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## Original article

## Arbuscular mycorrhizal fungi associated with wild forage plants in typical steppe of eastern Inner Mongolia

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## ABSTRACT

A preliminary investigation was conducted on the arbuscular mycorrhizal (AM) status of the dominant and common wild forage plants in typical steppe of eastern Inner Mongolia, a major semi-arid grassland region in China. Fifty-four wild forage plant species were collected and examined, and 27 of these were colonized by AM fungi. Some plants belonging to families that are presumed to lack mycorrhizas (Cyperaceae, Caryophyllaceae and Chenopodiaceae) were also found to be mycorrhizal. Higher proportions of arbuscular mycorrhizal plants were found in perennial (56.1%) and monocotyledonous (64.7%) forage species. However, neither percentage of root length colonized nor spore density varied significantly between the two life forms or cotyledon types. Twenty-seven species belonging to 7 genera of AM fungi were identified in total according to the morphological characteristics of the spores from field soil and trap cultures, and the results indicate that *Glomus* was the dominant AM genus and *Glomus geosporum* (Nicolson & Gerdemann) Walker and *Glomus mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe were the dominant species in field soil and trap cultures, respectively. *Glomus intradices* Schenck & Smith, *Glomus etunicatum* Becher & Gerdemann, *Glomus claroideum* Schenck & Smith emend Walker & Vestberg, *Glomus clarum* Nicolson & Schenck and *Scutellospora callospora* (Nicolson & Gerdemann) Walker & Sanders also occurred with high frequencies.

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### 1. Introduction

Typical steppe in eastern Inner Mongolia is one of the most representative and best preserved steppe ecosystems in China [18] and is also one of the largest remaining typical grassland ecosystems in the world [14]. Wild forage plants form an important component of the biodiversity of typical steppe. It has been reported that as much as 800 wild forage plant species belonging to 78 families are found in typical steppe of eastern Inner Mongolia [21]. The wild ancestors of most of the cultivars of forages that are widely grown around the world may be found in this grassland region [10]. As an important forage germplasm resource, the wild forage plants in the steppe are essential for the development of the stockbreeding industry in China. However, in recent decades increasing human disturbance, consisting mainly of excessive

cutting and over-grazing, has exerted great pressure on the steppe. Substantial degradation of the plant communities throughout most of the grassland areas has placed the native wild forage plant species in great danger [39].

Arbuscular mycorrhizal (AM) fungi are known to form an integral part of terrestrial plant communities, forming mutualistic associations with the roots of the majority of terrestrial plant species. In these plant–fungal relationships the fungus derives photosynthate from the host plant, which in turn gains greater quantities of nutrients from the association [32]. In addition, some studies have indicated that AM fungi have several beneficial functions on the water relations of plants [1,4] which suggest great potential of AM fungi for mitigating water stress of plants in drought regions. Formation of arbuscular mycorrhiza may therefore be an important growth strategy for wild forage plants in the natural semi-arid steppe ecosystems of eastern Inner Mongolia. Moreover, arbuscular mycorrhizal associations also provide an important approach for promoting seedling establishment [28]. However, to our knowledge information on the arbuscular mycorrhizal status of wild forage plants in the steppe is still lacking.

The aim of the present study was therefore to make a preliminary field survey of the AM root colonization status and species

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community associated with the common wild forage plants in the region to provide fundamental information necessary for wild forage plant re-establishment projects in degraded semi-arid steppe grassland ecosystems.

## 2. Materials and methods

### 2.1. Sampling area

Sampling was conducted at the Inner Mongolia Grassland Ecosystem Research Station (IMGERS), a research station established in 1979 by the Institute of Botany, Chinese Academy of Sciences in the Xilin River Basin (43°26′–44°34′N, 115°30′–117°12′E) in eastern Inner Mongolia. This region covers an area of about 10,000 km<sup>2</sup> and declines gradually topographically from the east (with a maximum elevation of 1505 m) to the west (with a minimum elevation of 902 m) [6]. The region has a semi-arid, continental, temperate steppe climate with dry springs and moist summers. The annual mean temperature increases from southeast to northwest, ranging from 0.5 to 2.1 °C [6]. The lowest average temperature (January) is –17 °C, and the highest (July) is 18 °C. Total annual precipitation decreases gradually from 400 mm in the southeast to 250 mm in the northwest [6]. More than 70% of the annual precipitation occurs from June to August. The growing season of perennial plant species in the Inner Mongolian grassland runs from early April to late September and annual species usually germinate in early July following the rains [2]. The soil types in this region are mainly Kastanozem and Luvic Kastanozem soils [37].

Two plots, each 500 m by 500 m, were selected as sample sites. Plot 1 was dominated by *Leymus chinensis* (Trin.) Kitag, a perennial rhizome grass, and plot 2 was dominated by *Stipa grandis* P. Smirn., a perennial bunchgrass. These two community types represent the most widely distributed grassland communities in the typical steppe region of Inner Mongolia [2]. Both plots were fenced-off in 1979 by staff of IMGERS and grazing was prohibited. Selected properties of the soil in plot 1 and plot 2 were as follows: pH value (H<sub>2</sub>O, 1: 2.5 soil/water ratio) was 7.37 and 7.03 units; organic matter content (Schollenberger method) 3.08 and 2.27%; available P (Olsen method) 4.30 and 3.51 mg kg<sup>-1</sup>; and available K (extracted with NH<sub>4</sub>AcO) 389.5 and 227.2 mg kg<sup>-1</sup> respectively.

