

# The Arbuscular Mycorrhizal Fungi's diversity in Fabaceous trees species of Northeastern Thailand

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Manuscript received: 11 January 2019. Revision accepted: 18 January 2019.

**Abstract.** Choosa-Nga P, Sangwanit U, Kaewgrajang T. 2019. The Arbuscular Mycorrhizal Fungi's diversity in Fabaceous trees species of Northeastern Thailand. *Biodiversitas* 20: 405-412. The species diversity of arbuscular mycorrhizal (AM) fungi, found in three Fabaceous tree species, namely, *Dalbergia cochinchinensis*, *Pterocarpus macrocarpus* and *Xylia xylocarpa*, was studied. Sixty rhizosphere soil samples were collected from the tree species plantations, and AM fungal spores were extracted and identified using their morphological characteristics. The spore density and species diversity of the fungi associated with these Fabaceous tree species was assessed. The 11 AM fungi morphospecies identified were *Acaulospora* (3 species), *Glomus* (6 species), *Sclerocystis* (1 species) and *Scutellospora* (1 species). Meanwhile, three species remained unidentified, which Unknown sp.2 was frequently found in all the three of plantations. The Shannon-Weiner diversity index of the fungal species ranged from 1.40 to 1.88, while the Evenness index ranged from 0.58 to 0.76. The diversity and Evenness index was the highest in the *D. cochinchinensis* plantation and lowest in the *X. xylocarpa* plantation. These differences may be due to differences in soil characteristics, including moisture, % organic matter, and nutrient composition. The species composition of AM fungi in the *P. macrocarpus* plantation was not similar with the *D. cochinchinensis* and *X. xylocarpa* plantations. It was indicated that different plant species were hosts to different AM fungal communities. Therefore, further studies are required to select the appropriate AM fungal species which have a positive effect on the growth of the three Fabaceous species when we would like to do the inoculating programs.

**Keywords:** *Dalbergia cochinchinensis*, endomycorrhiza, mycorrhiza, *Pterocarpus macrocarpus*, *Xylia xylocarpa*

**Abbreviations:** AM: Arbuscular Mycorrhiza, AMF: AM fungi, DC: *Dalbergia cochinchinensis*, PM: *Pterocarpus macrocarpus*, XX: *Xylia xylocarpa*, SD: spore density, RA: relative abundance, OM: organic matter, P: phosphorous, N: nitrogen, K: potassium

## INTRODUCTION

Arbuscular mycorrhiza (AM) is a symbiotic association between a fungus and the roots of a plant. The plant growth is enhanced by such mycorrhizal interactions with an increased access to soil resources, including the ability to trap water and alleviate nutrient stress by obtaining greater amounts of phosphorous (P), nitrogen (N) and potassium (K) (Smith and Read 2008; Lee et al. 2013; Cardoso et al. 2017). Other advantages of this symbiosis is increased resistance to root pathogens (Pozo et al. 2010), tolerance to environmental stresses such as salinity and drought (Hameed et al. 2014; Latef et al. 2016), and heavy metal contamination (Upadhyaya et al. 2010). AM fungi (AMF) are one of the most abundant organisms in the soil rhizosphere and are found in a wide range of host plants with ca. 70–90% of terrestrial plant species, from bryophytes to tracheophytes (Brundrett 2009). Therefore, their species diversity is low and they have indicated a lack of host specificity (Lee et al. 2013). Only 240 species of AMF exist, which belong to the phylum Glomeromycota, contain three classes, five orders, 14 families and 29 genera (Morton and Msiska 2010; Oehl et al. 2011; Krüger et al. 2012). In the tropical region, the AM plays an important role in both the natural and agricultural ecosystems, when compared with the other types of mycorrhizae (Cardoso et

al. 2017). However, all the studies related to AMF have focused on temperate ecosystems. Consequently, very little is known about the interaction between the fungi and host plants in extremely diverse communities such as tropical forests (Kiers et al. 2000; Husband et al. 2002). Previous studies conducted in tropical forests have indicated that the diversity of AMF is low and has no specific to their host plants. Marionho et al. (2018) reported 228 AMF in tropical forests, distributed in three classes, five orders, 14 families, and 35 genera. Among the AM fungal community, *Acaulospora* and *Glomus* were the dominant genera in tropical rhizosphere soils (Zhao et al. 2003; Marionho et al. 2018).

