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Infectivity and effectiveness of different species of arbuscular mycorrhizal fungi in micropropagated plants of Mr S 2/5 plum rootstock

P Fortuna [1], S Citernesi [2], S Morini [1], M Giovannetti [3], F Loreti [1]

[1] *Universita' di Pisa, Dipertimento di Coltivazione e Difesa delle Specie Legnose, Sezione di Coltivazioni Arboree, Pisa;*
[2] *Scuola Superiore di Studi Universitari e Perfezionamento "S Anna", Pisa;*
[3] *Universita di Pisa, Istituto di Microbiologia Agraria e Centro di Studio per la Microbiologia del Suolo, Pisa, Italy*

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Summary — Micropropagated plum plants (*Prunus cerasifera* Ehrh clone MrS 2/5) were inoculated with 4 different species of arbuscular mycorrhizal fungi (*Glomus mosseae, G caledonium, G coronatum* and *Glomus* strain A6) after transfer from an *in vitro* to an *in vivo* system. The most and the least infective fungi, *G mosseae* and *G coronatum* respectively, were tested for their growth-promoting abilities with respect to the micropropagated plum plants. Both fungi improved plant growth in comparison with uninoculated plants. *G coronatum* showed a prolonged lag phase during the early stage of mycorrhizal infection and affected growth less than *G mosseae* at the first harvest (4 wk). At the second harvest (14 wk), when mycorrhizal infection by both fungi was high, *G coronatum* was as effective as *G mosseae* in promoting plant growth of *P cerasifera*. The importance of a rapid mycorrhizal infection during the acclimatization phase of micropropagated plants is discussed in this paper.

infectivity / effectiveness / AM fungi / micropropagated plant / *Prunus cerasifera*

Résumé — Infectivité et efficacité de différentes espèces de champignons mycorhiziens arbusculaires chez les vitroplants de porte-greffe de prunier MrS 2/5. *Des vitroplants de prunier (*Prunus cerasifera *Ehrh clone Mr.S.2/5) ont été inoculés* post-vitro *avec 4 champignons endomycorhizogènes :* Glomus mosseae, G caledonium, G coronatum *et* Glomus *souche A6, dans le but d'évaluer les paramètres d'infection. Les champignons le plus infectieux* (G mosseae) *et le moins infectieux* (G coronatum) *ont été respectivement sélectionnés et leur capacité d'influencer la croissance de vitroplants de prunier a été évaluée. Ces 2 champignons ont un effet positif sur la croissance des plants inoculés.* G coronatum *montrait un temps de latence prolongé pendant les premiers stades d'infection mycorhizienne; on ne constate pas au premier prélèvement (4 semaines) une augmentation de croissance végétale comparable à celle obtenue avec* G mosseae. *Au deuxième prélèvement (14 semaines), quand les 2 champignons présentaient un taux élevé d'infection mycorhizienne,* G coronatum *se révélait aussi efficace que* G mosseae *pour stimuler la croissance de* P cerasifera. *Ce travail permet de discuter de l'importance d'une infection mycorhizienne rapide au cours de la phase d'acclimatation de vitroplants.*

***infectivité / efficacité / champignon mycorhizien arbusculaire / plant micropropagé /* Prunus cerasifera**

INTRODUCTION

In arbuscular mycorrhizal (AM) symbiosis, the ability of a fungal species to infect a host plant both rapidly and extensively may play an important role in determining the success of the relationship. The main parameters which can be utilized to measure this type of host affinity are fungal infectivity, plant mycotrophy and the growth promoting ability of AM fungi.

The effectiveness of combining different AM fungi and host plants has been largely studied in plants (Plenchette *et al,* 1982; Geddeda *et al,* 1984; Doud Miller *et al,* 1985; Schubert and Cammarata, 1986; Giovannetti and Lioi, 1987, including micropropagated plants (Morandi *et al,* 1979; Pons *et al,* 1983; Gianinazzi *et al,* 1986; Ravolanirina *et al,* 1989). Nevertheless, little is known about the early stages of AM infection in these plants, though early root colonization has been demonstrated to be very important during the delicate acclimatization phase (Ravolanirina *et al,* 1989; Gianinazzi *et al,* 1990).

In the present study we tested the fungal infectivity of 4 species of AM fungi, *Glomus caledonium* (Nicol et Gerd) Trappe et Gerd, *G coronatum* Giovannetti, *G mosseae* (Nicol et Gerd) Gerd et Trappe, and a strain of *Glomus*, denominated A6, on micropropagated plants of a rootstock clone (MrS 2/5) selected from *Prunus cerasifera* Ehrh seedlings. The most and the least infective fungi were selected to investigate whether and how a delay in mycorrhizal infection would affect their growth-promoting abilities.