### 2.2. Collection of root and soil samples

Root and soil sampling occurred from 13 to 15 July 2006 when the plants were in vegetative phase in the typical steppe. Dominant and more common forage plant species in the two steppe communities were selected. Three root samples representative of each plant species were collected where possible. During the collection of individual plants care was taken that the roots could be positively identified as belonging to a particular plant. The entire plant was dug out by trowel to ensure that the roots remained connected to the shoots after sample collection. Root samples were taken to the laboratory and stored frozen (0 °C) until AM root colonization was determined. Soil samples (each about 200 g) were collected by brushing soil off the roots and the root zone soil from each plant. Three soil samples from a particular plant species were air dried and mixed together to give one composite sample. The composite sample was divided into two parts, one of which was used for the measurement of AM fungal spore densities and species identification, and the other for establishment of trap cultures.

### 2.3. Assessment of AM colonization

Roots were washed carefully with tap water and cut into segments approximately 1 cm long. About 0.5 g root segments were

cleared in 10% (w/v) KOH at 90 °C in a water bath for 60 min. After cooling the root samples were washed and stained with 0.05% (w/v) Trypan blue [23]. Thirty 1 cm-long root segments were mounted onto slides in a polyvinyl alcohol–lactic acid–glycerol solution [16] and examined at 100–400× magnification under a Nikon YS100 microscope equipped with an automatic photomicrographic system for detecting the presence of AM structures. The percentage of root length colonized was calculated according to the method of Trouvelot et al. [35]. If at least one root segment was observed to contain hyphal coils or arbuscules, then the plant was recorded as an AM plant. If the root cortex was found to be colonized by fungal mycelia or vesicles but without coils/arbuscules, the plant was recorded as possibly AM.

### 2.4. Trap cultures

*Medicago sativa*, *Sorghum vulgare* and *Sorghum sudanense* were used as host plants. The soil sub-sample collected from the root zone of each plant species contained AM propagules in the form of spores, root fragments and hyphae were used as inoculum. Round plastic pots of 0.5 L capacity were used for trap cultures. The three trap plant species were grown together in each pot containing 250 g of soil and 250 g of autoclaved river sand for 5 months in a greenhouse. Pots were watered as required and no fertilizers were used.

### 2.5. Extraction and enumeration of AM fungal spores

Spores or sporocarps were extracted from 50 g of air-dried root zone soil of each wild forage plant species by wet sieving followed by flotation–centrifugation in 60% sucrose [8]. The finest sieve used was 40 µm and the spores were collected on a grid-patterned (4 × 4 mm) filter paper. After washing three times with distilled water to spread them evenly over the entire grid, the spores were counted using a dissecting microscope at 30× magnification. A sporocarp was counted as 1 unit.

### 2.6. Identification of AM fungal spores

Spores extracted from 50 g field soil and 150 g trap cultures were mounted on glass slides in polyvinyl alcohol–lactoglycerol (PVLG) + Melzer's reagent. Spores were identified to species level according to taxonomic criteria [29] and taxonomic information from websites on the internet (<http://invam.caf.wvu.edu> and <http://www.lrz-muenchen.de/~schuessler/amphylo/>).

With the data obtained either from field soil or trap cultures we calculated (1) frequency of occurrence, calculated as the percentage of samples from which a determined genus or species was isolated, and (2) relative abundance, calculated as the ratio between the number of spores of a particular genus or species to the total number of spores.

### 2.7. Statistical analysis

The root colonization data are presented as arithmetic mean values with standard errors. To analyze the root colonization or spore density among different cotyledon types and life forms, one-way analysis of variance was used and means were compared by least significant difference (LSD) at the 5% level (the AM colonization data were arcsine transformed prior to one-way analysis of variance). All the data were analyzed using the SPSS 13.0 software package. The Pearson correlation coefficient was employed to determine the relationships between AM spore density and colonization rate.

**Table 1**

Arbuscular mycorrhizal (AM) status of the dominant and common wild forage plant species collected in the typical steppe of eastern Inner Mongolia.

