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Arbuscular mycorrhizal associations in the Gurbantunggut Desert

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Abstract The plants in the Gurbantunggut Desert located in Xinjiang, China are drought adapted species and grow in generally nutrient-poor soils. A survey was conducted in the desert to determine the arbuscular mycorrhizal (AM) status associated with the desert plants which belong to 23 species from 11 families of annuals and perennials. Roots from all plants were examined for the presence of internal and external hyphae, vesicles and coils/arbuscules to determine the status and extent of mycorrhizal colonization. Of the plant species surveyed, 14 (61%) were found to form AM associations, 5 (22%) were possible AM colonized species, and 4 were non-mycorrhizal plants. The proportions of annuals and shrubs forming AM were significantly lower than those of perennials and herbs, respectively. Spore density varied from 5 to 21 per 20 g in soil of root zone. 14 AM fungal taxa in 3 genera were isolated and identified, 10 of which belonged to *Glomus*, 3 to *Acaulospora*, and 1 to *Archaeospora*. *Glomus* was the dominant genus in all genera identified. *G. desert-icola* and *G. etunicatum* were the most common taxa isolated, with occurrence frequencies of 77.4% and 74.8%, and relative abundances of 14.4% and 15.5%, respectively.

Keywords: xerophyte, arbuscular mycorrhiza, spore density, relative abundance, frequency, Gurbantunggut Desert.

Gurbantunggut Desert, located in the hinterland of the Junggar Basin in north-western China, lies between 44°11′—46°120′N and 84°31′—90º00′E. It covers an area of 48,800 km^2 and is the largest fixed and semi-fixed desert in China. Main morphological dune types in the desert are balk-hollow dune ridge and dentritic dune ridge, which are generally a few hundred meters to more than 10 km in length, 10—50 m in height and orientate from north to south. Interridge and middle to lower parts of a dune are stabilized but the crests of dune often have 10—40 m wide mobile zone. Under the action of bi-directional winds, sand materials move leftward and rightward and extend along the crest line of dune[1]. Annual accumulated temperature varies between 3000 and 3500℃, annual precipitation 70—150 mm and annual evaporation exceeds 2000 mm. It belongs climatically to the typical inland arid zone. There is a stable accumulated snow with a thickness of 20 cm in winter. Precipitation distribution is better in spring and summer than in autumn and winter, and such water and heat allocation pattern creates a favorable condition for the growth and development of plants. Soil profile differentiation is invisible and surface layer is poor in fertility. Interridge consists of mixed sand that includes coarse, medium, fine and silt sand, and there is little organic matter on soil surface. Dominant winds over the region are NW and NE winds caused by westerly circulation and Mongolian high pressure. Threshold wind ($\geqslant$6 m/s) occurs from April to September, mainly in April, May and June[2].

It is well known that drought and poor nutritions in soil are the basic limiting factors for the growth of plants in desert ecosystem. Arbuscular mycorrhiza (AM) has been proved as mutualistic associations which primarily assist plants in uptake of immobile nutrients such as phosphorus (P)[3]. In another aspect, there are increasing evidences that AM can improve plant drought tolerance via some mechanisms[4—8]. Therefore, AM fungi are crucially important for survival and growth of individual plant and stability of plant community structure, especially in a weak ecosystem[9—15]. AM fungal inoculum can promote revegetation in degraded or disturbed ecosystems. There has been increasing interest in the arbuscular mycorrhizae of desert plants in recent years[16—19]. In this study, a field survey was conducted to determine the mycorrhizal status of plant species in the Gurbantunggut Desert.

1 Materials and methods

1.1 Collection of soil and root samples

Twenty-three plant species belonging to 11 families were investigated. The biological characteristics of

each plant were listed in Table 1. The top ca. 1—2 cm of the soil surface was removed and soil and root samples were collected. Care was taken during collection of individual plants that the roots could be positively identified as belonging to a particular plant. Entire plants were dug out by trowel to ensure that the roots remained connected to the shoots after sample collection. Samples were taken to the laboratory for determination of AM root colonization. Soil samples (ca. 1000 g) associated with the plants removed were collected. The soil samples were air-dried prior to extraction, counting and identification of AM fungal spores.

