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Arbuscular mycorrhizal (AM) status and seedling growth response to indigenous AM colonisation of *Euryodendron excelsum* in China: implications for restoring an endemic and critically endangered tree

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Abstract. It is increasingly evident that the mycorrhizal colonisation of endangered species is of major importance for their restoration. In the present study, the symbiosis of arbuscular mycorrhizal fungi (AMF) and endangered species *Euryodendron excelsum* was investigated in 10 patches of a remnant population in south China. The presence of arbuscules and vesicles indicates that *E. excelsum* is a typical arbuscular mycorrhizal plant. Five genera were identified in the rhizosphere of *E. excelsum*, and the most common and frequent genus was *Glomus.* Root total colonisation intensity is negatively correlated with the available soil phosphorus and potassium content in the soil. In addition, we find no significant relationship between spore density and soil characteristics, or between spore density and total colonisation intensity. Furthermore, a greenhouse experiment under two soil types (humus: native soil = 3 : 1 ST1; humus: native soil = 1 : 3 ST2) was conducted to evaluate the effects of AMF inoculation on seedling growth. The levels of plant mycorrhizal response of *E. excelsum* seedlings under the ST1 and ST2 soil types were 136 and 413%, respectively. Although a significant growth enhancement was found in the ST1 soil type, seedling growth and survival rate were improved after AMF colonisation under both soil types. The results suggest that AMF colonisation may have practical implications in establishing effective conservation and restoration strategies for this critically endangered plant.

Introduction

Arbuscular mycorrhiza (AM) is an obligate symbiosis between arbuscular mycorrhizal fungi (AMF) and plant roots. It has been recognised as a highly complex network of plants and fungi interacting in a highly diverse environment in terrestrial ecosystems (Allen *et al*. 2003; Smith and Read 2008). In the past decades, mycorrhizal fungi ability to enhance plant growth and nutrition uptake, as well as to improve disease resistance, drought tolerance, and plant performance, has been well established (Ruiz-Lozano *et al*. 2001; Moora *et al*. 2004). It has also been demonstrated that AMF can affect plant community occurrence, succession, and structure and subsequently determine plant biodiversity, ecosystem variability, and productivity at the ecosystem level (Van der Heijden *et al*. 1998; Urcelay and Diaz 2003; Dhillion and Gardsjord 2004).

Although numerous studies highlight the key role of AM symbiosis in plant performance and ecosystem stability (Van der Heijden *et al*. 1998; Bashan *et al*. 2000), the mycorrhizal status of very few species are known with surprisingly little information on associations with rare and endangered species (Smith and Read 2008; Bothe *et al*. 2010). Studies to determine the mycorrhizal status and response of some endangered or endemic species have been conducted in natural ecosystems (Bethlenfalvay *et al*. 1984; Fuchs and Haselwandter 2004; Fisher and Jayachandran 2005; Bethlenfalvay *et al*. 2007) and greenhouse environments (Barroetavena *et al*. 1998; Gemma *et al*. 2002). For example, Bashan *et al*. (2007) demonstrated that the boojum tree *Fouquieria columnaris* endemic to Mexico is a typical AM plant and that mycorrhiza inoculation improves the physical, chemical and biological properties of soil, and consequently helps plant establishment in arid ecosystems (Carrillo-Garcia *et al*. 1999; Bashan *et al*. 2000). Fisher and Jayachandran (2002) found that native AMF inoculation can notably enhance the seedling growth of two endangered species, *Amorpha crenulata* and *Jacquemontia reclinata*, under nursery conditions. Turjaman *et al*. (2006) showed that the inoculation of *Dyera polyphylla* and *Aquilaria filarial* with AMF increased the survival and early growth of seedlings, while, Panwar and Tarafdar (2006*a*) verified the significance of AMF in the conservation and reestablishment of endangered medicinal plants in arid habitats. Hence, AMF inoculation has been regarded as a tool for the conservation and restoration of endangered plants or degraded forests (Koske and Gemma 1995; Fuchs and Haselwandter 2008; Sharma *et al*. 2008).

