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Analysing arbuscular mycorrhizal fungal diversity in shrub-associated resource islands from a desertification-threatened semiarid Mediterranean ecosystem

Nuria Ferrol*, Rut Calvente, Custodia Cano, José-Miguel Barea, Concepción Azcón-Aguilar

Departamento de Microbiología del Suelo y Sistemas Simbióticos, Estación Experimental del Zaidín, CSIC, Profesor Albareda 1, 18008 Granada, Spain

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Abstract

The diversity of arbuscular mycorrhizal (AM) fungal species was analysed in vegetation patches dominated by *Pistacea lentiscus*, one of the most representative shrub species from Mediterranean regions and a target plant currently used in revegetation programmes. The number of AM fungal spores in soil samples taken directly from the target ecosystem was relatively low, and the only AM fungal species recognised in the native soil was *Paraglomus occultum*. To bait the whole population of native AM fungi, trap cultures were established using different sources of AM fungal inoculum. External AM-mycelium appears to be the main source of AM inoculum in this ecosystem. After 3 years of trap culturing, only five distinguishable AM fungal species, *Glomus mosseae*, *Glomus claroideum*, *Glomus viscosum*, *Glomus constrictum* and *Paraglomus occultum*, were morphologically identified, indicating a relatively low AM fungal species richness. These isolates were also genetically characterised by sequence analysis of a portion of their SSU rRNA and their phylogenetic relationships were established. A functional diversity bioassay revealed the effectiveness of *G. constrictum* and *G. mosseae* as inoculants for the target shrub species.

Keywords: Arbuscular mycorrhizal fungi; Biodiversity; Desertification; Mediterranean ecosystems; Molecular characterisation; Trap cultures

1. Introduction

Mediterranean regions are characterized by a set of climatic conditions which include a long dry and hot summer with scarce, erratic, but often torrential rainfalls (López-Bermúdez and Albaladejo, 1990; Vallejo et al., 1999). These conditions make Mediterranean ecosystems fragile and susceptible to degradation. Man-mediated degradation activities (overgrazing, deforestation, etc.) exacerbate climatic constraints and have led to desertification processes in Mediterranean ecosystems, such as those present in southeast Spain (López-Bermúdez and Albaladejo, 1990). Disturbance of the vegetation cover is the first visible indication of a desertification process, but damages to physical, chemical and biological soil properties are known to occur concomitantly (Requena et al., 2001). With regard to biological components, land degradation is usually associated with reductions in below-ground microbial diversity and/or activities governing

* Corresponding author. Tel.: +34-958-181600; fax: +34-958-129600.
E-mail address: nferrol@eez.csic.es (N. Ferrol).

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the biogeochemical cycles of the major plant nutrients (Kennedy and Smith, 1995). In particular, desertification reduces the diversity and inoculum potential of one of the most important mutualistic plant microbial symbionts, namely arbuscular mycorrizal (AM) fungi (Jasper et al., 1991).

AM fungi belonging to the recently raised new fungal phylum the Glomeromycota (Schüβler et al., 2001b) are soil microorganisms known to establish a universally distributed mutual symbiosis with the majority of higher plants. AM fungi colonize root tissues biotrophically and form an extensive network of extra-radical mycelium, providing a direct physical link between soil and plant roots (Smith and Read, 1997). AM fungi are known to benefit plant establishment by increasing resistance to environmental stresses, enhancing plant nutrient acquisition and improving soil quality (Schreiner et al., 1997; Jeffries and Barea, 2001). Because of the key ecological functions played by AM symbiosis (Jeffries and Barea, 2001), loss or diminution of the mycorrhizal potential in degraded areas may limit the successful reestablishment of the native plants (van der Heijden et al., 1998; Requena et al., 2001). Therefore, a rehabilitation approach for revegetation of degraded ecosystems must begin with the evaluation of the mycorrhizal status as well as with the isolation, identification and characterisation of the native AM fungi in the target area, as a basis to produce AM inoculum for selected plant species to be used in the revegetation processes.

