

Natural Forest and Forest Plantation Affect Diversity of Arbuscular Mycorrhizal Fungi in the Rhizosphere of Dipterocarpaceae

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Abstract: The influences of natural forest and forest plantation of Dipterocarpaceae on arbuscular mycorrhizal (AM) fungal diversity were studied based on the investigation of *Hopea hainanensis* and *Vatica astrotricha* of Dipterocarpaceae grown in natural forest and forest plantation in Jianfengling Mountain of Hainan Island in South of China. The results showed that the percentage of root length colonized by AM fungal structures, spore density and species diversity of AM fungi were all higher in natural forest than in forest plantation. AM fungal species richness of *Hopea hainanensis* was significantly higher in natural forest than in forest plantation. However, species richness in the rhizosphere of *Vatica astrotricha* wasn't significantly different between natural forest and forest plantation. Twenty-one AM fungal species belonging to five genera were isolated and identified in rhizosphere soils of the two objective plants. Among them, 14 species are belonging to genus of *Glomus*, 4 for *Acaulospora* and 1 for each of *Archaeospora*, *Gigaspora* and *Scutellospora* respectively. *Glomus* species are found to be the dominant AM fungi in either natural forest or forest plantation.

Key words: Arbuscular mycorrhizal fungi · Dipterocarpaceae · diversity · natural forest · forest plantation

INTRODUCTION

As the most Widespread Symbiosis on Earth [1], Arbuscular mycorrhizal (AM) fungi evolved concurrently with the first colonization of land by plants some 450 to 500 million years ago and persist in most extant plant taxa [2]. 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 [3-7]. Recent studies have indicated that AM fungi are common and ecologically important in tropical ecosystems and that cooccurring 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 [8, 9]. Tropical rain forests display high plant species diversity and complex community structure [10].

The Dipterocarpaceae is one of the most important tree families in tropical rainforests both ecologically and economically. It is the symbol of tropical rainforest. Some trees are a major source of very valuable tropical hardwood timber such as *Hopea chinensis* and members of the family are also used for resin and gums. In recent years, there has been increasing interest in the arbuscular mycorrhizae of tropical rain forest plants including natural forests [11-13], secondary forest [14] and deforested forest [15]. Especially, the Arbuscular mycorrhizal fungi associated with Dipterocarpaceae also intrigued several researchers [13, 16]. As a kind of soil microorganisms, AM fungi do not avoid the influence of vegetation types [15, 17]. Zhang *et al.* have shown that the diversity of AM fungi were different in deforested and natural forest lands [15]. However, the effect of natural forest and forest plantation on diversity of AM fungi lacked systematic study, though there are fragmentary reports [13]. The purpose of present study is to investigate the effect of natural forest and forest plantation on AM fungal diversity by comparing the differences AM fungal

diversity in the rhizosphere of natural forest and forest plantation of Dipterocarpaceae based on a broad field survey in Jianfengling Mountain of Hainan Island in South of China.

MATERIALS AND METHODS

Site location: Jianfengling, located in the juncture of Ledong and Dongfang counties, is on the southwestern part of Hainan Island at 18°23'-18°52' N and 108°46'-109°02' E. The total area of Jianfengling forest region is 47227 km² and this makes the region one of the 5 largest forest areas on the island. The highest elevation of Mt. Jianfengling Peak is 1412.5 m. Jianfengling Primeval Tropical Forest is one of China's biggest and best-preserved primeval tropical forest areas. The samples of *Hopea hainanensis* and *Vatica astrotricha* in natural forest were collected from the Mt. Jianfengling Peak with the altitude of around 850 m and 500 m, respectively. The samples in forest plantation were collected from Jingfengling farms.

Collection of soil and root samples: Surface soil (approximately 1–2 mm) was removed and soil cores of 0 to 50 cm were collected including fine roots and rhizosphere soils of the host plants. Root samples were collected from a total of 2 objective species of *Hopea hainanensis* and *Vatica astrotricha* of Dipterocarpaceae. 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. A subsample of approximately 500 g was then taken for assessment of AM fungal colonization and extraction of AM fungal spores. Six individuals of each plant species were randomly selected for sampling of soil and roots. Samples of plant roots were taken to the laboratory for determination of root colonization. The soil samples were then air-dried in the shade at laboratory temperature (10-26°C) for spore extracting, counting and identification.