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Euryodendron excelsum H. T. Chang is a perennial woody species from the family Theaceae that is an endangered monotypic species endemic to China. As the sole member of a geographically isolated genus, *Euryodendron* plays an important part in the phylogeny and evolutionary of Theaceae (Shen *et al*. 2009). The plant is rarely distributed across the Bajia region of Guangdong Province with <200 extant plants making it the second most endangered plant in China and subject to national protection (Shen *et al*. 2009). In terms of the International Union for Conservation of Nature and Natural Resources (http://www.iucnredlist.org, accessed 8 July 2011) criteria, this plant has been listed as critically endangered meaning it faces a high risk of extinction. To determine the mechanisms causing the endangerment of this species, some studies on *E. excelsum* have been conducted (Wang *et al*. 2002; Shen *et al*. 2009), most of which have focussed on the species' distribution, biological and ecological characteristics and conservation genetics. These studies have demonstrated that *E. excelsum* exists as a single remnant population with a highly isolated and fragmented distribution pattern. The species is mainly distributed close to local villages and is exposed to human activities such as habitat destruction through wood cutting and land clearing for economic plant cultivation. This affects not only the current population size, but also future recruitment of *E. excelsum.* Thus, the conservation and restoration of this species is urgent. Although some studies show that AMF inoculation may be crucial for plant fitness and performance (Urcelay and Diaz 2003; Moora *et al*. 2004), no studies have been conducted on the mycorrhizal status and effects of AMF on *E. excelsum*.

In the present study, we hypothesised that *E. excelsum* is mycorrhizal and predicted that root colonisation of *E. excelsum* by native AMF will improve seedling growth. Specifically, the aims of this study were: (1) to assess the mycorrhizal status of *E. excelsum*, by examining the AMF colonisation, spore density and relationship between colonisation and soil characteristics, and (2) to determine the response of seedling growth to inoculation with native AMF.

Materials and methods

Study sites and sampling

The remnant *E. excelsum* population is distributed rarely across a very small part of the Bajia region (21°57′N, 111°24′E) in Yangchun County, Guangdong Province. Bajia has a humid subtropical monsoon climate with a mean annual temperature of ~22°C. Annual rainfall is more than 2000 mm. The main vegetation is composed of broad-leaved forest, in particular, plantation and secondary forests for wood production (Wang *et al*. 2002). Based on the latest survey, *E. excelsum* exhibits a fragmented pattern with 10 isolated patches in the Bajia region (Shen *et al*. 2009).

Rhizosphere samples (including root and soil adhering to the roots) were collected from all 10 known localities at 5–30-cm depths in December 2007 (dry season). Four replicates were collected in each patch. For the Helu, Muli, and Zhugenfu patches, we collected four replicates from four directions (east, west, north, and south) around each tree since only 1–2 plants were present (Table 1). Roots were washed with tap water, fixed in an FAA solution (5 mL formalin 10% + 5 mL glacial acetic acid + 90 mL 70% ethanol). Soils were stored in polyethylene plastic bags and transported to the laboratory.

Soil analyses

The soil in the study site is oxisolic. Soil analysis was conducted by the Supervision and Testing Centre for Farm Products Quality Ministry of Agriculture and included analysis of soil pH determined by the potentiometric method, total organic carbon (C) determined by the Walkley–Black wet combustion method, and nitrogen (N) determined by the Kjeldahl method. Available phosphorus (P) and potassium (K) were extracted with NH_4HCO_3 + DTPA (diethylenetriaminepentaacetatic acid 0.005 mol·L^{-1}) and were measured by inductively coupled plasma atomic emission spectrometry (Tan 1996).

