

เอกสารตีพิมพ์

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Diversity of Arbuscular Mycorrhizal Fungi in Forest Restoration Area of Doi Suthep-Pui National Park, Northern Thailand

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Abstract

Arbuscular Mycorrhizal (AM) fungal diversity was surveyed in the forest restoration area of Doi Suthep-Pui National Park, northern Thailand. Twenty four indigenous tree species, used for forest restoration in a degraded watershed area were examined. Rhizosphere soil samples were collected and AM spores were counted and identified morphologically. AM spores were found in the rhizosphere soils of all tree species. Twenty one AM species were identified: *Acaulospora* (6 species), *Glomus* (12 species) and *Scutellospora* (3 species). AM fungi belonging to the genera *Glomus* and *Acaulospora* were dominant. Abundant species present were *Acaulospora elegans*, *Glomus multicaule* and *Scutellospora pellucida*. These results showed that all 24 indigenous tree species were associated with AM fungi and some AM species had a broad host range.

Background

The tropical forests of Doi Suthep-Pui National Park are one of the most important watershed areas which composed of a number of indigenous tree species. Deforestation within the national park has had adverse consequences on biodiversity and environmental quality. One method of forest restoration, which involves planting mixtures of several indigenous tree species, has been used to counteract this problem (Goosem and Tucker, 1995; FORRU, 2006). Many indigenous species were selected and tested in the experimental plot, established in the north of national park (Elliott *et al.*, 2003). AM fungi are one of the beneficial soil microorganisms that play a crucial role in the mineral nutrition of forest trees (Koide and Mosse, 2004). Information on the capacity of indigenous tree species in association with AM fungi is very important to forest restoration. The purpose of this study was to obtain information on the diversity of AM fungi associated with indigenous tree species in the forest restoration plot.

Materials and Methods

Among the planted tree species in forest restoration plot, 24 potential indigenous species were selected for study. All tree species are reported to be multipurpose and suitable for acceleration of the forest regeneration. Rhizosphere soil samples (about 500 g) of each indigenous species were collected and stored at 4°C until analyzed. AM spores were extracted from 100 g air-dried soil samples by wet-sieving and 50% sucrose centrifugation (Brundrett *et al.*, 1996). Spores were recovered by filtering through a 53 µm sieve onto filter paper. The intact spores on filter

paper were counted under a stereomicroscope (Olympus SZ40). Spores were mounted on microscopic slides in polyvinyl lactic acid (PVA), with or without Melzer's reagent (Morton, 1988) and identified according to morphological characteristics of the originally published species descriptions under a light microscope (Olympus CH30). Light microscopic photographs were taken under an Olympus BX61.

Results and Discussion

The results of our study on the AM fungal diversity in the forest restoration area of Doi Suthep-Pui National Park showed that all 24 indigenous tree species are associated with AM fungi. Spores of AM fungi were found in the rhizosphere soils of all individual tree species. This reflects the mycotrophic nature of the plant species studied and the ability of AM fungi in soils to associate a wide range of host species. It has been reported that many tree species are highly associated with AM fungi (Janos, 1980; Onguene and Kuyper, 2001). Twenty one AM species were identified based on morphological characteristics of their spores according to published descriptions (Table 1). The diversity of AM species was varied among the different tree species (Table 2). Most of the isolated species belonged to the family *Glomaceae*, all of which were *Glomus* (12 species, 49.3%). Abundant species present was *G. multicaule* (14.2%) (figure 1). Six species were in the family *Acaulosporaceae*, all of which were in the genus *Acaulospora* (43.6%). Abundant species present was *A. elegans* (39.2%) (figure 2). Three species were members of the family

Gigasporaceae and belonged to the genus *Scutellospora* (7.1%). Abundant species present was *S. pellucida* (5.5%) (figure 3). Species in the genera *Archaeospora*, *Paraglomus*, *Entrophospora* and *Gigaspora* were not found. AM fungi belonged to the genera *Glomus* and *Acaulospora* were dominant. This fact must be related to their sporogenous characteristics, i.e. *Glomus* and *Acaulospora* species usually take a short time to produce small spores, compared with the large spores of *Gigaspora* and *Scutellospora* species in the same environment (Hepper, 1984; Bever *et al.*, 1996). *A. elegans*, *G. multicaule* and *S. pellucida* were the most commonly encountered species. This suggests that these species have a widespread and broad host range.

Conclusion

In the forest restoration area of Doi Suthep-Pui National Park, all surveyed indigenous tree species were associated with AM fungi. The AM fungal diversity in the plant rhizospheres was variable among the different tree species. Twenty one AM species were identified as 3 genera and 12 species of *Glomus*, 6 species of *Acaulospora* and 3 species of *Scutellospora*. *Glomus* and *Acaulospora* were the dominant genera. The present study obtains the information on the AM association of 24 potential indigenous trees used to restore tropical forest of Doi Suthep-Pui National Park.

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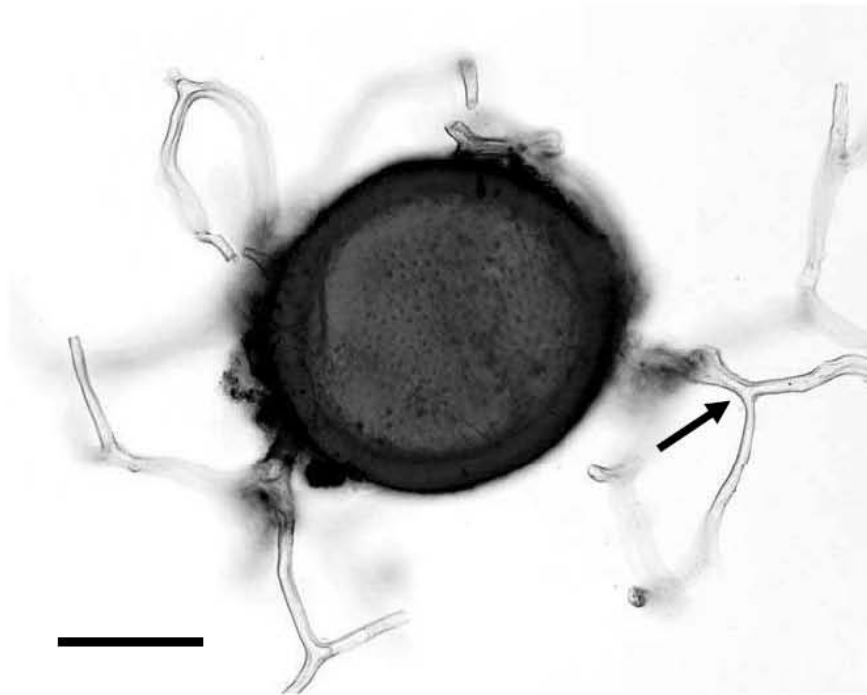


Fig. 1 *Glomus multicaulis*: Spore with multiple subtending hypha (arrow) and rounded projections on the surface, bar = 50 μ m.



Fig. 2 *Acaulospora elegans*: Cracked spore with sporiferous saccule (arrow) and crowded spines on the surface, bar = 50 μ m.

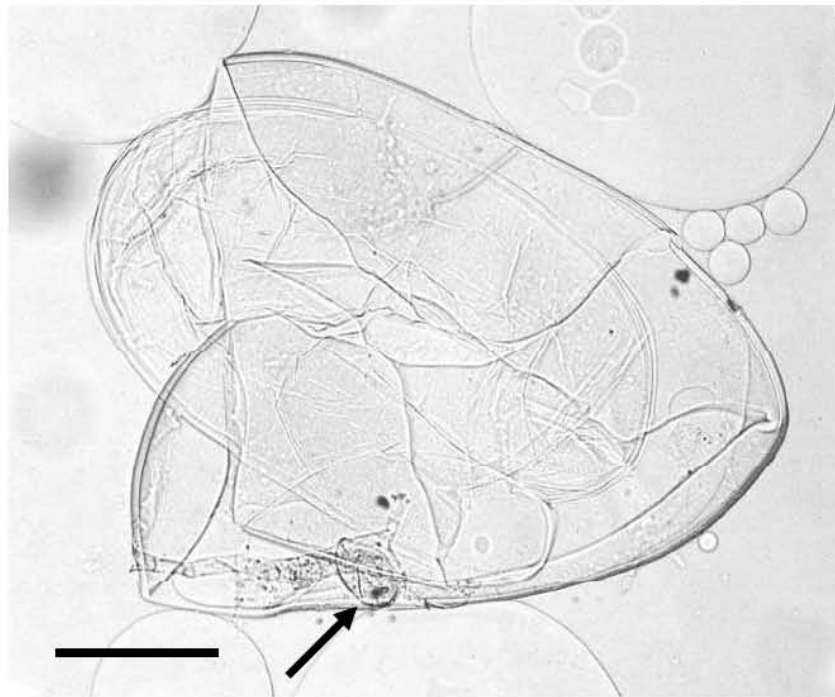


Fig. 3 *Scutellospora pellucida*: Cracked hyaline spore with hyaline bulbous subtending hypha, bar = 50 μ m.