Assessment of AM colonization: Fresh roots were processed by washing them to get rid of adhering soil particles and clearing in 10% (w/v) KOH: distilled water at 90°C 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 0.5% (w/v) acid fuchsin [18]. The percentage of root length colonized by AM fungal structures was determined using the magnified line-intersect method of McGonigle *et al.* [19].

Recovery and counting of AM fungal spores: Spores or sporocarps were extracted from 20 g air-dried subsamples of each soil sample in triplicate by wet sieving (53 µm) followed by flotation–centrifugation in 50% sucrose [20]. The spores were collected on a grid patterned (4×4 mm) filter paper, washed three times with distilled water to spread them evenly over the entire grid and counted using a dissecting microscope at 30× magnification. A sporocarp was counted as one unit. 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 [21] and information published by INVAM (<http://www.invam.caf.wvu.edu>).

Numbers and distribution of AM fungal spores: Spore density and species richness of AM fungi were expressed as follows:

$$\text{Spore density} = \text{No. of AM fungal spores in 20 g dry soil} \quad (1)$$

$$\text{Species richness} = \text{No. of AM fungal taxa found in 20 g dry soil} \quad (2)$$

Species diversity of AM fungi was assessed by the Shannon–Weiner index as follows:

$$\text{Shannon-Weiner index} = -\sum (P_i \ln[P_i]) \quad (3)$$

where $P_i = n_i/N$ and n_i = number of individuals in species i ; N is the total number of individuals in all species.

Statistical analysis: The data were subjected to one-way ANOVA using SPSS software version 11.0. The differences in percent root length colonized, spore density, species richness and species diversity were separated by least significant difference (LSD) test for significantly different means in all taxa.

RESULTS

Effect of natural forest and forest plantation on AM fungal colonization: The AM fungal colonization of *Hopea hainanensis* and *Vatica astrotricha* of

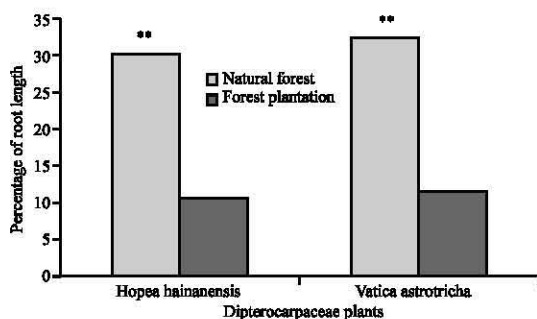


Fig. 1: Effect of natural forest and forest plantation on AM fungal colonization

Note: ** means significant difference at the level of $p=0.01$.

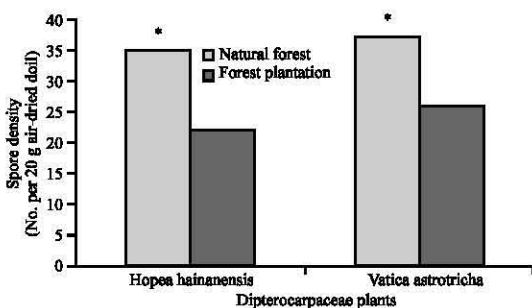


Fig. 2: Effect of natural forest and forest plantation on AM fungal spore density

Note: * means significant difference at the level of $p=0.05$.

Dipterocarpaceae in natural forest and forest plantation are presented in Fig. 1. The percentage of root length colonized of *Hopea hainanensis* and *Vatica astrotricha* are significantly higher in natural forest than in forest plantation.

Effect of natural forest and forest plantation on AM fungal spore density: The spores of AM fungi of 20 ml air-dried soil in rhizospheres of *Hopea hainanensis* and *Vatica astrotricha* of Dipterocarpaceae are isolated from natural forest and forest plantation, respectively. The spore densities of AM fungi are significantly higher in natural forest than in forest plantation in the rhizosphere either *Hopea hainanensis* or *Vatica astrotricha* (Fig. 2).

