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Mycorrhizal inoculation of micropropagated woody legumes used in revegetation programmes for desertified Mediterranean ecosystems

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Summary — Micropropagation of *Anthyllis cytisoides* (L) and *Spartium junceum* (L) 2 shrub legumes of ecological interest, was accomplished following standard procedures. Inoculation with the arbuscular mycorrhizal fungus *Glomus fasciculatum,* early during a *post vitro* weaning stage shortened the acclimatization process by 8 wk and particularly for *Anthyllis* improved survival, growth and nutrient status of microplants after outplanting. The level of mycorrhizal colonization of microplants was about the same as that for plants produced from seeds.

micropropagation / *Glomus fasciculatum* / *Anthyllis* / *Spartium* / desertification

Résumé — Inoculation mycorhizienne de légumineuses ligneuses micropropagées utilisées dans les programmes de remise en végétation des écosystèmes desertifiés méditerranéens. *La micropropagation d'*Anthyllis cytisoides *L, et de* Spartium junceum *L 2 légumineuses intéressantes du point de vue écologique a été réalisée suivant des techniques usuelles. L'inoculation avec* Glomus fasciculatum *tôt pendant le sevrage* post vitro *raccourcit l'acclimatation de 8 semaines, et en particulier pour* Anthyllis, *améliore la survie, la croissance et la nutrition des microplantes après leur plantation au dehors. Le niveau de mycorhization était à peu près le même que pour des plantes issues de semis.*

micropropagation* / Glomus fasciculatum / Anthyllis / Spartium / *désertification

INTRODUCTION

Micropropagation techniques have been recently applied for clonal selection and mass production of mycotrophic (arbuscular mycorrhizal) woody species of ecological interest. The available information mainly concerns tree species and reafforestation programmes in Mediterranean ecosystems (Morte *et al,* 1992; Nouaim *et al,* 1992). In this ecological context, and since it has been found that for some desertified semi-arid areas it would be more appropriate to develop shrublands rather than attempt afforestation (Francis and Thornes, 1990), production of plant material of the appropriate shrubs species to be used in revegetation has become critical. As far as we know there is no published information regarding micropropagation of shrubs of interest to recover degraded lands.

Previous studies have shown that 2 native shrub legumes, *Anthyllis cytisoides* (L) and *Spartium junceum* (L), could be good candidate species for a reclamation strategy in the Mediterranean region (Barea *et al,* 1990b). Mycorrhizal inoculation with Glomales fungi has been found highly effective in improving plant establishment and survival. Seeds of these plant species are not commercially available so that only field-grown individuals with recognized ecological value can be used as a seed source. Micropropagation therefore constitutes a promising technique for mass production. The aim of the present study was to develop *in vitro* propagation of these 2 species and to ascertain whether an effective mycorrhizal symbiosis could be obtained in rooted micropropagated plantlets by inoculation with appropriate arbuscular mycorrhizal (AM) fungi early on during a *post vitro* weaning stage.

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MATERIAL AND METHODS

Plant material and culture conditions

Anthyllis cytisoides (L) and *Spartium junceum* (L) are 2 shrub legumes that form part of the natural succession in southern Spain, and that are useful for land restoration (Barea *et al*, 1990b; Francis and Thornes, 1990). Seeds of these plant species were germinated on moistened sand and then grown on a soil–sand mixture in a greenhouse (Barea *et al*, 1990a).

Micropropagation of host plants

Axillary buds ≈ 0.3 cm long were excised from appropriate seedlings. These initial explants were surface disinfected (Morte *et al*, 1992) and then transferred to the basal medium of Murashige and Skoog (1962), supplemented with 6-benzyladenine (BA) (0.2 $mg.l^{-1}$) or indole-3-butyric acid (IBA) (0.1 $mg.l^{-1}$). The pH was adjusted to 5.7 before autoclaving. Cultures were grown at 25 °C with a 16-h photoperiod (Sylvania Gro-Lux fluorescent lamps) which provided a photosynthetic photon flux (PPF) of 45 $\mu E.s^{-1}.m^{-2}$. After 4 weeks' culture, shoots with visible roots were transferred to sealed glass flasks (500 ml) containing 200 ml peat–perlite medium (1:1 v/v) (Vidal *et al*, 1992). Four plantlets per flask were kept in a greenhouse for 4 or 6 wk. As indicated in figure 1, different acclimatization procedures and schedules were compared for AM inoculated plantlets and non inoculated controls.