1.2 Assessment of AM colonization

Fresh roots (approximately 0.2 g) were processed by washing them free of soil and clearing in 10% (w/v) KOH at 90℃ in a water bath for 15—30 min, the exact time depending on the degree of lignification of the roots and their pigmentation. The cooled root samples were washed and cut into 0.5 to 1.0-cm-long segments and stained with 0.5% (w/v) acid fuchsin[20]. Thirty root fragments (approximately 1 cm long) were mounted on slides in a polyvinyl alcohol-lactic acid-glycerol solution[21] and examined at 100—400× magnification under an Olympus BX50 microscope with an automatic photo micrographic system for the presence of AM fungal structures. The percentage of root length colonized by AM fungal structures was determined using the magnified line-intersect method of McGonigle *et al*.[22]. And also hyphae, coils/arbuscules and vesicles were examined and recorded as present '+' or absent '–' from each sample. Plants were not considered to be mycorrhizal if external hyphae were the only evidence of fungal colonization. If at least one root segment was observed to contain coils/arbuscules or vesicles, then the plant was recorded as an AM plant. If the root cortex was found to be colonized by fungal mycelia without coils/arbuscules or vesicles, the plant was recorded as possible AM. The extent of root length colonized was assessed for each sample and categorised as '0' (non-mycorrhizal), 'low' (< 10%), 'med' (< 30%) and 'high' (> 30%).

1.3 Extraction and counting of AM fungal spores

Three soil samples were randomly selected from the six original samples. Spores or sporocarps were ex-

Table 1 The biological characteristics of investigated plants in the Gurbantunggut Desert

	Plants	Life form	Habit
Chenopodiaceae	*Atriplex cana* C.A.Mey	herb	perennial
	Horaninowia ulicina Fisch. et Mey.	herb	annual
	Salsola laricifolia Turcz. Ex Litv.	shrub	perennial
	Salsola sp.	herb	annual
Compositae	*Artemisia desertorum* Spreng.	herb	perennial
	Seriphidium gracilescens (Krasch. Et Iljin) Poljak.	herb	perennial
	Seriphidium santolinum (Schrenk) Poljak.	herb	perennial
	Seriphidium terrae-albae (Krasch.) Poljak.	herb	perennial
Cruciferae	*Erysiumum* sp.	herb	annual
Ephedraceae	*Ephedra distachya* L.	shrub	perennial
Fabaceae	*Astragalus lithophilus* Kar. et Kir.	herb	annual
	Eremosparton songoricum (Litv.) Vass.	shrub	perennial
Gramineae	*Aristida heymannii* Rgl.	herb	annual
	Cleistogenes songorica (Rashev.) Ohwi	herb	perennial
Liliaceae	*Allium fetisovii* Rgl.	herb	perennial
	Allium sp.	herb	perennial
Polygonaceae	*Agriophyllum squarrosum* (L.) Moq.	herb	annual
	Calligonum junceum (Fisch. et Mey.) Litv.	shrub	perennial
	Calligonum sp.	shrub	perennial
Scrophulariaceae	*Pedicularis* sp.	herb	perennial
Tamaricaceae	*Tamarix* sp.	shrub	perennial
Zygophyllaceae	*Zygophyllum cuspidatum* Boriss.	herb	annual
	Zygophyllum macropodum Boriss.	herb	perennial

tracted from 20 g air-dried subsamples of each soil sample in triplicate by wet sieving followed by flotation-centrifugation in 50% sucrose[23]. The finest sieve used was 53 μm. The spores were collected on a grid patterned (4 mm × 4 mm) filter paper, washed three times with distilled water to spread them evenly over the entire grid, and counted using a dissecting microscope at 30× magnification. A sporocarp was counted as one unit. The number of spores is expressed as the mean of three replicates. For observation and identification of spore characters, spores were mounted on glass slides in polyvinyl alcohol-lactoglycerol (PVLG) and PVLG + Melzer's reagent and then identified to species level using current taxonomic criteria[24] and information published by INVAM (http://www.invam.caf.wvu.edu).

1.4 Numbers and distribution of AM fungal spores

AM fungal spore density and species occurrence frequency and relative abundance were expressed as follows: spore density (SD), number of AM fungal spores in 20 g dry soil; relative abundance (RA), (number of spores of a species or genus/total spores) × 100; and frequency (F), (number of samples in which the species or genus was observed/total samples) × 100.

2 Results

The fungal colonization status of 23 species of desert-fixing plants belonging to 11 families and the AM fungal spore density in rhizosphere soil of these plants were summarized in Table 2. Of the plant species surveyed, 14 (61% of the total plants) were found to form mycorrhizal structure, like coils/arbuscules and vesicles (Table 2). They belonged to families of Compositae, Fabaceae Gramineae, Lilium, Scrophulariaceae, Tamaricaceae and Zygophyllaceae. Five taxa (22% of the total plants surveyed) belonged to all members of Polygonaceae, and part members of Chenopodiaceae contained fungal hyphae but no coils/arbuscules or vesicles. These species were recorded as possible AM plant. No typical mycorrhizal structures were observed in all members of Cruciferae and Ephedraceae and 2 members of Chenopodiaceae. These plants were recorded as non-mycorrhizal plants. Among 14 mycorrhizal plant species, the percentage mycorrhizal colonization of 10 plant species was more than 30%.

