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# Arbuscular mycorrhizas in a valley-type savanna in southwest China

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**Abstract** The arbuscular mycorrhizal (AM) status of 67 plant species in a savanna community in the hot, dry valley of Jinsha River, southwest China was surveyed. It was found that about 95% of the plant species formed AM and 5% possibly formed AM. The composition of AM fungi (AMF) in the rhizosphere soils was also investigated. The AMF spore density ranged from 5 to 6,400 per 100 g soil, with an average of 1,530, and these spores/sporocarps were identified as belonging to six genera. Fungi belonging to the genera *Glomus* and *Acaulospora* were the dominant AMF. High densities of AMF spores in the rhizosphere soils, and the intensive colonization of the plant roots, indicated that plants grown in this valley-type savanna may be highly dependent on AM.

**Keywords** Arbuscular mycorrhiza · Savanna · Hot-arid ecosystems · Rhizosphere soils

## Introduction

Arbuscular mycorrhizas (AM) are important components in savanna ecosystems, as arbuscular mycorrhizal fungi (AMF) can contribute to plant growth by reducing stresses resulting from nutrient deficiencies (mainly P) and drought (Sanginga et al. 1999; Augé 2001). Beneficial effects on plant growth, biomass, drought resistance, and ecological restoration by inoculation with AMF have been reported with herbaceous host plants both in controlled environments using potted plants and in the field (Rao and Tarafdar 1993; Tarafdar and Praveen-Kumar 1996; Estaun et al. 1997; Gemma and Koske 1997; Cuenca et al. 1998; Smith et al. 1998; Frost et al. 2001; Caravaca et al. 2003).

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According to the vegetation classification of Walter (1979), a savanna usually develops where the evaporation is about 3–6 times the precipitation, 80–90% of precipitation is concentrated in the wet season, and the annual mean temperature is over 20°C. The characteristics of savanna vegetation are grasslands that are yellow in the dry season of the year, with rare trees. Typical savanna vegetation is distributed mainly in Africa. Valley-type savanna is a special type that develops in river valleys, such as those of different rivers in southwest China. The plant community developed in the hot-dry valley of Jinsha River, Yuamou County (101°35′E-102°06′, 25°23′N-26°06′) is the most typical of this special type of savanna. The annual average temperature is 21.8°C, the highest up to 43°C, and the mean annual precipitation is 634.0 mm, mainly between June and October. The wet season accounts for over 90% of the precipitation, while annual evaporation reaches 3,847.8 mm, about 6.1 times precipitation (data from Yuanmou County weather station over 24 years). Grasses form the background vegetation, which is withered and yellow in the dry season, and a scattering of trees and shrubs are distributed among the grassland. The dominant grass species are *Heteropogon contortus*, *Bothriochloa pertusa* and *Eriophorum comosum*, and the woody plants are *Phyllanthus emblica*, *Dodonaea angustifolia*, *Vitex negundo f. laxip* and *Jatropha curcas*. The arid soil of this area has the following chemical characteristics: total N 0.10±0.06 (N)%, total P 0.18±0.13 ($P_2O_5$)%, available P 2.8±0.7 ($P_2O_5$) mg/100 g soil, total K 2.1±0.7 ($K_2O$)%, and pH 8.2±0.6 (unpublished data, from 21 soil samples).

The Jinsha River is the upper reach of the Yangtse River, the longest river in China. It winds through the valleys of Yunnan and Sichuan provinces, southwest China, which are densely populated. Human activities have intensively interfered with the valley-type savanna ecosystem and the savanna is now facing desertification (Jin and Ou 2000). Much research has been done on the origin, development, evolution, structure and function of the savanna vegetation (Jin and Ou 2000; Jin 2002), but no reports on AM have been made. Here, we report the

AM status of the plants and the AMF composition in the valley-type savanna of the Jinsha River.