A key step in restoration strategies is re-establishment of adapted native plant species (Francis and Thornes, 1990; Vallejo et al., 1999). In the degraded semiarid Mediterranean ecosystems present in southeast Spain, plant communities are dominated by dwarf and tall shrub and grass species (López-Bermúdez and Albaladejo, 1990). Within the framework of revegetation programmes currently being developed in southeast Spain a number of drought-tolerant, deep-rooting shrub species are being assayed. These programmes include AM inoculation technologies to increase revegetation success. Shrub species in degraded semiarid ecosystems typically grow following a patchy distribution, with very low values of plant cover (López-Bermúdez and Albaladejo, 1990; Vallejo et al., 1999). The vegetation patches commonly constitute ‘resource islands’ (Garner and Steinberger, 1989; Reynolds et al., 1990), where facilitation among plants may be highly fostered (Callaway, 1997). These resource islands appear as targets for initiating AM fungal isolation and further identification processes.

The aim of the present work was to analyse the diversity of AM fungal species in the rhizosphere of one of the most representative shrub species from Mediterranean regions, *Pistacea lentiscus* L., which grows in typical patches in a representative semiarid ecosystem in southeast Spain. AM fungi were screened either directly in samples from the target soil or from long-term trap plant cultures established to increase AM fungal spore population, to reveal their maximum complement. To identify the different AM fungal isolates, morphological and developmental criteria were first applied. Since the low morphological diversity of AM fungal spores may not reflect their physiological and genetic diversity (Bachmann, 1998), molecular techniques based on the sequence analysis of a portion of the SSU rRNA were further used (Schüβler et al., 2001a; Redecker et al., 2000). The effectiveness of the native isolates as inoculants for the target shrub species was also assessed.

2. Materials and methods

2.1. The ecosystem

A representative area was chosen within a degraded semiarid Mediterranean ecosystem in the Aguas site (0°21′W, 38°31′N, 460 m above sea level), located in the Alicante province (east Spain). Very hot and dry summers, with irregular rainfall occurring mostly in autumn, are characteristic of the zone. Average annual precipitation is 288 mm. This experimental site has a loamy-silty loam *Lithic Calciorthid* soil derived from marls and limestones and represents an open degraded steppe site with a plant cover of 45% and a slope angle of 12°. The vegetation is dominated by grasses and with some very sparse patches of slow-growing shrubs dominated by *P. lentiscus* L., a drought-tolerant plant.

2.2. Field sampling

Three sampling sites were chosen in an area measuring approximately 4 ha. Three individual patches were selected in each site and three *P. lentiscus* plants

were randomly chosen within each patch. A soil sample from around the roots of each individual plant was collected, each sample consisting of three bulked sub-samples (200 cm^3 soil cores) randomly collected at 20–40 cm depths. Samples were stored in plastic bags at 4 °C until processed. Root fragments in these samples were examined for AM colonization (Phillips and Hayman, 1970).

2.3. Trap cultures

P. lentiscus and *Trifolium repens* were used as host plants. Three different types of AM propagule sources were used as starter inoculum: (i) spores isolated from the soil samples, (ii) root fragments taken from the corresponding soil sample and (iii) the native soil containing all types of AM propagules. The trap plants were grown in pots containing either the test soil pasteurised by steaming for 1 h for three consecutive days (for the control trap cultures and for inoculum sources (i) and (ii)), or transplanted directly to the native soil (for the inoculum source (iii)). Trap cultures were maintained for 3 years in a greenhouse to allow detection of slow-sporulating fungi. Environmental conditions were 16/8 h light/dark photoperiod, 25/18 °C, day/night temperature and 60/80% day/night relative humidity. Plants were watered when necessary and fertilized at fortnight intervals with a low-phosphorus-content (25%) Long Ashton nutrient solution (Hewitt, 1952).

2.4. Spore isolation and identification

AM fungal spores, either from the field collected soil or from the trap cultures, were isolated from a 100 g soil sample by the wet sieving and decanting method, followed by sucrose centrifugation (Sieverding, 1991). Trap cultures were sampled 4, 12, 24 and 36 months after their establishment, with the three last samplings carried out in late spring (May/June). After centrifugation, the supernatant was poured through a 50 μm mesh and quickly rinsed with tap water. Turgid spores (suggesting viability) were grouped under the dissecting microscope according to morphological characteristics. Diagnostic permanent slides were prepared for each different spore morphotype using either polyvinyl-alcohol alone or mixed with Melzer's solution (1:1). After confirming the uniformity of the morphological groups under the optical microscope, the different morphotypes were identified to genus and, when possible, to species. Spore identification was based mainly on spore size, colour, wall structure and hyphal attachment (Walker, 1983; Morton and Benny, 1990; Schenk and Perez, 1990). Spore identification was corroborated by comparisons with the descriptions from the reference cultures in the INVAM (the International Culture Collection of Arbuscular and Vesiculo Arbuscular Endomycorrhizal Fungi) and the BEG (International Bank for the Glomales, formerly named La Banque Europeene de Glomales).