Host plant species	Life form	Cotyledon type	AM or not	Percent root length colonized (%)	Vesicles	Arbuscules	Hyphal coils	Spore density (no. per 50 g air-dried soil)
<b>Asclepiadaceae</b>								
<i>Cynanchum thesioides</i> (Freyen) K.Schum.	P	D	Yes	12.1 ± 4.3	++	–	+	253
<b>Boraginaceae</b>								
<i>Lappula redowskii</i> (Hornem.) Greene	A	D	Yes	28.7 ± 4.6	+++	–	+	73
<b>Campanulaceae</b>								
<i>Adenophora stenanthina</i> (Ledeb.) Kitog.	P	D	?	10.5 ± 6.5	–	–	–	155
<b>Cannabiaceae</b>								
<i>Cannabis ruderalis</i> Janisch.	A	D	?	1.2 ± 0.9	+	–	–	253
<b>Caryophyllaceae</b>								
<i>Melandrium brachypetalum</i> (Hornem.) Fenzl.	P	D	Yes	4.5 ± 0.8	++	–	+	181
<b>Chenopodiaceae</b>								
<i>Axyris amaranthoides</i> L.	A	D	?	26.0 ± 10.4	+	–	–	93
<i>Chenopodium acuminatum</i> Willd.	A	D	No	0	–	–	–	15
<i>Chenopodium aristatum</i> L.	A	D	No	0	–	–	–	171
<i>Chenopodium glaucum</i> L.	A	D	?	0.3 ± 0.2	+	–	–	133
<i>Corispermum chinganicum</i> Iljin	A	D	?	4.5 ± 2.7	+	–	–	25
<i>Kochia prostrata</i> (L.) Schrad.	P	D	Yes	15.0 ± 0.2	++	+	+++	177
<i>Salsola collina</i> Pall.	A	D	?	2.1 ± 0.7	–	–	–	105
<b>Compositae</b>								
<i>Artemisia frigida</i> Willd.	P	D	Yes	28.0 ± 5.6	++	–	+	106
<i>Artemisia gmelinii</i> Webb ex Stechmann.	P	D	Yes	59.3 ± 2.4	+++	+	+	127
<i>Artemisia scoparia</i> Waldst.& Kit.	A	D	Yes	31.6 ± 1.0	++	+	+++	119
<i>Artemisia sieversiana</i> Ehrhart ex Willd.	A	D	Yes	16.8 ± 0.7	+	–	++	120
<i>Echinops sphaerocephalus</i> L.	P	D	?	12.8 ± 1.7	+	–	–	129
<i>Heteropappus altaicus</i> (Willd.) Novopokr.	P	D	Yes	53.3 ± 2.4	+++	+	–	183
<i>Leontopodium leontopodioides</i> (Willd.) Beauv.	P	D	?	8.1 ± 6.2	+	–	–	201
<i>Olgaea leucophylla</i> Iljin	P	D	Yes	43.9 ± 0.1	+++	+	++	106
<i>Serratula centauroides</i> L.	P	D	Yes	67.3 ± 3.5	+++	+	–	171
<b>Cyperaceae</b>								
<i>Carex duriuscula</i> C.A.Mey.	P	M	?	20.8 ± 3.4	++	–	–	138
<i>Carex korshinskyi</i> Kom.	P	M	Yes	16.3 ± 5.0	++	–	+	80
<b>Gramineae</b>								
<i>Agropyron michnoi</i> Roshev.	P	M	?	36.0 ± 10.6	+++	–	–	110
<i>Cleistogenes squarrosa</i> (Trin.) Keng	P	M	Yes	21.6 ± 5.1	+	+	–	136
<i>Koeleria cristata</i> (L.) Pers.	P	M	Yes	20.8 ± 3.4	++	–	+	135
<i>Leymus chinensis</i> (Trin.) Kitag	P	M	?	16.1 ± 0.04	+++	–	–	244
<i>Poa nemoralis</i> L.	P	M	?	22.8 ± 8	++	–	–	106
<i>Setaria viridis</i> (L.) Beauv.	A	M	?	2.3 ± 0.4	+	–	–	28
<i>Stipa grandis</i> P. Smirn.	P	M	Yes	39.1 ± 3.6	++	+	+++	233
<b>Iridaceae</b>								
<i>Iris tenuifolia</i> Pall.	P	M	No	0	–	–	–	116
<b>Labiatae</b>								
<i>Schizonepeta tenuifolia</i> Briq.	A	D	Yes	35.5 ± 1.8	+	+	++	90
<i>Scutellaria scordiifolia</i> Fisch.ex Schrenk.	P	D	Yes	23.4 ± 3.0	+	–	++	83
<b>Leguminosae</b>								
<i>Astragalus galactites</i> Pall.	P	D	?	39.7 ± 5.0	++	–	–	65
<i>Astragalus melilotoides</i> Pall.	P	D	No	0	–	–	–	246
<i>Caragana microphylla</i> Lam.	P	D	Yes	14.4 ± 4.8	+	–	+	50
<i>Melissitus ruthenica</i> (L.) C.W.Chang	P	D	No	0	–	–	–	20
<b>Liliaceae</b>								
<i>Allium anisopodium</i> Ledeb.	P	M	Yes	55.1 ± 5.2	+	+	+	88
<i>Allium bidentatum</i> Fisch.ex Prokh.	P	M	Yes	67.3 ± 6.2	+++	+	++	271
<i>Allium condensatum</i> Turcz.	P	M	Yes	27.6 ± 1.1	+	+	–	132
<i>Allium ramosum</i> L.	P	M	Yes	25.8 ± 9.6	++	+++	+++	256
<i>Allium senescens</i> L.	P	M	Yes	17.5 ± 3.4	+++	+	–	147
<i>Allium tenuissimum</i> L.	P	M	Yes	70.4 ± 0.6	+	+++	+	96
<i>Anemarrhena asphodeloides</i> Bunge.	P	M	Yes	28.7 ± 3.7	++	++	+++	162
<b>Polygonaceae</b>								
<i>Fallopia convolvulus</i> L.	A	D	No	0	–	–	–	95
<b>Ranunculaceae</b>								
<i>Thalictrum squarrosum</i> Steph. ex Willd.	P	D	?	3.7 ± 1.4	++	–	–	92
<b>Rosaceae</b>								
<i>Potentilla acaulis</i> L.	P	D	?	52.6 ± 11.7	+++	–	–	188
<i>Potentilla bifurca</i> L.	P	D	?	7.0 ± 1.4	++	–	–	59
<i>Potentilla nudicaulis</i> Willd. ex Schlecht.	P	D	Yes	2.5 ± 2.1	+	–	+	165
<i>Potentilla tanacetifolia</i> Willd. ex Schlecht.	P	D	?	23.9 ± 8.9	++	–	–	332
<i>Sanguisorba officinalis</i> L.	P	D	?	13.0 ± 0.4	++	–	–	217
<b>Rutaceae</b>								
<i>Haplophyllum dauricum</i> Juss.	P	D	No	0	–	–	–	145
<b>Scrophulariaceae</b>								
<i>Cymbaria dahurica</i> L.	P	D	?	20.8 ± 9.7	++	–	–	176
<i>Pedicularis verticillata</i> L.	P	D	Yes	3.0 ± 0.9	+	–	+	99

A, annual; P, perennial; D, dicot; M, monocot.