## Materials and methods

Roots and their rhizosphere soils were collected to a depth of 5–30 cm in July 2002 (wet season), making sure that the roots were connected to sampled plants and cleaning the trowels between samples. Part of the root system of each plant was fixed in 5 ml formalin, 5 ml acetic acid and 90 ml 70% alcohol, diluted 1:1 with water (1/2 FAA), and stored at 4°C. The remaining roots were air-dried with their rhizosphere soil (about 500 g) for 2 weeks, and then stored in sealed plastic bags at room temperature for up to 2 months until samples could be treated. Roots were taken from the 1/2 FAA, washed several times in tap water and cleared in 10% (w/v) KOH by heating to approximately 90°C in a water bath for 2–3 h, the time depending on the size/structure of the roots and their pigmentation. The cooled root samples were washed and cut into 0.5–1 cm segments and stained with 0.5% acid fuchsin according to the method of Berch and Kendrick (1982). Fifty 0.5–1 cm root fragments were examined per sample for their AM status under a compound microscope (×160–×880).

The rhizosphere soil samples were wet-sieved for spores using the method described by An et al. (1990). Soil (20 g) from each plant rhizosphere was independently suspended in 150 ml water, stirred with a magnetic stirrer for 10 min, sieved using 40 μm, 70 μm, 100 μm and 150 μm sieves with tap water, filtered onto a

**Table 1** Arbuscular mycorrhizal (AM) status of plants and spore densities of arbuscular mycorrhizal fungi (AMF) in the valley-type savanna soils. *M* AM status of plants (+ mycorrhizal, ± possibly mycorrhizal), *ASD* AMF spore density (number of AMF spores in 100 g soil from the corresponding plant rhizosphere), *ASD*[a] average AMF spore density

| Host plants | M | ASD | Host plants | M | ASD[a] |
|---|---|---|---|---|---|
| Acanthaceae | | | Gramineae | | |
| *Barleria cristata* | + | 235 | *Bothriochloa pertusa* | + | 2,130 |
| Adiantaceae | | | *Heteropogon contortus* | + | 555 |
| *Adiantum philipense* | + | 1,240 | *Neyraudia reynaudiana* | + | 3,210 |
| Agavaceae | | | *Paspalidium flavidum* | + | 5 |
| *Agave americana* | + | 525 | *Tripogon filiformis* | + | 1,340 |
| Amaranthaceae | | | Labiatae | | |
| *Achyranthes aspera* | + | 75 | *Elsholtzia cypriani* | + | 1,860 |
| *Amaranthus spinosus* | + | 10 | *Ocimum basilicum* | + | 1,810 |
| *A. viridis* | + | 1,750 | Malvaceae | | |
| Asclepiadaceae | | | *Abutilon indicum* | + | 1,065 |
| *Calotropis gigantea* | + | 4,355 | *Sida chinensis* | ± | 475 |
| Bignoniaceae | | | *S. szechuensis* | + | 2,310 |
| *Incarvillea arguta* | + | 115 | Mimosaceae | | |
| Bombacaceae | | | *Acacia farnesiana* | + | 4,695 |
| *Bombax malabaricum* | + | 2,075 | *Albizzia kalkora* | + | 320 |
| Boraginaceae | | | Moraceae | | |
| *Cynoglossum lanceolatum* | + | 4,375 | *Broussonetia papyrifera* | + | 2,220 |
| Caesalpiniaceae | | | Myrtaceae | | |
| *Tamarindus indica* | + | 1,555 | *Eucalyptus citriodora* | + | 70 |
| Chenopodiaceae | | | Nyctaginaceae | | |
| *Chenopodium ambrosioides* | + | 595 | *Boerhavia diffusa* | ± | 220 |
| Compositae | | | Papaveraceae | | |
| *Artemisia codonocephala* | + | 700 | *Macleaya cordata* | + | 90 |
| *Bidens pilosa* | + | 1,445 | Papilionaceae | | |
| *Blainvillea acmella* | + | 955 | *Alysicarpus vaginalis* | + | 580 |
| *Conyza blinii* | + | 1,380 | *Atylosia scarabaeoides* | + | 2,965 |
| *C. canadensis* | + | 490 | *Crotalaria medicaginea* | + | 80 |
| *Laggera pterodonta* | + | 1,810 | *Desmodium microphyllum* | + | 1,125 |
| *Parthenium hysterophorus* | + | 1,295 | *Tephrosia purpurea* | + | 535 |
| *Siegesbeckia orientalis* | + | 1,435 | *Vigna aconitifolius* | + | 30 |
| *Sonchus oleraceus* | + | 4,560 | Polygonaceae | | |
| *Xanthium sibiricum* | + | 245 | *Polygonum statice* | + | 330 |
| *Zinnia elegans* | + | 3,255 | *Rumex hastatus* | + | 570 |
| Cyperaceae | | | Rhamnaceae | | |
| *Cyperus niveus* | + | 1,560 | *Ziziphus mauritiana* | + | 1,815 |
| *C. rotundus* | + | 2,175 | Sapindaceae | | |
| *Eriophorum comosum* | ± | 2,355 | *Dodonaea viscosa* | + | 2,210 |
| Equisetaceae | | | Selaginellaceae | | |
| *Equisetum diffusum* | + | 2,015 | *Selaginella pulvinata* | + | 6,400 |
| Euphorbiaceae | | | *S. mairei* | + | 2,665 |
| *Acalypha acmophylla* | + | 530 | Solanaceae | | |
| *Euphorbia heterophylla* | + | 780 | *Datura stramonium* | + | 120 |
| *E. hirta* | + | 975 | *Solanum khasianum* | + | 65 |
| *E. royleana* | + | 765 | Tiliaceae | | |
| *E. thymifolia* | + | 2,645 | *Grewia biloba* | + | 2,900 |
| *Phyllanthus urinaria* | + | 3,330 | Verbenaceae | | |
| Gesneriaceae | | | *Vitex negundo* | + | 1,530 |
| *Corallodiscus flabellatus* | + | 4,505 | Zygophyllaceae | | |
| | | | *Tribulus terrester* | + | 80 |