2.5. Establishment of the AM fungal mono-specific cultures

Single species cultures of the different AM fungal morphotypes from the trap cultures were established in pots using a mixture of sterile soil/sepiolite/vermiculite (1:1:1 (v/v/v)) as growing substrate and *T. repens* as host plant. About 50 apparently healthy and viable spores of each AM fungal morphotype were used as inoculum by placing them close to the plant root system. Once multiplied and checked for purity, the different AM fungal isolates were registered in the BEG.

2.6. Molecular characterisation

AM fungal spores from the mono-specific cultures were collected to PCR amplify a portion of the SSU rRNA. DNA extracts were obtained by crushing surface-sterilized spores with a sterile disposable micro-pestle in 40 μl milli-Q water. Volume of 10 μl of 20% Chelex 100 iso-resin (Bio-Rad) (Wyss and Bonfante, 1993) were added to the crude extracts and the mixture was incubated at 95 °C for 5 min followed by 5 min on ice. The resin was removed by centrifugation at $13,000 \times g$ for 3 min and the supernatant stored at −20 °C and used as template for PCR. Partial small subunit (SSU) rRNA sequences were amplified using the universal primer NS31 (Simon et al., 1992) and the AM fungal specific primer AM1 (5′-GTTTCCCGTAAAGGCGGCGAA-3′) (Helgason et al., 1998). *Paraglomus occultum* SSU rRNA was amplified using the universal primers NS31 and NS41. Amplifications were carried out in a final volume of

25 μl using the 'ready to go' PCR beads (Amershan Pharmacia Biotech), 1 μM of each primer and 23 μl of the DNA spore extract. PCR was performed in an automated thermal cycler (Gene Amp PCR System 2400, Perkin-Elmer, Foster City, CA) with an initial denaturation for 10 min at 94 °C, followed by 40 cycles with denaturation for 1 min at 94 °C, annealing for 1 min at 58 °C and extension for 2 min at 72 °C, followed by a 8 min final extension at 72 °C. The PCR products were separated electrophoretically on 1.6% agarose, stained with ethidium bromide, visualized by UV illumination and the expected band was excised with a scalpel. The amplified DNA was isolated from the gel using the QIAEX gel extraction kit (Qiagen) following the manufacturer's protocols. Nucleotide sequences were determined by using *Taq* polymerase cycle sequencing and an automated DNA sequencer (Perkin-Elmer ABI Prism 373). All DNA fragments were sequenced in both strands.

2.7. Sequence analysis

The sequences were analysed using the Genetics Computer Group (Madison, WI) package (version 9.0). The sequence data were compared to gene libraries (EMBL and GeneBank) with BLAST programs (Altschul et al., 1990). Multiple sequence alignments of gene sequences were carried out with the program CLUSTALW, version 1.5 (Thompson et al., 1994). Genetic distances were estimated by using the Kimura two-parameter method employed by PHYLIP (Felstein, 1993). Phylogenetic analysis was performed by the neighbour-joining and evolutive parsimony methods by using PHYLIP (Felstein, 1993). The relative support for groups was determined based upon 1000 bootstrap trees.

