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Arbuscular mycorrhizal fungi associated with sedges on the Tibetan plateau

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Abstract The arbuscular mycorrhizal (AM) status of nine dominant sedge species and the diversity of AM fungi in Tibetan grassland were surveyed in the autumn of 2003 and 2004. Most of the sedge species and ecotypes examined were mycorrhizal, but *Carex moorcroftii* and *Kobresia pusilla* were of doubtful AM status, and *Kobresia humilis* was facultatively mycorrhizal. This is the first report of the mycorrhizal status of eight of the nine sedge species examined. Intraradical vesicles and aseptate hyphae were the structures most frequently observed. Appressoria, coils, and arbuscules were found in the roots of a few sedge species. A strong negative correlation was found between soil organic matter content and the extent of mycorrhizal colonization. Using trap cultures, 26 species of AM fungi belonging to six genera, *Glomus*, *Acaulospora*, *Paraglomus*, *Archaeospora*, *Pacispora*, and *Scutellospora*, were isolated from the soil samples collected. The frequency of occurrence of different taxa of AM fungi varied greatly. *Glomus* and *Acaulospora* were the dominant genera, and *Acaulospora scrobiculata* was the most frequent and abundant species. The species richness of AM fungi was 2.73 in the study area. Species richness and diversity index differed among the sedge species but were not correlated with soil factors such as pH, available P, or organic matter content.

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Introduction

About 80% of terrestrial plant species in natural ecosystems are mycorrhizal, and the associations may improve the fitness of the fungal and plant partners (Smith and Read 1997). Ecologists have taken increasing interest in the incidence and ecological role of arbuscular mycorrhizal (AM) fungi in natural plant communities in recent years (Bever 2002; Bever et al. 2001; Burrows and Pfleger 2002). Despite the occurrence of mycorrhiza in the great majority of modern plant taxa and in almost all ecosystems, there are still some plant families such as Cyperaceae, Brassicaceae, Caryophyllaceae, Juncaceae, and Amaranthaceae presumed to be nonmycorrhizal or rarely mycorrhizal (Newman and Reddell 1987; Peterson and Bradbury 1995; Hirsch and Kapulnik 1998; Regvar et al. 2003; Fuchs and Haselwandter 2004). The Cyperaceae have become particularly prominent in recent years for the many conflicting reports on their mycorrhizal status.

Sedges often dominate wetlands and arctic and alpine vegetation in which the mycorrhizal inoculum is often low or absent and were therefore often designated nonmycorrhizal (Harley and Harley 1987; Tester et al. 1987). However, Muthukumar et al. (2004) recently reviewed the current information on mycorrhizal associations in sedges and showed that the family can no longer be considered nonmycorrhizal. Indeed, the mycorrhizal status of its members seems to be greatly influenced by environmental conditions. Since 1987, information has become available for 221 sedge species, indicating that 88 (40%) are mycorrhizal, 109 (49%) are nonmycorrhizal, and 24 (11%) are facultatively mycorrhizal. There are still many sedge species that remain to be investigated, and detailed information on the AM fungal composition and community diversity in sedges is still unavailable. Further, functional aspects of the association in the extreme environments that sedges often inhabit remain unclear.

Tibet (26°44′–36°32′N, 78°25′–99°06′E) is the world's largest and highest plateau. The average altitude is about 4,500 m above sea level, and the weather differs sharply during day and night. Because of the different geographical conditions, contrasting temperatures are found in the north and south. The north has a continental climate, and the south is relatively warm and rainy. Grassland is very important because of the harsh geographical and climatic conditions, occupying 82.07 million hectares of the Tibetan plateau. The main types of grassland are alpine steppes, alpine meadow grasslands, alpine meadows, and montane scrub. Poaceae, Cyperaceae, and Asteraceae are the three most important plant families in terms of abundance, herbage quality, productivity, and distribution for pastoral agriculture. Most of the sedges, especially *Kobresia*, occur in the high quality pasture, and information is required on the mycorrhizal status and ecological role of AM associations in sedges. However, there are no reports on the mycorrhizal status of sedges on the Tibetan plateau.

