

五指山常见热带树种的丛枝菌根真菌多样性

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摘要 采用野外调查的方法, 分析了五指山不同海拔高度 7 个科 10 种常见热带树种形成丛枝菌根 (Arbuscular Mycorrhizal, AM) 的状况及其根际土壤中 AM 真菌的多样性。结果表明, 所调查的 10 种热带常见树种都能形成 AM 共生体, 其菌根侵染率随寄主植物的不同, 从 21.8 % ~ 90.5 % 变化不等, 同时, 在 10 种常见植物的根系中也都观察到了 AM 真菌的典型结构——丛枝和泡囊。从 10 种植物的根际土壤中共分离到 36 种 AM 真菌, 隶属于 *Acaulospora*, *Glomus*, *Gigaspora* 和 *Scutellospora* 4 个属, 其中, *Glomus* 属的真菌是该地区的优势类群, 其出现频度和相对多度分别为 84 % 和 56 %。在所调查的 10 种热带常见树种中, *Swietenia macrophylla* 根际 AM 真菌的孢子最丰富, 密度高达 7.32, *Machilus namu* 根际的 AM 真菌种类则最为丰富, 多样性指数达到 1.6548。通过对不同海拔高度 *Swietenia macrophylla* 根际 AM 真菌分布的分析表明, 海拔高度显著影响着 AM 真菌的分布, *Gigaspora* 属的真菌随海拔高度的增加显著升高, *Scutellospora* 属的真菌则显著降低。

关键词 丛枝菌根真菌; 多样性; 热带雨林; 五指山; 海拔高度

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Diversity of AM fungi associated with the common tropical tree species in Wuzhi Mountain of Hainan Island, China

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Abstract : Arbuscular mycorrhizal (AM) status and diversity of AM fungi were investigated on 10 indigenous tropical tree species at Wuzhi Mountain, central Hainan Island, China. All 10 plant taxa investigated were colonized by AM fungi, while the infective rates varied from 21.8 % to 90.5 % with a mean of 51.5 %. Arbuscules and vesicles were observed in all collected tree samples. A total of thirty-six AM fungi belonging to 4 genera, *Acaulospora*, *Glomus*, *Gigaspora* and *Scutellospora* were isolated from the rhizosphere of trees. *Glomus* was the most popular and dominant genus, closely followed by *Acaulospora*, and then *Scutellospora*. The highest spore density (7.32) was found in the soils of *Swietenia macrophylla*, while soils from the rhizosphere of *Machilus namu* were highest in species richness (5.37) and diversity index (1.6548) of AM fungi. A relationship between the frequency of occurrence and diversity of AM fungi and the elevation of the sampling sites for *Swietenia macrophylla* was observed. The elevation significantly affected the frequency of occurrence and diversity

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of *Gigaspora* and *Scutellospora* , but showed no significant influence to *Glomus* and *Acaulospora* .

Key Words : arbuscular mycorrhiza fungi ; diversity ; tropical rainforest ; Wuzhi Mountain ; elevation

Arbuscular mycorrhizal (AM) fungi belonging to the recently raised new fungal phylum the Glomeromycota are soil microorganisms known to establish a universally distributed mutualistic relationship with 80% of all terrestrial higher plant species. The associations formed between plant roots and AM fungi are of great interest because of their potential influence on ecosystem processes , their role in determining plant diversity in natural communities , and the capacity of AM fungi to induce a wide variety of growth responses in coexisting plant species ^[1,2]. Conversely , host plant diversity affects AM fungal community ^[3,4]. Besides host plants , climates , soils , latitudes and landscapes , environmental conditions may influence mycorrhizal formation , sporulation , distribution , and diversity of AM fungi ^[6-13].

Recent studies have indicated that AM fungi are common and ecologically important in tropical ecosystems , and that co-occurring plant species vary considerably in their germination , growth , and flowering responses to mycorrhizal colonization along a continuum from highly responsive , obligately mycotrophic species to facultatively mycotrophic and nonresponsive species ^[14,15].

Tropical rain forests display high plant species diversity and complex community structure ^[16]. In recent years there has been increasing interest in the arbuscular mycorrhizas of tropical rain forest plants ^[5,15,17-20]. Furthermore , China is a mountainous country with rich biodiversity resources. There have been many studies on aboveground plant diversity of China's mountains ^[21]. However , reports on belowground biodiversity are very few , limiting our understanding of aboveground ecological processes ^[22]. As important components of rhizosphere microbial communities in natural ecosystemsa , AM fungi play a key role in terrestrial ecological processes ^[23].