Effect of natural forest and forest plantation on AM fungal species richness: Figure 3 indicated the species richness of AM fungi in rhizospheres of *Hopea hainanensis* and *Vatica astrotricha* of Dipterocarpaceae in natural forest and forest plantation. AM fungal species richness in the rhizosphere of *Hopea hainanensis* is

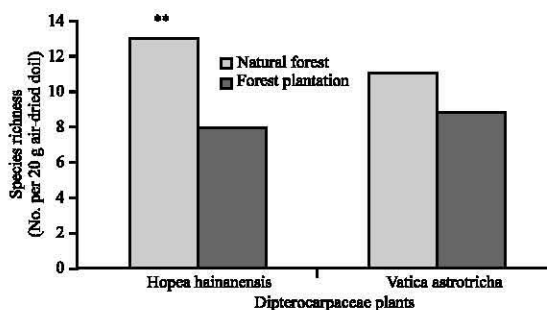


Fig. 3: Effect of natural forest and forest plantation on AM fungal species richness

Note: ** means significant difference at the level of $p=0.01$.

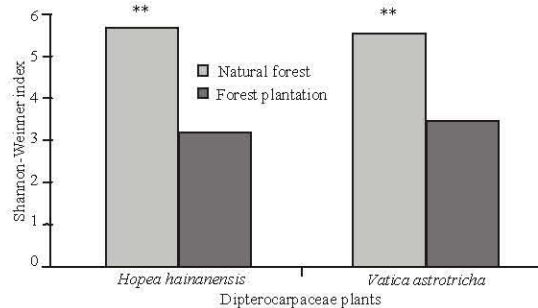


Fig. 4: Effect of natural forest and forest plantation on AM fungal species diversity

Note: ** means significant difference at the level of $p=0.01$.

higher in natural forest than in forest plantation significantly. However, no significant difference was observed in the rhizosphere of *Vatica astrotricha* between natural forest and forest plantation.

Effect of natural forest and forest plantation on AM fungal species diversity: The influences of natural forest and forest plantation on AM fungal species diversity were analyzed based on *Hopea hainanensis* or *Vatica astrotricha* (Fig. 4). The results showed that the diversity was more abundant in natural forest than in forest plantation in the rhizosphere either *Hopea hainanensis* or *Vatica astrotricha*.

Effect of natural forest and forest plantation on AM fungal community composition: Twenty-one AM fungal species belonging to five genera, including one species in *Archaeospora*, four in *Acaulospora*, one in *Gigaspora*, fourteen in *Glomus* and one in *Scutellospora*, were isolated and identified in the rhizosphere of *Hopea hainanensis* and *Vatica astrotricha* in natural forest and forest plantation (Table 1). Fifteen species representing

Table 1: Effect of natural forest and forest plantation on AM fungal community composition

AM fungi	Natural forest	Forest plantation
<i>Acaulospora appendicola</i> Rothwell and Trappe	# +	# +
<i>A. denticulate</i> Sieverd. and Toro	#	#
<i>A. foveata</i> Trappe and Janos	+	#
<i>A. rehmsii</i> Sieverd. and Toro	#	+
<i>Archaeospora leptoticha</i> (Schenck and Sm.) Morton and Redecker	#	#
<i>Gigaspora margarita</i> Becker and Hall	#	
<i>Glomus aggregatum</i> Schenck and Sm.	# +	# +
<i>G. caledonium</i> (Nicolson and Gerd.) Trappe and Gerd.	# +	# +
<i>G. chimonobambusa</i> Wu and Liu		#
<i>G. claroideum</i> Schenck and Sm. emend Walker and Vestberg	# +	+
<i>G. deserticola</i> Trappe, Bloss and Menge	#	#
<i>G. constrictum</i> Trappe	# +	+
<i>G. etunicatum</i> Becker and Gerd.	#	# +
<i>G. geosporum</i> (Nicol. and Gerd.) Walker	+	
<i>G. hoi</i> Berch and Trappe	+	
<i>G. macrocarpum</i> Tul. and Tul.	#	+
<i>G. microaggregatum</i> Koske, Gemma and Olexia	# +	# +
<i>G. microcarpum</i> Tul. and Tul.	+	+
<i>G. mosseae</i> (Nicol. and Gerd.) Gerd. and Trappe	#	# +
<i>G. reticulatum</i> Bhattacharjee and Mukerji	+	
<i>Scutellospora aurigloba</i> Walker and Sanders	# +	+