The objectives of the present study were to determine the mycorrhizal status of the dominant sedge species, *Kobresia humilis*, *Kobresia prainii*, *Kobresia pygmaea*, and *Carex moorcroftii*, and to assess the diversity of AM fungi in the rhizosphere of sedges in Tibetan grassland. The relationships between the extent of AM colonization and soil properties (soil pH, organic matter content, and available P) were also investigated.

Materials and methods

Sampling area and procedures

The investigation was conducted in the prefectures of Lhasa, Rikaze, Shannan, and Naqu (29°19′–32°52′N, 88°57′–92°20′E). Soils and roots were sampled in the grasslands at altitudes ranging from 3,798 to 5,220 m above sea level. The climate of this region from north to south varies from alpine plateau to a semiarid type of temperate climate. The mean annual temperature is −1.2 to 8°C, and annual precipitation is about 300–450 mm.

Vegetation types are mainly montane scrub, alpine steppe, alpine meadow grassland, and alpine meadow. The dominant sedge species in the different types of grassland were sampled in this study. In the autumn of 2003 and 2004, 22 soil samples were collected from the rhizosphere of nine sedge species in the main grasslands. The sedge species selected are often dominant and scattered among dense grasses in the sampling areas. Three or more plants were dug out together from each sampling point, and

Table 1 Sedges examined, vegetation type, altitude, soil characteristics, and AM status of Tibetan sedges

Host plants	Land type	Altitude (m)	Soil texture	Organic matter (g/kg)	Olsen-P (mg/kg)	pH	AM fungal structures	Colonization (%)	Spore density (per 20 g)
Carex capillacea var. *linzensis*	MS	3980	Loam	5.47	5.23	6.74	ar, v, h	30.3±8.5	66
C. capillacea var. *linzensis*	LM	3977	Loam	1.51	10.35	6.47	v, h	31.8±10.2	10
C. moorcroftii	AMG	5220	Sandy loam	1.82	14.99	6.67	v, h	22.7±5.9	30
C. moorcroftii	AT	4510	Gravel soil	3.68	17.08	8.19	v, h	22.1±4.1	79
C. tibetica	MS	3798	Sand	0.09	62.16	7.55	v, h, c	30.9±12.3	17
C. compressus	AM	4827	Loam	3.60	6.65	6.03	ar, v, h	25.3±6.7	10
C. compressus	AM	4827	Loam	5.46	5.25	6.01	ar, v, h	17.7±2.0	120
C. compressus	AM	4394	Loam	2.11	6.36	6.26	ar, v, h	51.3±15.7	48
C. compressus	AM	4857	Loam	8.29	8.44	6.02	ar, v, h	20.1±3.2	23
C. compressus	AM	4827	Loam	8.36	5.15	6.1	ar, v, h	19.9±1.9	42
K. humilis	AM	4385	Loam	2.51	15.2	7.03	ar, v, h	34.2±10.1	58
K. humilis	AMG	5138	Sand	2.58	9.67	6.86	v, h	37.9±3.8	16
K. humilis	AM	4789	Loam	11.56	12.45	6.97	ND	0	14
K. humilis	AS	4559	Loam	2.09	18.17	6.75	ap, v, h	42.7±14.7	72
K. humilis	AM	4455	Loam	3.57	11.45	6.37	v, h	35.2±8.9	24
K. prainii	AMG	4897	Loam	8.13	9.64	7.15	ap, ar, v, h	65.0±16.4	16
K. prainii	AS	4543	Gravel soil	0.82	1.78	7.39	ap, v, h	30.0±2.9	70
K. pusilla	AMG	4717	Loam	7.78	11.02	7.92	v, h	6.1±1.4	87
K. pusilla	AT	4837	Loam	3.06	7.49	6.61	v, h	48.1±12.9	24
K. pygmaea	AMG	5006	Gravel soil	0.36	2.94	8.18	ap, h	10.6±1.9	189
K. pygmaea	AS	4603	Silty soil	3.28	5.14	8.48	ar, v, h	15.3±3.2	8
K. tibetica	AMG	4738	Sand	1.76	9.25	8.11	ar, v, h	56.0±2.7	16