Table 2 Arbuscular mycorrhizal status of plant species and spore density in their root zone soil in the Gurbantunggut Desert

Family	Species	Colonization[a)]	Internal hypha	Coils/Arbuscules	Vesicles	Spore density
Chenopodiaceae	*Atriplex cana*	0	–[b)]	–	–	8
	Horaninowia ulicina	0	–	–	–	11
	Salsola laricifolia	low	+[c)]	–	–	10
	Salsola sp.	low	+	–	–	7
Compositae	*Artemisia desertorum*	high	+	+	–	16
	Seriphidium gracilescens	med	+	–	+	15
	Seriphidium santolinum	high	+	+	+	16
	Seriphidium terrae-albae	high	+	+	+	20
Cruciferae	*Erysiumum* sp.	0	–	–	–	5
Ephedraceae	*Ephedra distachya*	0	–	–	–	13
Fabaceae	*Astragalus lithophilus*	high	+	+	+	18
	Eremosparton songoricum	high	+	+	+	21
Gramineae	*Aristida heymannii*	med	+	–	+	10
	Cleistogenes songorica	med	+	–	+	11
Liliaceae	*Allium fetisovii*	high	+	+	+	15
	Allium sp.	high	+	+	–	18
Polygonaceae	*Agriophyllum squarrosum*	low	+	–	–	9
	Calligonum junceum	med	+	–	–	8
	Calligonum sp.	low	+	–	–	11
Scrophulariaceae	*Pedicularis* sp.	high	+	–	+	15
Tamaricaceae	*Tamarix* sp.	high	+	–	+	12
Zygophyllaceae	*Zygophyllum cuspidatum*	med	+	–	+	16
	Zygophyllum macropodum	high	+	+	+	19

a) Colonization: 0, no mycorrhiza found; low, <10%; med, <30%; high, >30%; b) "–" denotes absence; c) "+" denotes presence.

Coils/arbuscules were observed in 8 plants; vesicles were observed in 12 species. AM fungi spores were isolated from all investigated members although a few species were non-mycorrhizal plants (Table 2). The overall mean density of AM fungal spore was 13 per 20 g dried soil and ranged widely from 5 to 21.

There were significant differences in the proportions of annual or perennial plants which formed AM structures (Table 3; χ^2 = 13.89, d.f. = 1, P = 0.0002). The proportion of plant species forming AM structures differs significantly between herbs and shrubs (χ^2 = 6.04, d.f. = 1, P = 0.014).

A total of 14 taxa representing three genera of AM fungi were identified in the samples from 23 plant species. Of the 14 taxa, 3 belonged to the genus *Acaulospora*, 1 to *Achaeospora*, and 10 to *Glomus* (Table 4). The most abundant and most frequent AM fungal taxon present was *G. etunicatum* Becker & Gerdemann. The relative abundance of AM fungi varied from 2.1 (*Ar. leptoticha* (Schenck & Smith) Morton & Redecker) to 15.5% (*G. deserticola* Trappe, Bloss & Menge), with an average of 7%. The frequencies of AM fungi ranged from 12.2% to 77.4%, with an average of 38.2%.

3 Discussion

AM fungi play an important role in plant survival and in the community stability of vegetation in natural ecosystems[25−29]. Plant community structure and AM status and colonization might be used to monitor desertification and soil degradation. Several studies have been conducted in recent years on AM fungi in desert ecosystems[16−19].

No mycorrhizal structures were founded in the members in families of Cruciferae and Ephedraceae and part members of Chenopodiaceae in our survey. This result was reasonable because Cruciferae, Chenopodiaceae and Polygonaceae have been generally considered as non-mycorrhizal families[3]. However, two members of Chenopdiaceae were possible AM. The occurrence of mycorrhizal colonization of plant species

Table 3 Distribution of plant species that form arbuscular mycorrhiza (AM) among life forms and growth habits

	No. of species			Total no. of species	Species with AM (%)
	mycorrhizal	possible AM	non-mycorrhizal		
Habit					
Annual	3	2	2	7	43
Perennial	11	2	2	16	69
Life form					
Herb	12	3	3	17	71
Shrub	2	1	1	6	33