Many native fabaceous tree species in tropical forests are sources of the most valuable timber in the world. As a result, over the years, their numbers have been gradually declining due to their feeling for the valuable timber. The wood derived from the trees has been used traditionally for carving, making musical instruments, and luxury furniture in South America, Africa, and South East Asia (Jenkins et al. 2012). International trade of various species in the Fabaceae family has been increasing steadily and more and more species are being traded internationally, most of which is done in the black markets (CITES COP17 Prop.55). The Fabaceous trees species have a relatively low regeneration rate and slow growth in natural ecosystem.

Consequently, a proper balance of their populations in the conservation areas is not met leading to a gradual decline in numbers. Many shrubs and herbaceous plant species in Fabaceae family have been examined for the presence and diversity of AMF, while only some trees species in the family, i.e., *Acacia auriculiformis*, *A. mangium*, *Albizia saman* *Dalbergia sissoo*, *Paraserianthes falcataria*, and *Pongamia pinnata*, have been reported to have an association with the AM fungal species (Weravart 2003; Dhar and Mridha 2006; Wulandari et al. 2016; Ponpandian and Rose 2017). Dhar and Mridha (2006) showed that the dominant AMF found in the plants of the family Fabaceae was from the *Glomus* genus, while Ponpandian and Rose (2017) reported that *Acaulospora* was also abundant in the rhizosphere of the soils of *Pongamia pinnata* (Fabaceae). Currently, there is limited knowledge on AM fungal species in the rhizosphere of the soils of tropical forest tree species, especially for species with high economic value like *Dalbergia cochinchinensis* (DC), *Pterocarpus macrocarpus* (PM), and *Xylia xylocarpa* (XX). Therefore, the aim of this study was to investigate the species identity, richness, and diversity of AMF in the rhizospheres of the soils of *D. cochinchinensis*, *P. Macrocarpus*, and *X. xylocarpa*. Moreover, we aimed to determine the differences in the AMF communities found in the three plant species.

## MATERIALS AND METHODS

### Sampling sites

The sampling site was located in the Sakaerat silvicultural research station, Nakhon Ratchasima province, northeastern Thailand. The station is maintained by the Royal forest department (Figure 1). The Phu Luang forest reserve is located on the eastern side of the station with a total area of around 7,458 ha including the station area. The area around the station was covered with a dry evergreen and dry dipterocarp forest until the 1950s, until it was encroached by the local farmers and converted into agricultural land. Since 1967, over 2,000 ha (28 % of total area) of the area, which was abandoned and covered with tall grasses, has been under a re-forestation project with native and exotic fast-growing tree species been planted (Kamo et al. 2002; Visaratana et al. 2008).

### Soil sampling

The rhizosphere soils from the three Fabaceous plantations as treatments, namely *Dalbergia cochinchinensis* (DC) plantation; *Pterocarpus macrocarpus* (PM) plantation; *Xylia xylocarpa* (XX) plantation, were collected during the months from June to September 2016. Twenty trees were randomly selected from each plantation. DC and PM plantation's age is 30-year old, while XX plantation is 11-year old. Each plantation has total area 1.6 ha and is planted with spacing 2×4 m. One soil core (5 cm in diameter, 10 cm in depth), located 1 m away from the base of each tree, was collected and 20 soil cores were

collected from each plantation. In total, 60 soil samples were collected, placed in polyethylene bags, and transported to the laboratory, where they were stored at 4°C prior to spore extraction. Moreover, five disturbed soils samples (300-500 g/sample) were randomly collected from each plantation. These soil samples were analyzed to determine the soil properties, which included pH, soil moisture, organic matter, total N, available P, and exchangeable K content.