Plant roots with hyphae or vesicles but no coils/arbuscules are shown as “?” and are only possibly mycorrhizal.

Root colonization values are means ± standard error. *n* = 3.

+++ , always present in substantial numbers; ++ , always present; + , very rare; – , not detected.

### 3. Results

#### 3.1. AM colonization

Fifty-four wild forage plant species belonging to 18 families were collected in total and their AM root colonization status is shown in Table 1. Twenty-seven of the 54 species belonging to 12 families were confirmed to be arbuscular mycorrhizal, 7 species were not colonized by AM fungi, and 20 species were possibly arbuscular mycorrhizal because arbuscules or hyphal coils could not be found within the roots. The AM colonization rate of the mycorrhizal plants ranged from 2.5 to 70.4%, with an average value of 30.7%. More than half of the 27 mycorrhizal forage species belonged to three families: Compositae, Gramineae and Liliaceae. Most of the forage plants collected in this study were perennial or dicotyledonous plants. The proportion of perennial plants that were mycorrhizal (56.1%) was higher than the proportion of annual plants (30.8%), and the proportion was also higher in monocots (64.7%) than that in dicots (43.2%). However, there was no significant difference in average AM colonization rate between different life forms ( $P > 0.05$ ) or the two cotyledon types ( $P > 0.05$ ).

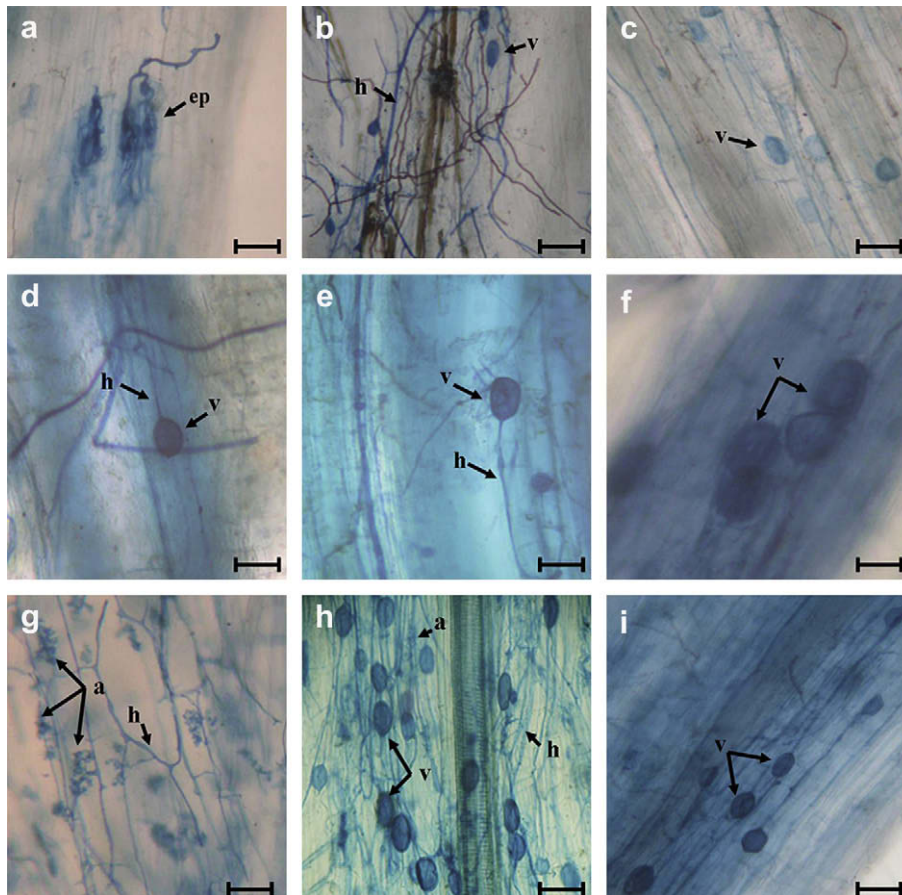
Some plant species that are often considered to be non-mycorrhizal (e.g. species belonging to the Caryophyllaceae, Chenopodiaceae and Cyperaceae) were found to be colonized by AM fungi (Table 1). One species (*Melandrium brachypetalum* (Hornem.) Fenzl.) in the Caryophyllaceae was arbuscular mycorrhizal with a colonization rate of 4.5% (Table 1). Seven forage

species belonging to the Chenopodiaceae were collected, one of which was found to be arbuscular mycorrhizal, with a colonization rate of 15.0%, two were non-mycorrhizal, and the remainder were possibly arbuscular mycorrhizal (Table 1; Fig. 1). One forage species (*Carex korshinskyi* Kom.) in the Cyperaceae was confirmed to be arbuscular mycorrhizal, with a colonization rate of 16.3% (Table 1). Only one plant species (*Fallopia convolvulus* L.) belonging to the Polygonaceae was collected but it was found not to be colonized by AM fungi.