2.8. Effectiveness of the native AM fungi

The native AM fungi successfully multiplied in the mono-specific cultures were assayed for their effectiveness as mycorrhizal inoculants for *P. lentiscus* plants using the living soil from the target ecosystem as growth substrate. *P. lentiscus* cuttings were inoculated with each of the following native AM fungi: *Glomus constrictum*, *Glomus mosseae*, *Glomus viscosum*, *Glomus claroideum* and *P. occultum*. The fungal inocula were obtained on a vermiculite/sepiolite mixture (1:1 (v/v)), using clover as host plant (Azcón-Aguilar et al., 2000), and consisted of rhizospheric samples containing spores, hyphae and mycorrhizal root fragments. The mycorrhizal potential in these inocula was determined by following a serial dilution technique (Sieverding, 1991) and further calculation of the most probable number (MPN) of infective mycorrhizal propagules (i.e. those able to develop colonization units in the root of a test plant). All AM inoculant were adjusted to supply around 375 propagules per plant by mixing the inoculum with the soil. Two types of controls were prepared. One using unsterilized native soil, as for the AM-inoculated plants, thus containing all naturally existing taxa. In the second one the soil was steam-sterilized (1 h, three consecutive days) to remove AM fungi. All control plants were supplied with a filtrate (<20 mm) of the AM inocula to provide the microbial populations accompanying the mycorrhizal fungi but free from AM propagules. Eight replicate plants were established per treatment. Each plantlet was individually transplanted to 2 l pots and grown in a greenhouse, with temperatures ranging from 18 to 25 °C and relative humidity from 80 to 60%. Plants were grown for 6 months and, before being transplanted to field conditions, non-destructive measurements were performed. Plant height was chosen as the response variable to evaluate AM fungal effectiveness. Measurements were at 3, 4, 5 and 6 months after inoculation. Data were processed by analysis of variance (ANOVA) for repeated measures using the SuperANOVA programme (version 1.11, Abacus Concepts Inc., Berkley, CA, USA). Sources of variability were partitioned into between-subjects factors (treatments) and within-subjects factors (date). One-way ANOVA was used to detect the treatment differences per measuring date, followed when appropriate, by the Fisher's Protected Least Significant Difference Test at $P < 0.05$.

3. Results

3.1. AM fungal diversity in natural communities and spore population changes in trap cultures

Although the root fragments present in the sampled soils showed a generalized presence of AM fungal structures, the number of AM fungal spores was

Table 1
AM fungal species recovered from soil samples around the root system of *P. lentiscus* collected directly from the field site (1998) or from *T. repens* trap cultures after different years of enrichment (1999–2001)

Site	1998	1999	2000	2001
1	*P. occultum* Unidentified	*P. occultum* *G. constrictum* *G. viscosum* *G. claroideum*	*P. occultum* *G. constrictum* *G. viscosum* *G. claroideum* *G. mosseae*	*P. occultum* *G. constrictum* *G. viscosum* *G. claroideum* *G. mosseae*
2	*P. occultum* Unidentified	*P. occultum* *G. constrictum* Unidentified	*P. occultum* *G. constrictum* Unidentified	*P. occultum* *G. constrictum* *G. viscosum*
3	Unidentified	Unidentified	*G. claroideum* Unidentified	*G. claroideum* *G. viscosum* *P. occultum*

relatively low (ca. 2–55 spores per 100 g of soil). No differences were found in the AM species present in the *P. lentiscus* rhizosphere of the three replicate patches within each sampling sites, therefore, data are given on a per sampling site basis. The only AM fungal species recognized in the native soils was *P. occultum*, previously considered as *Glomus occultum* (Morton and Redecker, 2001), which was detected in two of the three sampling sites (Table 1). The low number of spores of other taxa prevented their identification. To bait the whole population of native AM fungi, trap cultures were established using different sources of AM fungal inoculum. Neither the root fragments nor a mixture of the AM fungal spores isolated from the field samples were able to produce any AM-colonization in the roots of the trap plants (*P. lentiscus* and *T. repens*). All plants growing directly in the natural (unsterilized) soils showed AM colonization after 4 months. However, no spores were found in the rhizosphere of the plants at this sampling time.

In the subsequent AM fungal community analyses carried out 12, 24 and 36 months after establishment of the trap cultures, significant sporulation was found only in the rhizosphere of *T. repens*, a plant with a short life cycle (Table 1). Although *P. lentiscus* became mycorrhizal after 12 months of trap culturing, showing AM colonization of the *Paris*-type, sporulation was very low preventing any morphological or molecular characterisation. Therefore, diversity studies were restricted to *T. repens*-based trap cultures. Data summarized in Table 1 show that AM fungal species richness increased with time as plant cultures develop. Most of the species belonged to the genus *Glomus*, and one to *Paraglomus*. One year after establishment of the trap cultures, *P. occultum* was the most abundant AM fungus in the trap cultures from two of the sampled sites showing relative densities of 70.9% in site 1 and 72.8% in site 2, corroborating preliminary observations on spores directly extracted from the field sites. At this sampling time, *G. constrictum* was detected in the trap cultures from two of the sites while *G. claroideum* and *G. viscosum* were present in the trap cultures from one of the sites. A small number of spores still remained unidentified (Table 1). After 2 years of trap culturing, *G. mosseae* was detected in the trap cultures from site 1, and spores of *G. claroideum* were also found in trap cultures from site 3. At the last sampling time, 3 years after establishment of the cultures, no significant changes were detected in the trap culture from site 1, which had the highest AM fungal species richness. *G. viscosum*, previously detected in soil from site 1, also appeared in the other target sites. *P. occultum* was finally detected in cultures from the three sampling sites (Table 1).

