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SHORT NOTE

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Arbuscular mycorrhizal status of plants and the spore density of arbuscular mycorrhizal fungi in the tropical rain forest of Xishuangbanna, southwest China

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Abstract The arbuscular mycorrhizal status of 112 plant species and the spore density of arbuscular mycorrhizal fungi (AMF) in the rhizosphere soil of these plants in the tropical rain forest of Xishuangbanna, southwest China, were surveyed. It was found that 56% of the surveyed species were arbuscular mycorrhizal, 31% were possibly arbuscular mycorrhizal and 13% were non-mycorrhizal. The spore density of AMF ranged from 55 to 1,908 per 100 g soil, with an average of 476. The rhizosphere soil from the arbuscular mycorrhizal plants did not always have a higher AMF spore density than that from the possibly mycorrhizal and non-mycorrhizal plants. The clumped distribution of AMF spores and the complex structure of the underground component of the tropical rain forest may be two important factors that affected the spore density of AMF. Fungi belonging to the genera *Acaulospora* and *Glomus* are the dominant AMF in the soil of the tropical rain forest of Xishuangbanna.

Keywords Tropical rain forest · Arbuscular mycorrhiza · Arbuscular mycorrhizal fungi · Spore density

Introduction

The symbiosis formed between plant roots and arbuscular mycorrhizal fungi (AMF) is of great interest because of its potential influence on ecosystem processes, its role in determining plant diversity in natural communities and the ability of AMF to induce a wide variety of growth responses in coexisting plant species (Hartnett and Wilson 1999; Heijden et al. 1998a, 1998b; Klironomos et al. 2000; Sanders et al. 1996). Since over 80% of angiosperm plants are mycorrhizal, and most are qualified as arbuscular mycorrhizal plants (Trappe 1987), this symbiosis is a component which cannot be ignored in a terrestrial ecosystem. Despite their relatively low species diversity in the world, AMF show no special host specificity, which implies that the root systems in a natural forest ecosystem dominated by angiosperm species could be interconnected by a diverse population of mycelia. Groups of tree species joined together in this way have been recognized as functional guilds in which plants could exchange resources through a common hyphal network, or hyphal bridge (hyphal linkage) (Read 1997; Simard et al. 1997). Different plant species have differential growth responses to AMF, so that the composition and diversity of the AMF community in a natural ecosystem could potentially affect the way plant species coexist, and therefore be a determinant of plant community structure (Heijden et al. 1998b).

Tropical rain forests display high species diversity and complex community structure, and they are a major distribution area for AMF in the world (Read 1994). The tropical rain forest of Xishuangbanna is located at the northern margin of the tropical zone of Southeast Asia. Since it is a type of transitional forest (from tropical to subtropical zone), the tropical rain forest of Xishuangbanna contains more species diversity than the typical tropical rain forests of Southeast Asia (Jin and Ou 1997). Many studies have been made on the vegetation, plant flora and the biodiversity of the tropical rain forest of Xishuangbanna, but little attention has been paid to the underground characteristics of this ecosystem, especially with regards to the mycorrhizal status of plants and the diversity of AMF. Here we report on the arbuscular mycorrhizal status of the major plant species and the spore density of AMF in the tropical rain forest of Xishuangbanna.

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Table 1 Arbuscular mycorrhizal status of species and the spore densities of arbuscular mycorrhizal fungi (AMF) in the soils. *M* Arbuscular mycorrhizal status of plants, + mycorrhizal, ± possibly mycorrhizal, – non-mycorrhizal, *ASD* AMF spore density (number of AMF spores in 100 g soil from the corresponding plant rhizosphere)