Vesicles were found in the roots of 45 species but their abundance varied among different plant species (Table 1). Arbuscules were found in the roots of 16 plant species and higher abundance was found in the roots of species belonging to the Liliaceae (Table 1; Fig. 1). Hyphal coils were found in the roots of 22 plant species and they were abundant in *Allium ramosum* L., *Anemarrhena asphodeloides* Bunge., *Artemisia scoparia* Waldst. & Kit., *Kochia prostrata* (L.) Schrad., and *S. grandis* (Table 1).

#### 3.2. Spore density

The spore density of the root zone soil of the plant species ranged from 15 to 332 per 50 g of air-dried soil, with an average value of 139 per 50 g of air-dried soil (Table 1). There was no significant correlation between the AM colonization rate and spore density ( $r = 0.043$ ,  $P > 0.05$ ). There was also no significant difference between the different life forms or the cotyledon types ( $P > 0.05$ ).



**Fig. 1.** Photomicrographs of AM fungal structures in the roots of forage plants in the Cyperaceae (a, b, c), Chenopodiaceae (d, e, f), Liliaceae (g, h) and Caryophyllaceae (i). a, entry point (ep) in *Carex duriuscula*; b, intraradical hyphae (h) and vesicles (v) in *Carex duriuscula*; c, vesicles (v) in *Carex korshinskyi*; d, intraradical hyphae (h) and vesicles (v) in *Kochia prostrata*; e, intraradical hyphae (h) and vesicles (v) in *Chenopodium glaucum*; f, vesicles (v) in *Corispermum chinganicum*; g, intraradical hyphae (h) and arbuscules (a) in *Allium bidentatum*; h, intraradical hyphae (h), vesicles (v) and arbuscules (a) in *Allium senescens*; i, vesicles (v) in *Melandrium brachypetalum*. Bar = 100  $\mu$ m (b, c, e, g, h, i); bar = 30  $\mu$ m (a, d, f).



### 3.3. Identification of AM fungal spores

A total of 27 taxa representing seven genera of AM fungi were identified based on spore morphology (Table 2). Of these, five species belonged to *Acaulospora*, 2 to *Ambispora*, 14 to *Glomus*, 3 to *Scutellospora* and 1 each to *Entrophospora*, *Gigaspora* and *Paraglomus*. A total of seventeen species belonging to six genera occurred in field soils, while 24 species belonging to six genera were found in trap cultures. Ten AM species were not found in field soils but occurred after trap culture. However, there were also three species that were lost after trap culture (Table 2).

### 3.4. Relative abundance and frequency of AM fungi

Frequencies and relative abundance of the 7 genera and 27 AM fungal species are presented in Table 2. In the field soil, spores of *Glomus* were the most numerous, both in frequency (100%) and relative abundance (92.9%). *Acaulospora* ranked second in both frequency (14.8%) and relative abundance (3.5%). Of the 17 species, *Glomus geosporum* (Nicolson & Gerdemann) Walker was the most frequent (77.8%) and most abundant (38.0%) species. *Glomus etunicatum* Becher & Gerdemann, *Glomus claroideum* Schenk & Smith emend Walker & Vestberg, and *Glomus mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe also occurred frequently and abundantly. However, the result was much different in the trap cultures. *Glomus* was also the dominant genus among the six genera, but *G. mosseae* became the most frequent (92.6%) and most abundant species (42.4%) instead of *G. geosporum* in the field soil. The frequencies and relative abundance of *G. claroideum*, *G. etunicatum* and *G. geosporum* decreased after trap culture, but *Glomus*

*intraradices* Schenck & Smith, *G. mosseae* and *Scutellospora callospora* (Nicolson & Gerdemann) Walker & Sanders became much more frequent and abundant.

## 4. Discussion

In the present study 54 forage plant species belonging to 18 families growing in the typical steppe of eastern Inner Mongolia were examined. Half of the plant species were confirmed to be mycorrhizal (Table 1). Similar results have also been obtained in previous studies in arid or semi-arid grasslands [5,13]. Soil available P was rather low in the typical steppe of east Inner Mongolia, and the presence of arbuscules or hyphal coils in the mycorrhizal plants in the present study suggests that AM fungi may play an important role in helping the wild forage plant species with P uptake in this ecosystem. Some plant species belonging to the Cyperaceae, Chenopodiaceae and Caryophyllaceae, families that have often been presumed in the past to be non-mycorrhizal or rarely mycorrhizal, were found to be mycorrhizal in the present survey (Table 1). This is in accord with the conclusion that sedges are not strictly a non-mycorrhizal family as suggested by Muthukumar et al. [26] who reviewed the relevant studies published since 1987. Members of the Chenopodiaceae showed varied responses in our study, with some mycorrhizal and others not, and some were possibly mycorrhizal. Shi et al. [30] studied the AM status of some plant species in the Chenopodiaceae from the Junggar Desert in northwest China, and found only hyphae in the roots, so they were considered to be only possibly AM. One explanation might be the influence of particular soil conditions and vegetation composition. *M. brachypetalum*, belonging to the Caryophyllaceae, was also found to

**Table 2**

Occurrence, frequency (F) and relative abundance (RA) of the AM fungi in field and trap culture.

