, when inoculated in the weaning phase (Tables 1–3).

In the first experiment no significant effects were recorded in Citation (Table 1), even if a general reduction, especially in root parameters, was evident. A reduction in RL and SRL, which is an inverse, indirect

Table 2
Growth parameters observed in Citation in the second experiment

<table>
<thead>
<tr>
<th>Treatments</th>
<th>IT</th>
<th>Number of plants</th>
<th>Mycorrhizal infection (%)</th>
<th>SDW (mg)</th>
<th>RDW (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>–</td>
<td>9</td>
<td>0.0</td>
<td>269.0±46.9 ab</td>
<td>85.0±11.0 a</td>
</tr>
<tr>
<td><em>G. mosseae</em></td>
<td>I_W</td>
<td>4</td>
<td>0.0</td>
<td>213.5±20.4 a</td>
<td>97.9±9.5 ab</td>
</tr>
<tr>
<td></td>
<td>I_T</td>
<td>8</td>
<td>0.0</td>
<td>311.0±26.5 ab</td>
<td>134.2±10.8 bc</td>
</tr>
<tr>
<td></td>
<td>I_D</td>
<td>5</td>
<td>0.0</td>
<td>220.7±52.5 ab</td>
<td>93.0±12.2 ab</td>
</tr>
<tr>
<td><em>S. calospora</em></td>
<td>I_W</td>
<td>5</td>
<td>0.0</td>
<td>509.3±75.0 c</td>
<td>155.3±13.5 c</td>
</tr>
<tr>
<td></td>
<td>I_T</td>
<td>7</td>
<td>0.0</td>
<td>268.1±57.7 b</td>
<td>106.1±19.1 ab</td>
</tr>
<tr>
<td></td>
<td>I_D</td>
<td>7</td>
<td>0.0</td>
<td>376.2±57.7 b</td>
<td>138.4±18.8 c</td>
</tr>
</tbody>
</table>

*IT*=inoculation time; *I_W*=inoculation at the early weaning phase; *I_T*=inoculation at the transplant phase; *I_D*=double inoculation. SDW=shoot dry weight; RDW=root dry weight. Different letters indicate significant differences (*p*<0.05).
<table>
<thead>
<tr>
<th>Treatments</th>
<th>IT</th>
<th>Number of plants</th>
<th>Mycorrhizal infection (%)</th>
<th>Root mean diameter (mm)</th>
<th>RDW (mg)</th>
<th>Plant height (mm)</th>
<th>SDW (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>–</td>
<td>6</td>
<td>4</td>
<td>0.0</td>
<td>0.0</td>
<td>0.59±0.07 a</td>
<td>0.17±0.03 a</td>
</tr>
<tr>
<td><em>G. mosseae</em></td>
<td>Iw</td>
<td>5</td>
<td>6</td>
<td>0.0</td>
<td>0.0</td>
<td>0.89±0.13 bcd</td>
<td>0.55±0.05c</td>
</tr>
<tr>
<td></td>
<td>If</td>
<td>6</td>
<td>6</td>
<td>0.0</td>
<td>7.4</td>
<td>0.74±0.07 abc</td>
<td>0.16±0.03 a</td>
</tr>
<tr>
<td></td>
<td>Id</td>
<td>5</td>
<td>7</td>
<td>0.0</td>
<td>14.2</td>
<td>0.96±0.05 cd</td>
<td>0.30±0.10 ab</td>
</tr>
<tr>
<td><em>S. calospora</em></td>
<td>Iw</td>
<td>3</td>
<td>6</td>
<td>17.7</td>
<td>0.0</td>
<td>0.83±0.10 abcd</td>
<td>0.62±0.09 c</td>
</tr>
<tr>
<td></td>
<td>If</td>
<td>6</td>
<td>5</td>
<td>0.0</td>
<td>0.0</td>
<td>0.68±0.07 ab</td>
<td>0.44±0.07 bc</td>
</tr>
<tr>
<td></td>
<td>Id</td>
<td>4</td>
<td>6</td>
<td>23.9</td>
<td>0.0</td>
<td>1.05±0.18 d</td>
<td>0.66±0.07 c</td>
</tr>
</tbody>
</table>

*IT= inoculation time; Iw= inoculation at the early weaning phase; If= inoculation at the transplant phase; Id= double inoculation. RDW= root dry weight; SDW= shoot dry weight. Different letters indicate significant differences (p<0.05).
measure of mean root diameter, and an increase in LA/RL of inoculated plants were recorded. Such effects were statistically significant in mycorrhizal plants inoculated at the weaning phase with SCLS.