Total: plants =67 species; M ("+" =64, about 95%; "±" =3, about 5%);total spore specimens =102,485; ASD[a]=1,530

filter paper, and then placed in a 9 cm Petri dish for examination under a binocular stereomicroscope. AMF spores were counted in the four sieved samples. Some spores were tightly grouped in a sporocarp and it was difficult to count the number of spores per sporocarp; in these cases, a sporocarp was referred to as one spore.

Each spore type was mounted sequentially in water, lactophenol, PVA (polyvinyl lactic acid) and Melzer's reagent (Morton 1988), for identification. Identification was based on spore color, size, surface ornamentation and wall structure, with reference to the descriptions provided by the *International collection of vesicular and arbuscular mycorrhizal fungi* (http://invam.caf.wvu.edu) and the original species descriptions (Trappe 1977; Rothwell and Trappe 1979; Nemec et al. 1981; Walker and Trappe 1981; Smith and Schenck 1985; Morton 1988).

## Results

Under the microscope it was noted that hyphae, hyphal coils, vesicles and arbuscules were stained red while the root cortical cells were unstained or were pale red. If at least one root segment was found to contain arbuscules or vesicles, then the plant was noted as an AM plant, recorded as +. If the root cortex was found to be colonized by fungal mycelia, but without arbuscules or vesicles, the corresponding plant was noted as possibly AM, recorded as ±. Plants were recorded as non-mycorrhizal (−) when neither arbuscules/vesicles nor fungal mycelia were detected in their root cortical cells. Of the 67 plant species surveyed, 64 (about 95%) formed AM, and the roots were usually intensively colonized by the hyphae in these species. The possibly mycorrhizal species represented 5% (three samples), and no non-mycorrhizal plants were found. Furthermore, some plant species in families that are traditionally considered as non-mycorrhizal or rarely forming mycorrhizas were also colonized by AMF. For example, *Rumex hastatus* and *Polygonum statice* (Polygonaceae) were typically colonized by AMF (Table 1). Arbuscules were especially rich in the root cortical cells of *Parthenium hysterophorus*, *Zinnia elegans*, *Incarvillea arguta*, *Acalypha acmophylla*, *Abutilon indicum*, *Phyllanthus urinaria* and *Eucalyptus citriodora*. There were vesicles in *Xanthium sibiricum*, *Sida szechuensis*, *Artemisia codonocephala*, *Heteropogon contortus*, *Selaginella pulvinata* and *Bombax malabaricum*. Both arbuscules and vesicles displayed a high diversity in their morphologies.