Plants	M	ASD	Plants	M	ASD
Acronychia pedunculata (L.) Miq.	+	1,028	*Leea marcophylla* Roxb. ex Hornem. Hort. Hafn.	+	
Aglaonema modestum Schott ex Engl.	+	1,908	*Litsea dileniifolia* P.Y. Bai et P.H. Huang	+	278
Albizia corniculata (Lour.) Druce	+		*Litsea liyuyingi* Liou	+	438
Albizia lucidior (Steud.) I. Nielsen	±		*Litsea monopetala* (Roxb.) Pers.	±	
Alchornea tiliaefolia (Benth.) Muell.-Arg.	+	654	*Lycianthes biflora* (Lour.) Bitter	±	
Alocasia longiloba Miq.	+	566	*Macaranga indica* Wight	+	
Alpinia conchigera Griff.	+	1,436	*Macropanax dispermus* (Bl.) O. Ktze.	+	498
Amischotolype hispida (Less. et Rich.) Hong	+	610	*Magnolia henryi* Dunn	+	298
Angiopteris caudatiformis Hieron	+		*Mananthes patentiflora* (Hemsl.) Bremek.	±	
Anoectochilus tortus (King et Pantl.) King et Pantl.	+	388	*Mangifera sylvatica* Roxb.	±	
Ardisia solanacea Roxb.	±		*Measa permollis* Kurz	+	314
Ardisia tenera Mez	+	380	*Metadina trichotoma* (Zoll. et Mor.) Bakn. f.	+	
Baccaurea ramiflora Lour.	+	55	*Mitrephora calcarea* Diels et Ast	+	282
Barringtonia macrostachya (Jack) Kurz	+	368	*Mycetia gracilis* Craib	±	210
Barringtonia racemosa (L.) Preng	+		*Mycetia hirta* Hutch.	+	
Begonia dryadis Irmsch.	±		*Myristica yunnanensis* Y.H. Li	+	
Byttneria grandifolia DC.	±		*Neonauclea tsaiana* S.Q. Zou	+	115
Carlemannia tetragona Hook. f.	+	384	*Oroxylum indicum* (L.) Vent.	+	
Caryota monostachya Becc.	+	264	*Pandanus furcatus* Roxb.	–	1,079
Castanopsis indica (Roxb.) A. DC	+	908	*Paramignya retispina* Craib	+	321
Celastrus paniculatus Willd.	±		*Paramomum petaloideum* S.Q. Tong	+	475
Celtis wightii Planch.	±	1,026	*Paraphlomis javanica* (Bl.) Prain	+	
Chesalia curviflora Thw.	+		*Peliosanthes sinica* Wang et Tang	±	554
Chloranthus spicatus (Thunb.) Makino	±	204	*Phaius mishmensis* (Lindl.) Rchb. f.	+	424
Clausena excavata Burm. f.	±		*Phaphidosperma vagabunda* (R. Ben.) C.Y. Wu	±	
Cleidion brevipetiolatum Pax et Hoffm.	+		*Phlogacanthus curviflorus* (Wall.) Nees.	+	286
Cleistanthus sumatranus (Miq.) Muell.-Arg.	–	292	*Phoebe lanceolata* (Wall. ex Nees) Nees	±	274
Colebrookea oppositifolia Sm.	+	1,040	*Phrynium capitatum* Willd.	±	314
Costus tonkinensis Gagnep.	±	116	Phrynium placenium (Lour.) Merr.	±	
Croton argyratus Bl.	±		*Piper longum* L.	+	662
Cudrania fruticosa Wight ex Kurz	±	100	*Piper polysyphorum* C. DC.	±	
Curculigo orchioides Gaertn.	±	396	*Pittosporopsis kerrii* Craib	±	420
Dalbergia obtusifolia Prain	+		*Pometia tomentosa* (Bl.) Teysm. et Binn.	–	166
Diospyros nigrocartex C.Y. Wu ex Wu et Li	+	318	*Pseudoranthemum palatiferum* (Nees) Radlk.	+	332
Drypetes hoaensis Gagnep.	–		*Pseudoranthemum polyanthum* (C.B. Clarke) Merr.	±	
Duperrea pavettaefolia (Kurz) Pitard	+	266	*Pseuduvaria indochinensis* Merr.	–	
Elaeocarpus varunua Buch.-Ham. ex Mast.	±	178	*Psychotria calocarpa* Kurz	+	316
Epiprinus silhetianus (Baill.) Croiz	–	162	*Psychotria henryi* Levl.	±	242
Ficus callosa Willd.	±	662	*Psychotria siamica* (Craib) Hutch.	–	306
Ficus cyrtophylla (Wall. ex Miq.) Miq	–	354	*Pteris venusta* Kunze	+	
Ficus hispida L. f.	+	526	*Pterospermum yunnanensis* Hsue	+	
Ficus langkokensis Drake	+	193	*Rhynchotechum obovatum* (Griff.) B.L. Burtt	±	148
Garcinia cowa Roxb.	–		*Schzomussaenda dehiscens* (Craib) H.L. Li	–	
Geophila herbacea (Jacq.) O. Ktze.	+	340	*Sterculia brevissima* Hsue	±	
Glochidion assamicum (Muell.-Arg.) Hook. f.	–	188	*Sumbaviopsis albicans* (Bl.) J.J. Sm.	±	276
Gomphostemma microdon Dunn	+	392	*Syzygium polypetaloideum* Merr. et Perry	±	1,540
Goniothalamus griffithii Hook. f. et Thoms.	+	382	*Tacca chantrieri* Andre	–	150
Harpullia cupanioides Roxb.	+		*Tetracera asiatica* (Lour.) Hoogl.	±	
Horsfieldia pandurifolia Hu	+	1,226	*Tetrameles nudiflora* R. Br.	±	
Jasminum laurifolium Roxb.	+	348	*Trevesia palmata* (Roxb.) Vis. var. *costata* H.L. Li	–	118
Knema globularia (Lamk.) Warb.	±		*Trigonostemon lii* Y.T. Chang	±	242
Lasianthus sikkimensis Hook. f.	+	892	*Ventilago calyculata* Tul.	+	1,560
Lasianthus verticillatus (Lour.) Merr.	+	570	*Vitex vestita* Wall. ex Schau	+	294
Leea compactiflora Kurz	+	296	*Wallichia mooreana* Basu	–	
Leea indica Merr.	+		*Zizyphus mauritiana* Lam.	+	462
Total: plants=112 species; M (+=63, ±=35, –=14); ASD=32,810					
Average or percentage: M (+56%, ±31%, –13%); ASD[a]=476					