Mycorrhizal inoculum

Glomus fasciculatum (Thaxter *sensu* Gerd) Gerd and Trappe was chosen among various AM fungal isolates after a previous selection for functional compatibility with the micropropagated plant species (Barea *et al*, 1990b). The inoculum consisted of washed mycorrhizal roots with the external mycelium and spores attached, but free from soil particles. Inoculum (1 g fresh weight) cut into 1-cm fragments was applied to each plantlet close to the root system. As figure 1 indicates, the rooted plantlets were inoculated when transferred from the flask to 250-ml pots. The potting medium consisted of a soil–sand mixture (5:2, v/v). Plants were transferred to a greenhouse and gradually exposed to reduced relative humidity by progressively removing a plastic cover during a 1–2-wk period. The non inoculated plants treated in the same way quickly died and were therefore kept for a further period of 6 wk in a misting tunnel before being transferred to the greenhouse.

Growth conditions

Inoculated and non-inoculated (control) plants (20 replicated per treatment) were grown in a greenhouse under controlled conditions with day–night temperatures ranging from 19–25 °C. Daylength was extended to 16 h by cool white fluorescent lamps, providing a PPF of 650 $\mu E.s^{-1}.m^{-2}$. Plantlets were fertilized weekly with 10 ml full strength Long Ashton nutrient solution (Hewitt, 1952) and watered daily. Percent survival, dry matter yield, P concentration in plant shoots and the degree of mycorrhizal colonization were recorded after an additional 2-wk growing period in an open greenhouse (Vidal *et al*, 1992).

Student's *t*-test was used for statistical analysis of data. In the case of the values given as percentages, data were subjected to an arcsin square-root transformation to ensure homogeneity of variances.

RESULTS

The micropropagation procedure used resulted in a 60% rooting rate. Five subcultures from the initial stock still yielded suitable plant material. As figure 1 indicates, inoculation with the arbuscular mycorrhizal fungus *Glomus fasciculatum* early on during the *post vitro* weaning shortened the acclimatization process by 8 wk.

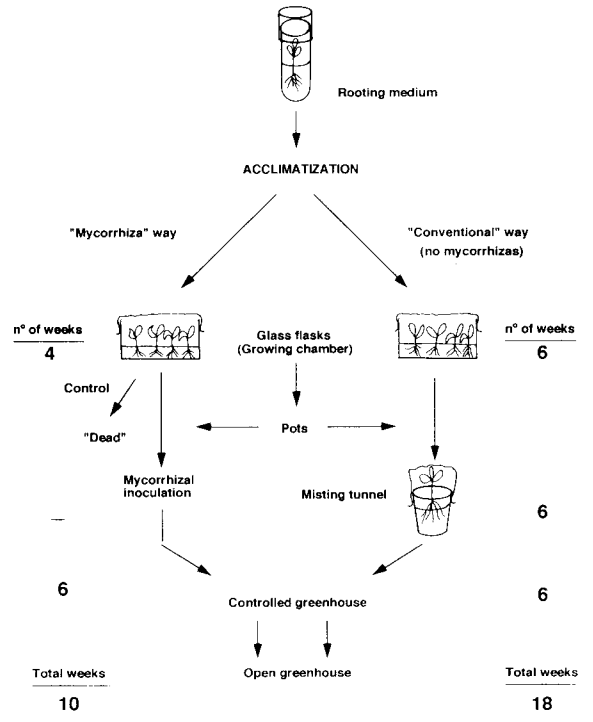

Fig 1. Acclimatization procedure and schedule.

Data summarized in figure 2 show that the survival of microplants after outplanting was generally good but AM inoculation still improved outplanting performance in *Anthyllis*. Growth, P concentration and content in this species were also significantly improved by mycorrhizal inoculation (fig 2). The degree of mycorrhizal colonization developing in roots of the vitroplants was about the same as for plantlets of the same age but propagated from seeds (fig 3).