AM fungus	Field soil		Trap culture	
	RA (%)	F (%)	RA (%)	F (%)
<i>Acaulospora</i>	3.5	14.8	1.4	22.2
<i>A. delicata</i> Walker, Pfeiffer, & Bloss	2.3	11.1	0.5	7.4
<i>A. longula</i> Spain & Schenck	0	0	0.2	3.7
<i>A. polonica</i> Blaszkowski	0	0	0.2	3.7
<i>A. rehmi</i> Sieverd. & Toro	0.6	3.7	0.2	3.7
<i>A. spinosa</i> Walker & Trappe	0.6	3.7	0.2	3.7
<i>Ambispora</i>	1.2	3.7	3.5	11.1
<i>Am. gerdemannii</i> Walker, Vestberg & Schuessler	0	0	3.5	11.1
<i>Am. fecundispora</i> Walker	1.2	3.7	0	0
<i>Entrophospora</i>	1.2	7.4	0	0
<i>E. infrequens</i> (Hall) Ames & Schneider	1.2	7.4	0	0
<i>Gigaspora</i>	0	0	0.5	7.4
<i>Gi. albidia</i> Schenck & Smith	0	0	0.5	7.4
<i>Glomus</i>	92.9	100	91.8	100
<i>G. aggregatum</i> Schenck & Smith emend Koske	2.3	14.8	0.7	7.4
<i>G. claroideum</i> Schenk & Smith emend Walker & Vestberg	8.2	33.3	1.2	11.1
<i>G. clarum</i> Nicolson & Schenck	0	0	12.7	37.0
<i>G. constrictum</i> Trappe	0	0	0.7	7.4
<i>G. deserticola</i> Trappe, Bloss & Menge	0	0	0.5	7.4
<i>G. etunicatum</i> Becher & Gerdemann	26.9	66.7	6.3	37.0
<i>G. geosporum</i> (Nicolson & Gerdemann) Walker	38.0	77.8	5.6	37.0
<i>G. glomerulatum</i> Sieverding	0.6	3.7	0	0
<i>G. heterosporum</i> Smith & Schenck	1.8	7.4	0.7	7.4
<i>G. hoi</i> Berch & Trappe	0	0	1.2	11.1
<i>G. intraradices</i> Schenck & Smith	2.3	14.8	14.8	74.1
<i>G. mosseae</i> (Nicolson & Gerdemann) Gerdemann & Trappe	7.6	25.9	42.4	92.6
<i>G. tortuosum</i> Schenck & Smith	0.6	3.7	2.8	11.1
<i>G. versiforme</i> (Karsten) Berch	2.3	14.8	0.7	11.1
<i>Paraglomus</i>	2.3	7.4	1.4	7.4
<i>P. occultum</i> (Walker) Morton & Redecker	2.3	7.4	1.4	7.4
<i>Scutellospora</i>	1.2	7.4	3.2	40.7
<i>S. callospora</i> (Nicolson & Gerdemann) Walker & Sanders	1.2	7.4	2.3	29.6
<i>S. minuta</i> (Ferrer & Herrera) Walker & Sanders	0	0	0.2	3.7
<i>S. pellucida</i> (Nicolson, & Schenck) Walker & Sanders	0	0	0.7	11.1

be arbuscular mycorrhizal (Table 1). Some members of the Caryophyllaceae were also found to be mycorrhizal in Hawaiian angiosperms [15]. However, the Polygonaceae, another family that typically lacks mycorrhizas, was not found to be colonized by AM fungi in our study (Table 1).

In the present study higher percentage of root length colonized, abundance of colonization structures within the roots, and spore densities were found in the root zone soils of Compositae and Liliaceae (Table 1; Fig. 1), suggesting a relatively high AM dependency of these plant families for survival in the natural steppe ecosystem, and also observed in some other regions [25]. We also found that the proportion of plant species with mycorrhizal associations was higher for perennial plants than for annuals, which is in accord with the findings of Fontenla et al. [12]. Some previous studies indicate that the AM colonization rate was also closely related to plant life form, and perennial plants often have higher AM colonization rates than annuals [7,12]. We did not find a similar trend. However, a higher proportion of mycorrhizal monocotyledonous species than dicotyledonous species was observed in the present study (Table 1). Demars studied the mycorrhizal status of spring ephemerals in two Ohio forests and found that 100% of monocots were mycorrhizal, with a corresponding value for dicots of only 25% [9].

No significant correlation was found between the percentage of root length colonized and spore density ( $r = 0.043$ ,  $P > 0.05$ ). A previous study supports this result [19]. The relationship between spore number and percentage of root length colonized by AM fungi is complicated and may be influenced by numerous environmental and biological factors [20]. In our study a high spore density of AM fungi occurred even in the rooting zone soil collected from non-mycorrhizal plants and similar observations have also been made in other studies [40]. This may be the result of the intermingling of roots from different plant species in the same sample: mycorrhizal plants may influence AM fungal spore numbers in the rooting zone of non-mycorrhizal plants [41].

A total of 27 taxa representing seven genera of AM fungi were identified morphologically from field soil and trap cultures (Table 2). Similar results have been obtained in other semi-arid zones. For example, 21 AM fungal species were identified in semi-arid areas in Brazil [31] and Namibia [33], and 20 species were isolated from a semi-arid region of Rajasthan [27]. The present study also reveals that the genus *Glomus*, which included 14 AM species, was the most dominant AM genus both in field soil and in trap cultures. The dominance of *Glomus* species in semi-arid areas is a common finding in numerous studies [25,27,31], and they have also been reported to be the most common AM fungi occurring throughout the world [3,17,34]. *G. geosporum* and *G. mosseae* dominated the field soil and trap culture soil respectively. These two species were also often found in the semi-arid environment of Jordan [24].