AM, Arbuscular mycorrhizal, *Ap* appressoria, *ar* arbuscules, *h* intercellular aseptate hyphae, *v* vesicles, *c* coils, *ND* not detected, *AMG* alpine meadow grassland, *AS* alpine steppe, *AM* alpine meadow, *MS* montane scrub, *LM* lowland meadow

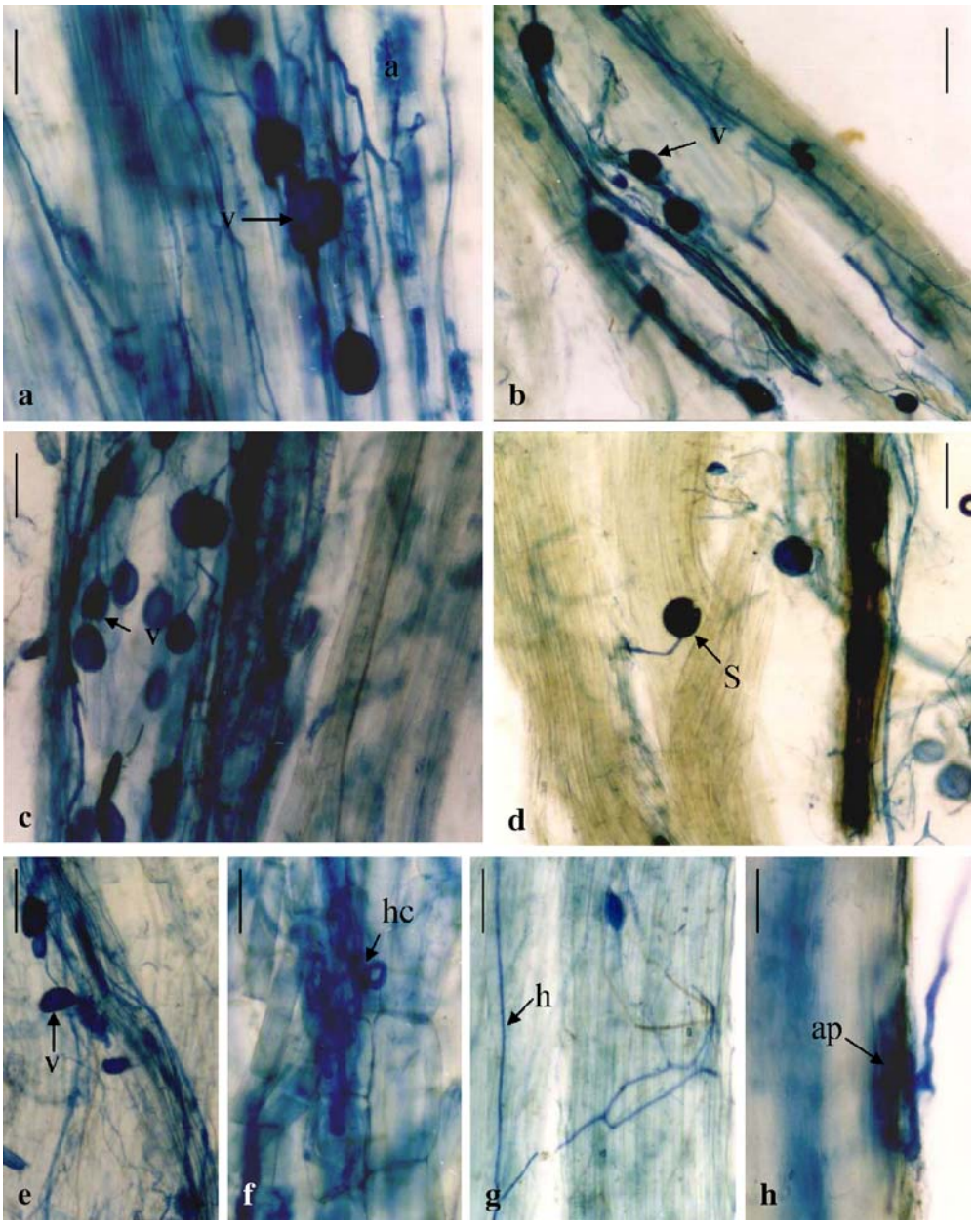

Fig. 1 Fungal colonization in Tibetan grassland sedges. **a** Arbuscule (a), intraradical hyphae, and vesicle (v) in root of *K. prainii*. **b** Intraradical hyphae and vesicle (v) in cells of *C. moorcroftii.* **c** Vesicle (v) and intraradical hyphae in cells of *K. humilis*. **d** Extramatrical spores (s) attached to *K. pygmaea* root. **e** Intraradical hyphae and vesicle (v) in root of *Carex capillacea* var. *linzensis*. **f** Hyphal coil (hc) formation on *C. tibetica* root. **g** Intraradical hyphae (h) and vesicle in root of *C. compressus*. **h** Appressorium (ap) formation in *K. pygmaea*. Bar=50 μm (**a**, **d**, **h**); bar=100 μm (**b**, **c**, **f**); bar=150 μm (**e**, **g**)

approximately 2 kg soil was collected from the rooting zone of the sedges to a depth of 20 cm. Care was taken to avoid contamination from other plants. The soil samples were taken back to the laboratory, and soil texture and soil pH were determined immediately. After air-drying, the soil samples were passed through a 2-mm sieve and stored at room temperature. Soil available P and organic matter content were determined using the methods described by Lu (2000).

Table 2 Isolation frequency, species richness, and relative abundance of the five AR fungal genera in Tibetan grassland sedges

Genus	F (%)	Richness (mean±SE)	RA (mean±SE)