Spore extraction

Soil samples were wet-sieved for spores according to the method described by Li and Zhao (2005). For each soil sample, 20 g soil was independently suspended in 150 mL water, stirred with a magnetic stirrer for 10 min, and the spores collected using 76-, 105-, 150- and 900-μm sieves. The spores on each sieve were filtered onto filter paper, and AMF spores were counted under a stereoscopic microscope (7–45×). A sporocarp was counted as one spore, and spore density was defined as the number of spores per 100 g of soil. Each spore type was mounted both in PVA (polyvinyl lactic acid) and PVA with Melzer's reagent (1 : 1 v/v)

Table 1. Number of individuals and soil physicochemical properties in studied 10 patches of *Euryodendron excelsum* population

Patches	Coordinates	No. plants	Soil physicochemical characteristics				
			pH	Organic C (%)	Available N (mg kg^{-1})	Available P (mg kg^{-1})	Available K (mg kg^{-1})
Chendong	21°55.88′N, 111°24.50′E	5	4.8	1.54	59.90	8.26	39.60
Chongtian	21°55.97′N, 111°24.92′E	59	4.2	2.20	83.10	8.20	29.60
Helu	21°56.34′N, 111°23.16′E	1	5.3	4.38	143.50	12.30	38.40
Muli	21°55.73′N, 111°22.74′E	1	6.4	1.26	63.30	30.90	391.00
Jietang	21°58.28′N, 111°27.52′E	62	4.3	2.24	103.00	10.80	19.30
Shankou	21°57.62′N, 111°27.04′E	7	4.7	1.24	63.30	5.97	19.50
Shanye	21°55.64′N, 111°23.93′E	14	4.4	2.11	67.70	6.99	19.70
Shijiao	21°55.69′N, 111°23.03′E	12	5.3	3.27	139.10	8.37	86.50
Tangchong	21°57.62′N, 111°27.15′E	16	4.5	1.36	63.30	8.14	23.80
Zhugenfu	21°56.21′N, 111°25.04′E	2	4.2	0.99	64.00	5.99	11.70

for identification (Panwar and Tarafdar 2006*b*; Bashan *et al*. 2007). Identification to the generic level was based on spore size, colour, surface ornamentation and wall structure, with reference to the descriptions provided by the International Collection of Vesicular and Arbuscular Mycorrhizal Fungi (http://invam.caf.wvu.edu, accessed 8 July 2011) and original species descriptions.

Root colonisation by AMF

Roots were washed several times in tap water and cleaned with 10% (w/v) KOH in heated water for 2–3 h. Afterward the cooled fine roots were cut into ~1-cm fragments and stained with 0.5% acid fuchsin (Berch and Kendrick 1982). We quantified the percentage of hyphae, hyphal coils, arbuscules, vesicles, and total AM colonisation using the magnified intersection method (McGonigle *et al*. 1990) under a compound-light microscope (OLYMPUS-BX51, Tokyo, Japan) at a magnification of 200×. A total of 150 intersections were examined for each root sample.

Native AMF inoculum

The native soil was initially sieved through a 5-mm mesh to remove stones and large root segments, and was subsequently placed in a 4-L pot for native AMF nurse culture in the greenhouse according to the method described by Fisher and Jayachandran (2002). *Zea mays* was used as the host plant. When the root AMF colonisation reached at least 80% and many spores (>1600 spores 100 g^{-1} of soil) in the soil (~12 weeks), soils with AMF spores, external mycelium, and colonised root fragments were mixed fully and used as an indigenous AMF inoculum.

AMF inoculation and seedling culture

Seed of *E. excelsum* were surface sterilised with 0.3% potassium permanganate solution and then cleaned with distilled water several times. The seed were sown in a polyvinyl chloride box (28 × 17.5 × 12 cm) with sterilised sand (~2 kg in each box) for germination. A preliminary experiment showed that the purified native soil was not suitable for *E. excelsum* seedling growth due to its poor water-holding capacity. Consequently, we mixed the native soil with humus (3 : 1 and 1 : 3, v/v) to increase the water-holding capacity (Brady and Weil 1996). The humus soil characteristics were: pH 3.6, available P 264.87 mg/kg, available N 1156.24 mg/kg, and available K 701.89 mg/kg. The native soils were collected from the Jietang patch, and the soil properties are listed in Table 1.