### AM fungal spore extraction and identification

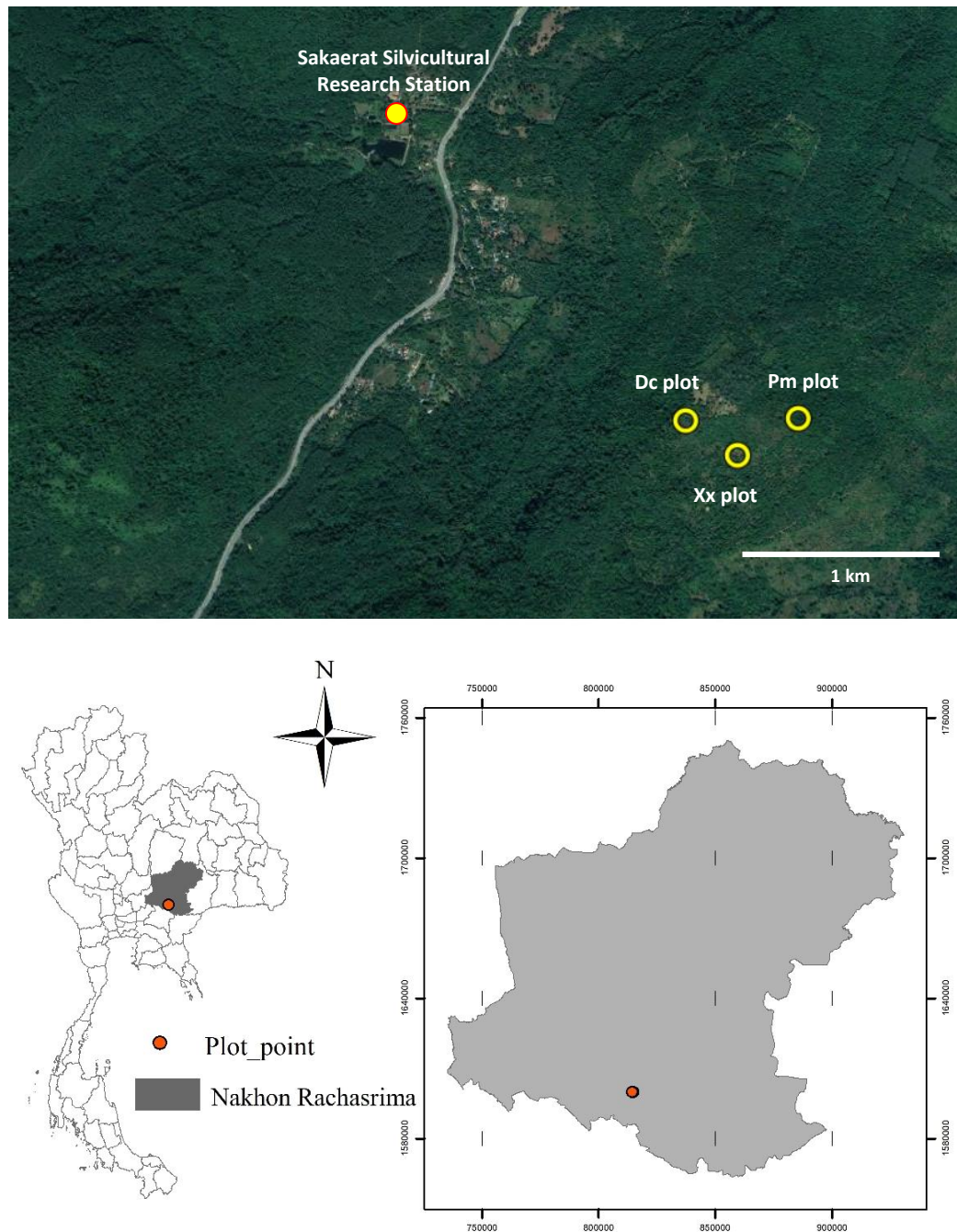
Spores were isolated from a 4 g subsample obtained from each soil sample using the modified wet sieving method (Brundrett et al. 1996) and sucrose centrifugation (Daniels and Skipper 1982). Each subsample was first placed in a bucket containing 2 L of tap water, swirled, and the resulting soil suspension was then passed through three nested sieves, with an aperture of size 150, 75, and 45 µm. The material remaining on each sieve was transferred to a separate tube for each sieve, containing 50% sucrose gradient. Then each tube was centrifuged at 4,000 rpm for 5 mins. The supernatant was transferred to a new test tube containing 50% sucrose gradient and centrifuged at 3,000 rpm for 1 min. It was then poured onto a sterile filter paper and rinsed with sterile distilled water several times and the spores remaining on the filter paper were inspected under a stereomicroscope (Zeiss Stemi 508).

### Species identification

All the spores were isolated, mounted on a slide with polyvinyl alcohol-lactic acid-glycerol (PVLG), for the identification of the AM fungal species. The AM fungal spores were distinguished for their morphotype by observing the macroscopic features under a compound microscope (Zeiss Axioskop 40) from 10-100X magnification. These included size, color, hyphal attachment, shape, type, and the number of spore walls. The species were identified using the manual recommended for the identification of Vesicular-arbuscular (VA) mycorrhizal fungi of Schenck and Perez (1988). Furthermore, online databases of species description at the West Virginia University, USA (<http://invam.caf.wvu.edu>) and the Department of Plant Pathology, University of Agriculture in Szczecin, Poland (<http://www.agro.ar.szczecin.pl/~jblaszkowski/>), were used to identify the AM species.