[a] The average ASD over all soils

Materials and methods

Roots and their rhizosphere soil were collected to a depth of 5–30 cm in January 2000 (dry season), making sure that the roots were connected to sampled plants and cleaning the trowels between samples. A part of the root of each plant was fixed in 5 ml formalin, 5 ml acetic acid, and 90 ml of 70% alcohol, diluted twice when used (1/2 FAA), and stored at 4°C. The remaining roots were airdried with the 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 approximate 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-to 1-cm segments and stained with 0.5% acid fuchsin according to Berch and Kendrick's method (1982). Fifty 0.5- to 1-cm root fragments were examined per sample for their arbuscular mycorrhizal status under a compound microscope (×160–×800).

The rhizosphere soil samples were wet-sieved for spores using the method described by An et al. (1990). Twenty grams of soil from each plant rhizosphere was independently suspended in 250 ml water, stirred with a magnetic stirrer for 10 min and the suspension sieved. Spores and debris were collected on 40-µm, 70-µm, 100-µm and 150-µm sieves with tap water, filtered onto a filter paper, then placed in a 9-cm Petri dish for examination under a binocular stereomicroscope. AMF spores were counted in the four sieve samples. Some spores were tightly grouped in sporocarps and it was difficult to count the number of spores per sporocarp, so, in these cases, a sporocarp was referred to as one spore.

Each spore type was mounted in water, lactophenol, PVA and Melzer's reagent, respectively (Morton 1988), for identification. The identification was based on spore colour, 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 originally published species descriptions.

Results

The arbuscular mycorrhizal status of 112 plant species was surveyed. If at least one root segment was found to contain arbuscules or vesicles, then the plant was noted as an arbuscular mycorrhizal plant. If the root cortex was found to be colonized by fungal mycelia, but no arbuscules or vesicles were observed, the corresponding plant was noted as possibly arbuscular mycorrhizal. Plants were recorded as non-arbuscular mycorrhizal plants when neither arbuscules/vesicles nor fungal mycelia were detected in their root cortex. Results are summarized in Table 1. Sixty-three out of the 112 plant species surveyed were arbuscular mycorrhizal (Table 1). The possibly mycorrhizal and non-mycorrhizal plants represented 31% and 13% of the total sample, respectively.

