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Preparation of gel-entrapped mycorrhizal inoculum in the presence or absence of *Yarowia lipolytica*

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Abstract

Two arbuscular mycorrhizal fungi (*Glomus deserticola* and *Glomus fasciculatum*) were entrapped in calcium alginate, alone or in combination with a phosphate-solubilizing yeast (*Yarowia lipolytica*) and, after storage for 60 days, were inoculated into soil microcosms with tomato as the test plant. The average extent of root colonization by gel-entrapped *G. deserticola* and *G. fasciculatum* were 32 ± 5.6 and 24 ± 12.1%, respectively. Improved infective potential and colonization efficiency were observed when *Y. lipolytica* was co-entrapped with the mycorrhizal fungi. The best value, 49%, of mycorrhizal colonization was in roots of plants inoculated with *G. deserticola* co-entrapped with *Y. lipolytica*.

Introduction

Arbuscular mycorrhizal (AM) fungi are ubiquitous in soils and serve as a natural link between the roots of most of the major plant families and soil (Smith & Read 1997). Introducing a source of AM fungal inoculum improves the efficiency of agricultural and horticultural practices. However, establishment of AM fungi depends on abiotic factors such as soil pH, nutrient availability, temperature, etc. In addition, soil microbial commuties also affect root mycorrhizal colonization. Application of mixed inoculants resulted in synergistic effects benefiting plant growth by a number of activities which also improve the physiological and biochemical characteristics of all microorganisms involved (Bashan 1998).

The aim of this study was to determine the infective potential of *Glomus deserticola* and *Glomus fasciculatum* entrapped in calcium-alginate in the presence or absence of the phosphate-solubilizing yeast cells of *Yarowia lipolytica*.

Materials and methods

Microorganisms

The arbuscular mycorrhizal (AM) fungi used in this experiment were *Glomus deserticola* and *Glomus fasciculatum*. Mycorrhizal roots of stock pot cultures of lettuce (*Lactuca sativa* L.) colonized to between 80 and 90% by each one of the mycorrhizal fungi were used for immobilization. The yeast culture, applied as a mycorrhiza-helper microorganism, was *Yarowia lipolytica*, which was grown further and prepared for gel entrapment as described previously (Vassileva *et al.* 2000, Vassilev *et al.* 2001).

Immobilization procedure

Glomus deserticola and *Glomus fasciculatum* infected roots of *L. sativa*, cut to 0.1 mm fragments, were sterilized in 100 ml water containing 40 mg streptomycin, 2 g Chloramine-T trihydrate and 0.1 ml Tween 80. Root fragments containing each one of the AM fungi, alone or in combination with *Y. lipolytica* cell biomass, were mixed in 1.5% (w/v) sodium alginate which was further poured in a sterile Petri dish to form a thin,

2 mm, layer. The mixture was hardened by adding 20 ml 0.5M $CaCl_2$ and after 30 min washed with sterile distilled water. Alginate material was then cut to 3 × 2 mm pieces and stored in Petri dishes at 4 °C for 60 days.

Soil-plant system and experimental design

The soil used was the top 0–20 cm of a Granada province (Spain) field soil with a pH of 7.5 containing 8 μg phosphate g^{-1} soil (Olsen test), organic carbon 0.46%, and total N 0.046%. The soil was sieved (mesh, 2 mm) and steam-sterilized.

Tomato (*Lycopersicon esculentum*) was the test plant. Three-day-old uniform seedlings obtained from surface-sterilized seeds were transplanted to 300-ml-capacity pots (one per pot). Mycorrhizal inoculum (*G. deserticola* or *G. fasciculatum*) was applied at a rate of 0.5 g alginate-entrapped root fragments per pot. When necessary *Y. lipolytica* was applied at a rate of 1.7×10^6 cells g^{-1} soil co-entrapped in the carrier containing the respective AM fungi. Plants were grown in a greenhouse with a 16/8 h light/dark cycle at 25/19 °C and 50% relative humidity. The pots were weighed and watered to field capacity daily.

Analytical methods

After 30 days, the plants were harvested and root samples were stained with trypan blue (Phillips & Hayman 1970). A gridline intersect technique was used to assess percent root length colonized by AM fungi (Giovanetti & Mosse 1980). Data were processed by analysis of variance and Duncan's test ($P \leq 0.05$).