### Data analysis

Soil characteristics at the three plantations were compared using one-way analysis of variance (ANOVA). Additionally, the number of AM species was determined by directly counting the number of fungal species per soil sample. The spore density (SD) of the AM fungal species was defined as the number of spores in 100 g of soil sample. It was assessed using a one-way ANOVA to compare the spore density in different plantations. All the statistical analyses were performed using the R Statistical software (Version R386.3.5.1).



**Figure 1.** Location of the Sakaerat Silvicultural Research Station, Nakhon Ratchasima province, Thailand indicating the sampling sites. Dc plot: *Dalbergia cochinchinensis* plantation, Pm plot: *Pterocarpus macrocarpus* plantation, Xx plot: *Xylia xylocarpa* plantation

Relative abundance (RA) was defined as the percentage number of spore of a species divided by the total spores observed. The dominant AM fungal species was determined by SD and RA. The Shannon-Weiner's index ( $H'$ ) (Magurran 1988) was chosen to measure the fungal species diversity in different plantations using the equation  $H' = -\sum p_i (\ln p_i)$ , where  $p_i$  is the ratio of the number of spores in the  $i$ th species to the total number of spores,  $i = 1, 2, 3, \dots, n$ , and the summation was done over the total

number of species  $n$ . In addition, the Pielou's evenness index (E) (Pielou 1966) was evaluated using the equation  $E = H' / \ln S$ , where  $H'$  is the number derived from the Shannon-Weiner's index and  $S$  is the total number of species in a sample, across all samples in a dataset. Moreover, the two-way clustering analysis, with Sorensen (Bray-Curtis) distance was implemented in PC-ORD version 6.08 (Peck 2010) to classify group of AM species.

## RESULTS AND DISCUSSION

### Soil characteristics

The soil pH ranged from 4.07 to 4.48, organic matter (OM) between 1.8 to 6.76%, N between 0.10 to 0.21%, P between 5.77 to 15.72 mg/kg, K between 32.60 to 136.24 mg/kg and soil moisture content between 7-23%, across the plantations (Table 1). It indicated that the soil has strong acid and contained moderate chemical properties. The soil characteristics were significantly different among the plantations ( $P < 0.05$ ), except pH and the level of P. DC plantation, had the highest soil moisture content, OM, and total N, while all soil characteristics were the lowest in the XX plantation.

### AM species diversity

Spore density, in the soil samples, ranged from 1125 to 9350 spores  $100\text{ g}^{-1}$  soil (with a mean of  $4156 \pm 2001$  spores). The spore density was not significantly different across plantations ( $P > 0.05$ ). However, the maximum spore density was measured in the XX plantation ( $4313 \pm 1646$ ), while it was the lowest in the PM plantation ( $4017 \pm 1970$ ) (Figure 2).

Fourteen morphospecies of AMF, recovered from the 60 soil samples, were identified using their spore characteristic features. There were 3 species in *Acaulospora*, 6 species in *Glomus*, 1 species in *Sclerocystis*, 1 species in *Scutellospora*, and 3 unidentified species in the three plantations (Figure 3). There were 12, 13, and 11 AM species found in the DC, PM and XX plantations, respectively. The Shannon-Weiner's index was the highest in the DC plantation ( $H' = 1.8$ ) and lowest in the XX plantation ( $H' = 1.40$ ). Similarly, E ranged from 0.58 to 0.76, being the highest in the DC plantation and lowest in the XX plantation (Table 2).

### Two-way cluster analysis

The similarity index based on two-way cluster analysis indicated to two clear clusters distinguished as Group 1, consisting of AM fungal communities in the rhizosphere soil of DC and XX plantation and Group 2, consisting of AM fungal communities in the rhizosphere soil of PM plantation (Figure 4). There were several dominant species in Group 1, such as *Acaulospora* sp.1 (Acau1), *Acaulospora* sp.3 (Acau3), *G. aggregatum* (Gloag), *G. fasciculatum* (Gloffa), *G. pustulatum* (Glofpu), *Glomus* sp.1 (Glo1), *S. pachycaulis* (Scipa), *Acaulospora* sp.2 (Acau2), Unknown sp.1 (Unk1), Unknown sp.2 (Unk2), and *G. maculosum* (Gloma). On the other hand, *G. maculosum* (Gloma), Unknown sp.3 (Unk3), *Glomus* sp.2 (Glo2) (Scusp), and Unknown sp.2 (Unk2) were dominant in the rhizosphere of Group 2. These AM fungal species were mostly found in Group 2 (XX plantation), except Unknown sp.2 (Unk2) and *G. maculosum* (Gloma), which were dominant in both the groups.

