Arbuscular mycorrhizal fungi associated with the clonal plants in Mu Us sandland of China*

Zhao Jinli and He Xueli**

(College of Life Sciences, Hebei University, Baoding 071002, China)

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Abstract Clonal plants in Mu Us sandland change the sandy enviroment. The clonal plant is a kind of resource in restoration of the Mu Us sandy landscape. Soil samples at depth of 50 cm in the rhizosphere of the clonal plants were collected in 4 replicates at each location and divided into sections corresponding to 0—10, 10—20, 20—30, 30—40 and 40—50 cm depths in two representative sites from north to south in Mu Us sandland, northwestern China, in July 2005. Clonal plants included *Psammochloa villosa* and *Hedysarum laeve*. The colonization and ecological distribution of arbuscular mycorrhizal (AM) fungi were investigated in the rhizosphere of clonal plants in Mu Us sandland. The results showed that the clonal plants established well symbiosis with AM fungi; AM fungal species and spatial distribution were significantly related with the host plants and soil factors. Of 16 AM fungal taxa in three genera isolated and identified, *Glomus multicaule* was only observed in the rhizosphere of *Psammochloa villosa*; *Glomus aggregatum*, *Glomus hydembadensis*, *Glomus constrictum* and *Acaulospora rehmii* only appeared in the rhizosphere of *Hedysarum leave*. The depth of soil layers observably affected the spore density and the frequency of colonization (%F). The maximal %F and spore density occurred in the 10—20 cm layer at the site of Ordos Sandy Land Ecological Station, but which occurred in the 0—10 cm layer in Shanxi Yulin Rare Sandy-plants Conversation Field. AM fungal status and colonization might be used to monitor desertification and soil degradation.

Keywords: AM fungi, clonal plants, Mu Us sandland.

In sandland, vegetation and soil microorganisms play a fundamental role in sand stabilization. Arbuscular mycorrhizal (AM) fungi evolved concurrently with the first colonization of land by plants some 450 to 500 million years ago and persist in most extant plant taxa[1]. In particular, arbuscular mycorrhizas are ubiquitous symbioses between fungi and plants. The associations formed between plant roots and AM fungi are of great interest because of their potential influence on important processes in the soil-plant interface, such as improving plant nutrition by increased nutrient and water uptake[2]; enhancing establishment, growth, and survival of seedlings due to improved stress tolerance[3—6]; binding sand grains into large aggregates[7]; and improving soil structure that can influence plant succession[8, 9].

Mu Us sandland is the biggest mobile sandland in dry and nutrient-poor grassland of China, whose complex dynamics mainly depends on wind exposure, sand deposition, sand texture and fluctuations in soil moisture and temperature. These characteristics influence plant colonization in accordance with microenvironmental condition, where the plants that are established may exhibit adaptations to survive in these extreme environments.

Clonal plants are those which reproduce asexually by means of branches that remain attached to the parent during their establishment. Once established, branches form new potentially independent units (ramets). However, ramets often stay connected to the parent for a time, resulting in a group of connected ramets (a clonal fragment). Clonal plants possess wider ecological neighborhood than no-clonal plants. They are the drivers of changes in community environment and make great contribution to the maintenance of community function in the restorational process of vegetation. The clonal plants can considerably improve the ability of self-rehabilitation of a sandy landscape.

The rhizomatous clonal semi-shrub and clonal grass are dominant plant species and important for vegetation restoration in the Mu Us sandland. Clonal physiological integration often helps clonal plants buffer local environmental stresses encountered by the

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** To whom correspondence should be addressed. E-mail: xuelh1256@yahoo.com.cn

ramets. Previous studies on mycorrhizas have shown that plants of sandland are abundantly colonized by AM fungi[10, 11]. The present study is carried out to better understand the arbuscular mycorrhizal status in rhizosphere of clonal plants in Mu Us sandland. At the same time, the results of this study will provide more information for rehabilitating vegetation and resuming environment in desert regions.

1 Materials and methods

1.1 Study site

Two study sites (Fig. 1) were selected, Ordos Sandy Land Ecological Station of the Institute of Botany, the Chinese Academy of Sciences (OSES) and Shanxi Yulin Rare Sandy-plants Conversation Field (RSCF). OSES (39°29′N; 110°11′E) is located in the northeast of Ordos Plateau of Nei Mongol, with altitude of 1300—1400 m, mean annual temperature of 6.0—8.5 ℃, and mean annual rainfall of 370 mm. Ecological environment is very brittle and impressible. RSCF (38°21′N; 109°40′E) is located in the south of Mu Us sandland, with altitude of 1200 m, mean annual temperature of 10.7 ℃, and mean annual rainfall of 412.4 mm. Several decades of sand-binding plants species were introduced.