AMF spores were counted in the 67 rhizosphere soil samples. The spore density was highly uneven, ranging from 5 to 6,400 spores per 100 g rhizosphere soil. The average spore density was 1,530 (Table 1). From the 67 rhizosphere soil samples, 844 AMF spore (or sporocarp) samples were obtained by wet-sieving. Some spore types were identified to species by their morphological characters (Fig. 1) but most of the specimens were identified only to genus level because they lacked distinguishable, fine taxonomic characters. Accordingly, the frequency of AMF occurrence could be statistically analyzed only at the genus level. *Acaulospora* and *Glomus* were the dominant genera; their frequencies of occurrence were 22.4% and 67.0%, respectively, in all the spore samples examined. The frequency of occurrence of AMF from the other four genera was very low (Table 2).

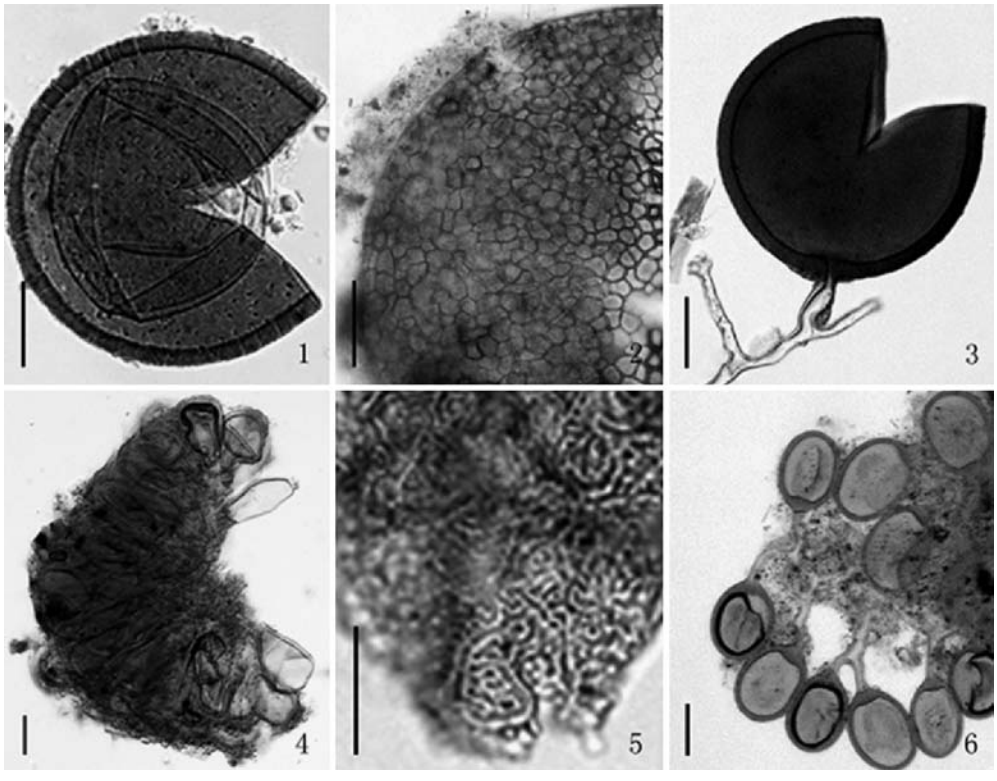


**Fig. 1** Some identified species of AMF. **1.** *Acaulospora sinosa* **2.** *A. bireticulata* **3.** *Glomus constrictum* **4.** *G. coremioides* **5.** *G. sinuosa* **6.** *G. rubiformis.* Bar=30μm

