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Greenhouse inoculation of psammophilic plant species with arbuscular mycorrhizal fungi to improve survival and early growth

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ABSTRACT

Psammophilic plants are characterized for their adaptability to the hard environmental conditions of the coastal sand dune habitats that limit the survival of most vegetation. Considering that mycorrhizal plants are known to be effective colonizers of degraded habitats, the influence of the arbuscular mycorrhizal (AM) symbiosis in plant growth was studied in fifteen psammophilic plant species: *Lotus creticus*, *Dorycnium pentaphyllum*, *Otanthus maritimus*, *Thymbra capitata*, *Armeria maritima*, *Halimium halimifolium*, *Ononis natrix*, *Medicago marina*, *Genista umbellata*, *Crucinella maritima*, *Elymus farctus*, *Ammophila arenaria*, *Pancratium maritimum*, *Glaucium flavum* and *Limoniastrum monopetalum*. Wild seeds were used in this study to compare the effect of plant inoculation with a consortium of native AM fungi isolated from a coastal sand dune and with the selected isolate *Glomus intraradices* BEG 72 versus non mycorrhizal plants. After six months growth, we found that eleven of the fifteen psammophilic species responded positively to AM fungi inoculation and both root and shoot development benefited considerably from the presence of the AM fungi. We can conclude that many of the psammophilic species produced after the germination of the wild seeds collected in their natural habitats show limited growth due in part to the lack of adequate AM fungal inoculum in the plants growing substrate. AM inoculation seems to be critical for their survival.

1. Introduction

Psammophilic plant species are tolerant to sandy nutrient deficient soils and can withstand wind and salt influences, burial by sand accumulation, root exposure by erosion, heat and intensive sunlight. The stabilization of coastal degraded ecosystems depends on the success in the establishment of a sustainable plant community adapted to these conditions [1]. Coastal sand dunes soils are characterized by a low level of organic matter decomposition and thus low soil fertility. In these soils the upper edaphic layers are often dry due to the high temperatures registered during the day and are subjected to severe wind-driven sand accumulation [2]. Psammophilic plants are characterized for their adaptability to the coastal sand dune environments that limit the survival of other plant species. Propagation of the majority of psammophilic plants is based on the collection of wild seeds from natural habitats and not all plant species survive in the nursery or in the field. Despite the growing demand in the market to use them in revegetation activities some of these species are not available in commercial nurseries because growers do not succeed to produce them. Seeds germinate but the plantlet dies after a limited growth period.

Mycorrhizal colonization plays a vital role in plant community structure and diversity along the primary and secondary successions, especially in low-nutrient ecosystems [3,4]. The use of arbuscular mycorrhizal (AM) fungi in programs for conservation of targeted habitats has been evaluated in experimental projects and is known to be a successful methodology for the establishment of plant species in degraded soils [5,6]. The beneficial effects of this symbiosis include an increased water and nutrient absorption, a higher tolerance to salinity and to soil borne plant pathogenic infections, and an effective formation of soil aggregates by the extraradical hyphal network of the fungus surrounding the mycorrhizal plants [7,8]. Mycorrhizal associations affect plant nutrient uptake and nutrient cycling and bacterial community composition in the rhizosphere [9,10]. AM fungi also play an important role as colonizers of both root and soil, influencing the development and stability of the plant-soil system [11]. It has been reported that dependency on mycorrhiza varies between and within plant species [12] and no information is available on the level of dependency of the psammophilic plants.

This paper reports a greenhouse experiment designed to assess the effect of inoculation with the AM fungus *Glomus intraradices* Schenck & Smith and with a consortium of native fungi obtained

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from a coastal sandy soil. The growth performance of fifteen psammophilic plant species was determined and the relative mycorrhizal dependency of these species was evaluated to improve their early establishment in the nursery and in the field.

2. Materials and methods

To isolate the native arbuscular mycorrhizal fungi, soil and root samples were collected from the rhizosphere of psammophilic plants (*Ammophila arenaria* (L.) Link, *Medicago marina* L., *Lotus creticus* L., *Elymus farctus* (Viv.) Runemark, *Calystegia soldanella* (L.) R. Br. and *Pancratium maritimum* L.) in three Mediterranean coastal sand dunes in the northeast of Spain [13]. Spores present in the soil samples were extracted using the wet sieving and decanting method [14] and observed under a stereoscopic microscope. Spores with the same morphology were mounted in water, in Polyvinyl-Lactoglycerol (PVLG) and in PVLG with a drop of Mezler's reagent for microscopic examination. Spores mounted in Mezler's reagent were crushed in order to observe the staining of the different spore wall layers for morphological identification after the original descriptions [15] and also with internet published reference culture data bases. Spores of *G. intraradices*, *Glomus ambisporum* Smith & Schenck, *Glomus diaphanum* Morton & Walker, *Glomus clarum* Nicolson & Schenck, *Glomus microaggregatum* Koske, Gemma & Olexia, *Gigaspora margarita* Becker & Hall and *Scutellospora persica* (Koske & Walker) Walker & Sanders were identified. To obtain pot cultures of the native arbuscular mycorrhizal fungi from the coastal sand dunes, a mixture of these fungi was used to inoculate *Allium porrum* L. plantlets under greenhouse conditions in an autoclaved sandy soil.