The pot experiments followed a 2 × 2 randomised complete block design with five replicates per treatment. The treatments consisted of two types of soils [humus : native soil = 3 : 1 (ST1) and humus : native soil = 1 : 3 (ST2)] with or without mycorrhizal inoculation. The two soil types were autoclaved at 121°C for 45 min then placed into 4-L pots (18 × 24 cm), and three seedlings at the second true leaf stage were transplanted into each pot. Approximately 20 mL of the AMF inoculum was added to each pot 1–3 cm below the seedlings. The uninoculated treatment consisted of 20 mL autoclaved (45 min at 121°C) native AMF inoculum. To ensure that both substrates had the common native microflora, we added a filtrate free of AMF obtained from a suspension of the mycorrhizal inoculum in water before sterilisation to the uninoculated substrate (Aguín *et al*. 2004). Seedlings were grown under natural light greenhouse conditions. Temperatures varied from 18 to 25°C, relative humidity was 80–85%, and the photoperiod was ~12 h. Seedlings were watered as required, and no additional nutrition was applied during the experiment. Cross contamination was prevented by pot separation and care in watering.

Seedlings of *E. excelsum* were harvested after 48 weeks of growth. The height and basal diameter were measured. Dry weight of shoots and roots were examined after drying at 80°C for 72 h. Seedling survival was based on the rate of harvested seedlings compared with the initial number of transplanted seedlings. Each seedling's percentage root colonisation was determined using the above mentioned method. The response of the plant to mycorrhiza was defined as the ratio of dry weight of the inoculated seedlings to that of the uninoculated seedlings (Janos 2007).

Statistical analysis

All data were statistically analysed with SPSS software (version 13.0: SPSS Inc., Chicago, IL, US). Percentage root colonisation data and the spore densities were analysed by one-way ANOVA. Root colonisation was arcsine-transformed and spore density was $\log(x+1)$ transformed to meet ANOVA assumptions although reported means are untransformed. Comparison of means was done using the least significant differences method at the 0.05 level of significance unless reported otherwise. The relationships between soil characteristics and root colonisation, as well as from root colonisation to spore density, were determined by Pearson's correlation analysis. A two-way full factorial ANOVA with replication was performed to determine the effect of indigenous AMF inoculation on seedling growth under the two different soil types.

Results

Soil properties

Soil physicochemical properties of each patch are presented in Table 1. The soils of the 10 patches were all slightly acidic in the range of pH 4.2–6.4. Soil organic C ranged from 0.99 to 4.38%, with the Helu patch being the highest. Available P in most patches contained less than 12 mg/kg, suggesting a low P status in the soil according to the definition of Havlin *et al*. (2005). Available N ranged from 59.9 to 143.5 mg/kg, while available K was from 11.7 to 391.0 mg/kg. In general, available N and P were low at most patches.

AMF colonisation and spore density

In total, 10 patches of *E. excelsum*, all with AM structures including intracellular hyphae, hyphal coils, arbuscules, and vesicles, were examined in the root samples (Fig. 1 and Table 2). Roots had an AMF colonisation ranging from 19 to 59% with an average of 43%. Hyphae were found mostly in root samples of each patch, ranging from 15 to 46%. The hyphal coils and arbuscules were also found frequently with a range of 5–27% and 3–21%, respectively. Vesicles were mainly found in older regions of roots, and consequently had relatively lower colonisation intensity ranging from 1 to 14%. The density of viable AMF spores recovered from the rhizosphere soil samples