As the symbol of Hainan Island , Wuzhi Mountain has been listed by the International Tourism Organization in recognition of its natural scenery which comprises one of the few natural rain forests surviving in the world today. Numerous studies on plant and soil characteristics have been carried out on Wuzhi Mountain. However , as far as we aware , there have been no published reports on AM diversity in Wuzhi Mountain.

To enhance our understanding of the mycorrhizal status of AM fungi on some indigenous trees in tropical Hainan Province , a survey was carried out in an autumn season and first targeted area in this research was Wuzhi Mountain located on the central island. Rhizosphere soils and intact roots of 10 selected dominant tree species were collected and examined to analyze the AM status on each host and the richness of fungal species.

1 Materials and methods

1.1 Site location and description

Wuzhi Mountain is located in central Hainan island (18°49'—18°59'N ,109°40'—109°48'E). The mean annual temperature is 22.5 ℃. The average annual precipitation is 2350.7 to 2488.3 mm and the rainy season is from May to October with a long dry season. Soils were derived from lateritic red earths (Elevation 500—700m) , yellow lateritic red earths (700—1100m) , yellow podzolic soil (1100—1600m) , and southern mountain shrubby-meadow soil (>1600 m).

1.2 Sampling procedures

The indigenous tree species from 7 families were selected , i. e. Annonaceae (*Uvaria calamistrata*) , Dipterocarpaceae (*Vatica astrotricha*) , Fagaceae (*Castanopsis fabric* and *Castanopsis hystrix*) , Hamamelidaceae (*Liquidambar formosana*) , Lauraceae (*Machilus namu*) , Meliaceae (*Aphanamixis polystachya* , *Melia azedarach*

and *Swietenia macrophylla*) and Podocarpaceae (*Podocarpus imbricatus*). Surface soil (approximately 1 — 2 cm) was removed , and soil cores of 0 to 50 cm were collected including fine roots and rhizosphere soils of the host plants. Roots were traced back to the stem of the host plants to ensure that the roots were indeed connected to the plants selected for sampling. Three rooting-zone soil samples (each approximately 1000 g) with fine roots were collected in three different directions from each plant , and the three samples were mixed thoroughly. The fine roots were used to analyses AM fungal colonization. A subsample of approximately 500 g was then taken for extraction of AM fungal spores. Six individuals of each plant species were randomly selected for sampling of soil and roots.

1.3 Measurement of AM colonization

Fresh roots were processed by washing them free of soil and clearing in 100g/L KOH at 90℃ in a water bath for 30 — 60 min , the exact time depending on the degree of lignification of the roots and their pigmentation. The cooled root samples were washed and cut into 0.5 to 1.0-cm-long segments and stained with 5g/L acid fuchsin after acidification in thin HCl [24]. Thirty root fragments (approximately 1 cm long) were mounted on slides in a polyvinyl alcohol lactic acid glycerol solution and examined at 100 — 400 magnification under an Olympus BX50 microscope with an automatic photomicrographic system for the presence of AM fungal structures. The percentage of root length colonized by AM fungal structures was determined using the magnified line-intersect method of McGonigle *et al.* [25]. Hyphae , arbuscules , and vesicles were recorded when any of them were present at an intersection.

1.4 Isolation and identification of AM fungi

Spores or sporocarps were extracted from 20 ml air-dried subsamples of each soil sample by wet sieving followed by flotation centrifugation in 50% sucrose [26]. A sporocarp was counted as one unit. The number of spores is expressed as the mean of three replicates. For observation and identification of spore characters , spores were mounted on glass slides in polyvinyl alcohol lactoglycerol (PVLG) and PVLG + Melzer's reagent and then identified to species level using current taxonomic criteria [27] and information published by INVAM (http ://www.invam.caf.wvu.edu).

1.5 Measurement of the species richness of AM fungi in soils

Species richness was measured as the number of species compared with the number of individuals in the community. It is given by : $d = (S - 1) / \log N$, where d is the richness index , S is the number of species , and N is the number of individuals [28]. Relative abundance , spore density and frequency of occurrence were determined according to Allaby [28] and website information (www. si. edu/crc/ep/forest/bin-pdf/ 8Biodiversity/Calc _ Biodiversity_index. pdf). Species diversity of AM fungi was measured by Shannon-Weiner index. The formula for the Shannon-Weiner biodiversity index was $H = - [\sum (Pi) (\ln Pi)]$, where H represents the symbol for the amount of diversity in an ecosystem , Pi is the proportion , or relative abundance of , each individual species to the total , and $\ln Pi$ is the natural logarithm of Pi . (refer to : www. si. edu/crc/ep/forest/bin-pdf/ 8Biodiversity/Calc _ Biodiversity_index. pdf).