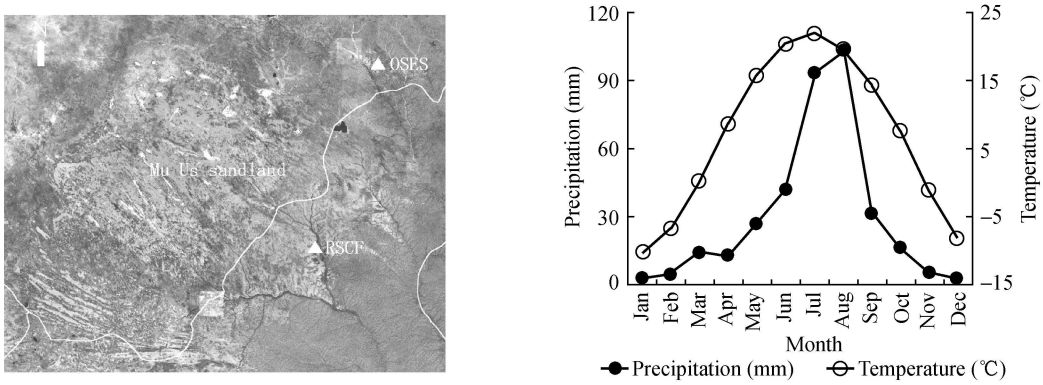

Fig. 1. Sampling sites in Mu Us sandland.

1.2 Collection of soil and root samples

Soil samples were collected in 4 replicates from the rhizosphere of *Psammochloa villosa*, *Hedysarum laeve*. Surface soil (approximately 1—2 mm) was removed, and soil cores of 0 to 50 cm that were divided into 5 sections for interval 10 cm were collected including fine roots and rhizosphere soils of the host plants in July, 2005. Each layer from each replicate was placed in an individual plastic bag and transported to the laboratory. Air-dried soil samples were sieved (2-mm mesh size) and root segments were collected from each sample.

1.3 Soil chemical analysis

Subsamples from each replicate were used for soil chemical analysis. pH was measured in water (1∶5). Organic matter content was assessed using the Walkey-Black method[12] and available N was measured using alkali hydrolysis diffusion method. Available P was assessed by the method of Murphy and Riley[13] after the extraction with $NaHCO_3$ according to Olsen et al.[14]. Exchangeable K^+ was measured by atomic absorption spectrophotometry.

1.4 Assessment of AM colonization

Fresh roots were cut into 0.5 to 1.0 cm long segments and processed by washing them free of soil and clearing in 10% KOH at 90 ℃ in a water bath for 15—30 min, the exact time depending on the degree of lignification of the roots. The root subsamples were cooled, washed and stained with 0.5% acid fuchsin. Thirty root fragments were examined at 100—400× magnification using a Nikon YS100 microscope with an automatic photomicrographic system for the presence of AM fungal structures. The frequency of colonization (%F), the proportion of hyphae (%H), arbuscules (%A) and vesicles (%V) present in the roots were recorded.

1.5 Identification and extraction of AM fungal spores

Spores or sporocarps were extracted from 100 g air-dried soil of each soil sample by wet sieving fol-

lowed by flotation-centrifugation in 50% sucrose[15]. The spores were collected on a filter paper, washed several times with distilled water, and counted using a dissecting microscope at 75× magnification. A sporocarp was counted as one unit. For observation and identification of spore characters, spores were mounted on glass slides in PVLG and PVLG+Melzer's reagents and then identified to species using current taxonomic criteria[16] and information published by INVAM (http://www.invam.caf.wvu.edu).

1.6 Numbers and distribution of AM fungal spores

Spore density, species richness, frequency and relative abundance of AM fungi were expressed as follows: spore density (SD), number of AM fungal spores in 100 g air-dried soil; species richness (SR), number of AM fungal taxa found in 100 g air-dried soil; frequency (F), (number of samples in which the species or genus was observed/total samples) × 100; and relative abundance (RA), (number of spores of a specie or genus/total spores) × 100.

1.7 Statistical analysis

The data were subjected to one-way ANOVA using SPSS software version 13.0.

2 Results

2.1 Soil chemcial analysis

Soil characteristics of the two topographic units were similar. All soils were sandy and strongly infertile. Soil N, P, K and organic matter were low. In the 0—10 cm layer, available N, P, K and organic matter were of the maximal value, then decreased slightly alone with depth. Soils in OSES were more infertile than that in RSCF. But soils in OSES were alkaline and soils in RSCF were weakly acidic (Table 1).