3.2. Phylogenetic relationships between isolates

To further purify and verify identification of all the isolated AM fungal spores, single species pot cultures were established. Spores from these cultures were collected for PCR amplification of a portion of their SSU rRNA. Sequence analysis of the PCR fragments revealed that the amplified DNA shows more than 95% identity to the corresponding portion

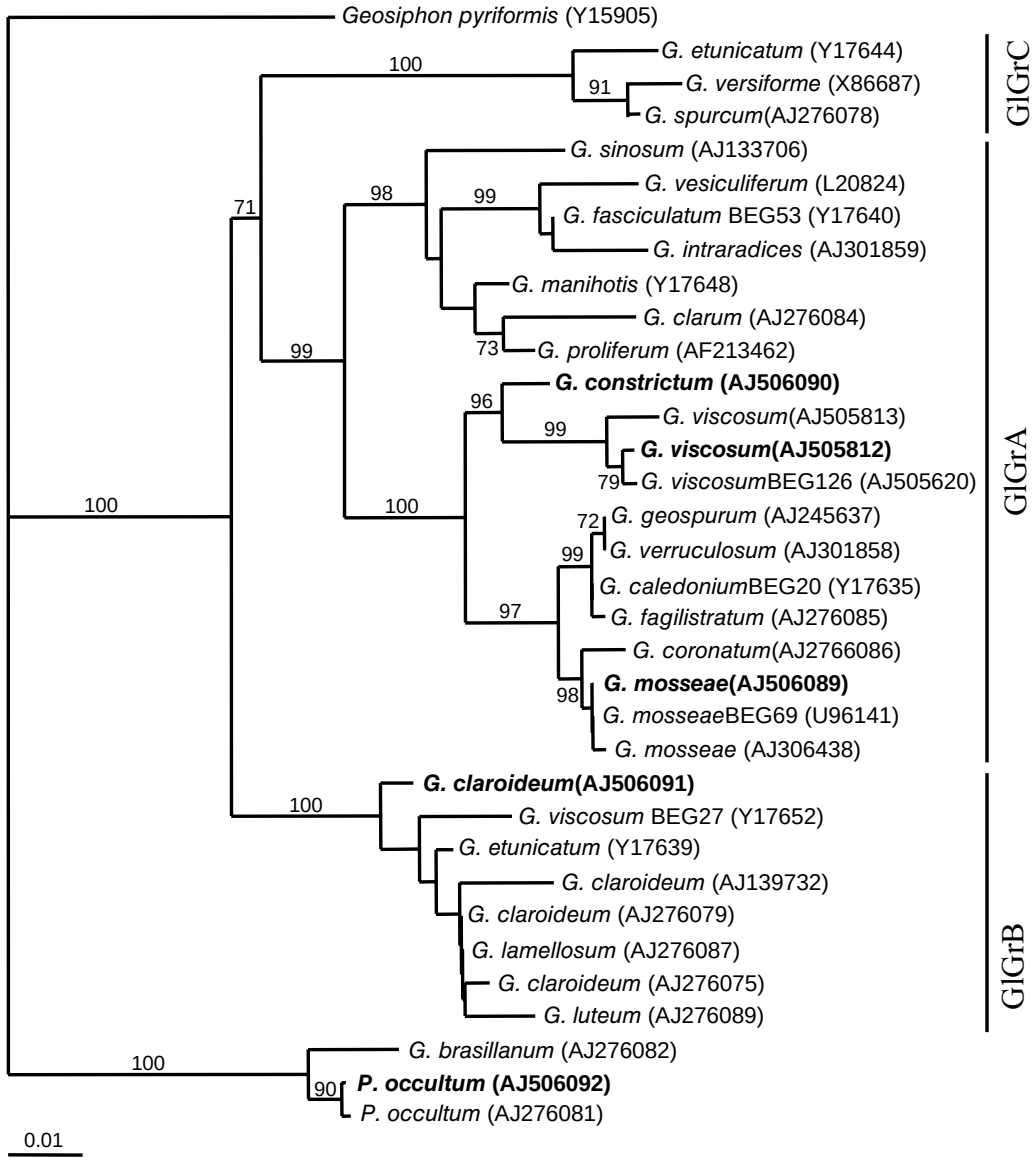

Fig. 1. Neighbour-joining analysis of a portion of the SSU rDNA of the Glomaraceae and Paraglomeraceae families of AM fungi. New sequences obtained in this study are in bold. Accession numbers are indicated in brackets. Bootstrap values (in %) of the neighbour-joining analysis (1000 bootstraps) are shown above the branches. Only topologies with bootstrap support of at least 60% are shown.

of the 18S rRNA of the different AM fungi present in the database, indicating the AM fungal origin of the sequences obtained. Phylogenetic analysis of the obtained sequences and other selected AM fungal SSU rRNA genes from the database was performed with neighbour-joining and parsimony methods, using *Geosiphon piriforme* as outgroup. However, since the same topology was observed with both methods, only the neighbour-joining tree is included (Fig. 1). The new sequences obtained for the isolates of *G. mosseae*, *G. claroideum* and *P. occultum* cluster with the sequences obtained from these fungi in other

Table 2