### AM fungal spore density and relative abundance

The SD and RA of AM fungal species are shown in Table 3. The results indicate that the Unknown sp.2 was the most dominated species in all three plantations (RA=34.2

in DC; 51 in PM; 54.7 in XX), followed by *Glomus maculosum* (RA=18.4 in DC; 19.5 in PM; 9.6 in XX), and *Acaulospora* sp.2 (RA=17.5 in DC; 14.3 in PM; 21.8 in XX). There were some species which were rarely found in any of the plantations. Some AMF appeared only in the PM plantation, for e.g., *Glomus* sp.2 and *Scutellospora* sp. Moreover, *Glomus fasciculatum* was found in both DC and PM plantations and *Sclerocystis pachycaulis* was found in DC and XX plantations.

**Table 1.** Soil characteristics of the three Fabaceous tree plantations at Sakaerat silvicultural research station, Thailand

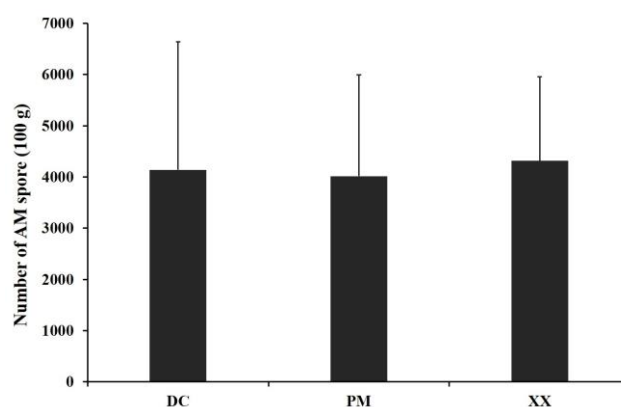
Soil characteristics (mean±SD)	DC	PM	XX
pH	4.31a±0.16	4.16a±0.09	4.19a±0.05
Soil moisture (%)	19.42a±1.91	17.53a±1.43	7.84b±1.38
OM (%)	5.41a±1.84	2.52b±0.96	1.78b±0.23
Total N (%)	0.20a±0.02	0.15ab±0.03	0.11b±0.02
Available P (mg/kg)	8.48a±2.35	8.53a±0.72	10.10a±4.87
Exchangeable K (g/kg)	78.73a±1.91	107.65a±27.33	32.81b±0.22

Note: DC: *Dalbergia cochinchinensis* plantation; PM: *Pterocarpus macrocarpus* plantation; XX: *Xylia xylocarpa* plantation; SD : standard deviation; and means in row with similar letters (a, b, c) are not statistically significant ( $P > 0.05$ ).

**Table 2** Total number of species (n), Shannon-Wiener's diversity index and E of AMF in the three Fabaceous tree plantations at Sakaerat silvicultural research station, Thailand

	DC	PM	XX
Total number of species (n)	12	13	11
Shannon-Wiener's Index ( $H'$ )	1.88	1.49	1.40
Evenness Index (E)	0.76	0.58	0.59

Note: DC: *Dalbergia cochinchinensis* plantation; PM: *Pterocarpus macrocarpus* plantation; XX: *Xylia xylocarpa* plantation