1.6 Statistical analysis

The data were analyzed using the SPSS software package version 11.0.

2 Results

2.1 Frequency of occurrence and relative abundance of AM fungi

Species of *Acaulospora* and *Glomus* were very frequently in collected form the soils. The frequencies occurrence (OF) of the two genera was 81% and 84% respectively. The relative abundance (RA) was 32% (*Acaulospora*) and 56% (*Glomus*). In terms of species , *Glomus claroideum* had the highest values for both testing index (OF and RA). *Gigaspora* was less abundant than the other fungal genera in this survey (Table 1).

Table 1 Frequency (F) and relative abundance (RA) of AM fungi in tropical rainforest in Wuzhi Mountain area of Hainan Island								
AM fungi	<i>Glomus</i>	<i>Acaulospora</i>	<i>Gigaspora</i>	<i>Scutellospora</i>	<i>A. spinosa</i>	<i>G. claroideum</i>	<i>Gi. sp. 1</i>	<i>Scu. sp. 1</i>
F (%)	84	81	9	26	18	28	7	16
RA (%)	56	32	3	9	5	10	1	4

2. 2 Species richness , spore density and species diversity of AM fungi

There were significant differences in species richness and spore density between host plant species (Table 2). The highest spore density occurred in soils of *Swietenia macrophylla* (7. 32) , while *Castanopsis hystrix* contained much less spores (3. 01) (Table 2). The species diversity (Shannon-Weiner index) varied from 1. 1 (*Castanopsis hystrix*) to 1. 6 (*C. fabri*).

2. 3 Effect of host plants on colonization and distribution of AM fungi

The AM status of each of the 10 tree species is shown in Table 3. AM fungi colonized all plant species examined. However , there were significant differences among different host plants. The overall mean percentage of root length colonized was 51. 5% and ranged widely from 21. 8% to 90. 5% . Arbuscules and vesicles were observed in all plant samples collected (Table 3).

The distribution of different genera of AM fungi varied with different host plants. For instance , the o frequencies of ccurrence of *Glomus* and *Scutellospora* was 57. 3% and 3. 5% on *Melia azedarach* but 43. 6 and 9. 4 on *Uvaria calamistrata* , respectively (Table 4).

2. 4 Elevation effect on distribution of AM fungi

Variation was observed in the frequency of occurrence (OF) and diversity of AM fungi on *Swietenia macrophylla* from sites at different elevations. There was a positive correlation between OF values and diversity index and the elevation levels for *Gigaspora* , while with the increment of elevation the OF and diversity of *Scutellospora* declined (Table 5). There was no such clear effect of elevation on the OF value or diversity index of either *Glomus* or *Acaulospora* on *S. macrophylla*.

2. 5 Tentative species of AM fungi

Thirty six AM fungi ,belonging to *Acaulospora* (9) , *Glomus* (20) , *Gigaspora* (3) and *Scutellospora* (4) , were isolated from soils , including two unidentified species belonging to *Gigaspora* and *Scutellospora* , respectively (Table 4).

Table 2 Species richness , spore density and species diversity of AM fungi in tropical rainforest in Wuzhi Mountain area of Hainan Island

Host plant species	AM species richness	Spore density	
		(Degrees/ No. of samples)	<i>H</i> *
<i>Aphanamixis polystachya</i>	4. 31 d **	3. 94 d	1. 3786bc
<i>Castanopsis fabri</i>	5. 03 b	3. 87 d	1. 6081a
<i>Castanopsis hystrix</i>	3. 76 e	3. 01 e	1. 0975d
<i>Liquidambar formosana</i>	4. 55 cd	3. 49 de	1. 3927b
<i>Machilus namu.</i>	5. 37 a	3. 54 de	1. 6548a
<i>Melia azedarach</i>	4. 85 bc	6. 51 b	1. 5180ab
<i>Podocarpus imbricatus</i>	4. 62 c	5. 68 c	1. 4103b
<i>Swietenia macrophylla</i>	4. 16 de	7. 32 a	1. 3721bc
<i>Uvaria calamistrata</i>	3. 92 e	5. 33 c	1. 3263c
<i>Vatica astrotricha</i>	3. 88 e	4. 28 d	1. 1876cd