Summary of repeated measures analysis of variance (ANOVAR) of the height of *P. lentiscus* plants grown in the target soil containing natural AM populations inoculated with different native AM fungi

Source of variation	d.f.	*F*-value	*P*
Between-subjects			
Treatment (*T*)	6	8.0	0.0001
Error	47		
Within-subjects			
Measuring date (*D*)	3	250.9	0.0001
$D \times T$	18	11.7	0.0001
Error	147		

studies. Surprisingly, the new sequence of *G. viscosum* fell in a different cluster than the *G. viscosum* sequence present in the database. However, it forms a highly bootstrapped clade with the sequences of two *G. viscosum* ecotypes isolated in our laboratory from a different semiarid Mediterranean ecosystem in northeast Spain (Accession number, AJ505813) and from an agro-system in south Spain (Accession number, AJ505620), and with the new sequence of *G. constrictum* from this study.

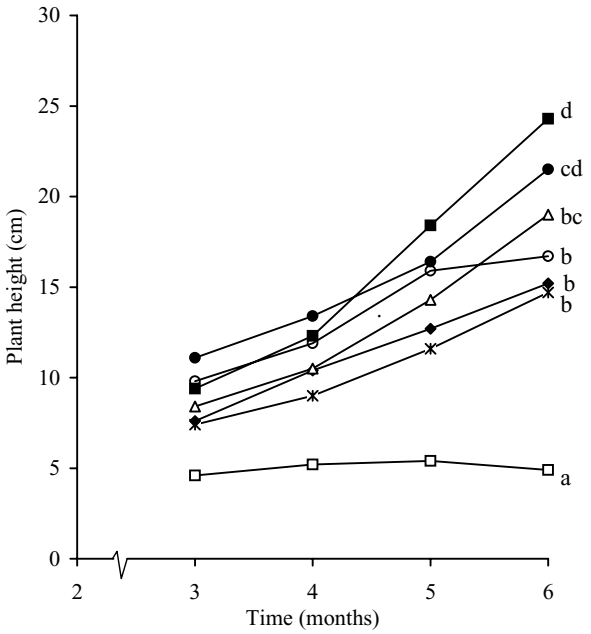

Fig. 2. Time-course effect of the inoculation with the different native AM fungi on the height of *P. lentiscus* plants grown in the target soil containing natural AM populations. (△) control (with all naturally existing taxa); (■) *Glomus constrictum*; (●) *Glomus mosseae*; (○) *Glomus viscosum*; (◆) *Paraglomus occultum*; (✱) *Glomus claroideum*; (□) AM fungi-free soil. Data not sharing a letter in common differ significantly at $P < 0.05$ by the Fisher's Least Significant Difference test. Comparison was limited to the last measuring date (6 months) before transplanting the plants to field conditions.