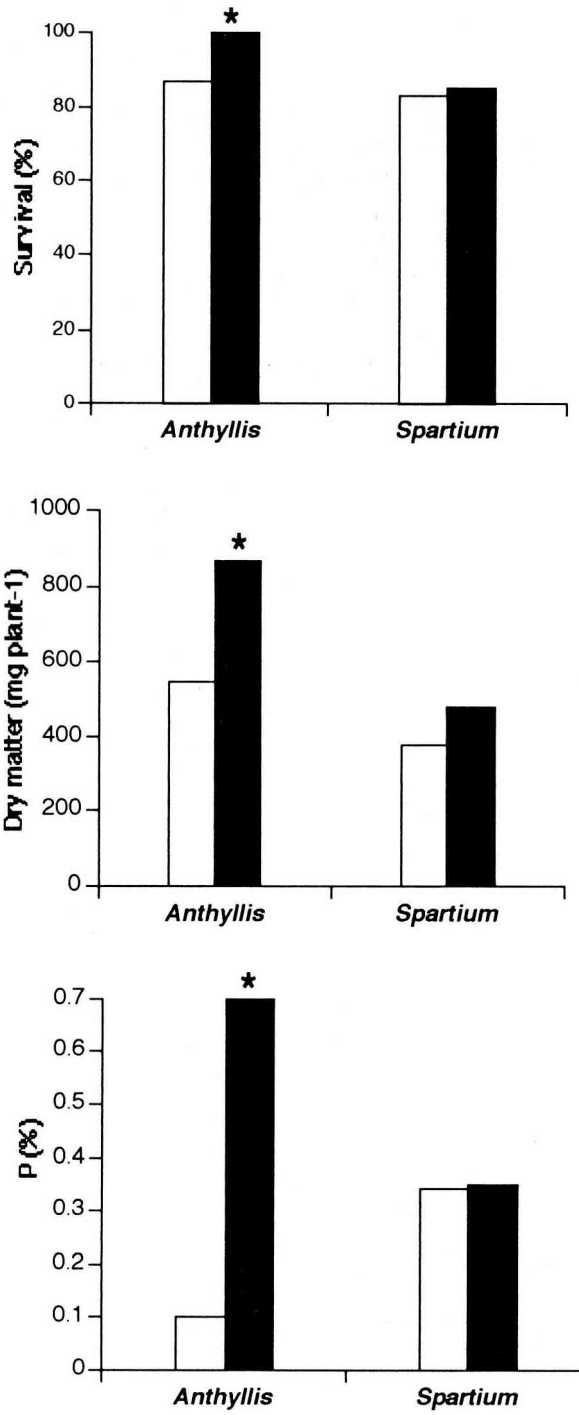

Fig 2. Effect of *Glomus fasciculatum* on survival, growth and nutrient status of microplants of *Anthyllis* and *Spartium*. Mycorrhizal plants (■) were 12-wk old, and non mycorrhizal ones (❑) 20 wk old. * Significant effects at the 5% level.

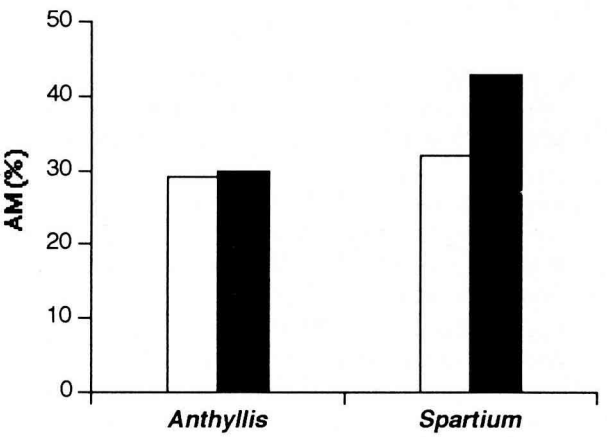

Fig 3. Comparison of the mycorrhizal colonization (percent) (AM%) of microplants (❑), and plantlets of the same age grown from seeds (■).

DISCUSSION

It is obvious that inoculation of *Anthyllis* microplants with the AM fungus *Glomus fasciculatum* early on during a *post vitro* weaning stage improved plant growth and nutrition. However, the most noticeable effect of AM inoculation which also applied to the other shrub legume, *ie Spartium*, was the shortening of the acclimatization process (fig 1). This is an important factor in micropropagation (Ravolanirina *et al,* 1989). Since the ideal situation is to induce mycorrhizal status as early as possible during the micropropagation schedule (Gianinazzi *et al,* 1990), AM inoculation was also attempted directly *in vitro* after transfer to the flask stage (fig 1). Although mycorrhizal establishment was obtained in a few cases with concomitant plantlet growth improvement, the percentage of microplant survival was in fact very low, as was the level of mycorrhizal establishment. Work is now in progress to test different inoculum formulations and substrate combinations in order to improve AM colonization at the flask stage.

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