Table 1 Diversity of AM fungi from the rhizosphere soils of 24 indigenous tree species in the forest restoration area of Doi Suthep-Pui National Park, northern Thailand.

Code of AM species	Genus	Species
<i>Acaulospora</i>		
1	<i>A. bireticulata</i>	Rothwell & Trappe
2	<i>A. elegans</i>	Trappe & Gerd.
3	<i>A. foveata</i>	Trappe & Janos
4	<i>A. laevis</i>	Gerd. & Trappe
5	<i>A. mellea</i>	Spain & Schenck
6	<i>A. scrobiculata</i>	Trappe
<i>Glomus</i>		
7	<i>G. aggregatum</i>	Schenck & Smith
8	<i>G. ambisporum</i>	Smith & Schenck
9	<i>G. clavisporum</i>	Trappe
10	<i>G. coremioides</i>	Berk. & Broome
11	<i>G. intraradices</i>	Schenck & Smith
12	<i>G. microaggregatum</i>	Koske, Gemma & Olexia
13	<i>G. microcarpus</i>	Iqbal & Bushra
14	<i>G. mosseae</i>	(Nicol. & Gerd.) Gerd. & Trappe
15	<i>G. multicaule</i>	Gerd. & Bakshi
16	<i>G. rubiforme</i>	Gerd. & Trappe
17	<i>G. sinuosum</i>	Gerd. & Bakshi
18	<i>G. viscosum</i>	Nicol.
<i>Scutellospora</i>		
19	<i>S. gregaria</i>	(Schenck & Nicol.) Walker & Sanders
20	<i>S. heterogama</i>	Walker & Sanders
21	<i>S. pellucida</i>	(Nicol. & Schenck) Walker & Sanders

Table 2 Diversity of AM fungi found in the rhizospheres of 24 indigenous tree species in the forest restoration area of Doi Suthep-Pui National Park, northern Thailand.

Indigenous tree species	AM species*
<i>Acrocarpus fraxinifolius</i> Wight ex Arn. (Caesalpinoideae)	3, 4, 14, 15
<i>Balakata baccata</i> (Roxb.) Ess. (Euphorbiaceae)	12, 20
<i>Castanopsis acuminatissima</i> (Bl.) A. DC. (Fagaceae)	1, 15
<i>Erythrina subumbrans</i> (Hassk.) Merr. (Papilionoideae)	2, 7, 12, 19
<i>Ficus altissima</i> Bl. (Moraceae)	2, 6, 10
<i>Ficus benjamina</i> L. var. <i>benjamina</i> (Moraceae)	6, 19, 20
<i>Ficus glaberrima</i> Bl. var. <i>glaberrima</i> (Moraceae)	16
<i>Ficus hispida</i> L. f. var. <i>hispida</i> (Moraceae)	2, 5, 16, 17
<i>Ficus racemosa</i> L. var. <i>racemosa</i> (Moraceae)	2, 8, 14, 16, 18
<i>Ficus subulata</i> Bl. var. <i>subulata</i> (Moraceae)	2, 14, 16, 20
<i>Glochidion kerrii</i> Craib (Euphorbiaceae)	21
<i>Gmelina arborea</i> Roxb. (Verbenaceae)	2, 6, 13, 15
<i>Heynea trijuga</i> Roxb. ex Sims (Meliaceae)	2, 3, 8, 10, 20
<i>Hovenia dulcis</i> Thunb. (Rhamnaceae)	2, 7, 15
<i>Macaranga denticulata</i> (Bl.) M.-A. (Euphorbiaceae)	9, 10, 16, 20
<i>Machilus bombycina</i> King ex Hk.f. (Lauraceae)	2, 10, 16, 20
<i>Melia toosendan</i> Sieb. and Zuc. (Meliaceae)	2, 9, 20
<i>Michelia baillonii</i> Pierre (Magnoliaceae)	16, 17
<i>Nyssa javanica</i> (Bl.) Wang. (Nyssaceae)	6, 15, 20
<i>Prunus cerasoides</i> D. Don (Rosaceae)	7, 10, 13, 15, 16
<i>Rhus rhesoides</i> Craib (Anacardiaceae)	2, 6, 13, 16
<i>Sapindus rarak</i> DC. (Sapindaceae)	2, 20
<i>Sarcosperma arboreum</i> Bth. (Sapotaceae)	10, 11, 20
<i>Spondias axillaris</i> Roxb. (Anacardiaceae)	15

* Numbers in column refer to the codes of AM species in Table 1.

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Shifting Cultivation System and Crop Symbiosis with Arbuscular Mycorrhizal Fungi

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ABSTRACT

*Farmers of the Karen ethnic group who live in Huai Tee Cha village, Mae Hong Son province in northern Thailand, still practice the rotational shifting cultivation or swidden agriculture system for food and some cash crops. This study investigated the association of upland rice (*Oryza sativa* cv. Bue Bang), other food crops [Job's tears (*Coix lachryma-jobi*), corn (*Zea mays*), sesame (*Sesamum indicum*) and sorghum (*Sorghum bicolor*)] and pada (*Macaranga denticulata*) with AM fungi in farmers' fields. Soils in the farmers' fields were mildly acidic to neutral (pH 5.2 to 7.0) and showed diversity in P status (6.8-271 mg kg⁻¹ soil, Bray II) but not in N (0.29-0.35% total N) or K (103-130 mg kg⁻¹). The roots of all plants investigated were colonized by AM fungi with upland rice and corn the most infected (≥ 90%), followed by Job's tears (75%), then sorghum (50%) and sesame (45%). Rhizosphere spore density ranged from 160 spores 100 g⁻¹ soil for pada and sorghum, to 120 for sesame and half of this in Job's tears, corn and upland rice.*

This study suggests that swidden crops in northern Thailand have a strong relationship with indigenous AM fungi.

Key words: Arbuscular mycorrhizal fungi, Shifting cultivation system, Swidden crops

INTRODUCTION

Karen is the largest of the minority groups living in the mountainous areas of northern Thailand. Karen farmers in Huai Tee Cha village, Sob Moei district, Mae Hong Son province, located at 19° 78' N, 93° 84' E, 700 MASL, manage fields ranging in altitude from 600 to 900 m with steep slopes (Rerkasem and Rerkasem, 1994). These people have lived in this neighborhood for more than 200 years. Crop

production in this area is generally referred to as rotational shifting cultivation. It involves clearing land for crop production by slashing and burning the forest. After one year of cropping, the field is left in fallow for several years, and then cleared and cropped again when the rotation cycle is completed. The Karen farmers at Huai Tee Cha village grow over 50 crops including upland rice (the major staple crop), maize, sorghum, sesame, cowpea, Job's tears, vegetables, some cash crops (passion fruit, coffee, chili, etc.) and other traditional crops in their swidden fields. Most soils in this region are reddish clay loams (Yimyam et al., 2003) and the climate is tropical monsoon with wet, cool and hot seasons. The shifting cultivation cycle at Huai Tee Cha village has been reduced from 10-15 to 7 years. In spite of this, farmers appear to have been able to maintain rice yields by managing their short fallow with *Macaranga denticulata* (local name is pada), one of the pioneer tree species in the area (Rerkasem et al., 2002; Yimyam et al., 2003). The successful management of this local fallow species by farmers is evident by the higher grain yield and grain N content in upland rice grown after dense pada stands (Yimyam et al., 2003). Pot trials have shown that pada is highly dependent on arbuscular mycorrhizal (AM) fungi in Huai Tee Cha field soil (Youpensuk, 2004). However, it is unknown whether these AM fungi also directly benefit the food crops and other crops in the farmers' fields. This field study was undertaken to provide baseline data on AM fungi and crops in Huai Tee Cha fields.