3.3. Functional diversity

Mycorrhizal treatments applied had significant effects on plant growth (Table 2). Control plants growing in AM fungi-free soil were significantly smaller than all the others across all sampling dates. The significant 'treatment by time' ($D \times T$) interaction indicates that the growth rate of the plants over time differs depending on the mycorrhizal treatment applied. Data of the *P. lentiscus* growth as affected by the inoculation with native AM fungi are summarised in Fig. 2. *G. constrictum* and *G. mosseae* were the most effective species for improving plant growth, although only *G. constrictum* exerted a significant effect.

4. Discussion

Using successive and extended trap cultures and applying both molecular and morphological approaches, it was shown that five species of AM fungi were present in the representative *P. lentiscus*-dominated vegetation patches, characteristic of the target ecosystem. The number of AM fungal spores and species richness in the root-associated soil samples taken directly from the target vegetation patches was found to be low. This study confirms that the use of successive and extended trap cultures detects a higher species richness than extraction of spores directly from field soil, as seen in other ecological situations (Stutz and Morton, 1996; Stutz et al., 2000). Previous surveys of AM fungi-species richness in degraded warm arid and semiarid environments worldwide could have reported an underestimation of the actual species richness in these ecosystems if a prolonged trap culturing was not performed (Bethlenfalvay et al., 1984; Requena et al., 2001; Azcón-Aguilar et al., 2002). Actually, it is accepted that the low species richness generally reported for arid and semiarid ecosystems by extractions of spores from field soil, reflects limitations in the sporulation patterns under field conditions (Stutz and

Morton, 1996; Stutz et al., 2000). As Stutz and Morton (1996) discussed, AM fungi must wait for 'windows of opportunity' in the environmental conditions to multiply and sporulate in arid and semiarid ecosystems.

As is known, plant species can influence the multiplication pattern of AM fungi in the rhizosphere (Allen et al., 1995; Bever et al., 1996; del Val et al., 1999). The high sporulation level induced by *T. repens* contrasts with the lack of sporulation in soil associated with *P. lentiscus*. This could be explained by the different nature of these plant species, the woody and slow-growing *P. lentiscus* versus the herbaceous and relatively fast-growing *T. repens*. In fact, *T. repens* is a plant species with a short life cycle, but able to maintain an active vegetative re-growth upon successive development and sporulation. Whereas *T. repens* forms *Arum*-type mycorrhiza, *P. lentiscus* forms those of the *Paris*-type. *Paris*-type mycorrhiza, commonly found in Mediterranean ecosystems (Bedini et al., 2000), are known to allocate most of their carbon to intra-radical rather than extra-radical development, which can prevent an efficient sporulation (Brundrett, 1991; Smith and Read, 1997).

The fact that inoculation with spores isolated directly from the ecosystem failed to colonize plant roots in the trap cultures suggests that this form of AM propagule is not the main inoculum source in the target ecosystem. Instead, the rapid initiation of the AM colonization when the trap plants grew in the living soil from the target area, containing all type of AM propagule sources, indicates that the soil-based AM-mycelium, rather than the spores, may be the main source of AM inoculum in this area. These findings agree with previous observations emphasizing the ecological role of the AM mycelium as inoculum source in arid and semiarid ecosystems (Jasper et al., 1991; Requena et al., 1996; Azcón-Aguilar et al., 2002). The AM mycelium developing around the root system is known to play an important ecological role in connecting the root system of plants growing nearby (Allen and Allen, 1992; Bethlenfalvay and Schüepp, 1994; Azcón-Aguilar et al., 2002). Therefore, it could be suggested that the network of AM mycelium colonizing the soil in the *P. lentiscus*-dominated patches would be a functional component for the development and functioning of these resource islands to enhance an early integration of mycotrophic seedlings into the system, as previously shown for other plant species (Carrillo-García et al., 1999, 2000; Azcón-Aguilar et al., 2002).