**Table 2** Identified AMF genera and the frequency of occurrence in rhizosphere soils. *IT* Number of times corresponding AMF isolated from the soils, *F* frequency of occurrence

| AMF genus | IT | F (%) |
|---|---|---|
| *Acaulospora* | 189 | 22.4 |
| *Archaeospora* | 16 | 1.9 |
| *Entrophospora* | 9 | 1.1 |
| *Gigaspora* | 21 | 2.5 |
| *Glomus* | 566 | 67.0 |
| *Scutellospora* | 43 | 5.1 |
| Total | 844 | 100 |

## Discussion

It has been demonstrated that indigenous AMF can increase the growth of their host plants in hot-dry areas (Requena et al. 2001). Water is a major stress for plants grown in hot-dry valley ecosystems. Though mycorrhizal effects on plant water relations are not as direct and consistent as those on P acquisition and host growth, the results of this study suggest that plants in the valley-type savanna may have a strong dependency on AM, as 95% of the surveyed plants formed AM, and their roots were intensively colonized. There were apparently more arbuscules and vesicles in the roots of the hot-dry valley plants than generally found in previous studies in tropical areas. Onguene and Kuyper (2001) reported that 79% of the samples surveyed in the rain forest of South Cameroon were colonized by AMF. AM plant species were only 56% of the total number of species sampled in Xishuangbanna rain forest in southwest China (Zhao et al. 2001). In the present study, most of the plants surveyed in the families of Brassicaceae, Polygonaceae, Cyperaceae, etc., which are usually considered to be non-mycorrhizal, also commonly formed AM; examples were *Cyperus rotundus*, *Polygonum statice* and *Rumex hastatus*. A similar situation has been found in the savanna of Venezuela (Lovera and Cuenca 1996) and in Indian semi-arid tropical grassland (Muthukumar and Udaiyan 2002), and indicates that plants grown in these stress environments may depend more on AM.

It has been reported that both spore production and species richness of AMF are lower in arid climates than in other ecosystems (Rose 1981; Pond et al. 1984), and decrease as aridity increases (Stahl and Christensen 1982; Stutz and Morton 1996). However, a short-term, transient decrease in soil moisture may promote spore production (Jacobson 1997; Guadarrama and Alvarez-Sanchez 1998). An average spore density of 1,530 spores per 100 g soil in our study was higher than previous reports in some other areas (Walker et al. 1982; Sylvia 1986; Friese and Koske 1991; Bever et al. 1996). Our results strongly support the hypothesis of Redhead (1975) that the amount of soil water optimal for plant growth may also be optimal for AMF sporulation. The samples were collected in the wet season (plant growth season) in this study, and the abundance of AMF spores and colonization has been reported to be coordinated with growth stages of plants during the long stress of a strong regimen of drought and rain (Kennedy et al. 2002). Some researchers have also shown that much less sporulation occurs in either extremely dry soils (Stahl and Christensen 1982; Stutz and Morton 1996) or flooded and permanently waterlogged soils (Khan 1974).

AMF in the genera *Acaulospora* and *Glomus* are dominant in the valley-type savanna of the Jinsha River, with frequencies of occurrence around 90%. *Glomus* species with sporocarp and periderm, such as *Glomus sinuosa*, and the small-spored *Acaulospora* and *Glomus* species were the most common. Stutz et al. (2000) also reported that AMF in arid regions of southwestern North America and Namibia, Africa were limited mostly to small-spored fungi in Glomaceae and Acaulosporaceae. It is possible that the plants in valley-type savanna and arid regions have a short growth period in the year, and that formation of small spores takes a shorter time than that of large spores in AMF in Gigasporaceae, which may not have time to form and mature prior to the dry season (Brundrett et al. 1999; Boddington and Dodd 2000).

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