Table 1. Comparison of the samples in different soil layers

Plant species	Soil depth (cm)	OSES					RSCF				
		pH	N available (mg/kg)	P available (mg/kg)	K available (mg/kg)	Organic matter (%)	pH	N available (mg/kg)	P available (mg/kg)	K available (mg/kg)	Organic matter (%)
Psammochloa villosa	0—10	7.99a	1.31a	2.74a	32.90a	0.13a	6.97a	2.27a	7.67a	110.32a	0.23a
	10—20	8.03a	0.98a	2.25a	24.66a	0.08a	6.69b	1.13b	4.84b	61.07b	0.08b
	20—30	8.21a	0.89a	2.11a	23.57a	0.07a	6.66b	0.98b	3.97b	42.40b	0.05bc
	30—40	8.26a	0.71a	2.06a	23.47a	0.07a	6.78b	0.75b	3.69b	36.55b	0.04bc
	40—50	8.29b	0.66a	1.95a	22.89b	0.06a	6.70b	0.70b	3.48b	33.57b	0.03c
Hedysarum laeve	0—10	8.31a	2.43a	3.11a	80.20a	0.16a	6.75a	4.52a	4.63a	123.43a	0.61a
	10—20	8.39a	2.34a	2.48a	27.89ab	0.14a	6.80a	2.32b	3.20ab	84.08b	0.25b
	20—30	8.52a	2.06a	2.19a	24.59b	0.13a	6.81a	1.11bc	2.77ab	49.78c	0.14b
	30—40	8.56a	1.78a	2.18a	24.08b	0.09a	6.88a	0.77c	2.41ab	49.35c	0.13b
	40—50	8.63b	1.68a	1.74a	23.42b	0.09a	7.35b	0.38c	2.04b	42.16c	0.09b

Note: Different letters in the some column are considered significantly different.

2.2 Arbuscular mycorrhizal colonization

The AM status of the clonal plants is shown in Table 2. Arbuscular mycorrhizal fungi colonized two plant species in every layer. Frequency of colonization of *Psammochloa villosa* had no significant differences from 79.6% to 100% among different layers. Frequency of colonization of *Hedysarum laeve* was significantly lower in the 40—50 cm layer than that in the 0—40 cm layer. However, frequency of colonization of *Psammochloa villosa* and *Hedysarum laeve* was both higher in OSES than in RSCF. The arbuscular, vesiclar and hyphal infection showed a similar pattern of development (Table 2).

Table 2. Arbuscular mycorrhizal status of the samples in different soil layers

Plant species	Soil depth (cm)	OSES						RSCF					
		%A	%V	%H	%F	Spore number /100 g soil	Species richness	%A	%V	%H	%F	Spore number /100 g soil	Species richness
Psammochloa villosa	0—10	10.4a	52.4a	58.1a	70.8b	82a	3.5a	12.6a	38.6a	83.7a	92.4a	761a	7.8a
	10—20	20.6a	61.9a	85.6a	100.0a	197b	6.9a	5.9a	35.3a	76.3a	89.3a	607a	6.7a
	20—30	15.8a	53.3a	89.2a	100.0a	153b	5.3a	19.1a	32.8a	81.5a	88.2a	333b	6.4a
	30—40	18.7a	62.6a	87.4a	98.5a	89a	4.6a	12.5a	45.8a	73.6a	82.3a	220b	6.2a
	40—50	9.8a	62.0a	82.9a	97.8a	49a	3.2a	9.8a	29.4a	70.9a	79.6a	283b	5.3a
Hedysarum laeve	0—10	13.6a	44.6a	80.3b	99.2a	613a	12.6a	15.8a	45.1a	69.2a	80.0a	605a	14.2a
	10—20	17.3a	52.7a	87.2a	99.2a	1066b	13.8a	19.6a	46.7a	58.4a	77.5a	469a	9.6a
	20—30	14.8a	59.6a	89.6a	99.2a	907b	12.9a	21.5a	42.8a	60.8a	65.8b	265b	11.2a
	30—40	9.8b	38.5ab	90.1a	96.7ab	590a	8.7a	15.2a	27.2b	47.3b	65.0b	104b	10.3a
	40—50	7.6b	27.8b	79.7b	91.7b	500a	4.3b	3.4b	21.7b	46.6b	56.7c	79b	2.4b

Note: Different letters in the some column are considered significantly different.