MATERIALS AND METHODS

Soil properties, plant sampling and spore density

In the 2005 cropping year, at the end of the hot season, about 2 months after upland rice had been sown, when the crop was approximately 20 cm high, 34 soil samples (0-15 cm depth) were collected by randomly coring (4.5 cm diameter and 15 cm deep) 3 farmers' fields (Kayo, Takae and Murkur) for determining soil properties [pH (water, 1:1); Bray II phosphorus (Wanatabe and Olsen, 1962); Kjeldahl nitrogen (Jackson, 1967); and extractable potassium (1 M NH_4OAc , pH7)] and for spore density assessment. Fine root samples from the root zone of five common upland crops, grown after slashing and burning the forest [Job's tears (*Coix lachryma-jobi* L.), corn (*Zea mays* L.), sesame (*Sesamum indicum* L.), sorghum (*Sorghum bicolor* L.) and upland rice cv. Bue Bang (*Oryza sativa* L.)] and seedlings of one fallow-enriching tree, pada (*Macaranga denticulata* (Bl.) Muell. Arg) were obtained by digging part of the root systems (15 cm depth; 10 cm from the base) of three plants species⁻¹ from each farmer's field. Roots and soils were transported to the laboratory for determining root colonization and examination of spore density. Youngest fully-expanded leaf (YFEL) samples of each crop were taken from the farmers' fields to the laboratory and were dried at 75°C for 48 hours and then analysed: N by the Kjeldahl method (Jackson, 1967); P by dry ashing followed by the molybdovanado phosphorus acid method (Murphy and Riley, 1962) and K by dry ashing and atomic absorption spectrophotometry.

Arbuscular mycorrhizal fungi assessment

a) Determination of arbuscular mycorrhizal colonization

The root system was separated from the soil, washed over a 106 μm mesh sieve, then subsampled. Roots in the subsample were cut into pieces 1-2 cm in length, cleared in 10% KOH at 121°C, rinsed with water on a sieve and stained with 0.05% trypan blue in lactoglycerol at 121°C (Brundrett et al., 1996). Thirty root pieces were taken at random from each sample, mounted on glass slides and AM colonization determined, using the gridline intersect method (McGonigle et al., 1990) under a compound Olympus microscope, model CX41RF.

b) Determination of arbuscular mycorrhizal spore density

Spores of AM fungi in 50 g soil were obtained by wet sieving through 710, 250, 106 and 53 μm mesh sieves. The 250, 106 and 53 μm fractions were centrifuged for 5 minutes at 2000 r min^{-1} to remove floating debris, the spores were resuspended in 50% sucrose with vigorous shaking and centrifuged for 1 minute at 2000 r min^{-1} . The spores were washed with water, transferred to filter paper with gridlines and counted under a stereomicroscope (Brundrett et al., 1996).

Effect of soil profile on spore density

Soil pits were dug at random locations at high, middle and low slope positions in Kayo fields. Soil samples were taken at 0-5, 5-10, 10-15, 15-20, 20-30, 30-40 and 40-50 cm depth and spores were obtained by wet sieving (see above).

Yield and crop use

Grain yield and crop use data were obtained from farmer interviews after they finished crop harvesting.

Data analysis

Data are presented as means and standard errors (S.E.), rice yield of each farmer was explored as standard deviation (S.D.).

RESULTS

Soil properties

Soil pH_{water} in the farmers' fields was mildly acidic to neutral, ranging from 5.2 to 7.0 and soils varied considerably in their Bray II P status, ranging from 6.8 to 271 mg kg^{-1} soil. There was a wide range in the soil P among farmers' fields: it ranged from 53.5-271.0, 6.8-65.3 and 12.4-27.8 mg kg^{-1} soil in the fields of Takae, Kayo and Murkur, respectively. By contrast, the levels of N and K laid within a narrow range, 0.29-0.35% for N and 103-130 mg kg^{-1} for K (Table 1).

Leaf nutrient concentrations

Leaf nitrogen (N) concentrations were 2.10 to 2.46 %, P concentrations were 0.18 to 0.33 % and K concentrations were 1.83 to 8.44 %. There was a narrow range in N concentration for all crops sampled whereas the P concentration was lower in

upland rice and pada (0.18, 0.20%) than in corn or Job's tears (0.33, 0.30%), respectively. Sesame and sorghum had intermediate foliar P concentrations. By contrast, corn and upland rice had higher K concentrations (6.74 and 8.44 %, respectively) than the other crop species (pada, Job's tears, sesame and sorghum: 2.08, 1.83, 3.05 and 2.08 %, respectively) (Figure 1).

Spore number with soil depth

Abundance of AM spores varied with depth with most concentrated in the 0-20 cm part of the profile. The highest spore density was at 5-10 cm [225 spores 100 g⁻¹ soil], followed by 0-5 and 15-20 cm [36 and 27 spores 100 g⁻¹ soil, respectively]. Spore numbers declined in soil deeper than 20 cm. Spore density differed with position in the landscape. Higher spore numbers occurred at the upper slope with 758 spores 100 g⁻¹ soil than at the middle and low slopes, 109 and 105 spores 100 g⁻¹ soil, respectively (Table 2).

Root colonization and spore density

The roots of all plants sampled were infected with AM fungi. The extent of root colonization was highest in upland rice, corn and pada (90-95%), followed by Job's tears (75%), then sorghum (50%) and was lowest in sesame (45%). Rhizosphere spore density was about 160 spores 100 g⁻¹ soil for pada and sorghum, 120 spores 100 g⁻¹ soil for sesame and half of this in Job's tears, corn and upland rice (Table 3).

Crop yield and usage

The dominant crop in the field area was upland rice and other swidden crops were sown as intercrop with rice in the main fields. Rice and sorghum were harvested at grain maturity and used for food and ceremonies. Some corn was harvested for eating at the green ear stage and the remainder harvested dry for animal feed. Seeds of Job's tears were collected for ornamental decoration of clothes. Job's tears and sorghum were also used for cooking by mixing with rice and for animal feed (Table 4). However, in this cropping year, the farmers left the sorghum in the field for birds as they believe that birds will eat sorghum in preference to eating rice. All farmers keep swidden crop seeds for growing the next crop. Rice yields from the fields of Kayo, Murkur and Takae were 555, 360 and 200 kg rai⁻¹ or 3.47, 2.25 and 1.25 ton ha⁻¹, respectively (Figure 2).

Table 1. Properties of the field soils at Huai Tee Cha village.

<i>Soil property^a</i>	
Texture	Sandy loam
pH (water)	5.2 – 7.0 (6.2)
Bray II P (mg kg ⁻¹)	6.8 – 271 (81.9)
N (%)	0.29 – 0.35 (0.31)
K (mg kg ⁻¹)	103 – 130 (122)

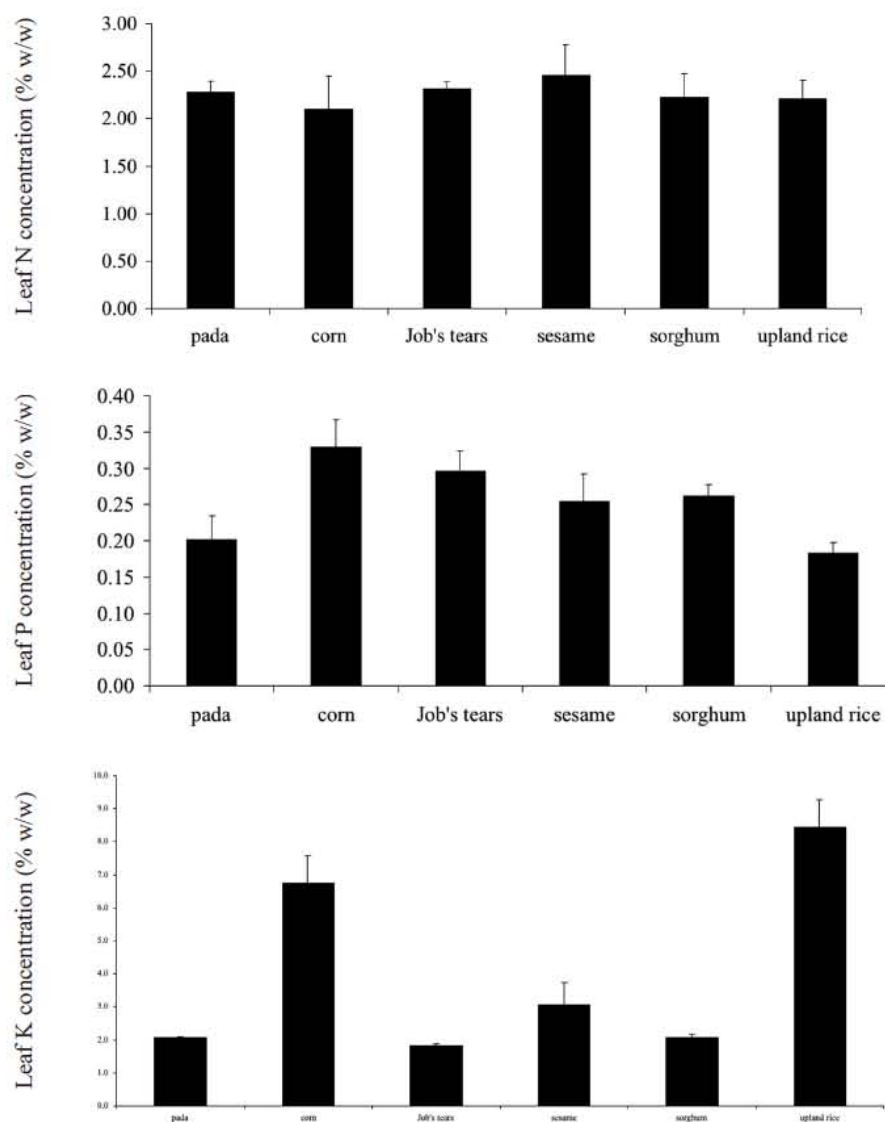
^a Values are the range with the mean in brackets**Figure 1.** Foliar nutrient concentrations (N, P, K) in pada and swidden crops at Huai Tee Cha fields (vertical bar above each column represents one S.E.).