Glomus	81.8	1.364±0.229	19.0±5.8
Acaulospora	63.6	0.955±0.184	17.4±6.1
Scutellospora	13.6	0.136±0.077	0.7±0.4
Pacispora	13.6	0.136±0.077	5.6±3.2
Paraglomus	9.1	0.091±0.064	5.5±4.4
Archaeospora	4.5	0.045±0.045	0.1±0.1

F Frequency, *RA* relative abundance

Root staining

Roots were gently washed with water to remove adhering soil and carefully separated according to their morphological characteristics to avoid misinterpretation. Washed root samples were cleared in 10% (w/v) KOH for 30 min at 90°C, acidified in 2% (v/v) lactic acid for 10 min, and stained for 30 min at 90°C with 0.05% (w/v) Trypan blue (Phillips and Hayman 1970). Roots that remained dark after clearing were bleached in alkaline H_2O_2 prior to acidification with lactic acid. Fifty 0.5- to 1-cm root fragments from each sample were examined under a compound microscope (100–400×) for AM fungal structures. The presence of arbuscules was used to designate AM associations (Brundrett 1991), and the presence of an AM association was considered doubtful when arbuscules were absent. The proportion of root length colonized was estimated by the root slide technique (Brundrett et al. 1996).

Spore extraction and counting

Spores were isolated from air-dried soil using the method of Daniels and Skipper (1982). Twenty grams of soil taken from each sample was suspended in 1 l water and left to stand for 20 min. The suspensions were passed through nested 500- and 45-μm sieves and wet sieved, followed by sucrose density gradient centrifugation. The AM fungal spores were counted on a grid-patterned dish under a binocular stereomicroscope.