MATERIALS AND METHODS

Experiment 1

Shoots of the MrS 2/5 plum clone were grown for 3 wk *in vitro* in an appropriate rooting medium (Morini *et al,* 1990). Microplants were then transplanted into pots filled with a substratum composed of 1 part sterile sand and 1 part crude AM inoculum (v:v). Crude AM inoculum consisted of infested soil which contained (as infective propagules) spores, external mycelium and infected root fragments obtained from alfalfa pot-cultures inoculated with *G caledonium, G coronatum, G mosseae* or *Glomus* A6. Plants were grown in a growth chamber with a 14-h day according to the procedure generally adopted for acclimatization (Morini and Barbieri, 1986). Two wk after inoculation, plantlets were harvested and roots stained with trypan blue in lactic acid (Phillips and Hayman, 1970). Infected root pieces, selected under the dissecting microscope, were mounted on slides and the morphology and frequency of appressoria were observed using a Reichert–Jung Polyvar microscope.

Experiment 2

Micropropagated plantlets of the MrS 2/5 plum clone were transplanted into 0.1-l pots containing a substratum consisting of 1 part crude AM inoculum, 1 part sterile peat and 1 part sterile perlite (v:v:v). The crude inoculum was obtained from sunflower pot cultures inoculated with *G coronatum* and *G mosseae*. In uninoculated control plants, the AM inoculum was replaced by the same sterilized soil utilized for pot culture production (a sandy soil, pH 7.3, 16.4 ppm available P). Plants were maintained in a greenhouse, from June to September, adopting a suitable procedure for acclimatization (Morini and Barbieri, 1986). One month after transplanting, the content of each pot was transferred into a 1-l pot containing the same soil used in the control treatment. Plants were fertilized twice a week with 50 ml/pot of half-strenght Hoagland solution without phosphorus. The number of surviving plants was recorded after 30 d. Two harvests were performed, 4 and 14 wk after transplanting. At each harvest, fresh shoot and root mass, and height of 10 replicate plants per treatment were measured. AM infection was assessed after staining the roots with trypan blue (Phillips and Hayman, 1970; Giovannetti and Mosse, 1980). Dry shoot mass, measured at the second harvest, was obtained after drying the shoots for 48 h at 90 °C. All data from the 2 experiments were statistically analyzed using 1-way analysis of variance and averages were separated by Tuckey's test.

RESULTS

Experiment 1

Two wk after inoculation, *G caledonium, G mosseae* and *Glomus* A6 had already infected plum roots, and many appressoria were clearly visible on the root surface. In contrast *G coronatum* had developed a much lower number of appressoria than that formed by the other AM fungi (table I).

Three main shapes of appressoria were identified: lenticular, amoeboid and mamillary. The mamillary shape was characteristically formed by *Glomus* A6. Significant differences in the length of appressoria, formed by different endophytes, were found in the lenticular and amoeboid shape. The number of appressoria/mm root formed by *G mosseae* was significantly higher than those of the other fungal species (table I).

Table I. Shape, number and occurrence of appressoria formed by *Glomus mosseae*, *G caledonium*, *Glomus* A6 or *G coronatum* on roots of plum rootstock 2 wk after inoculation.

Appressoria	Glomus mosseae	Glomus caledonicum	Glomus *A6*	Glomus coronatum
Number (per mm root)	9.72[a]*	6.60[b]	6.76[b]	0.25[c]
Lenticular				
Occurrence (%)	56	55	53	–
Length (μm)	28.75[a]	21.27[b]	27.18[a]	–
Width (μm)	11.50[a]	11.69[a]	11.09[a]	–
Amoeboid				
Occurrence (%)	30	37	–	–
Length (μm)	30.53[a]	22.49[b]	–	–
Width (μm)	20.49[a]	17.02[a]	–	–
Mamillary				
Occurrence (%)	–	–	11	–
Length (μm)	–	–	62.80	–
Width (μm)	–	–	15.33	–
Others				
Occurrence (%)	14	8	36	–

* Values in rows followed by the same letter do not differ significantly at $P = 0.05$.