Table 2. Spore density of AM fungi in three soil profiles in Huai Tee Cha fields.

Soil depth (cm)	Spore numbers 100 g ⁻¹ soil				
	Upper slope	Mid slope	Lower slope	Average	S.E.
0-5	30	24	54	36	9.1
5-10	650	17	9	225	212.4
10-15	10	10	14	11	1.4
15-20	46	15	19	27	9.7
20-30	15	9	4	9	3.1
30-40	1	18	4	8	5.2
40-50	5	16	1	8	4.4
Total spore	758	109	105		
CV	221	32	121		

Table 3. Root colonization by AM fungi and spore density of pada and five swidden crops in farmers' fields at Huai Tee Cha village.

Plant species	Root colonization (%)	Spore numbers 100 g ⁻¹ soil
Pada	95 ± 2.1	163.9 ± 49.0
Corn	90 ± 2.8	64.4 ± 12.6
Job's tears	75 ± 10.2	82.8 ± 13.9
Sesame	46 ± 14.5	122.2 ± 40.3
Sorghum	50 ± 8.1	151.7 ± 60.8
Upland rice	95 ± 1.8	63.9 ± 11.5

values are mean ± S.E.

Table 4. The use of swidden crop seed in Huai Tee Cha village.

Common name or local name	Scientific name	Main use			
		F1	F2	Or	SC
Job's tears	<i>Coix lachryma-jobi</i> L.	*	*	*	
Glutinous corn	<i>Zea mays</i> L.	*	*		
Sorghum	<i>Sorghum bicolor</i> L.	*	*		
Rice	<i>Oryza sativa</i> L.	*			*
White/black seed sesame	<i>Sesame indicum</i> L.	*			*

Sources: household interview in 2005 (after crop harvests)

F1=Food, F2=Animal feed, Or=Ornamental, SC=Spirit ceremony

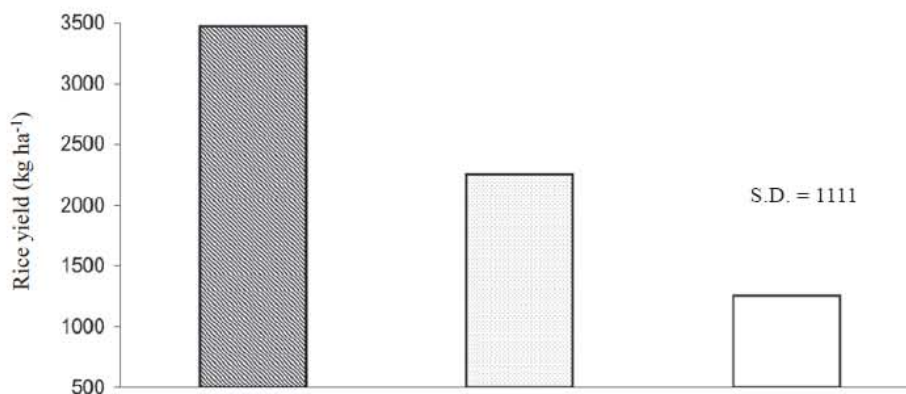


Figure 2. Rice yield (kg ha⁻¹) of Kayo, Murkur and Takae fields at Huai Tee Cha village in cropping year 2005.

DISCUSSION

Available soil P as measured in samples taken from Huai Tee Cha fields in 2005 were higher (average 81.9 mg kg⁻¹ soil) and wide-ranging (6.8-271 mg kg⁻¹ soil), compared to an earlier study of Yimyam et al., (2003) who reported 2-4 mg kg⁻¹ soil. Yimyam sampled fields before burning and 30 days after sowing rice in 2000. The differences in soil P measured in the two studies may be due to location or crop rotation. Because the fields are used once and then returned to forest succession, different fields were sampled in these two studies. The fields sampled by Yimyam were also more acidic than those used in the present study. The rice fields of Takae were located near a valley floor that is the lowest point of the village's land use area, so the source of high P accumulation in these fields may have resulted from leaching by rain from fields higher up. Another factor likely to influence the soil P reserves is the distribution of pada trees in the fields before the cropping period. Yimyam (2006) found that the distribution of pada between shifting cultivation fields varied greatly, in 2000 was mostly dense whereas in 2003 was sparse, so the distribution of pada may have been dense in 1998, resulting in very high soil fertilities.

The percentage root colonization by AM fungi was lowest in sorghum (50%) and highest in pada and upland rice (95%). In a previous study at the same village, Youpensuk et al., (2004) reported that 81% of the fine roots of pada were colonised by AM fungi, and the spore density in pada rhizosphere was four times more than what is found in this study. These differences can be attributed to sampling time and variation between mountain slopes (the fields were different in the two studies).

Upland rice yields varied among farmers' fields, Kayo had the higher rice yield compared to Murkur and Takae. Rice yield of Takae was lowest although this soil had high P levels. The farmers in Huai Tee Cha village grow both glutinous and non-glutinous rice, and use 3-5 varieties each, depending on the conditions

of the field and their preference. Rice yield of farmers was estimated for the total yield, and some of the difference between seed yield of each farmer may be due to differences in rice variety.

Another factor affecting yield may be weed control. The common practice for weed control is by hand, and is normally done three times during the entire cropping phase (Yimyam, 2006). Hence, farmers who are able to control weeds on time may achieve higher crop yield than farmers who have poorer weed control. Soil analysis revealed that the fields varied considerably in available phosphorus. It is not known whether the density of spores or the extent of root colonization by AM fungi varies with soil fertility within a field, and this is an area where further work is needed.

CONCLUSION

Although the addition of fertilizer P is probably a simple way for improving crop productivity on soils low in available P, most farmers in this area have severe poverty and have weak purchasing power to buy artificial fertilizers. Fortunately, the farmers in Huai Tee Cha village have tacit knowledge of using pada as a fallow-enriching tree species in their rotational shifting cultivation system as it helps benefit their crops. As this tree has high dependence on AM fungi and there is high diversity of AM fungi associated with its root system, it is possible that these fungi may also be contributing to nutrient uptake by the swidden crops, thus assisting farmers to increase their yields and decrease inorganic fertilizer inputs. This small field study has shown that swidden crops are also colonized by AM fungi. However, the dependence of swidden crops on AM fungi is yet to be determined.

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เอกสารตีพิมพ์

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The mycorrhizal status of indigenous arbuscular mycorrhizal fungi of physic nut (*Jatropha curcas* L.) in Thailand

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The dependence of physic nut (*Jatropha curcas* L.) on beneficial soil fungi for growth promotion is less to know. Therefore, the spore density and species diversity of arbuscular mycorrhizal fungal (AMF) associated with physic nut was assessed by extracting spores from physic nut plantations from 10 sites representing 6 provinces in Northern and North eastern of Thailand. Sixty hundred and ninety nine AMF spores were obtained using the wet sieving and sucrose gradient centrifugation methods. AMF colonization was also examined by staining root samples in trypan blue and observed under compound microscope. The following 34 morphospecies of AMF were identified: *Acaulospora* (16 species), *Entrophospora* (1 species), *Gigaspora* (2 species), *Glomus* (10 species) and *Scutellospora* (5 species). The diversity index ranged from 0.28 to 0.86 (average 0.64) and the species richness of AMF varied from 3 to 11 (average 6.2). Root colonization was exceeding 90% suggesting that physic nut is highly dependent on AMF.