2.3 Correlation analysis between AM fungi and soil factors

The results of correlation analysis (Table 3) showed that alkali solution N was significantly positively correlated with spore density ($P<0.01$); pH was significantly positively correlated with %V, %H and %F ($P<0.01$). The correlation between other factors was not significant.

Table 3. Correlation coefficient between AM fungi and soil factors

	Alkali solution N	Available P	Available K	Organic matter	pH	Spore density
%A	0.196	−0.028	0.094	0.205	−0.020	0.051
%V	0.105	−0.228	−0.220	0.032	0.530*	0.022
%H	0.135	0.023	−0.260	−0.184	0.561*	0.427
%F	0.174	−0.027	−0.214	−0.152	0.610*	0.418
Spore density	0.678*	0.350	0.323	0.345	0.078	1.000

* means the correlation is very significant at $p<0.01$.

2.4 Spore density and species richness of AM fungi

The spore density in the rhizosphere of the clonal plants ranged from 49 to 1066 per 100 g air-dried soil. Significant differences were observed among different layers. In OSES, the spore density in the rhizosphere of *Psammochloa villosa* and *Hedysarum laeve* was significantly higher in the 10—30 cm than that in the 0—10 cm and 30—50 cm and reached the maximal value in the 10—20 cm layer. In RSCF, the spore density in the rhizosphere of *Psammochloa villosa* and *Hedysarum laeve* was significantly higher in the 0—20 cm than that in the 20—50 cm and had the maximal value in the 0—10 cm layer. The spore density in the rhizosphere of *Psammochloa villosa* was lower than that in the rhizosphere of *Hedysarum laeve* at two study sites (Table 2). The species richness of AM fungi ranged from 3.2 to 7.8 in the rhizosphere of *Psammochloa villosa* and had no significant difference among different layers. The species richness of AM fungi in the rhizosphere of *Hedysarum laeve* was higher than that in the rhizosphere of *Psammochloa villosa* and ranged from 2.4 to 14.2 and was significantly lower in the 40—50 cm than in the 0—40 cm layer.

2.5 Genera and species of AM fungi

A total of 16 taxa belonging to three genera of AM fungi were distinguished in the soil samples in the rhizosphere of clonal plants (*Psammochloa villosa* and *Hedysarum leave*) in Mu Us sandland. Among them, 11 (68.75%) were identified up to the species level and 5 (31.25%) up to the genus level. Of the 16 taxa, 11 belonged to the genus *Glomus*, 4 to *Acaulospora* and 1 to *Gigaspora*. The species diversity of AM fungi in the rhizosphere of *Psammochloa villosa* was more abundant than in the rhizosphere of *Hedysarum leave*, only 8 species in the rhizosphere of *Psammochloa villosa* and 15 species in the rhizosphere of *Hedysarum leave*. *Glomus hydembadensis* and *Glomus multicaule* only occurred in RSCF, whereas *Glomus aggregatum* only appeared in OSES (Table 4).

Table 4. Relative abundance (%) and frequency of occurrence (%) of AM fungal species in the rhizospheres of the clonal plants in Mu Us sandland

Host plant species	AM fungal species	OSES		RSCF	
		Relative abundance	Frequency of occurrence	Relative abundance	Frequency of occurrence
Psammochloa villosa	*Glomus intraradices* Schenck & Smith	4.5	6.7	3.9	8.3
	Glomus melanosporum Gerdemann & Trappe	25.8	63.3	23.7	68.3
	Glomus multicaule Gerdemann & Bakshi	0	0	4.7	5.0
	Glomus mossae Nicolson & Gerdemann	43.7	81.7	39.8	83.6
	Glomus sp. 1	4.7	18.2	4.5	14.2
	Acaulospora excavata Ingieby & Walker	8.3	21.7	7.3	23.3
	Acaulospora sp. 1	5.9	12.6	4.7	16.8
	Gigaspora decipiens Hall & Abbott	7.1	25.0	10.4	29.7
Hedysarum laeve	*Glomus intraradices* Schenck & Smith	3.5	8.9	3.8	7.1
	Glomus melanosporum Gerdemann & Trappe	12.4	53.0	13.3	56.6
	Glomus mossae Nicolson & Gerdemann	25.6	72.8	22.6	69.4
	Glomus geosporum Walker	12.3	52.6	15.1	51.7
	Glomus hydembadensis Rani, Prasad & Manoharachary	0	0	2.9	6.0
	Glomus constrictum Trappe	8.7	32.1	7.4	36.9
	Glomus aggregatum Schenck & Smith	3.5	11.3	0	0
	Glomus sp. 1	2.6	9.0	1.9	12.8
	Glomus sp. 2	3.7	12.5	2.5	11.9
	Glomus sp. 3	2.2	8.7	2.6	7.6
	Acaulospora excavata Ingieby & Walker	9.1	26.8	10.2	22.0
	Acaulospora rehmii Sieverding & Toro	7.8	14.9	6.8	10.7
	Acaulospora sp. 1	2.9	13.6	3.7	11.3
	Acaulospora sp. 2	1.5	7.1	1.9	6.9
	Gigaspora decipiens Hall & Abbott	7.1	18.5	8.3	19.6