Culture establishment

Trap cultures were established from fresh soil samples mixed with autoclaved sand in a ratio of 2:1. Clover (*Trifolium repens* L.) and sorghum (*Sorghum vulgare* Pers.) were used as host plants. Cultures were grown in a greenhouse at China Agricultural University for 5 months with a temperature regime of 28°C (day) and 15°C (night) and a 14-h photoperiod at a light intensity of 250 μmol m^{-2} s^{-2} provided by supplementary illumination.

Identification of AM fungi

Spores of AM fungi isolated from the field soils and trap cultures were mounted on glass slides in polyvinyl–lacto–glycerol (PVLG) or PVLG + Melzer's reagent (1:1, v/v). The spores were examined microscopically and identified according to current taxonomic criteria (Schenck and Péréz 1990) and using the Internet information from the INVAM website (http:// invam.caf.wvu.edu).

Data analysis

AM fungal diversity was evaluated by spore density, relative abundance, isolation frequency (F), and species richness. Spore density was expressed as numbers of AM fungal spores per 20 g dried field soil. Relative abundance was defined as the percentage of numbers of spores of the particular species or genera in the field soil. Data from field soil and trap cultures were combined to determine species composition. Isolation frequency was calculated as the percentage of samples in which the particular genus or species was present. Species richness was defined as numbers of AM fungal species per soil sample. Species diversity was measured by the Shannon–Weiner index as follows:

$$\text{Shannon} - \text{Weiner index} = -\sum (\text{Pi } \ln [\text{Pi}])$$

where:

$$\text{Pi} = \text{ni}/\text{N}$$

and n_i = number of spores in species i and N = total spore number of all species.

Results

Mycorrhizal status of sedges

AM fungal structures were observed in all the sedge roots examined, except for one ecotype of *K. humilis* (Table 1; Fig. 1). At species level, six species in our survey were mycorrhizal; *K. humilis* was facultatively mycorrhizal, and *C. moorcroftii* and *Kobresia pusilla* were of doubtful mycorrhizal status. The colonization pattern varied among the plant species and even among the ecotypes of individual species. Vesicles and intercellular aseptate hyphae were the most frequently observed structures present in the sedge roots examined. Vesicles were observed in 20 root samples (90%), whereas arbuscules were observed in only 10 samples (46%). Appressoria were present in *K. humilis* sampled from alpine steppe, *K. prainii* from both alpine meadow grassland and alpine steppe, and *K. pygmaea* from alpine meadow grassland. Intracellular coils were found only in *Carex tibetica*. No AM fungal structures were observed in the root sample of *K. humilis* from alpine meadow.

Colonization levels in sedges varied from 0 to 65%, spore density from 8 to 189 per 20 g dried soil, and both varied greatly with plant species and ecotype (Table 1).

Table 3 Isolation frequency (≥15%) and relative abundance of arbuscular mycorrhizal fungal species

Species	F (%)	RA (mean±SE)
A. laevis	22.7	3.5±1.6
A. scrobiculata	31.8	5.8±2.2
G. claroideum	18.2	5.3±4.2
G. intraradices	22.7	2.4±1.5
G. mosseae	27.3	2.8±1.5

F Isolation frequency, *RA* relative abundance

Table 4 Arbuscular mycorrhizal species richness and diversity in different sedge species

Host plants	Species richness	Diversity
C. capillacea var. *linzensis*	3.5±2.0	0.143±0.135
C. moorcroftii	3.5±1.0	0.201±0.120
C. tibetica	1.0±0.0	0.067±0.000
C. compressus	2.4±0.6	0.169±0.062
K. humilis	2.0±0.4	0.137±0.042
K. prainii	4.5±2.1	0.177±0.150
K. prattii	3.0±0.0	0.195±0.123
K. pygmaea	3.0±1.4	0.252±0.252
K. tibetica	2.0±0.0	0.070±0.000

Further, Pearson's correlation analysis of colonization levels and soil factors (soil pH, organic matter, and available P) revealed a strong negative correlation between organic matter and colonization rate ($r=-0.733$, $P<0.00$, $n=22$).