AMF spore numbers were counted in 69 samples of the 112 rhizosphere soils (Table 1). The remaining 43 soil samples weighed <20 g as they were taken from the plants growing in rock crevices (*Ardisia solanacea* Roxb., *Harpullia cupanioides* Roxb and *Pteris venusta* Kunze etc.) or epiphytically to other plants [*Celastrus paniculatus* Willd., *Chesalia curviflora* Thw. and *Tetrameles asiatica* (Lour.) Hoogl. etc.] and so were insufficient for the estimation of spore density. However, AMF spores were identified in these soil samples.

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

AMF genus	IT	F (%)
Acaulospora	184	52
Gigaspora	1	0.3
Glomus	145	41
Sclerocystis	16	4.5
Scutellospora	8	2.2
Total	354	100

Three hundred and fifty four specimens of typical AMF spores were identified down to the genera *Acaulospora*, *Gigaspora*, *Glomus*, *Sclerocystis* and *Scutellospora*. Some specimens were identified to species, but identification of most of the specimens was unreliable because the field-sieved spores usually lacked distinguishable, fine taxonomic characters, or only a few spores were isolated. The occurrence of AMF was therefore only statistically analysed at the genus level. The genera of AMF identified and the frequency of their occurrence in the rhizosphere soils are listed in Table 2. AMF in the genus of *Acaulospora* and *Glomus* were the most frequently encountered, their occurrence frequency being 52% and 41%, respectively, in all the analysed rhizosphere soils. The occurrence of AMF from the other three genera was very low.

Discussion

Trappe (1987) concluded that about 62% of angiosperms were arbuscular mycorrhizal, and most of these were crops or weeds. Gemma et al. (1992) reported that arbuscular mycorrhizas were present in 66 of the 89 (74%) fern species examined in Hawaii. In our survey of a tropical rain forest, only 56% of plants had arbuscular mycorrhiza. However, this is probably an under-estimate, and with a large sample size of surveyed plant species and soil, the proportion of arbuscular mycorrhizal plants in the tropical rain forest of Xishuangbanna may well increase. Furthermore, sampling through different periods may reveal arbuscules or vesicles in some of the root systems colonized by a fungal mycelium.

AMF belonging to the genera *Acaulospora* and *Glomus* are dominant in the tropical rain forest of Xishuangbanna, since 93% of the encountered AMF are within these two genera, whilst *Gigaspora*, *Sclerocystis* and *Scutellospora* represent only 0.3%, 4.5% and 2.2%, respectively. Spore density ranged from 55 to 1,908 per 100 g soil, with an average of 476, for rhizosphere soils sampled in the present study, and it was not always related to the arbuscular mycorrhizal status of the corresponding plant. Relatively high spore densities were found in rhizosphere soils of some possibly mycorrhizal or nonmycorrhizal plants, and they were sometimes higher than those associated with mycorrhizal plants. In some cases this may have been due to the fact that plant roots

were interwoven in the same field sample so that mycorrhizal plants may have influenced sporulation in the rhizosphere of a plant that was non-mycorrhizal. There are many factors that could affect spore proliferation in a given host rhizosphere, and values for AMF spore density associated with different plants and at different sites have also varied a lot in previous reports (see for examples, Koske 1987; Sylvia 1986; Walker et al. 1982). Seasonality, edaphic factors, host-dependence, age of the host plants, the sporulation abilities of AMF, and the dormancy and the distribution patterns of AMF spores in the soils, have been previously reported to be amongst these factors (Bever et al. 1996; Gemma and Koske 1988; Greipson and El-Mayas 2000; Koske 1987; Koske and Halvorson 1981; Sylvia 1986; Walker et al. 1982; Zhao 1999). Guadarrama and Alvarez-Sanchez (1999) pointed out that disturbance, but not seasonality, affected abundance and richness of mycorrhizal spores in a tropical wet forest in Mexico. Our observations suggest that the uneven spatial distribution (clumped distribution) of AMF spores and the complex structure of the underground component of the tropical rain forest should also be considered as major factors affecting the spore density of AMF.

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