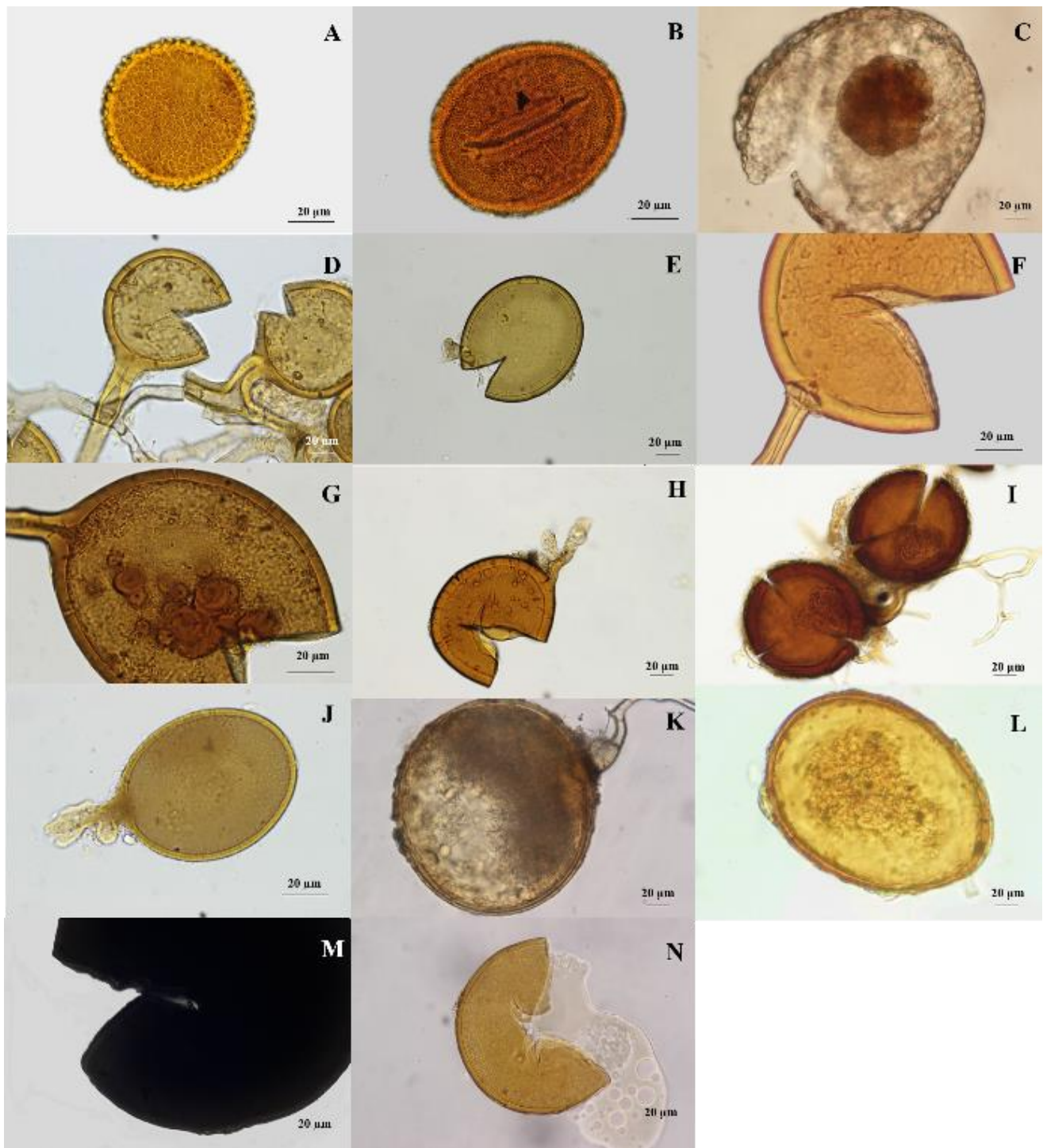


**Figure 2** Spore density of AMF in the Sakaerat silvicultural research station, Thailand (black filled columns). DC: *Dalbergia cochinchinensis* plantation, PM: *Pterocarpus macrocarpus* plantation, XX: *Xylia xylocarpa* stand). The error bars indicate standard deviation (as obtained from Duncan's new multiple range test ( $P > 0.05$ ))

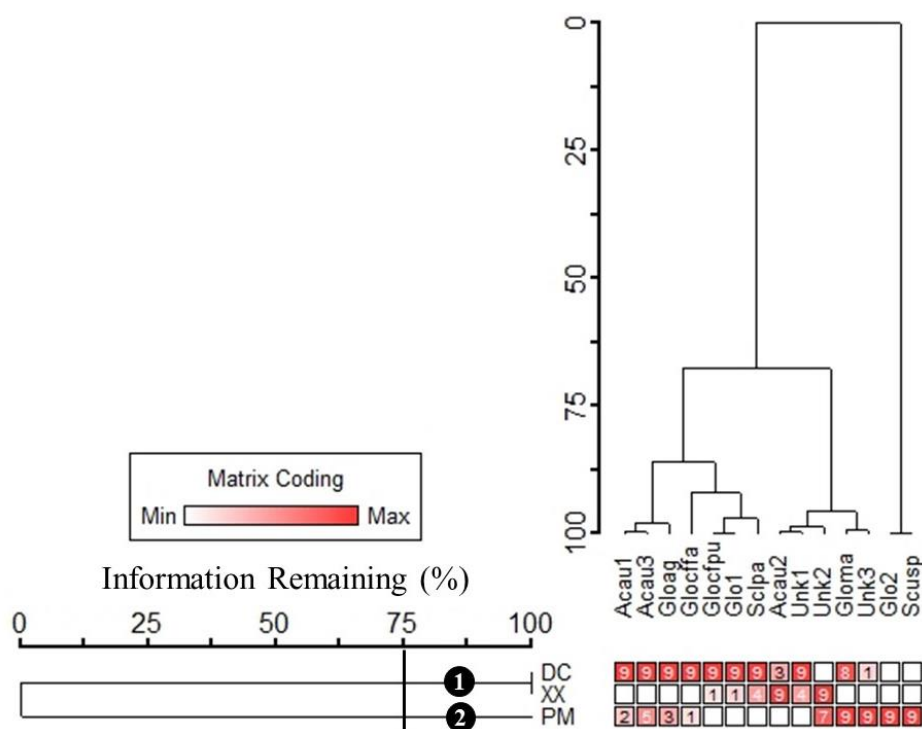
### Discussion

Members of the Fabaceae plant family are known to have an AM symbiosis (Weravart 2003; Dhar and Mridha 2006; Washa et al. 2012). In this study, we firstly identified the species, richness, SD, and species composition of AMF in the rhizosphere soils of three important Fabaceous tree