Experiment 2

The most and the least infective AM fungal species in Experiment 1, *G mosseae* and *G coronatum* respectively, were tested for their ability to improve plant growth after transplanting microplants from the *in vitro* to an *in vivo* culture. After 4 wk growth the number of surviving plants was ≈ 100% in all treatments. The fresh mass of the plant roots inoculated with *G mosseae* was significantly higher than that of the uninoculated controls and of plant roots uninoculated with *G coronatum* (table II). The fresh mass and height of shoots did not differ, regardless of the treatment (tables II, III). Percentage of mycorrhizal infection was 8 and 38.5% of root length in *G coronatum* and in *G mosseae* infected plants respectively (table III). At the second harvest (14 wk growth), control plants showed a significantly lower fresh and dry mass whereas mycorrhizal plants showed comparable growth increases (table II). It is important to note that the growth of control plants was blocked after transplanting, and shoot apices failed to resume activity during the entire experiment. At the final harvest, the percentage of mycorrhizal infection in plants inoculated with *G coronatum* was similar to that obtained in plants inoculated with *G mosseae* (table III).

Table II. Growth of plum rootstock 4 and 14 wk after inoculation with *Glomus mosseae* or *G coronatum* as compared to uninoculated control plants.

Treatment	*Fresh mass (g)*		*Dry mass (g)*
	Shoot	*Root*	*Shoot*
4 wk			
G mosseae	0.65[a]*	0.45[a]	–
G coronatum	0.57[a]	0.34[b]	–
Control	0.55[a]	0.34[b]	–
14 wk			
G mosseae	3.97[a]*	3.75[a]	1.20[a]
G coronatum	3.76[a]	3.58[a]	1.11[a]
Control	0.70[b]	1.45[b]	0.27[b]

* For each growth period values in columns followed by the same letter do not differ significantly at $P = 0.05$.

Table III. Height and percentage of infected root length in plum rootstock 4 and 14 wk after inoculation with *Glomus mosseae* or *G coronatum* as compared to uninoculated control plants.

Treatment	Height (cm)		% infection	
	After 4 wk	After 14 wk	After 4 wk	After 14 wk
Glomus mosseae	5.7[a*]	20.6[a]	38.5[a]	71.0[a]
Glomus coronatum	6.5[a]	21.0[a]	8.0[b]	66.0[a]
Control	5.5[a]	5.3[b]	0[c]	0[b]

* Values in columns followed by the same letter do not differ significantly at $P = 0.05$.

DISCUSSION AND CONCLUSION

The results of this experiment show that a delay in mycorrhizal infection can negatively affect the growth of the micropropagated plum rootstock clone MrS2/5 during the early acclimatization stages. *G coronatum* infected roots very slowly, forming a low number of appressoria on the root surface and colonizing only 8% of the length of the plum roots after 4 wk. These data confirm previous results concerning the erratic germinability and the low infectivity of this endophyte (Giovannetti *et al,* 1991). The prolonged lag phase at the beginning of mycorrhiza establishment led to poor root infection, which failed to give early growth increases. In fact, only 4 wk after transplanting, fresh root mass of plantlets inoculated with the highly infective endophyte *G mosseae* were significantly higher than those of plants inoculated with *G coronatum*. However, at the later harvest (14 wk), infection by *G coronatum* (66%) was as extensive as that of *G mosseae* (71%) and both fungi were equally effective in promoting plant growth. Growth differences between mycorrhizal and control plants were very large, confirming the importance of mycorrhizal inoculation for micropropagated plants (Ravolanirina *et al,* 1989; Gianinazzi *et al,* 1990). The high amount of inoculum used in both experiments overcame problems linked to differences in the infection potential of the fungi and which can occur when low concentrations of inoculum are used (Porter, 1979; Daniels *et al,* 1981; Lioi and Giovannetti, 1987).

The large differences obtained in fresh and dry mass between mycorrhizal and uninoculated control plants also resulted from differences in the growth behaviour of the plants. In fact, micropropagated plants frequently show blocked apical growth just after transplanting ("transplant shock"), and renewed growth occurs at different periods, depending on nutritional and environmental conditions as, for example, reported for peach plants (Morini and Concetti, 1984). The plum rootstock clone MrS 2/5 showed similar behaviour, but with mycorrhizal inoculation renewed apical growth began in most plants the first month after transplanting, whilst uninoculated controls did not actively grow and apices remained blocked up to the end of the experiment. This is a very interesting result, probably indicating that additional effects other than nutritional ones such as hormone balance modifications are produced by mycorrhizal symbiosis as suggested by Allen *et al* (1980, 1982). Nevertheless, further studies are necessary to determine the physiological basis of the positive role of the mycorrhizal infection in promoting apical activity of transplanted micropropagated plum plants.

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