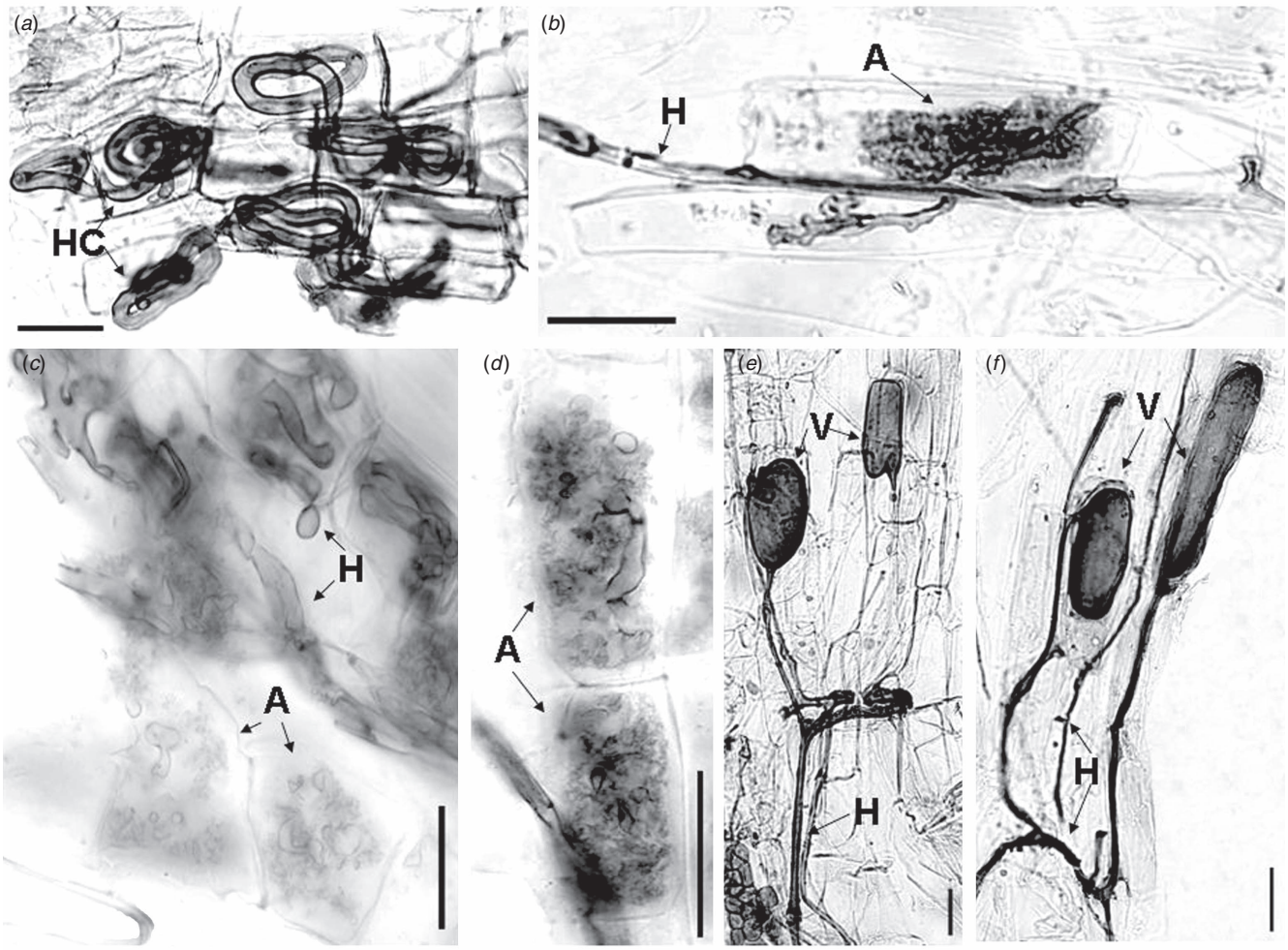

Fig. 1. Arbuscular mycorrhizal fungi colonisation structures in the roots of *Euryodendron excelsum* in the natural conditions: (*a*) hyphal coil (HC); (*b*) hyphae (H) and arbuscule (A); (*c*) H and A; (*d*) A; (*e*) H and vesicle (V); (*f*) H and V. Bar = 10 µm.

Table 2. Arbuscular mycorrhizal fungi (AMF) colonisation and spore density in 10 patches of *Euryodendron excelsum* population
Mean values ± standard error followed by the different letters (a–f) in each column are significantly different within different patches according to l.s.d. test at the 0.05 level of probability