Some AM species not found in the field soil were recovered successfully in the trap cultures (Table 2). Similar findings were also reported by Ferrol et al. [11]. However, there are also some species present in the field soil that were lost after trap culture, and the frequency and relative abundance of some species were quite different before and after trap culture, an effect also observed in some previous studies [22,38]. This may be due to differences in the ability of AM species to sporulate [36], and the host plants used in the trap cultures may also be an important factor influencing mycorrhizal development, spore formation and distribution of AM fungi [20]. In the present study only three trap culture host plant species were used, and these were distinctly different from the plant community composition in the typical steppe. Many AM species which colonize the roots of plants but do not sporulate in the soil may have remained undetected in the present study. Molecular

methods that can detect the AM communities within the roots should therefore be included in future studies.

Some of the 54 forage plant species examined in this study are rare species native to the eastern Inner Mongolian steppe (for example *Corispermum chinganicum* Iljin and *Melissitus ruthenica* L.) and many are recognized as high quality forage plants (for example *L. chinensis* and *Agropyron michnoi* Roshev) [10]. These plant species play an important role not only in the development of the local forage industry but also in the restoration and environmental improvement of degraded semi-arid ecosystems. Investigation of the AM status of the wild forage plants in the present study provides some preliminary information for the protection and utilization of these important plant resources.

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## References

- [1] G. Al-Karaki, N. Abu-Qobah, Y. Othman, Influence of mycorrhizal fungi and water stress on growth and yield of two onion cultivars, Arab Gulf J. Sci. Res. 24 (2006) 206–214.
- [2] Y.F. Bai, X.G. Han, J.G. Wu, Z.Z. Chen, L.H. Li, Ecosystem stability and compensatory effects in the Inner Mongolia grassland, Nature 431 (2004) 181–184.
- [3] J. Blaszkowski, *Acualaospora cavernata* (Endogonaceae) new species from Poland with pitted spores, Crypt. Bot. 1 (1989) 201–207.
- [4] S. Bolandnazar, N. Aliasgarzad, M.R. Neishabury, N. Chaparzadeh, Mycorrhizal colonization improves onion (*Allium cepa* L.) yield and water use efficiency under water deficit condition, Sci. Hortic. 114 (2007) 11–15.
- [5] M.S. Chaudhry, Z. Batool, A.G. Khan, Preliminary assessment of plant community structure and arbuscular mycorrhizas in rangeland habitats of Cholistan Desert, Pakistan, Mycorrhiza 15 (2005) 606–611.
- [6] Z.Z. Chen, Topography and Climate of Xilin River Basin, Science Press, Beijing, 1988 (in Chinese).
- [7] S.C. Collier, C.T. Yames, R.P. Herman, Mycorrhizal dependency of Chihuahuan Desert plants is influenced by life history strategy and root morphology, J. Arid Environ. 55 (2003) 223–229.
- [8] Y. Dalpé, Vesicular–arbuscular mycorrhiza, in: M.R. Carter (Ed.), Soil Sampling and Methods of Analysis, Lewis Publishers, Boca Raton, FL, 1993, pp. 287–301.
- [9] B.G. Demars, Vesicular–Arbuscular mycorrhizal status of spring ephemerals in two Ohio forests, Ohio J. Sci. 96 (1996) 97–99.
- [10] H.J. Ding, N.E. Wu, S. Ha, B. Yu, G.L. Qing, The utilization and protection of wild forage germplasm resources in Inner Mongolia, Anim. Husb. Feed Sci. 1 (2007) 38–40 (in Chinese).
- [11] N.R. Ferrol, R. Calvente, C. Cano, J.M. Barea, C. Azcón-Aguilar, Analysing arbuscular mycorrhizal fungal diversity in shrub-associated resource islands from a desertification-threatened semiarid Mediterranean ecosystem, Appl. Soil Ecol. 25 (2004) 123–133.
- [12] S. Fontenla, J. Puntieri, J.A. Ocampo, Mycorrhizal associations in the Patagonian steppe, Argentina, Plant Soil 233 (2001) 13–29.
- [13] J.P. Gai, G. Feng, X.B. Cai, P. Christie, X.L. Li, A preliminary survey of the arbuscular mycorrhizal status of grassland plants in southern Tibet, Mycorrhiza 16 (2006) 191–196.
- [14] K. Kawamura, T. Akiyama, H.O. Yokota, M. Tsutsumi, T. Yasuda, O. Watanabe, S.P. Wang, Quantifying grazing intensities using geographic information systems and satellite remote sensing in the Xilingol steppe region, Inner Mongolia, China, Agric. Ecosyst. Environ. 107 (2005) 83–93.
- [15] R.E. Koske, J.N. Gemma, T. Flynn, Mycorrhizae in Hawaiian angiosperms: a survey with implications for the origin of the native flora, Am. J. Bot. 79 (1992) 853–862.
- [16] R.E. Koske, B. Tessier, A convenient, permanent slide mounting medium, Mycol. Soc. Am. Newsl. 34 (1983) 59.
- [17] R.E. Koske, Gigaspora gigantean observations on the spore germination of a VA mycorrhizal fungus, Mycologia 73 (1981) 289–300.
- [18] B. Li, S.P. Yong, Z.H. Li, The vegetation of Xilin river basin and its utilization, Res. Grassland Ecosyst. 3 (1988) 84–183 (in Chinese).
- [19] L.F. Li, Y. Zhang, Z.W. Zhao, Arbuscular mycorrhizal colonization and spore density across different land-use types in a hot and arid ecosystem, Southwest, China, J. Plant Nutr. Soil Sci. 170 (2007) 419–425.