Note: # present in the rhizosphere of *Hopea hainanensis*. + present in the rhizosphere of *Vatica astrotricha*.

5 genera AM fungi presented in rhizosphere of *Hopea hainanensis* in natural forest comparing to 12 fungal species within 3 genera in forest plantation. As to AM fungi associated with *Vatica astrotricha*, the same number species presented in natural forest and forest plantation. They included 12 species in *Acaulospora*, *Glomus* and *Scutellospora*.

DISCUSSION

Most trees in tropical forest might be associated with arbuscular mycorrhizas [9, 11, 12, 22-25]. As a symbol of tropical rainforest, the AM fungal diversity associated with Dipterocarpaceae plants attracted more attention of many researchers [13, 16, 26, 27]. Furthermore, AM fungal diversity, e.g., spore formation, distribution, species community composition and mycorrhizal development, is affected by vegetation types or life formations [15, 17, 28]. The present study has indicated natural forest and forest plantation affected diversity of AM fungi in the Rhizosphere of Dipterocarpaceae.

The percentage of root length colonized by AM fungal structures, spore densities and species diversities connected with *Hopea hainanensis* and

Vatica astrotricha were higher in natural forest than in forest plantation. This is in agreement with the previous reports [13]. The possible reasons are that although the tree species selected are same, the annual or perennial herbaceous plant species existed more in natural forest than in forest plantation. These annual or perennial herbaceous plants, as AM fungal hosts, are associated with more AM fungal spore production than are evergreen broad-leaved trees [29, 30]. Moreover, the plant diversity affected the diversity of AM fungi [31-33]. As to the species richness of AM fungi, the significant difference was observed in rhizosphere of *Hopea hainanensis* between natural forest and forest plantation. However, species richness associated with *Vatica astrotricha* wasn't significant different between natural forest and forest plantation. The possible explanation is that the samples collecting error caused the results. This result was presented by species community composition in Table 1. As far as Figure 3 of species richness associated with *Vatica astrotricha* is concerned, although there were no significant differences between natural forest and forest plantation as statistical analysis stated, Figure 3 showed the species richness in natural forest is little higher than in forest plantation. However,

Table 1 indicated the same number of species were isolated and identified in the rhizosphere of *Vatica astrotricha* between natural forest and forest plantation. Figure 3 and Table 1 seem incompatible, but in fact they are accordant, because the data in Fig. 3 are the average of six random tree individuals, while the data in Table 1 are the maximal data presented in the rhizosphere of *Vatica astrotricha* six random tree individuals.

Twenty-one AM fungal species representing five genera were isolated from the rhizosphere soils of *Hopea hainanensis* and *Vatica astrotricha* in natural forest and forest plantation. Fourteen *Glomus* species appeared to be dominant in the rhizosphere soils of *Hopea hainanensis* and *Vatica astrotricha* in natural forest and forest plantation in Jianfengling Mountain. In contrast, *Acaulospora*, *Archaeospora*, *Gigaspora* and *Scutellospora* represented only 19.0, 4.8, 4.8 and 4.8% of the species present, respectively. This provides strong support for the conclusions drawn by other workers who have suggested that *Glomus* species tend to be the dominant AM fungi in tropical rainforest ecosystems [11-13]. In addition, the genus *Glomus* is the largest of all the AM fungal genera in the Glomales [34].

Additionally, not only the forest type but seasonality, host-dependence and age of the host plants, etc. of Dipterocarpaceae can influence the AM fungal colonization, spore density, species richness and diversity and species community composition. These aspects still need further research.

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