Key words –ecology–species diversity–spore density–taxonomy

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Introduction

Physic nut (*Jatropha curcas* L.) is a multipurpose plant and is grown in many parts of the world for example Brazil, India, Mexico, Nicaragua and Thailand (Foidl et al. 1996, Heller 1996, Prueksakorn et al. 2006, David et al.

2009). It is a widely used species for traditional medicine, hedging fences and preventing soil erosion. The specie is originated from Central America. It belongs to the botanical family Euphorbiaceae, which has 300 genera and around 7,500 species. Most species

are tropical trees and shrubs which grow in the lower storey of forests. Many members of this family are known to be dependent on AMF, for example species of *Euphorbia*, *Glochidion*, *Hevea* and *Manihot* (Tawaraya et al. 2003, Zhao & Zhiwei 2007, Straker et al. 2010). Currently, physic nut is becoming an increasingly attractive plant for producing biofuels. Yield estimated currently for physic nut is 1,300 litre of oil per hectare behind oil palm but higher than rapeseed (Anonymous 2007). As diesel fuel prices continue to escalate, opportunities will open for a conversion from crude-oil into bio-diesel based fuel consumption. Several countries, domestic organizations and international have proposed greatly increasing the area under physic nut cultivation for oil production. It is therefore one of the most selected crops in commercial agriculture.

Arbuscular mycorrhizal fungi (AMF) are abundant and ubiquitous in almost all natural terrestrial communities and form obligate symbiotic associations with 80% of vascular plants (Harley & Smith 1983, Smith & Read 1997). It is apparent that these fungal symbionts became an integral component of plant communities in both natural and agricultural ecosystems. They play a vital role in sustaining plant diversity, increasing plant productivity and maintaining ecosystem processes by promoting plant fitness through a range of mechanisms including protecting the host from pathogens, improving soil structure, and enhancing water and nutrient uptake (Borkowska 2002, Jansa et al. 2002, Kapoor et al. 2004, Pasqualini et al. 2007). Several authors have documented that associations

between agronomic plants species and AMF are likely to increase the efficiency of fertilizer use and plant growth (Schreiner 2007, Tewari 2007, Porras-Soriano et al. 2009).

It has been reported that members of the plant family are highly dependent on AMF (Chen et al. 2005). However, there is limited knowledge of AMF status in the rhizosphere of physic nut. This study was undertaken to determine the diversity of AMF in physic nut plantings in Northern and North eastern of Thailand. We hypothesized that the mycorrhizal status and species richness differ from site to site. Furthermore, soil conditions and plantation age are likely to play a key role for determining species diversity on root tips of physic nut.

Methods

Sample collection

A total of 10 physic nut plantation sites in Chiang Rai (CR1, CR2), Chiang Mai (CM1, CM2, CM3 and CM4), Loei (LO1), Lumphun (LP1), Khon Kaen (KK1) and Nong Kai (NK1) province were selected as study site for AMF diversity (Table 1, Fig.1). Ninety five soil samples were collected from beneath physic nut in the planting row during October-December 2007. At each of the field sites, 4 soil core samples per tree were taken at a depth of 5-30 cm using a soil corer. Approximately 1 kg total of rhizosphere soil from each site was collected. Soil samples were carefully grounded, air dried and mixed into composite samples. Each composite sample representing one plot was a mixture of four soil core samples. The samples were kept in an ice-box and

transport by car to a laboratory. All soil samples were kept in a cold room and processed within one month. The analyses of soil samples included AMF spore isolation and enumeration, identification of species; and determination of the following chemical soil parameters: soil humidity (Lambe & Whitman 1969), pH by water extraction (Thomas 1996), organic matter using wet oxidation (Nelson & Sommers 1996), available phosphorus (P) using the Olsen method (Kuo 1996), extractable potassium (K) using the molybdenum blue method and stannous chloride as the reducing agent and ammonium acetate (NH_4OAc) as extractant (Helmer & Sparkers 1996, Helrich 1990), and total soil nitrogen (N) content using the Kjeldahl method (Bremner 1996). Soil nutrient analysis (Table 2) was conducted by the Department of Soil Science, Faculty of Agriculture, Chiang Mai University. Roots from each composite sample were removed from the soil by washing and fixed in 70% ethanol.

AMF spore isolation and identification

AMF spores occurring in the rhizosphere soil samples were extracted by wet sieving and sucrose density gradient centrifugation (Brundrett et al. 1996) methods. 100 g of each soil sample was suspended in 500 ml of water and stirred for 10 mins. Sieve sizes ranging from 250 μm , 106 μm and 45 μm , were used for spore collection. The spores retained on each sieve size were filtered onto filter paper and subsequently examined under a stereomicroscope (Olympus CX31) at a magnification of up to 400X and

identified based on spore morphology. Each spore morphotype was mounted in polyvinyl-lacto-glycerol (PVLG) and PVLG mixed with Meltzer's reagent in 1:1 (v/v) ratio (Morton 1988). Identification was based on current species descriptions and identification manuals (International Culture Collection of Vesicular and Arbuscular Endomycorrhizal Fungi [http://invam.caf.wvu.edu/Myc_Info/Taxonomy/species.htm]).

Spore density (SD) is the number of spores in 100 g soil. Relative abundance (RA) was defined as the percentage of spore numbers of a species divided by a total of spore observation (Dandan & Zhiwei 2007). The frequency isolation of each AMF species was calculated by the percentage of the number of the samples in which the species or genus observed per total samples. The dominant AMF species according to relative abundance ($\text{RA} > 6\%$) and spore density of in 100 g soil (spore density higher than 40 spores) and species richness were determined in each sampling site.

Mycorrhizal root colonization assessment

Roots fixed in 70% ethanol were cleared in 10% (w/v) KOH solution and autoclaved at 121°C and 15 lb/inch^2 for 15 minutes. Then, roots were washed with distilled water to remove KOH, stained with 0.05% trypan blue dye (C.I. 23850) and reautoclaved. Thirty stained roots (each about 1 cm in length) were assessed for colonization using the intercept method under a compound Olympus CX31 microscope (Brundrett et al. 1996).

Diversity index and concentration of dominance

AMF diversity was evaluated using the Shannon-Weiner diversity index which has two main components, evenness and number of species (Shannon & Weiner 1963). The Shannon-Weiner index (H') was calculated according to the formula $H' = -\sum(n_i/N) \log_2(n_i/N)$, where n_i represents individuals of a species and N represents the total number of species. Concentration of dominance (C) was also measured by the Simpson's index

(Simpson 1949) using the formula $C = \sum(n_i/N)^2$, where n_i and N are the same as for Shannon-Weiner diversity index.

Statistical analysis

The percentage of infection was arc sin transformed prior analysis. One-way analysis of variance (ANOVA) was carried out for root colonization and spore density. Statistical analyses were performed with the Statistical Package for Social Sciences version 11.5 (SPSS Inc., Wacker Drive, Chicago, IL). All factors were analyzed at $\alpha = 0.05$.

Table 1 Geographic coordinates and number of soil sampling in each sites.

Geography	Site *									
	CR1	CR2	CM1	CM2	CM3	CM4	LO1	LP1	KK1	NK1
Latitude	E99°48'	E99°26'	E98°55'	E98°30'	E98°55'	E98°54'	E101°21'	E99°07'	E102°53'	E102°43'
Longitude	N19°54'	N19°52'	N18°45'	N18°09'	N18°45'	N18°44'	N17°27'	N18°34'	N16°23'	N17°51'
MSL**(m)	398	399	340	1,137	340	360	800	337	150	163
Sampling no.	5	10	10	10	10	10	10	10	10	10

*Chiang Rai site 1 (CR1), Chiang Rai site 2 (CR2), Chiang Mai site 1 (CM1), Chiang Mai site 2 (CM2), Chiang Mai site 3 (CM3), Chiang Mai site 4 (CM4), Loei (LO1), Lumphun (LP1), Khon Kean (KK1), and Nong Khai (NK1).

**MSL=Mean Sea Level



Fig.1. Ten sampling sites in 6 provinces of Thailand. (a) CR2: Chiang Rai site 2, (b) CM1: Chiang Mai site 1, (c) CM2: Chiang Mai site 2, (d) CM3: Chiang Mai site 3, (e) CM4: Chiang Mai site 4, (f) LP1: Lumphun, (g) CR1: Chiang Rai site 1, (h) KK1: Khon Kean, (i) LO1: Loei, (j) NK1: Nong Khai.