To assess the effects of arbuscular mycorrhizal fungi on the growth of fifteen psammophilic plant species the inoculation with *G. intraradices* (BEG 72) [16], a fungus from IRTA (Institut de Recerca i Tecnologia Agroalimentaria) collection effective in a wide range of experimental agricultural situations [17–19], and the inoculation with the mixed inoculum of AM species isolated from the Mediterranean coastal sand dune were evaluated. The inocula consisted of rhizosphere soil and colonized roots of *A. porrum* recovered from pot cultures obtained after nine months growth in a sandy soil.

Seeds of *Lotus creticus*, *Dorycnium pentaphyllum* Scop., *Otanthus maritimus* (L.) Hoffmanns & Link, *Thymbra capitata* (L.) Cav., *Armeria maritima* (Mill.) Willd., *Halimium halimifolium* (L.) Willk, *Ononis natrix* L., *Medicago marina*, *Genista umbellata* (L'Hér.) Dum. Cours., *Crucinella maritima* L., *Elymus farctus*, *Ammophila arenaria*, *Pancratium maritimum*, *Glaucium flavum* Crantz. and *Limoniastrum monopetalum* (L.) Boiss, were surface sterilized with a 0.5% NaClO solution for 10 min, rinsed with sterile water and sown in seedbed containers on autoclaved sandy soil in the greenhouse. The sandy soil selected as a substrate had similar physical and chemical characteristics to the coastal sand dunes soils. Twenty five plants of each plant species and for each treatment were inoculated in individual pots (1L) filled with autoclaved sandy soil (8 ppm P content) with the selected AM fungi and a noninoculated treatment was included as a control. To ensure a sufficient number of AM propagules, 20 g of inoculum from the corresponding treatments were placed below the seedling roots at transplanting. The pots were placed in a greenhouse with 16 h daylight and mean temperature of 20 °C ± 5 °C per day. Water was added when needed.

Plant height was measured monthly. After six months growth plants were harvested, the root systems were washed and root and shoot dry weights were determined after one week at 70 °C. To evaluate the mycorrhizal colonization, roots were cleared and stained with 0.05% trypan blue in lactic acid [20,21] and the percentage of AM root colonization was estimated using the grid line intersect method [22]. The relative mycorrhizal dependency (RMD) was determined for each inoculum and plant species by expressing the difference between the average dry weight of the mycorrhizal plants and the average dry weight of the non-mycorrhizal plants as a percentage of dry weight of the mycorrhized plants [23]. The calculated values stand between 0% (no AM dependency) to 100% (total AM dependency). Data were analyzed with one-way analysis of variance and means were compared using Tukey Test ($P \leq 0.05$).

3. Results

Growth of shoots and roots of *T. capitata*, *D. pentaphyllum*, *L. creticus*, *H. halimifolium*, *M. marina*, *P. maritimum* and *G. umbellata* was significantly enhanced by inoculation with both, *G. intraradices* and native dune fungi inocula (Table 1). The effect of AM inoculation on the height increment was clear on visual comparison during and at the end of the experiment. The biomass of *O. natrix* and *A. maritima* was significantly increased by inoculation with *G. intraradices* but not by the consortium of the AM dune fungi, while the growth of *O. maritimus* and *E. farctus* was significantly increased by inoculation with the native dune fungi but not by *G. intraradices* (Table 1). The other plant species: *C. maritima*, *A. arenaria*, *G. flavum* and *L. monopetalum*, did not respond positively to AM fungi inoculation. These plants achieved low percentages of root colonization except for *A. arenaria* that did not show any distinctive difference compared with the AM dependent plants (Table 1).

Thymbra capitata and *H. halimifolium* achieved the maximum RMD values, reaching 99% irrespective of AM inoculum (Fig. 1). They were totally dependent on arbuscular mycorrhization and did not grow without the symbiosis. *Dorycnium pentaphyllum*, *L. creticus*, *M. marina*, *P. maritimum* and *G. umbellata* had a RMD over 75% for both AM inocula and showed limited growth without mycorrhization. *Ononis natrix* and *O. maritimum* had only a RMD over 75% when they were colonized either by *G. intraradices* or by the consortium of dune fungi respectively. *Elymus farctus* and *A. arenaria* had a RMD above 25% for the dune consortium inoculum as well as *A. maritima for G. intraradices*. *Crucinella maritima*, *G. flavum* and *L. monopetalum* had a RMD below 25%.

We found three patterns of psammophilic plant development according to plant growth parameters and to the AM dependency in low fertility soils: a) Plants that did not grow without mycorrhizas, b) plants that could grow without the symbiosis, although the mycorrhizal colonization increased their growth and c) plants exhibiting similar growth despite their mycorrhizal status.

4. Discussion

Eleven of the fifteen psammophilic plant species inoculated with *G. intraradices* or with native dune fungi responded positively to AM fungi inoculation and both root and shoot development benefited considerably from the presence of the AM fungi. We found that *L. monopetalum* exhibited little dependence on AM symbiosis, although plant roots were colonized by the arbuscular mycorrhizal fungi. On the other hand *A. arenaria*, specie belonging to the Graminaceae family, achieved a high mycorrhizal colonization and has also been reported as highly mycorrhizal under the sand dune natural conditions [13]. But in our study, under greenhouse conditions, the presence of mycorrhizal colonization did not improve their growth response (Table 1). According to Koske et al. [24] the presence of mycorrhiza does not necessarily indicate a direct relationship between beneficial association and plant growth. It has been described [25] that the plant ability to adapt to partial or complete burial in sand and to withstand sand blasting seems to be the most important characteristic for the establishment of the plants in coastal sand dunes. *Ammophila arenaria* is resistant to sand burial and we need to study if the arbuscular mycorrhizal root colonization has a direct influence on the anchorage of these plants.