In the second experiment (Table 2), although no plants were mycorrhized, their growth increased significantly in the soils inoculated with SCLS in the weaning phase (Fig. 2), and also in the case of double inoculation. Plants grown on SCLS inoculated soil had a higher SDW and RDW.

In the first experiment on GF677, infection seemed to induce a general growth promotion, especially in the case of double inoculation (Table 3).

The most evident effects on growth parameters were seen in the second experiment (Table 3), where the plant height and the shoot dry weight as well as the root diameter increased in the inoculated plants, independently of the establishment of the symbiosis. The effects were significant when the inoculum was repeated; better performances were always associated to SCLS also in the weaning phase (Fig. 2). In M51 no growth effect was recorded in either experiment (Fig. 2).

4. Discussion

The beneficial effects of AMF inoculation of micro-propagated plants are widely documented throughout the literature. The technique to inoculate at the start of the acclimatization stage is normally employed, due to its easier feasibility (Ravolanirina et al., 1989). In some cases a better plant response is produced when the inoculation is carried out at the transplant phase (Vidal et al., 1992). The colonization of the roots takes place only in young secondary roots and these can be produced later during the ex vitro development (Azcón-Aguilar and Barea, 1997). In our experiments, the frequency of infection is seen to be very low. Possible causes could be insufficient time for the development of infection before data collection (8–10 weeks when applied in the weaning phase, just 1 month when applied at transplanting). In fact the time course for development of the infection and the entity of the infection can vary depending the fungal species (Berta et al., 1995) and the mycorrhizal effects may become evident in later stages (de la Viña et al., 1996).

It is possible that a longer period of time is needed to observe visible effects. A low root colonization percentage is reported for GF677 infected with *G. mosseae* after 72 days (Schubert et al., 1996), while other authors reported that the frequency of infection after 7 months can reach 92% (Rapparini et al., 1994).

Another aspect to consider is the effect of the amount of inoculum, which can affect root infection (Schubert et al., 1992). The volume ratio of soil/inoculum used could be insufficient for a massive mycorrhizal establishment. Moreover the characteristics...
of the substrates (pH, phosphorous content) used in the acclimatization can affect the success of the mycorrhization: they must ensure the spore germination and the mycelial growth as well as the plant development (Azcón-Aguilar and Barea, 1997).

The failure of mycorrhization can probably explain the absence of significant differences on survival rate between inoculated and uninoculated plants.

Nevertheless, some differences could be observed among treatments, in terms of plant growth, except than for M51. The main effect was on root diameter that was generally increased by the inoculum, as shown by the reduced specific root length. This is also a commonly reported mycorrhiza-effect, observed in micropropagated plants (Berta et al., 1995; Azcón-Aguilar et al., 1996). The root diameter increase was significant in Citation in the mycorrhizal plants (first experiment) and also in GF677 in inoculated but not mycorrhizal plants. In the second experiment the inoculum seemed to positively influence the biomass of mycorrhizal plants. In the micropropagated plants (Berta et al., 1995; Azcón-Aguilar et al., 1994). In our experiment this behaviour was more evident in SCLS inoculated plants.

An increased development of the shoots, in terms of height and weight, was evident especially for double inoculated plants, independently on the establishment of the symbiosis. The shoot elongation has been reported for GF677 infected with *G. mosseae* (Rapparini et al., 1994). In our experiment this behaviour was more evident in SCLS inoculated plants.

The fact that the plant growth parameters in GF677 and Citation were affected by the presence of inocula, even in the absence of mycorrhization, suggested a role attributable to the microflora commonly associated to AMF inocula, even inside spores (Bianciotto et al., 1996).

The choice of AM strain could be critical for a successful establishment of a symbiosis: further research should consider the isolation and use of the mycorrhizal strains from the same plant species on which it is intended to carry out the inoculation trials.

5. Conclusions

Mycorrhizal inocula confirm again as a proper tool for establishing the “beneficial rhizosphere”. Such procedures are even more valuable in micropropagated plants, especially in the acclimatization phase, when Plant Growth Promoting Rhizobacteria (PGPR) and symbiotic fungi may start to act as protective agents against pathogens and as plant growth promoters. Nevertheless, effective practices must consider not only the compatible fungal strain and associated microflora, but also plant characteristics, to ensure the most beneficial effects.

References