Patches	Spore density ($100\ g^{-1}$ soil)	AMF colonisation (%)				
		Total colonisation	Hyphae	Hyphal coils	Arbuscules	Vesicles
Chendong	1762 ± 190a	59 ± 3.1a	45 ± 2.7a	20 ± 2.9a	21 ± 3.6a	11 ± 0.4b
Chongtian	426 ± 69ef	52 ± 3.0ab	36 ± 1.6b	27 ± 2.8a	7 ± 1.0bc	3 ± 0.3cd
Helu	601 ± 87de	37 ± 4.8b	22 ± 0.4c	11 ± 2.5a	6 ± 1.6bc	5 ± 0.4c
Muli	81 ± 6f	19 ± 3.2c	15 ± 1.9d	5 ± 2.0c	3 ± 0.4c	1 ± 0.4d
Jietang	1357 ± 92b	40 ± 3.6b	24 ± 1.8c	8 ± 0.3b	19 ± 2.6a	3 ± 0.4cd
Shankou	1331 ± 147b	41 ± 5.4b	39 ± 1.7ab	7 ± 0.4b	4 ± 0.1c	12 ± 2.0ab
Shanye	928 ± 52cd	50 ± 5.1ab	38 ± 2.7b	17 ± 0.8a	21 ± 3.4a	13 ± 1.4ab
Shijiao	811 ± 29cde	41 ± 5.5b	26 ± 5.4c	16 ± 2.4a	7 ± 1.0bc	2 ± 0.47cd
Tangchong	999 ± 66bc	58 ± 1.8a	46 ± 1.5a	19 ± 1.2a	18 ± 1.9a	14 ± 1.5a
Zhugenfu	576 ± 39de	34 ± 2.7b	23 ± 0.5c	13 ± 0.8a	11 ± 1.5b	5 ± 1.3c

ranged from 81 to 1762 spores $100\ g^{-1}$ soil. The average is 887 spores $100\ g^{-1}$ soil. Significant differences in AMF variables among study patches were verified (Table 2). Five AMF genera were identified in the rhizosphere soils. *Glomus* species were most dominant followed by *Acaulospora*, *Entrophospora*, *Gigaspora* and *Scutellospora*.

Correlation analysis

No significant correlations between soil physicochemical characteristics and AMF colonisation structures were observed ($P > 0.05$), and no significant relationship existed between total colonisation and spore density ($r = 0.612$, $P = 0.060$). Total AMF colonisation was significantly negatively correlated with available P ($r = –0.704$, $P = 0.023$) and available K ($r = –0.688$, $P = 0.028$) content in the soil, but was positively correlated with hyphal coil ($r = 0.791$, $P = 0.006$), arbuscule ($r = 0.636$, $P = 0.048$), and vesicle colonisation ($r = 0.636$, $P = 0.048$). Moreover, the relationship between total colonisation and hyphae was very significantly positively correlated ($r = 0.921$, $P < 0.001$).

Effect of indigenous AMF inoculation on seedling growth

The seedlings grown both in ST1 and ST2 soil types with AMF inoculation were colonised by indigenous AMF with colonisation under ST2 (42%) being doubly higher than that of ST1 (21%) ($F_{1,4} = 51.838$, $P = 0.002$). No AMF colonisation was observed in the root of the uninoculated seedlings (Table 3). Under the ST1 soil type, inoculated plant height was 16% higher relative to uninoculated seedlings ($F_{1,17} = 7.198$, $P = 0.016$), while no significant variation in plant basal diameter ($F_{1,17} = 0.251$, $P = 0.623$), shoot ($F_{1,4} = 1.438$, $P = 0.297$) and root dry weight ($F_{1,4} = 1.191$, $P = 0.336$), and R/S (root : shoot ratio) ($F_{1,4} = 0.170$, $P = 0.701$) were established between inoculated and uninoculated AMF seedlings. Under the ST2 soil type, AMF colonisation notably increased the height ($F_{1,13} = 7.019$, $P = 0.020$), basal diameter ($F_{1,13} = 5.829$, $P = 0.031$), and R/S ($F_{1,4} = 11.053$, $P = 0.029$). There were no significant interactions between soil type and indigenous AMF inoculation except in the R/S ratio. The response of *E. excelsum* seedlings to mycorrhiza under ST2 was more than three times higher than that of ST1 (Table 3). The inoculation of *E. excelsum* with indigenous AMF increased seedling survival rates compared with that of non-AM seedlings under both soil types, albeit at a higher rate under ST1. The highest seedling survival occurred in the ST1 soil type with indigenous AMF colonisation.