species, i.e., *D. cochinchinensis*, *P. macrocarpus*, and *X. xylocarpa*, growing in various plantations in northeastern Thailand (located in the Sakaerat silvicultural research station, Nakhon Ratchasima Province).



**Figure 3.** AMF found in plantations of *D. cochinchinensis*, *P. macrocarpus* and *X. xylocarpa*. A. *Acaulospora* sp.1, B. *Acaulospora* sp.2, C. *Acaulospora* sp.3, D. *Glomus aggregatum*, E. *Glomus fasciculatum*, F. *Glomus maculosum*, G. *Glomus pustulatum*, H. *Glomus* sp.1, I. *Glomus* sp.2, J. *Sclerocystis pachycaulis*, K. *Scutellospora* sp.1, L. Unknown sp.1, M. Unknown sp.2, and N. Unknown sp.3



**Figure 4.** Cluster analysis of AMF in the three plantations. DC: *Dalbergia cochinchinensis* plantation; PM: *Pterocarpus macrocarpus* plantation; XX: *Xylia xylocarpa* plantation

**Table 3** Spore density (SD) and relative abundance (RA) of AM fungal species in plantations at the Sakaerat silvicultural research station, Thailand

AM fungal species	DC		PM		XX	
	SD	RA (%)	SD	RA (%)	SD	RA (%)
<i>Acaulospora</i> sp.1	323	7.8	178	4.4	133	3.1
<i>Acaulospora</i> sp.2*	725	17.5	575	14.3	940	21.8
<i>Acaulospora</i> sp.3	28	0.7	20	0.5	10	0.2
<i>Glomus aggregatum</i> N.C. Schenck & G.S. Sm	60	1.4	28	0.7	8	0.2
<i>Glomus fasciculatum</i> (Thaxter) Gerd. & Trappe	25	0.6	3	0.1	-	-
<i>Glomus maculosum</i> * D.D. Miller & C. Walker	760	18.4	783	19.5	413	9.6
<i>Glomus pustulatum</i> Koske, Friese, C. Walker & Dalpé	425	10.3	98	2.4	150	3.5
<i>Glomus</i> sp.1	75	1.8	15	0.4	30	0.7
<i>Glomus</i> sp.2	-	-	8	0.2	-	-
<i>Scutellospora pachycaulis</i> C.G. Wu & Z.C. Chen	40	1	-	-	20	0.5
<i>Scutellospora</i> sp.	-	-	3	0.1	-	-
Unknown sp.1	135	3.3	110	2.7	128	3.0
<b>Unknown sp.2*</b>	1415	34.2	2048	51	2358	54.7
Unknown sp.3	128	3.1	153	3.8	125	2.9
Total	4138	100	4018	100	4313	100

Note: DC: *Dalbergia cochinchinensis* plantation; PM: *Pterocarpus macrocarpus* plantation; XX: *Xylia xylocarpa* plantation; \*: dominant species.

In a tropical rainforest, the SD of AMF is low, at the same time, being the most abundant, when comparing with the other organisms (Zhao et al. 2003). Additionally, *Acaulospora* and *Glomus* were the dominant genera of AM fungal community in tropical rhizosphere soils (Zhao et al. 2003; Marionho et al. 2018). In our study, a total number was 14 AMF species was observed. Four genera were isolated from the rhizosphere soils of different plantations. *Glomus* was found to be the dominant genera in terms of

spore diversity and SD (Table 4). Moreover, our study indicated that *G. maculosum* *G. pustulatum* and Unknown sp.2 were frequently found and dominant in all three plantations. These results are similar with the previous study of Dhar and Mridha (2006), who reported that the major AMF in the rhizosphere of other Fabaceous tree species, such as *Acacia auriculiformis*, *A. mangium*, and *Dalbergia sissoo*, was *Glomus*. Weravart (2003) also reported that *Glomus* was the dominant genus among the