2.6 Relative abundance and frequency of AM fungi

Relative abundance and frequency of all the species of AM fungi are shown in Table 4. Spores of the genus *Glomus* were the most numerous in both relative abundance and frequency, followed by genus *Acaulospora*. The most abundant and frequent AM fungal taxon present was *Glomus mossae*. *Glomus melanosporum* and *Glomus mossae* were dominant species in the rhizosphere of *Psammochloa villosa*, frequency exceeded 50%. *Glomus melanosporum*, *Glomus mossae* and *Glomus geosporum* were dominant species in the rhizosphere of *Hedysarum leave*, frequency exceeded 50%. *Glomus constrictum* was frequent species in the rhizosphere of *Hedysarum leave*, frequency ranged from 32.1% to 36.9%. *Glomus aggregatum* was observed only in the rhizosphere of *Hedysarum leave* in OSES, but *Glomus multicaulei* and *Glomus hydembadensis* were observed in the rhizosphere of *Psammochloa villosa* and *Hedysarum leave* in RSCF respectively.

3 Discussion

AM fungi play an important role in plant survival and the community stability of vegetation in natural ecosystems[17—20]. The present study has indicated the predominance of arbuscular mycorrhizae in the clonal plants in Mu Us sandland, which showed that the associations might be one of the valid countermeasures to acclimatize the clonal plants to the arid and infertile environments. 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[21—23].

The distribution and activity of AM fungi were tightly correlated with host plants and soil conditions. In the present study, the spatial distribution and the variety of colonization of AM fungi were different in the rhizosphere of two species clonal plants at different study sites. The frequency of colonization in OSES was higher than that in RSCF, while the frequen-

cy of colonization of *Psammochloa villosa* had no significant differences among different layers and the frequency of colonization of *Hedysarum laeve* was significantly lower in the 40—50 cm layer than in other layers. The spore density in the the rhizosphere of *Psammochloa villosa* in OSES was lower than that in RSCF and the spore density in the rhizosphere of *Hedysarum leave* in OSES was higher than in RSCF. Values for spore density were higher than the values for the other sandlands[24, 25]. AM fungal infection levels are generally inversely correlated with nutrient availability[26, 27]. At the same time, the mobile and semimobile dunes that possess well aeration of the soil are dominant in OSES. AM fungi are impressible to the anoxic enviroment[28].

AM fungi and host plants selected each other. The community members and abundence of AM fungi are different in the rhizosphere of different plants. The host plant species may generate a variety of mechanisms to affect AM fungi, including variation in host plant and their phenology, mycorrhizal dependency, host plant-mediated alteration of the soil microenvironment, or other unknown host plant traits[29, 30]. *Glomus multicaule* isolated in the study only occured in the rhizosphere of *Psammochloa villosa*; *Glomus aggregatum*, *Glomus hydembadensis*, *Glomus constrictum* and *Acaulospora rehmii* were only distributed in the rhizosphere of *Hedysarum leave*.

There were significant differences in species richness of the AM fungi in the rhizopheres of the clonal plants. The richness values were relatively high and varied with host plant species, but not in relation to soil properties. Other ecological factors could have affected the development and distribution of AM fungi, for example, seasonality, host-dependence, age of the host plants, sporulation capability of the AM fungi, and the dormancy and distribution patterns of AM fungal spores in soils[31, 32].

Arbuscular mycorrhizal fungi belonging to the *Glomus* genera were dominant in the rhizopheres of the clonal plants in our study, with 68.75% of the AM fungi identified in the genera. In contrast, *Acaulospora* and *Gigaspora* represented only 25% and 6.25%. This provides strong support for the conclusions of other work that AM fungi belonging to *Glomus* tend to be dominant in arid ecosystems[33, 34].

It is well known that AM fungi can increase plant nutrient uptake[35], water use efficiency[36] and resistance to abiotic stress under certain conditions. Our study indicate that the clonal plants in Mu Us sandland may establish good symbiosis with AM fungi, the symbionts may depend on each other for survival in these extreme environments, but further studies will be required to elucidate the mechanisms operating in desert ecosystem.

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