* *H* , Shannon-Weiner index ; * * Values in columns followed by the same letter are not significantly different by LSD (*p* > 0. 05) ; The same as below

Table 3 Effect of host plants on colonization status of AM fungi in root samples

Host plant species	Colonization (%)	Arbuscules	Vesicles
<i>Aphanamixis polystachya</i>	45. 6 c *	+ + + **	+ + +
<i>Castanopsis fabri</i>	68. 7 b	+ + +	+ + +
<i>Castanopsis hystrix</i>	21. 8 d	+	+
<i>Liquidambar formosana</i>	64. 2 b	+ + +	+ + +
<i>Machilus namu</i>	66. 9 b	+ + +	+ + +
<i>Melia azedarach</i>	41. 3 cd	+ +	+ +
<i>Podocarpus imbricatus</i>	90. 5 a	+ + +	+ + +
<i>Swietenia macrophylla</i>	39. 6 c	+ +	+ +
<i>Uvaria calamistrata</i>	30. 6 d	+ +	+ + +
<i>Vatica astrotricha</i>	45. 3 c	+ +	+ +

* Values in columns followed by the same letter are not significantly different by LSD (*p* > 0. 05) ; * * Relative development of structures shown as + + + , always present in substantial numbers ; + + , always present ; + , very rare

Table 4 Effect of host plant species on distribution of genera of AM fungi

Host plants	AM fungi *			
	<i>Glomus</i>	<i>Acaulospora</i>	<i>Gigaspora</i>	<i>Scutellospora</i>
<i>Aphanamixis polystachya</i>	<i>caledonium claroideum clarum</i> , <i>etunicatum geosporum</i> ; (43.7%)	<i>elegans excavata rehmi</i> , <i>scrobiculata spinosa</i> ; (42.8%)	sp. 1 (3.5%)	<i>calospora minuta</i> ; (10.0%)
<i>Castanopsis fabri</i>	<i>aggregatum dolichosporum etunicatum</i> , <i>glomerulatum liquidambaris</i> , <i>fasciculatum microaggregatum</i> ; (46.4%)	<i>denticulata elegans</i> , <i>lacunosa spinosa</i> ; (48.7%)	<i>albida</i> (2.5%)	sp. 1 (2.4%)
<i>Castanopsis hystrix</i>	<i>citricolum etunicatum mosseae</i> , <i>fasciculatum macrocarpum</i> , <i>microcarpum claroideum</i> ; (54.5%)	<i>denticulata rehmi</i> , <i>spinosa</i> ; (35.3%)	<i>albida</i> (2.5%)	<i>calospora</i> (7.6%)
<i>Liquidambar formosana</i>	<i>caledonium claroideum clarum</i> , <i>geosporum microaggregatum hoi</i> , <i>mosseae</i> ; (47.3%)	<i>bireticulata elegans</i> , <i>excavata rehmi</i> ; (41.2%)	sp. 1 (2.8%)	sp. 1 (8.7%)
<i>Machilus namu</i>	<i>aggregatum caledonium hoi claroideum</i> , <i>constrictum clarum etunicatum</i> , <i>formosanum persiforme</i> ; (48.1%)	<i>bireticulata excavata</i> , <i>foveata lacunosa</i> ; (37.6%)	sp. 1 (4.2%)	<i>aurigloba</i> sp. 1 ; (10.1%)
<i>Melia azedarach</i>	<i>caledonium chimonobambusa clarum</i> , <i>fasciculatum formosanum macrocarpum</i> , <i>microcarpum mosseae persiforme</i> ; (57.3%)	<i>denticulata elegans</i> , <i>rehmi scrobiculata</i> , <i>spinosa</i> ; (39.2%)	0.0	<i>calospora</i> (3.5%)
<i>Podocarpus imbricatus</i>	<i>caledonium citricolum claroideum</i> , <i>clarum mosseae dolichosporum</i> , <i>geosporum</i> ;(48.6%)	<i>denticulata rehmi</i> , <i>foveata elegans</i> , <i>excavate</i> ; (42.3%)	sp. 1 (2.3%)	sp. 1 (6.8%)
<i>Swietenia macrophylla</i>	<i>hoi chimonobambusa mosseae</i> , <i>citricolum clarum geosporum</i> , <i>macrocarpum</i> ; (49.9%)	<i>rehmi scrobiculata</i> , <i>elegans</i> ; (45.4%)	<i>margarita</i> (2.2%)	sp. 1 (2.8%)
<i>Uvaria calamistrata</i>	<i>claroideum constrictum etunicatum</i> , <i>mosseae microaggregatum</i> ; (43.6%)	<i>scrobiculata excavata</i> , <i>bireticulata elegans</i> , <i>spinosa</i> ; (46.3%)	sp. 1 (0.6%)	<i>minuta</i> sp. 1 ; (9.4%)
<i>Vatica astrotricha</i>	<i>aggregatum claroideum clarum</i> , <i>etunicatum hoi persiforme</i> ; (54.3%)	<i>bireticulata excavata</i> , <i>lacunosa spinosa</i> ; (38.4%)	<i>margarita</i> (1.2%)	<i>aurigloba</i> (6.1%)