27 AMF in the rhizosphere of *A. mangium*. Additionally, we found that *Acaulospora* was second most dominant genus following *Glomus*. Similar results were obtained by Ponpandian and Rose (2017), who reported 19 AMF species in the rhizosphere soils of *Pongamia pinnata* (Fabaceae) as well as *Acaulospora*, which was the predominant genus following *Glomus*. It was indicated that genus *Acaulospora* was found to be one of the dominant genera in other host species. Charoenpakdee et al. (2010) reported that *Acaulospora* was the major AMF found in the rhizosphere soils of physic nut (*Jatropha curcas* L.). Bever et al. (1996) and Hepper (1984) stated that *Glomus* and *Acaulospora* species grow in clusters, produce smaller-size of spores and sporulate more frequently, while other species like *Gigaspora*, *Scutellospora*, etc., sporulate singly. Therefore, a larger population of *Glomus* and *Acaulospora* species should have a higher chance to be detected in the rhizosphere soil.

In this study, the Shannon-Weiner's index and Evenness index were highest in the DC plantation and the lowest in XX plantation. The difference may be due to the nutrient composition of the soil. In our study, we observed that the soil physical properties were different in the three plantations. When we compared the soil physical properties among the three plantations, the XX plantation had the lowest soil moisture, OM, N and K content, but the highest P content. On the contrary, DC plantation had the highest level of soil moisture, OM, and N content, and the lowest P content. Visaratana et al. (2004) who studied litter production in the DC, PM and XX plantation at Sakaerat silvicultural research station (the same plantation with our sampling site), reported that the DC plantation was the highest in amount of litterfall, when was compared with the PM and XX plantations. Therefore, these results may be due to the amount of OM in the DC plantation was higher than the other two plantations. Additionally, owing to the older plantation age and closer crown canopy, the soil moisture of the DC plantation was higher the XX plantation. Previously, Nongkling and Kayang (2017) had indicated that the OM, soil moisture, and nutrient content were the factors responsible for AMF species richness in the soils. Similar with Khaekhum et al. (2017), found that the number of AM fungal spores in the rhizosphere of eucalyptus was higher soil moisture content (rainy season) and lower in low soil moisture content (dry season). Furthermore, a high level of P had a negative effect on AM fungal distribution (Rubio et al. 2003; Alguacil et al. 2010). Wang et al. (2009) indicated that increasing the P content significantly reduced the species diversity of AMF. Our study showed no significant difference in spore density across the plantations. However, we found that the species composition of AMF in the rhizosphere of PM plantation was not similar to the other two plantations (Figure 4). Several studies revealed that factors related to the host plant can affect community structure of the AM fungal species (Chaturvedi et al. 2009; Pérez and Urcelay 2009; Torrecillas et al. 2012). Chaturvedi et al. (2009) reported that a high diversity of hosts, such as a natural forest, had a higher diversity of AMF in rhizosphere of the soils than a single host plant. Moreover, Torrecillas et al. (2012) found

that different plant families were hosts to different AM fungal communities and that the roots of annual plants had a higher number of AM fungal spores compared to the perennial plant species. In general, the roots of different plants produce different types of organic chemicals and volatile compounds, which could contribute to the activity and affect the life cycles of AMF in a natural ecosystem (Dhar and Mridha 2006). Therefore, further studies are required to select the appropriate AM fungal species which have a positive effect on the growth of *D. cochinchinensis*, *P. macrocarpus*, and *X. xylocarpa*. The study can also be used in inoculation programs in order to restore AM fungal diversity in degraded lands.

In conclusion, AM fungi could be classified in three genera, which were 3 species of *Acaulospora*, 7 species of *Glomus*, and 1 species of *Scutellospora*. Meanwhile, three species remained unidentified. The DC plantation soils had the highest species diversity index and the lowest in the XX plantation due to the differences in soil characteristics, including moisture, %OM, and nutrient concentration. In this study, *G. maculosum*, *G. pustulatum* and Unknown sp.2 were the majority in all three plantations. Moreover, AM fungal communities of three Fabaceous trees species were different. These differences may be due to the different types of organic chemicals and volatile compounds from their roots.

## ACKNOWLEDGEMENTS

This work was financed by the Kasetsart University Research and Development Institute and research fund for supporting assistant researcher from Faculty of Forestry, Kasetsart University, Thailand (OPORA-2015). We would like to sincerely thank the head of Sakaerat silvicultural research station for all kind supports. The manuscript has been thoroughly reviewed with the kind help of Dr. Tushar Andriyas from Center for Material Sciences, Institute of Interdisciplinary Studies, University of Allahabad, India.

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