- [20] R.J. Liu, F.Y. Wang, Selection of appropriate host plants used in trap culture of arbuscular mycorrhizal fungi, *Mycorrhiza* 13 (2003) 123–127.
- [21] Y.Q. Ma, Inner Mongolia Flora, People of Inner Mongolia Press, Hohhot, 1994 (in Chinese).
- [22] F. Oehl, E. Sieverding, K. Ineichen, P. Mäder, T. Boller, A. Wiemken, Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of Central Europe, *Appl. Environ. Microbiol.* 69 (2003) 2816–2824.
- [23] J.M. Phillips, D.S. Hayman, Improved procedures for cleaning and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection, *Trans. Br. Mycol. Soc.* 55 (1970) 158–160.
- [24] M.J. Mohammad, S.R. Hamad, H.I. Malkawiz, Population of arbuscular mycorrhizal fungi in semi-arid environment of Jordan as influenced by biotic and abiotic factors, *J. Arid Environ.* 53 (2003) 409–417.
- [25] T. Muthukumar, K. Udaiyan, Arbuscular mycorrhizas of plants growing in the Western Ghats region, Southern India, *Mycorrhiza* 9 (2000) 297–313.
- [26] T. Muthukumar, K. Udaiyan, P. Shanmughavel, Mycorrhiza in sedges: an overview, *Mycorrhiza* 14 (2004) 65–77.
- [27] M. Pande, J.C. Tarafdar, Arbuscular mycorrhizal fungal diversity in neem-based agroforestry systems in Rajasthan, *Appl. Soil Ecol.* 26 (2004) 233–241.
- [28] G.S. Pattinson, K.A. Hammill, B.G. Sutton, P.A. McGee, Growth and survival of seedling of native plants in an impoverished and highly disturbed soil following inoculation with Arbuscular mycorrhizal fungi, *Mycorrhiza* 14 (2004) 339–346.
- [29] N.C. Schenck, Y. Perez, Manual for the Identification of Vesicular–arbuscular Mycorrhizal Fungi, INVAM, Univ of Florida, Gainesville, FL, USA, 1990.
- [30] Z.Y. Shi, G. Feng, P. Christie, X.L. Li, Arbuscular mycorrhizal status of spring ephemerals in the desert ecosystem of Junggar Basin, China, *Mycorrhiza* 16 (2006) 269–275.
- [31] G.A. Silva, S.F.B. Trufem, O.J. Saggin Junior, L.C. Maia, Arbuscular mycorrhizal fungi in a semiarid copper mining area in Brazil, *Mycorrhiza* 15 (2005) 47–53.
- [32] S.E. Smith, D.J. Read, *Mycorrhizal Symbiosis* London, UK, second ed. (1997).
- [33] J.C. Stutz, R. Copeman, C.A. Martin, J.B. Morton, Patterns of species composition and distribution of arbuscular mycorrhizal fungi in arid regions of southwestern North America and Namibia, Africa, *Can. J. Bot.* 78 (2000) 237–245.
- [34] N.C. Talukdar, J.J. Germida, Occurrence and isolation vesicular arbuscular mycorrhizal fungi in cropped field soils Saskatchewan, Canada, *Can. J. Microbiol.* 39 (1993) 567–575.
- [35] A. Trouvelot, J.L. Kough, V. Gianinazzi-Pearson, Mesure dutaux de mycorrhization VA d'un systeme racinaire. Recherche de methods d'estimation ayant une signification fonctionnelle, in: V. Gianinazzi-Pearson, S. Gianinazzi (Eds.), *Physiological and Genetic Aspects of Mycorrhizae*, INRA, Paris, 1986, pp. 217–221.
- [36] K. Turnau, P. Ryszka, V. Gianinazzi-Pearson, D. van Tuinen, Identification of arbuscular mycorrhizal fungi in soils and roots of plants colonizing zinc wastes in southern Poland, *Mycorrhiza* 10 (2001) 169–174.
- [37] J.W. Wang, W.Q. Cai, *Studies on Genesis, Types and Characteristics of the Soils of the Xilin River Basin*, Science Press, Beijing, 1988 (in Chinese).
- [38] Y.Y. Wang, M. Vestberg, C. Walker, T. Hume, X.P. Zhang, K. Lindström, Diversity and infectivity of arbuscular mycorrhizal fungi in agricultural soils of the Sichuan Province of mainland China, *Mycorrhiza* 18 (2008) 59–68.
- [39] Z.Z. Xu, G.S. Zhou, Effects of water stress and nocturnal temperature on carbon allocation in the perennial grass *Leymus chinensis*, *Physiol. Plantarum* 123 (2005) 272–280.
- [40] Y. Zhang, L.D. Guo, R.J. Liu, Arbuscular mycorrhizal fungi associated with common pteridophytes in Dujiangyan, southwest China, *Mycorrhiza* 14 (2004) 25–30.
- [41] Z.W. Zhao, Y.M. Xia, X.Z. Qin, X.W. Li, L.Z. Cheng, T. Sha, G.H. Wang, Arbuscular mycorrhizal status of plants and the spore density of arbuscular mycorrhizal fungi in the tropical rain forest of Xishuangbanna, southwest China, *Mycorrhiza* 11 (2001) 159–162.