* Data under each AM genera are the means of their occurrence frequency on each host tree

Table 5 Effect of elevation on frequency of occurrence (OF) and diversity of genera of AM fungi *

Elevation (m)	<i>Glomus</i>		<i>Acaulospora</i>		<i>Gigaspora</i>		<i>Scutellospora</i>	
	OF	<i>H</i> **	OF	<i>H</i>	OF	<i>H</i>	OF	<i>H</i>
> 1300	55.6 a	1.5125 a	34.9 a	1.3188 a	4.6 a	1.008 a	3.9 c	0.8812 b
800 ~ 1000	53.6 a	1.5087 a	32.5 a	1.3525 a	3.2 b	0.8712 ab	10.7 b	1.1208 a
< 500	50.5 ab	1.4879 a	33.5 a	1.2916 a	2.0 c	0.8538 b	14.0 a	1.2031 a

* Data presented here were obtained from the rhizosphere of *Swietenia macrophylla* only ; * * *H* means Shannon-Weinner index

3 Discussion

The present study has indicated the predominance of arbuscular mycorrhizae in the tropical forests of Wuzhi Mountain. This is in agreement with the observations made on other mountains with tropical forests [29]. The fungi in genera *Glomus* were dominant with higher frequency of occurrence and relative abundance (Table 1). This provides strong support for the conclusions of other workers who have suggested that *Glomus* species tend to be the dominant AM fungi in tropical forest [5, 18–20]. The predominance of *Glomus* species on Wuzhi Mountain is in accord with the

observation that they are the most common AM fungi isolated throughout the world^[30].

Although many AM fungi were thought to have a broad host range , in general fungal species were found in specific soils or on specific host plant species. This may be related to diversity and the degree of AM colonization depending on host plant species in some extent. This study indicates that the host plants contributed to the abundance and colonization rate of AM fungi in soils ,although there was a great variation of mycorrhizal colonization rate , spore density , species richness and diversity of AM fungi in this study area. This is accorded with previous researches in tropical forest^[5, 31-33] and other ecosystems^[34, 35].

As to *Scutellospora* and *Gigaspora* , their occurrence frequency and relative abundance were much low than those of *Glomus* fungi. However , comparing these two genera , the isolation frequency and relative abundance of *Scutellospora* were higher than those of *Gigaspora*. The former may be better adapted to the soil and host conditions on the island than the latter since the numbers of the former isolated on and near islands were greater than those of the latter^[36]. As environmental conditions on marine islands may often be relative unpolluted , with rich vegetation of high biodiversity and fertile forest soils , thus the colonization , spore production , and species diversity of AM fungi may be promoted. This may also emphasise the important role of environmental protection in the conservation of biodiversity.

In addition , elevation effects on fungal diversity have been repotted previously^[37]. The frequency of occurrence and diversity index of *Glomus* and *Acaulospora* were not markedly different with changing elevation in the rhizosphere of *Swietenia macrophylla*. However , *Gigaspora* and *Scutellospora* were correlated with elevation. One possible explanation is that the range of elevation was quite narrow. Zhang *et al.* found that *Glomus* and *Acaulospora* were not significantly different below 3000 m and *Scutellospora* was distributed mainly below 1500 m^[38]. Further investigations are required to examine this relationship on a broad range of host trees in the area.

Identification of AM fungi has traditionally relied on the morphological and developmental characteristics of their large multinucleate spores^[39]. In the present study the predominant AM fungi were isolated and identified according to the morphological characteristics of the AM fungal spores. However , we do not know whether all the AM fungi isolated from the soils associated with the roots colonized the plants studied because of intersecting plant roots in the tropical forest. Furthermore , AM fungal diversity differed among the different host plant species and elevation. molecular approaches will be necessary to circumvent this problem and to define and relate taxa in the Glomeromycota

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