AM fungal diversity

Twenty-six taxa of AM fungi were isolated from the soil samples belonging to *Glomus*, *Acaulospora*, *Paraglomus*, *Archaeospora*, *Pacispora*, and *Scutellospora* (Table 2). Species richness in the survey areas was 2.73. *Glomus* and *Acaulospora* were the dominant genera, both in frequency and relative abundance. The five most commonly observed species were *Acaulospora scrobiculata*, *Glomus mosseae*, *Glomus intraradices*, *Acaulospora laevis*, and *Glomus claroideum*. Of these, *A. scrobiculata* was the most frequent and abundant species (Table 3).

The species richness and fungal diversity of nine sedge species differed substantially (Table 4). Species richness varied from 1.0 (*C. tibetica*) to 4.5 (*K. prainii*) and diversity from 0.070 (*Kobresia tibetica*) to 0.252 (*K. pygmaea*). No relationship existed between AM fungal diversity (diversity index, spore density) and soil factors (soil pH, organic matter, and available P).

Discussion

Here, we report for the first time the mycorrhizal status of eight of the nine sedge species investigated. Only *Cyperus compressus* has previously been reported as mycorrhizal (Allsopp and Stock 1993; Muthukumar and Udaiyan 2000) and was defined as facultatively mycorrhizal when Muthukumar et al. (2004) summarized the published information on the mycorrhizal status of sedges. *C. compressus* was mycorrhizal in all ecotypes collected in our study. Only intercellular aseptate hyphae and vesicles were found in *C. moorcroftii* and *K. pusilla*. It has been reported that vesicles and hyphae are found in nonmycorrhizal hosts such as *Cyperus iria* and *Cyperus rotundus* (Koske et al. 1992; Giovannetti and Sbrana 1998). These plants can therefore be referred to as of doubtful AM status. *K. humilis* was found to be facultatively mycorrhizal in our survey. The incidence of mycorrhizal species in the present study was much higher (67%) than the average level of 40% reported. Tibetan sedges therefore appear to be readily colonized by AM fungi.

Vesicles and intercellular aseptate hyphae were the most frequent structures present in the sedge roots, and this accords with earlier reports (Muthukumar et al. 2003; Muthukumar and Udaiyan 2000). The incidence of arbuscules was 46%, not a very low value compared with other surveys (Muthukumar et al. 2003; Muthukumar and Udaiyan 2000). However, the percentage of root length colonized by AM fungi was very low in most root samples, and hyphal coils and appressoria were found in only a few specimens. The potential nutritional benefit of the mycorrhizal association in sedges therefore has yet to be ascertained. More detailed studies are necessary to ascertain the exact degree of mycorrhizal dependency of Cyperaceae and the functional aspects of the association in these Tibetan grassland communities.

The rarely colonized or nonmycorrhizal status of sedges is often attributed to the small amount or absence of mycorrhizal propagules in the soils that sedges inhabit (Peat and Fitter 1993). However, many sedges tend to be nonmycorrhizal even in the presence of AM fungal propagules (Van der Heijden et al. 1998), indicating that absence of AM fungal inoculum may not always explain the low incidence of AM in these plants. Spore densities in the rhizosphere of sedges were determined in our study, and there was no correlation between spore density and colonization rate. Other important factors likely to influence root colonization are soil properties. A strong negative correlation was found between soil organic matter content and root colonization rate. This is in agreement with the analysis of Muthukumar et al. (2004). However, root colonization was not related to important factors such as soil pH or soil available P, which are shown to influence the mycorrhizal status of sedges (Lovera and Cuenca 1996; Muthukumar et al. 2004). One likely explanation could be the narrow data range in a particular habitat of this study. The mechanisms by which soil properties and other factors influence mycorrhizal associations still remain unclear and require more detailed research.