Results and discussion

The two AM fungi which were assayed colonized plant roots, independently of the presence or absence of *Y. lipolytica* in the gel carrier. The average levels of root mycorrhization showed that in the absence of *Y. lipolytica G. deserticola* produced a more extensive root colonization (32 ± 5.6%) than *G. fasciculatum* (24 ± 12.1%). Similar rates of root colonization have been reported with alginate-encapsulated spores of AM fungi applied immediately after the encapsulation procedure (Calvet *et al.* 1996). The percentage of mycorrhizal colonization was enhanced when *G. deserticola* and *G. fasciculatum* were introduced into the soil microcosms, co-entrapped in the gel carrier

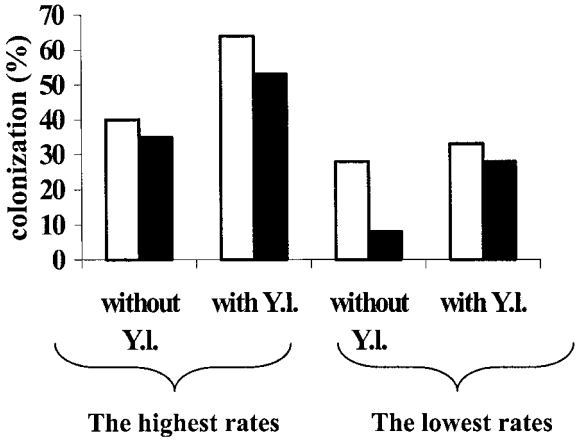

Fig. 1. Colonization of tomato roots by *Glomus deserticola* (open bars) and *G. fasciculatum* (filled bars) gel-entrapped alone or co-entrapped with *Y. lipolytica* (Y.l.). The experiment consisted of four treatments (5 repetitions): alginate-entrapped *G. deserticola*; *G. desericola*, co-entrapped with cells of *Y. lipolytica*; alginate-entrapped *G. fasciculatum*; *G. fasciculatum* co-entrapped with cells of *Y. lipolytica*. Only the highest and lowest rates of colonization are given using results obtained from single pots with each treatment.

with *Y. lipolytica* (48.6 ± 11.5% and 39.0 ± 11.3%, respectively).

Taken individually (results of single pots within each treatment), the highest values of root colonization did not differ significantly for both AM fungi applied either alone or co-entrapped with *Y. lipolytica* (Figure 1). Similar results were observed analyzing the lowest rates of root mycorrhization by the AM fungi co-entrapped in the gel carrier with *Y. lipolytica*, whereas the difference between the colonization potentials of *G. deserticola* and *G. fasciculatum* was significant in the absence of *Y. lipolytica*.

Immobilization (attachment or entrapment) of viable cells of microorganisms, plants and animals has been shown to offer numerous advantages over the use of free cells in a variety of laboratory and industrial processes. However, only recently the importance of entrapped soil microorganisms was widely accepted as an alternate technology for agricultural and environmental purposes (Cassidy *et al.* 1996, Bashan 1998). Since the early work of Ganry *et al.* (1982) and based on some recent reports (Declerck *et al.* 1996, Calvet *et al.* 1996), there seems to be no doubt that gel-entrapped AM fungi are able to colonize plant roots. Therefore, our results with entrapped *G. deserticola* and *G. fasciculatum*, introduced even after storage for 60 days, support these findings. The colonization efficiency of the immobilized mycorrhizal

fungi was enhanced by the inclusion of *Y. lipolytica* in the gel carrier. Live and dead baker's yeast cultures were reported to increase AM fungal establishment and development and possibly stimulate soil microbial activity (Larsen & Jakobsen 1996, Ravnskov *et al.* 1999). An additional advantage in the case of *Y. lipolytica* should be noted related to its ability to solubilize insoluble inorganic phosphates as it was recently reported (Vassileva *et al.* 2000, Vassilev *et al.* 2001). AM fungi are known to facilitate the transport of nutrients, including soluble phosphates (Joner *et al.* 2000). Further studies should be performed in order to evaluate the effect of inoculum formulations containing co-entrapped AM and phosphate-solubilizing microorganisms on nutrient cycling in soils amended with rock phosphate.

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