Table 4 Relative abundance (%) and frequency of occurrence (%) of AM fungal species in the rhizospheres of investigated plants

AM fungal species	Relative abundance	Frequency of occurrence
Acaulospora (3)		
A. delicata Walker, Pfeiffer & Bloss	3.2	15.7
A. elegans Trappe & Gerdemann	5.0	12.2
A. rehmii Sieverding & Toro	4.5	18.3
Achaeospora (1)		
Ar. leptoticha (Schenck & Smith) Morton & Redecker	2.1	15.7
Glomus (10)	0	
G. aggregatum Schenck & Smith	9.7	42.6
G. caledonium (Nicol. & Gerd.) Trappe & Gerd.	5.1	27.8
G. claroideum Schenck & Smith	7.4	43.5
G. deserticola Trappe, Bloss & Menge	15.5	74.8
G. dolichosporum Zhang & Wang	7.1	46.1
G. etunicatum Becker & Gerdemann	14.4	77.4
G. geosporum (Nicol. & Gerd.) Walker	3.0	37.4
G. microaggregatum Koske, Gemma & Olexia	8.8	49.6
G. microcarpum Tulasne & Tulasne	11.7	52.2
G. mosseae (Nicol. & Gerd.) Gerd. & Trappe	2.4	20.9

in the Chenopodiaceae has occasionally been re- ported[16,30−32] although this family is usually thought to be non-mycorrhizal. Moreover, O'Connor *et al*. observed vesicles in *Salsola kali* and *Sclerolaena diacantha* of Chenopodiaceae[16]. In the present study, no coils/arbuscules and vesicles were observed in Chenopodiaceae possibly due to the seasonal influence. Seasonal patterns have been shown to influence the formation of arbuscules in coastal sand-dune plants[33] and arbuscules in Chenopodiacean plants have been observed to be short lived[30,31]. Arbuscules might occur in Chenopodiacean plants in this study but were not detected because of limitations in the number of samples and the time of sampling. The occurrence of mycorrhizal colonization on plant species in the Zygophyllaceae was observed in this study although this family is usually thought to be non-mycorrhizal. This result was contrary with the conclusion made by O'Connor *et al*.[16]. However, it accorded with the results obtained from desert in Israel[34]. *Ephedra distachya* belonging to the family of Ephedraceae was found as a non-mycorrhizal plant in this study. This is inconsistent with the observation in another species of *Ephedra trifurca* in the family reported by Collier *et al*. in the Chihuahuan Desert[18]. The possible explanation might be the difference of sampling time and sites because the opportunities for sampling were restricted to short surveys and the quantity of roots collected was small, so the absence of mycorrhizal colonization could not be the conclusive evidence that a species is non-mycorrhizal. O'Connor *et al*.[16] showed that the same plant species may be considered as mycorrhizal or non-mycorrhiza species due to the difference of distribution in dune's position.

The proportion of plant species surveyed that did not form AM structures was lower than that of mycorrhizal plants. This is consistent with previous reports that the proportion of non-mycorrhizal plants is relatively lower in arid ecosystems[35−38]. The proportions of annuals and shrubs forming AM were significantly lower than that of perennials and herbs, respectively. These results supported previous report obtained from the Simpson Desert[16].

It has been reported that both spore production and species richness of AM fungi are lower in arid environments than in others[39,40], and they usually decrease with aridity increasing[41,42]. The mean spore density in our study was lower than reports from other ecosystems, like the tropical rain forest of southwest China[43,44] and semiarid regions of Nambia[45]. However, our results are similar to those from other desert ecosystems such as the Mojave[17] and Negev deserts[34].

Fourteen AM fungal taxa were isolated and identified in the study area. This number was basically accordant with the species isolated from the Chihuahuan Desert (12 species)[18]. AM fungi belonging to the genus *Glomus* seem to be dominant in the root zone of investigated plants in the Gurbantunggut Desert. This provides strong support for the conclusions of other researchers who suggested that AM fungi belonging to *Glomus* tend to be dominant in arid ecosystems[46,47].

In summary, plant species growing on sand dunes have a high proportion of forming mycorrhizal association with AM fungi[48,49] and therefore mycorrhizal inoculum has been considered as agents for promoting revegetation on sand dune[50,51]. Given the low fertility of these dune sands it is likely that AM association improves the P-uptake capacity of many of these species[16]. To our knowledge, there is no report on AM association in the Gurbantunggut Desert, the second largest sandy desert in China. The relationship between plant distribution and mycorrhizal colonization needs to be further studied in this area.

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