Results

Soil characteristics

Soil samples of each study site were pooled, mixed and determined for chemical properties (Table 2). Soil pH ranged from 5.3 and 8.0, OM 0.63-

7.22%, N 0.02-0.44%, P 11.5-175.5 ppm, K 22.2-1058.0 ppm and soil humidity 7-23% at different sites.

Table 2 Soil Characteristic of 10 physic nut plantations in Northern and North eastern of Thailand.

site	plantation age (year)	pH	H (%)	OM (%)	N (%)	P ppm	K ppm
CR1	>1	6.0	13.7	1.54	0.09	111.8 ^{VH}	158.0
CR2	5	5.3	17.0	7.22	0.44	121.4 ^{VH}	1058.0
CM1	<1	6.0	14.0	3.30	0.15	94.3 ^{VH}	198.5
CM2	10	6.0	18.7	6.94	0.26	150.5 ^{VH}	521.4
CM3	10	6.9	9.5	4.30	0.19	75.2 ^{VH}	204.0
CM4	10	5.9	23.0	2.42	0.07	19.8 ^M	171.0
LO1	4	5.9	13.8	2.68	0.15	147.4 ^{VH}	232.4
LP1	1	6.1	10.0	1.86	0.07	11.5 ^M	171.8
KK1	5	8.0	16.4	0.63	0.02	11.8 ^M	22.1
NK1	5	6.0	18.8	1.74	0.12	175.5 ^{VH}	746.3

Remark: According to Land Development Department, Thailand (Phosri et al. 2010); P<10ppm means Low (L), P ranging between 11 and 25ppm means Medium (M), P ranging between 26 and 45ppm means High (H), P>45ppm means Very High (VH).

AMF status

In total 699 AMF spores and sporocarps were derived using wet sieving and sucrose gradient centrifugation methods from 95 rhizosphere soil samples of physic nut. Spore density in the rhizosphere of physic nut ranged from 19 to 163 spores 100 g⁻¹ soil (mean 70.0±22.9 spores) (Table 3). Maximum spore density was observed in NK1 (163.0±1.5) and minimum in CM1 (66.0±4.9). There was a significant difference ($P < 0.05$) in spore density between 10 sites (Table 3).

Thirty four morphospecies of AMF were identified using spore characteristics. Species richness of AMF varied from 3 to 11 (average 6.1). In the present study we found 3 species from CR1, 4 species from CR1, 6 species from CM1, 7 species from CM2, 5 species from CM3, 11 species from CM4, 7 species from LO1, 6 species from LP1, 4 species from KK1 and 8 species from KK1 (Table 4). *Acaulospora* and *Glomus* occurred most frequently and overall, the most prevalent species. Among them 16 species were in the genus *Acaulospora*

and 10 species in *Glomus*. Only 5 species in *Scutellospora*, 2 species in *Gigaspora* and 1 species in

Entrophospora were found from 10 sampling sites (Table 4).

Table 3 spore density (SD), Shannon-Weiner index (H'), Simpson's index (D) and Root colonization by AMF of each sampling site.

Site	SD*	H'	D	Colonization (%)*
CR1	27±0.9a ²	0.43	0.61	37.7±5.9a ¹
CR2	45±1.4bc	0.28	0.32	85.8±1.3d
CM1	19±0.6a	0.75	0.86	66.0±4.9b
CM2	34±0.7ab	0.78	0.84	87.5±1.5d
CM3	150±2.0f	0.52	0.64	93.2±2.7e
CM4	86±1.8d	0.83	0.81	94.3±2.8e
LO1	112±0.6e	0.65	0.71	64.4±4.2b
LP1	42±0.6bc	0.68	0.77	76.5±5.5c
KK1	21±0.6a	0.30	0.35	77.3±8.7b
NK1	163±1.5f	0.60	0.69	66.4±5.4c

*The same letter in each column indicates that there is no significant difference at $\alpha = 0.05$

¹mean±SD, n = 30

²mean±SD, n = 2

Table 4 (cont.) Spore density (S) and relative abundances (RA) of AMF in each sample sites.

Code	Species	Sample site*																							
		CR1		CR2		CM1		CM2		CM3		CM4		LP1		LO1		KK1		NK1					
		S	RA	S	RA	S	RA	S	RA	S	RA	S	RA	S	RA	S	RA	S	RA	S	RA				
Glomus		15	55.5	0	0	5	26.3	14	41.2	6	4	3	3.5	22	52.4	3	2.7	19	90.5	3	1.8				
CMU17	Glomus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	2.7	2	9.5	-	-				
CMU18	Glomus etunicatum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	1.8				
CMU19	Glomus sp.	-	-	-	-	-	-	-	-	-	-	-	-	6	14.3	-	-	-	-	-	-				
CMU20	Glomus sp.	-	-	-	-	-	-	-	-	-	-	-	-	16	38.1	-	-	-	-	-	-				
CMU21	Glomus Gigasporum	-	-	-	-	-	-	10	29.4	-	-	-	-	-	-	-	-	-	-	-	-				
CMU22	Glomus sinuatum	-	-	-	-	5	26.3	4	11.8	-	-	-	-	-	-	-	-	-	-	-	-				
CMU23	Glomus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	17	80.9	-	-				
CMU24	Glomus sp.	-	-	-	-	-	-	-	-	-	1	1.2	-	-	-	-	-	-	-	-	-				
CMU25	Glomus sp.	-	-	-	-	-	-	-	-	6	4.0	2	2.3	-	-	-	-	-	-	-	-				
CMU27	Glomus fulvum	15	55.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
Gigaspora		0	0	0	0	0	0	0	0	51	34	2	2.3	0	0	51	45.5	0	0	0	0				
CMU28	Gigaspora sp.	-	-	-	-	-	-	-	-	-	-	2	2.3	-	-	51	45.5	-	-	-	-				
CMU29	Gigaspora rosea	-	-	-	-	-	-	-	-	51	34.0	-	-	-	-	-	-	-	-	-	-				
Scutellospora		7	25.9	0	0	6	31.6	7	20.6	0	0	7	8.1	10	23.8	0	0	0	0	4	2.4				
CMU30	Scutellospora sp.01	-	-	-	-	-	-	-	-	-	-	4	4.7	-	-	-	-	-	-	-	-				
CMU31	Scutellospora pellucida	7	25.9	-	-	3	15.8	-	-	-	-	3	3.5	-	-	-	-	-	-	4	2.4				
CMU32	Scutellospora sp.02	-	-	-	-	-	-	7	20.6	-	-	-	-	-	-	-	-	-	-	-	-				
CMU33	Scutellospora heterogamia	-	-	-	-	3	15.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
CMU34	Scutellospora sp.03	-	-	-	-	-	-	-	-	-	-	-	-	10	23.8	-	-	-	-	-	-				
Total spore		27	100	45	100	19	100	34	100	150	100	86	100	42	100	112	100	21	100	163	100				
Species richness		3		4		6		7		5		11		7		6		4		8					

Herein *A. scrobiculata* was the most widely distributed species. It was found in 7 out of 10 sampling sites including CR1, CR2, CM1, CM2, CM3, CM4 and NK1 (70% IF) following by *A. excavata* which appeared in 5 sites including CM1, CM3, CM4, LO1 and LP1 (50% IF). Some species were only found in specific site (10% IF) for example *G. fulvum* in CR1, *Acaulospora* sp.02 and *Acaulospora* sp.03 in CR2, *G. clavisporum* and *Scutellospora* sp.02 in CM2, *E. colombiana* and *Gi. rosea* in CM3, *A. denticulate*, *Glomus* sp.05 and *Scutellospora* sp.01 in CM4, *Glomus* sp.01, *Glomus* sp.02 and *Scutellospora* sp.03 in LP1, *A. spinosa* in KK1, *Acaulospora* sp.01, *Acaulospora* sp.04 and *G. etunicatum* in NK1 (Table 4).