Discussion

Arbuscular mycorrhizal status of E. excelsum

The results of AMF colonisation and spore density in this study showed that *E. excelsum* is a typical mycorrhizal plant under natural conditions. The colonisation of AMF structures and spore density differ greatly among patches. In general, AMF colonisation intensity and spores density are influenced by many factors including soil properties, environmental conditions, plant phenology, fungal predation, and propagule availability (Muthukumar *et al*. 2003; Li *et al*. 2007). In the present study, there are no correlations of spore density to root colonisation or to soil physicochemical characteristics. There is also no correlation of AMF colonisation structure to soil physicochemical characteristics. This result probably indicates that the factors influencing the colonisation of *E. excelsum* in natural habitats are numerous and complex requiring further elucidation of the factor that drives the variance of AMF colonisation in different patches.

Mean AMF spore density in soil associated with *E. excelsum* was 887 per 100 g of soil which is higher than spore densities recorded for other endangered plants in Desert ecosystems (Carrillo-Garcia *et al*. 1999; Panwar and Tarafdar 2006*a*, 2006*b*). This may be attributed to the variations in plant phenology and environmental properties. Furthermore, samples were collected in the dry season (December) when the highest spore density is to be expected (Guadarrama and Álvarez-Sánchez 1999). Previous studies have found that the *E. excelsum* population is mainly distributed around local villages where human activity is high. In such places, the rhizosphere of this species can be disturbed by native land use and agricultural practices (Shen *et al*. 2009) and it is generally considered that high disturbance can decrease soil AMF spore density (Guadarrama and Álvarez-Sánchez 1999; Enkhtuya *et al*. 2000). In our study, however, the relatively higher spore density in these disturbed patches contradicts the view that disturbance reduces spore density, but is consistent with other studies that show AMF spore density was not influenced during the conversion of tropical forest to agriculture in Costa Rica (Johnson and Wedin 1997; Picone 2000). This implies that the relationship between habitat and AMF spore density is not simple, and requires further investigation.

Table 3. Effects of arbuscular mycorrhizal fungi (AMF) inoculation on *Euryodendron excelsum* seedling growth

Mean values ± standard error followed by the different letters (a–d) in each column are significantly different within different treatment according to l.s.d. test at the 0.05 level of probability. * $P < 0.05$, ** $P < 0.01$, n.s.: not significant

Soil type	AM treatment	Height (cm)	Basal diameter (cm)	Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})	Root : shoot ratio	Colonisation rate (%)	Plant mycorrhizal response (%)	Survival rate (%)
ST1	–AM	50.3 ± 2.43b	0.7 ± 0.05a	5.7 ± 1.37a	1.3 ± 0.37ab	0.2 ± 0.01b	–	–	46.67
	+AM	58.4 ± 1.82a	0.7 ± 0.02a	8.0 ± 1.45a	1.8 ± 0.30a	0.2 ± 0.01b	21 ± 2.22b	136	80.00
ST2	–AM	15.9 ± 2.09d	0.3 ± 0.02c	0.6 ± 0.03b	0.2 ± 0.01c	0.3 ± 0.01b	–	–	46.67
	+AM	25.1 ± 2.68c	0.4 ± 0.05b	2.3 ± 0.06b	0.8 ± 0.03bc	0.3 ± 0.02a	42 ± 1.83a	413	53.33
ANOVA									
Soil		**	**	**	**	**	–	–	–
AM		**	*	n.s.	*	*	–	–	–
ST*AM		n.s.	n.s.	n.s.	n.s.	*	–	–	–

Previous studies have demonstrated that soil P can affect AMF colonisation under different conditions (Li *et al*. 2007). In general, high soil P can inhibit germ tube growth and reduce the production of root exudates that stimulate hyphal branching near roots, reducing AMF colonisation (Smith and Read 2008). In this study, total AMF colonisation was negatively correlated with available P and K, suggesting a low AMF response when plants have high nutrient availability. The result is similar to previous studies, which showed that AMF colonisation is negatively correlated with soil P (Fisher and Jayachandran 2002; Panwar and Tarafdar 2006*a*, 2006*b*). No significant relation between total AMF colonisation and spore density was observed in our study. Similar results were also examined by Carrillo-Garcia *et al*. (1999) and Bashan *et al*. (2000) when studying the effect of mycorrhiza on plant establishment in Desert ecosystems. This result supported the view that no relationship could be established between colonisation and AMF spore density throughout the course of a year (Daniell *et al*. 2001; Li *et al*. 2007). Moreover, sporulation of some AMF species, especially those from the genera *Scutellospora* and *Gigaspora*, is always preceded by a prolonged phase of root colonisation (Camargo-Ricalde and Dhillion 2003; Landis *et al*. 2004). Therefore, the abundance of spores produced in the rhizosphere soil does not always coincide with AMF colonisation in the root.