AM fungi are known to exhibit ecological specificity (McGonigle and Fitter 1990). There are no published reports on the AM fungal community associated with sedges under the climatic conditions prevailing on the Tibet plateau. Only a few studies have addressed the ability of AM fungi to survive winter freezing or drought stress (van der Heijden et al. 1998; Addy et al. 1998). The predominance of *Glomus* and *Acaulospora* is in accordance with Klironomos et al. (2001), who studied interspecific differences in AM fungal tolerance to freezing and dry conditions. These authors found that *Glomus* and *Acaulospora* were more frequently isolated in the field, and *Glomus* species were the least affected by freezing in pot experiment conditions, while drying gave more variable responses in colonization by AM fungi. Soil properties are important factors influencing the AM fungal community (Bever et al. 2001). *Acaulospora* species are often associated with acid soils. Most of the soils

in our study were acidic, and this could be one explanation for our frequent detection of *Acaulospora*.

The colonization pattern varied among plant species, and even among ecotypes of particular plant species. *K. humilis* was mycorrhizal or nonmycorrhizal, which demonstrates that mycorrhizal fungi and their hosts occupy different positions on the "mutualism–parasitism continuum" under different environmental conditions (Johnson et al. 1997). The AM fungal community composition of particular sedge species varied with habitat type, similar to the results of Brundrett et al. (1999). *G. mosseae* and *G. intraradices* were also found by Klironomos et al. (2001) in similar environmental conditions. This indicates that some species have higher specific ecological adaptability.

Host species is an important factor influencing AM fungal species diversity (Brundrett 1991; Bever et al. 2001). Species richness and fungal diversity differed among the nine sedge species in our study. It has been shown that AM fungal diversity has a strong effect on the plant community, on plant productivity, and on succession (Allen 1991; Bever et al. 2001). The available data suggest an important ecological role for AM fungi in the Tibetan sedge community. However, full elucidation of the effects of AM fungi on the sedges still remains unclear, and the ecology and importance of AM associations require further detailed research.

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Appendix

AM fungal species and their frequency of isolation from rhizosphere soils of sedges

Species	Frequency
Acaulospora appendicula Spain, Sieverding & Schenck	4.5
Acaulospora dilatata Morton	9.1
Acaulospora laevis Gerd. & Trappe	22.7
Acaulospora mellea Spain & Schenck	4.5
Acaulospora scrobiculata Trappe	31.8
Acaulospora spinosa Walker & Trappe	9.1
Acaulospora sp. 1	9.1
Acaulospora sp. 2	4.5
Archaeospora gerdemanni (Rose, Daniels & Trappe) Morton & Redecker	4.5
Glomus claroideum Schenck & Sm. emend Walker & Vestberg	18.2
Glomus convolutum Gerd. & Trappe	4.5
Glomus clarum Nicolson & Schenck	4.5
Glomus diaphanum Morton & Walker	9.1
Glomus etunicatum Becker & Gerdemann	9.1
Glomus geosporum (Nicol. & Gerd.) Walker	9.1
Glomus glomerulatum Sieverd.	4.5
Glomus intraradices Schenck & Smith	22.7
Glomus luteum Kennedy, Stutz, et Morton	4.5
Glomus manihotis Howeler, Sieverd. & Schenck	4.5
Glomus mosseae (Nicol. & Gerd.) Gerd. & Trappe	27.3
Glomus verruculosum Błaszk	9.1
Glomus sp. 1	9.1
Pacispora scintillans (Rose & Trappe) Oehl & Sieverd	13.6
Paraglomus occultum (Walker) Morton & Redecker	9.1
Scutellospora calospora (Nicol. & Gerd.) Walker	9.1
Scutellospora verrucosa (Koske & C. Walker) Walker & Sanders	4.5

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