Based on spore density and relative abundance, 7 species were dominant (> 40 spores 100 g^{-1} soil, $RA \geq 6\%$): *A. dilatata* (51 spores, 7.3%), *A. excavata* (42 spores, 6%), *A. foveata* (74 spores, 10.6%), *A. lacunosa* (74 spores, 10.6%), *E. colombiana* (74 spores, 10.6%), *Gigaspora* sp.01 (53 spores, 7.6%) and *Gi. rosea* (51 spores, 7.3%) (Table 5). The morphological characteristics of some dominant AMF were illustrated in Fig. 2

Species diversity was calculated using 2 indices. Both of diversity indices ranged from 0.28 to 0.86 (average 0.64). Shannon–Weiner diversity index ranged from 0.28 to 0.83. The highest was presented in CM4 ($H' = 0.83$) and the lowest was occurred in CR2 ($H' = 0.28$). Similarly, Simpson's index ranged from 0.32 to 0.86 where the highest was shown in CM1 and the lowest was obtained in CR2 (Table 3). All samples of physic nut roots were colonized by AMF (Fig. 3a-d), the mean percentage of root length infection ranged from 38% in CR1 to 94% in CM4 ($P < 0.05$), and generally was exceedingly 60% (Table 3). Significant differences in percentage of root colonization occurred between sampling sites ($P < 0.05$). Physic nut appears to be readily colonized by AMF under a range of field conditions in acidic and calcareous soils, in low to moderate organic matter and in low to high available P (Table 2). A few months old seedlings were moderately colonized by AMF (66% root length in CM1).

Discussion

Member of plant species in the family Euphorbiaceae are known to form mycorrhizal symbioses (Tawarayama et al. 2003; Youpensuk et al. 2004; Zhao & Zhiwei 2007; Straker et al. 2010). In this study we reported for the first time the population density, composition of AM fungi and root colonisation in selected physic nut plantations in Thailand. AMF were presented in all the study sites both in physic nut roots and in soil with moderately to high level of colonisation regardless of plantation age. In contrast Narendra et al. (2009) observed higher colonisation in older plants in India. Nevertheless, this suggested that the plant is strongly mycorrhizal dependent.

The number of AMF species obtained in the present study (34 species) is similar to several reports in Thailand (Table 6). Weerawat (2003) identified 27 AMF species from *Acacia mangium* in the North and Northeastern part of Thailand whilst Nandakwamg et al. (2008) described 24 AMF species from indigenous forest trees in the North of Thailand. The numbers of AMF species in the present study were doubled the number of AMF species detected in continuous maize cropping in the central of Thailand where high P input was applied for long term fertilization (Nabhadaluang et al. 2005). However, several AMF species were observed from only a few sampling sites for example Youpensuk et al. (2004) reported 29 AMF species associated with *Macaranga denticulate* in an upland shifting agriculture with low P level in the North of Thailand. In the tropical rain forests of China, Zhao et al. (2003) identified 27 AMF species

Further Dandan & Zhiwei (2007) recorded 43 AMF morphospecies from the hot and dry valley of south west China. Further, Singh et al. (2008) detected a total of 51 AMF morphospecies associated with the rhizosphere of tea growing in natural and cultivated ecosystems. In comparison, from our study 34 AMF species (average 3.4 AMF species per site) haboured in physic nut roots were obtained. This could be considered as low species richness.

Table 5 Identified AMF and their spore density (S), isolation frequency (IF) and relative abundances (RA) under physic nut rhizosphere (dominant species are written in bold).

Code	Species	S	IF	RA
CMU04	<i>Acaulospora colossica</i> P.A. Schultz, Bever & J.B. Morton	10	10	1.4
CMU08	<i>Acaulospora denticulate</i> Sieverd. & S. Toro	2	10	0.3
CMU09	<i>Acaulospora dilatata</i> J.B. Morton	51	20	7.3
CMU12	<i>Acaulospora excavate</i> Ingleby & C. Walker	42	50	6.0
CMU02	<i>Acaulospora foveata</i> Trappe & Janos	74	20	10.6
CMU14	<i>Acaulospora lacunosa</i> J.B. Morton	74	30	10.6
CMU16	<i>Acaulospora morrowiae</i> Spain & N.C. Schenck	19	20	2.7
CMU11	<i>Acaulospora nicolsonii</i> C. Walker, L.E. Reed & F.E. Sanders	6	30	0.9
CMU10	<i>Acaulospora rehmsii</i> Sieverd. & S. Toro	4	20	0.6
CMU06	<i>Acaulospora scrobiculata</i> Trappe	29	70	4.1
CMU01	<i>Acaulospora spinosa</i> C. Walker & Trappe	1	10	0.1
CMU03	<i>Acaulospora tuberculata</i> Janos & Trappe	34	20	4.9
CMU07	<i>Acaulospora</i> sp.01	2	10	0.3
CMU13	<i>Acaulospora</i> sp.02	2	10	0.3
CMU15	<i>Acaulospora</i> sp.03	3	10	0.4
CMU26	<i>Acaulospora</i> sp.04	37	10	5.3
CMU05	<i>Entrophospora colombiana</i> Spain & N.C. Schenck	74	10	10.6
CMU29	<i>Gigaspora rosea</i> T.H. Nicolson & N.C. Schenck	51	10	7.3
CMU28	<i>Gigaspora</i> sp.01	53	20	7.6
CMU21	<i>Glomus claviforme</i> (Trappe) R.T. Almeida & N.C. Schenck	10	10	1.4
CMU18	<i>Glomus etunicatum</i> W.N. Becker & Gerd.	3	10	0.4
CMU27	<i>Glomus fulvum</i> (Berk. & Broome) Trappe & Gerd.	15	10	2.1
CMU22	<i>Glomus sinuosum</i> (Gerd. & B.K. Bakshi) R.T. Almeida & N.C. Schenck	9	20	1.3
CMU17	<i>Glomus</i> sp.01	5	20	0.7
CMU19	<i>Glomus</i> sp.02	6	10	0.9
CMU20	<i>Glomus</i> sp.03	16	10	2.3
CMU23	<i>Glomus</i> sp.04	17	10	2.4
CMU24	<i>Glomus</i> sp.05	1	10	0.1
CMU25	<i>Glomus</i> sp.06	8	20	1.1
CMU31	<i>Scutellospora pellucida</i> (T.H. Nicolson & N.C. Schenck) C. Walker & F.E. Sanders	17	40	2.4
CMU30	<i>Scutellospora</i> sp.01	4	10	0.6
CMU32	<i>Scutellospora</i> sp.02	7	10	1.0
CMU33	<i>Scutellospora heterogama</i> (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders	3	10	0.4
CMU34	<i>Scutellospora</i> sp.03	10	10	1.4
Total: AMF 34 species		699		100



Fig.2 Spore characteristics of the dominant AMF collected from physic nut rhizosphere under light (a-g) and scanning electron (h) microscopes. *Acaulospora foveata* (CMU02) [a], *Entrophospora colombiana* (CMU05) [b, h], *A. dilatata* (CMU09) [c], *A. lacunosa* (CMU14) [d], *Gigaspora rosea* (CMU29) [e], *Gigaspora* sp.01 (CMU28) [f], and *A. scrobiculata* (CMU06) [g] with Melzer's reagent. Bars: a, b, c, d, g = 38 μ m (40 $\times$); e, f = 150 μ m (10 $\times$); h = 50 μ m.