In the target ecosystem generic diversity was confined to *Glomus* and *Paraglomus*. This contrasts with descriptions from habitats with more moderate temperatures and better moisture conditions, in which generic diversity is higher (Johnson and Wedin, 1997). With the exception of *P. occultum*, the species recorded are typical of semiarid Mediterranean ecosystems (Dodd and Krikun, 1984; Jeffries et al., 1988; Requena et al., 1996). The finding that *P. occultum* was the most abundant species suggests that this fungus should have an ecological role in the target ecosystem. The effects of local environmental processes on AM fungal community structure in the target ecosystem were most evident in the selection of small-spored species, especially *P. occultum*. A similar restriction in species composition to mostly small-spored fungi belonging to *Glomus* spp. was previously reported in arid and semiarid ecosystems (Jacobson, 1997; Stutz et al., 2000). These species may be more adaptable in adjusting patterns of sporulation to environmental stress conditions (Jacobson, 1997).

Identification of AM fungi has traditionally relied on the morphological and developmental characteristic of their large multinucleate spores (Walker, 1983; Morton and Benny, 1990). However, there is increasing evidence that morphology-based identification of AM fungi has limited use in ecological settings since the low morphological diversity of AM fungal spores may not reflect their physiological and genetic diversity (Bachmann, 1998). To circumvent this problem, molecular approaches are being incorporated to define and relate taxa in the Glomeromycota (Schüβler et al., 2001a; Redecker et al., 2000). The analysis of a portion of the SSU rRNA gene of the different AM fungal ecotypes isolated from the target ecosystem has allowed establishing of their phylogenetic relationships. The use of an AM fungal specific primer in the PCR has avoided DNA amplification of contaminant organisms. However, the divergence present in the ribosomal sequences of some of the AM fungi required the use of two different sets of primers. The divergence found between the *P. occultum* sequence and the ribosomal sequences of the *Glomus* species supports the placement of *P. occultum* in a different family (Redecker

et al., 2000), and even in a different order (Schüβler et al., 2001a,b). Recent analyses of AM fungal SSU rRNA gene sequences have shown that the examined *Glomus* species separate naturally into three major clades (GlGrA, GlGrB and GlGrC, as given in Fig. 1) representing at least three different families (Schüβler et al., 2001a,b; Schwarzott et al., 2001). The *Glomus* sequences obtained in this study are members of two of the major clades of *Glomus* GlGrA and GlGrB, following the currently accepted terminology of AM fungi (Schüβler et al., 2001a,b). The new sequences of *G. mosseae* and *G. claroideum* group with the sequences obtained for these fungi in other studies, confirming their morphological identification. Although this is the first 18S rRNA sequence obtained for a *G. constrictum* isolate, its placement in the '*Glomus* group A' agrees with the observation that the large subunit rRNA gene of *G. constrictum* (BEG130) is closely related to the species in this group (Clapp et al., 2001). Placement of the *G. viscosum* sequences obtained in our laboratory in a different group than the sequence of the *G. viscosum* (BEG27) present in the database indicates, as previously pointed out (Schüβler et al., 2001a), that more sequences of this species are necessary to determine the phylogenetical relationships with other *Glomus* species.

Different AM fungal species have different effects on plant performance and nutrient cycling (Newsham et al., 1995; van der Heijden et al., 1998; Jeffries and Barea, 2001). In fact, particular AM fungal species, and not all the components of any AM fungal population, appear to play a role at a given stage of plant performance (Jeffries and Barea, 2001). Functional diversity tests show the effectiveness of the indigenous ecotypes, particularly those of *G. mosseae* and *G. constrictum*, to improve performance of the target plant species. Although it is difficult to know whether plant growth promotion was due either to the consistent presence of one dominant species in the root or to interactions between the inoculated and indigenous species, our data revealed the effectivity of *G. constrictum* and *G. mosseae* as inoculants for the target shrub species. This study shows the importance of including native AM fungal isolates, which appear to be physiologically and genetically adapted to the stress conditions of the target environment, in the inoculation strategies used in revegetation programs of degraded ecosystems.

5. Conclusions

Our data indicate that AM fungal sporulation in the target ecosystem appears rather limited, suggesting that the AM-mycelium serves as the main source of AM propagules. At least five species of AM fungal ecotypes constitute the actual AM fungal population in the resource islands of the *P. lentiscus*-dominated vegetation patches. Taxonomic diversity in the target area has been assessed using morphological and molecular approaches. The functional diversity bioassay performed supports the potential for exploiting the natural AM fungal diversity as inoculum sources for the formulation of AM inoculants to be used in revegetation programmes in the region.

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