Effect of indigenous AMF colonisation on seedling growth

As expected, although the ST1 soil markedly stimulated seedling growth compared with the ST2 soil, seedlings inoculated with indigenous AMF had enhanced height, basal diameter and plant biomass compared with uninoculated seedlings regardless of soil types. These results are similar to other reports, including *A. crenulata* and *J. reclinata* (Fisher and Jayachandran 2002), *D. polyphylla* and *A. filarial* (Turjaman *et al*. 2006), and *Curculigo orchioides* (Sharma *et al*. 2008). Seedling growth and survival rate is a crucial measure of success in the conservation and restoration of endangered plants (Bashan *et al*. 2009). In the present study, seedling survival was higher for the ST1 soil, indicating an important role of soil properties (i.e. soil nutrition and water-holding capacity) for seedling propagation of *E. excelsum*. Moreover, survival rates in both soil types increased after AMF colonisation, making it is reasonable to assume that seedling survival is influenced by soil nutrition and will be improved by AMF colonisation, especially in poor soils.

It is acknowledged that soil nutrient levels (especially P) can influence host plant response to AMF, and that high soil P concentrations can reduce or eliminate mycorrhizal colonisation (Bethlenfalvay and Linderman 1992; Smith and Read 2008). In our study, no growth differences were found between inoculated and uninoculated seedlings under ST1 soil conditions. Compared with the ST2 soil, the plant mycorrhizal response was only 136% in the ST1 soil, suggesting a relatively lower response of seedling growth to mycorrhizal colonisation. Under ST2 soil conditions, AMF colonisation rate and mycorrhiza response level of *E. excelsum* seedlings reached about two and three times higher than the ST1 soil, suggesting that given the native soil with even less nutrients, mycorrhiza should be even more important in the wild. Overall, seedling growth and survival were improved by indigenous AMF inoculation under both ST1 and ST2 soil conditions even though this study used a substrate with sufficient soil nutrient (ST1 soil). This is similar to the results presented by Aguín *et al*. (2004), who found that grapevine growth can be enhanced by AMF inoculation even when sufficient levels of P occur. Furthermore, it has also been demonstrated that the plant response to mycorrhizal colonisation varies according to host, soil nutrient levels and mycorrhizal species (Nogueira and Cardoso 2007; Zangaro *et al*. 2007). Therefore, soil nutrient levels that ensure efficient improvement of *E. excelsum* seedling growth without deterring AM symbiosis need to be evaluated in future experiments.

The uninoculated seedlings also showed normal growth, with survival rates reaching 46.67% under both ST1 and ST2 soil conditions. The results support the opinion of Pattinson *et al*. (2004), who pointed out that inoculating a seedling with AMF is not essential for its establishment and survival when adequate minerals are supplied exogenously. Bashan *et al*. (2000) also found that AMF inoculum is not the primary factor for the establishment of cactus seedlings in Desert ecosystems. However, considering the seedling survival, we suggest that nursery propagation of this endangered plant should use relatively higher nutrient soils as well as AMF, although higher nutrient soils reduced AMF colonisation.

The results obtained from this study indicate that *E. excelsum* is a characteristically mycorrhizal plant under natural conditions but that colonisation and spore density varied among the 10 known patches. Seedling growth and survival of *E. excelsum* were enhanced by native AMF colonisation grown in two different soil types under greenhouse conditions. The restoration of *E. excelsum* proposed in China includes augmentation to reinforce remnant populations or to create new populations into similar protected habitats. Our results suggest that the use of indigenous AMF inoculation is a sound method that can be applied in designing the restoration and conservation strategies of this endangered plant. However, the AMF colonisation for seedling growth under natural conditions still needs further evaluation.

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