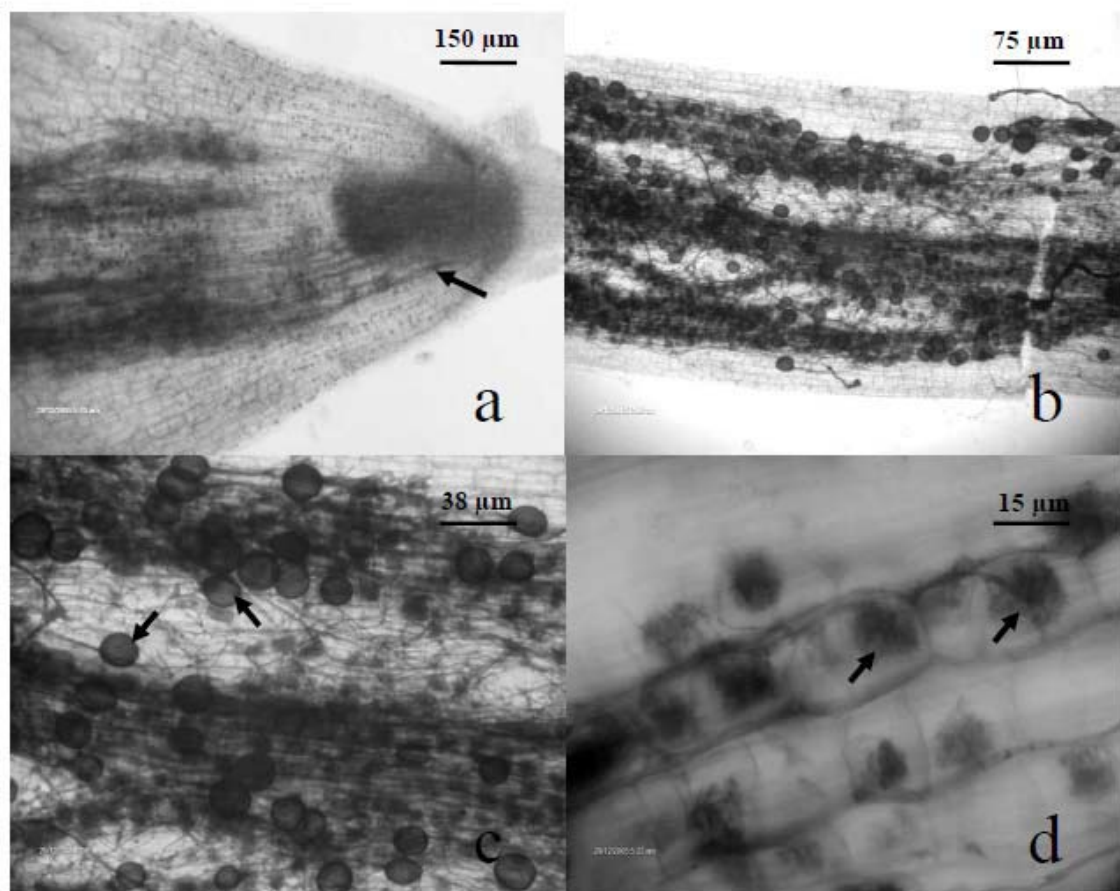


Fig.3. AMF colonization in physic nut roots collected from the field. (a) AMF structures near the root tip (arrow), (b) highly infected root, (c) vesicles (arrows), and (d) arbuscules (arrows).

Previous studies have shown that agricultural management practise for example tillage, quality and quantity of fertilizer applied, cropping systemes have a negative impact on the AMF association with temperate and tropical agronomic plant species (van der Heijden et al. 1998; Douds & Millner 1999; Cardoso & Kuyper 2006; Wang et al. 2009). Fertilization is one of an important abiotic factor influencing growth, colonisation, sporulation, composition and distribution of AM fungi (Johnson 1993; Egerton-Warburton & Allen 2000; Na Bhadalung et al. 2005; Zhang et al. 2006; Wang et al. 2009). In general high level of P fertilization applied negates the mycorrhizal effect in the field both infection of roots and sporulation by majority of AM fungi (Douds & Schenck 1990; Tang et al. 2001; Rubio et al. 2003; Alguacil et al. 2010). Several authors indicated that increasing P fertilization significantly reduced the species diversity of AM fungi and altered the species composition (Johnson 1993; Kahiluoto et al. 2001; Wang et al. 2009). Alguacil et al. (2010) clearly demonstrated that P fertilization affected AM fungi diversity and composition in a tropical savana forage system when different sources and high dose of P were applied. Apart from P content, high N content in soil can also influence the species composition of AM fungi and colonisation (Blanker et al. 2005, Treseder & Allen 2002, Wang et al. 2009). In our case most soil samplings contained from medium P to very high. Therefore this result could suggest that P content attribute to low species richness and diversity. However, other factors can also affect AMF diversity and

community structure such as vegetation type, host specificity between fungi and plants and temporal variation (Johnson et al. 1992; Barni & Siniscalco 2000; Boddington & Dodd 2000; Burrows & Pfleger 2002; Husband et al. 2002; Narendra et al. 2009). Gaidashova et al. (2009) demonstrated that AM fungi diversity varied considerably depending on other edapho-climatic conditions i.e. rain fall, soil texture and soil management practise. In addition large samples are likely to contain more AMF species which could result in a high species diversity including species richness, Shannon-Weiner index and Simpson's index (Barrow et al. 1997; Nandakwang et al. 2008). Therefore a greater sampling effort would be required to prove this.

The present study represented that *Acaulospora* was predominant genus in term of spore density and species diversity (Table 4). A similar finding was obtained from the rhizosphere soil under food crops planted into an upland swidden farm and in dry tropical forests in northern Thailand (Nandakwang 2008; Wangmo 2008). Members of *Acaulospora* have been identified mainly in low input farm, forest and grassland soils. They are considered as facultative symbionts and adapted to wide array of soil and host species, appearing in soil of widely different pH and nutrient availability (Sieverding 1991; Shepherd et al. 1996; Straker et al. 2010). Moreover, *Acaulospora* species are frequently associated with acidic soil (Abbott and Robson, 1991). Our study indicated that *A. scrobiculata* was frequently found in many sites. This is in agreement with several reports (Shepherd et al. 1996;

Jefwa et al. 2006; Straker et al. 2010). Therefore this may account for the appearance of *Acaulospora* species in many sites where our studied undertaken.

Glomus species are considered as cosmopolitan fungi in many ecosystems (Sýkorová et al. 2007). They has dominated in various habitats and dominate communities both in the cold or temperate and in the tropical region and subtropical vegetation. Members of them are usually occur in neutral and slightly alkaline soil (Mukerji et al. 2002) in particular *G. etunicatum* is a worldwide distributed species and can be found in many ecotypes (Becker & Gerdemann, 1977). In other reports from Thailand indicated that *Glomus* was prevalent AMF genus as in *Macaranga denticulate* (Youpensuk et al. 2004) and indigenous tree of North of Thailand (Nandakwang et al. 2008). Most of the soils in our study sites were acidic. Therefore this could explain our less frequent detection of *Glomus*. Moreover frequency of occurrence also varies drastically at the species level (Rubio et al. 2003). Eventhough *Glomus* has been found at almost all sites investigated but is found at lower frequencies.

Other genera seem to be less common in the present study, with only a few examples of species, such as *Entrophospora colombiana*, *Gigaspora rosea*, *Scutellospora pellucida* and *S. heterogama*. The results shown that the number of species in Gigasporaceae was less than other AMF. It may cause from the sampling soil posses a very fine grain as clay. Normally, *Gigaspora* species predominate in soil with sand content as dunes (Lee & Koske 1994). *Scutellospora* is ancestor of *Gigaspora*

(Walker 1992). Both *Gigaspora* and *Scutellospora* produce large spores and these require a longer period to develop than the small-spored species (Hepper 1984). It has been suggested that the latter are therefore more adaptive to changing environmental conditions (Stutz 1996). It also appears that *Scutellospora* might be a poor competitor in colonizing plant roots that the host plant favors fungi from Glomerales (Sýkorová et al. 2007). Moreover species in *Gigaspora* and *Scutellospora* were much more frequently associated with wild plants than with field crops (Gai et al. 2006).

Obviously physic nut was heavily colonized by AMF in all field locations studied. The ability of this crop to harbor AMF across a wide range of site conditions makes it a good potential crop for large scale plantations. To our knowledge the establishment of physic nut plantation in Thailand is often planted into degraded land that previously supports other crops or was forested. The degraded land resulted in erosion of top soils by rain and severe leaching of the soils. It can also result in loss of indigenous AMF that inhabited in the top soils. This means that the land has a limited capability to support growth of either indigenous or agronomic plants species without addition of considerably amount of fertilisers. One of our conclusions is that application of correct AMF inoculation could be benefit in the establishment of physic nut plantation on degraded land with poor and infertile soil and especially where benefit soil microorganisms are being lost from top soils.

Table 6 Comparison of AMF diversity in the current study with previous studies in Thailand.

Host plant	AMF genera*								Total AMF species
	A	Ar	E	Gi	G	Pg	S	U	
<i>M. denticulata</i> (Youpensuk et al. 2004)	6	1	-	2	17	1	2	-	29
<i>A. mangium</i> (Weravart 2003)	6	-	-	7	7	-	6	1	27
<i>Z. mays</i> (Nabhadalung et al. 2005)	2	-	2	-	9	-	1	2	16
Indigenous trees (Nandakwang et al. 2008)	6	-	-	-	15	-	3	-	24
<i>J. curcas</i> (current study)	16	-	1	2	10	-	5	-	34

*A = *Acaulospora*, Ar = *Archaeospora*, E = *Entrophospora*, Gi = *Gigaspora*, G = *Glomus*, Pg = *Paraglomus*, S = *Scutellospora*, and U = unknown

However, the fact that physic nut grows abundantly in the wild. The specie has never really been domesticated. There is certainly doubt for the conditions that best suitable for its growth. This could lead to unproductive agriculture. However, if we are discuss in moving to more sustainable practise, AMF inocula could have real potential to improve physic nut productivity in established plantation as evident from our study that physic nut is very responsive to mycorrhizas. For this reason, further research is required to identify effective AMF for physic nut in Thailand that can be used in inoculation program in order to restore AMF diversity in degraded land.

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