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ผลสัมฤทธิ์

การจัดอบรมเชิงปฏิบัติการ

1. เรื่อง "การใช้มายคอร์ไรซ่าเพื่อเพิ่มผลผลิตของพืช"
   ระหว่างวันที่ 7-9 มีนาคม 2550
   ณ อาคาร 40 ปี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่
2. เรื่อง "การใช้มายคอร์ไรซ่าเพื่อเพิ่มผลผลิตของพืช"
   วันที่ 25 เมษายน 2551
   ณ อาคาร 40 ปี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่
3. เรื่อง "การอบรมมายคอร์ไรซ่าเพื่อเกษตรอินทรีย์"
   ระหว่างวันที่ 22—23 เมษายน 2552
   ณ อาคาร 40 ปี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่
4. เรื่อง "การใช้ประโยชน์จากไมคอร์ไรซ่าเพื่อการอนุรักษ์และการเกษตร"
   ระหว่างวันที่ 13-14 พฤษภาคม 2553
   ณ อาคาร 40 ปี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่

การนำเสนอผลงาน

1. Chanthiboon, P. and S. Lumyong. 2006. Biodiversity of mycorrhiza in coffee seedling. 5th International Symbiosis Society Congress, 4-10 August 2006, Vienna, Austria.
2. Charoenpakdee, S., B. Dell and S. Lumyong. 2006. Biodiversity of Arbuscular Mycorrhizal Fungi (AMF) associated with physic nut (*Jatropha curcas* L.) in some areas of Thailand. Crop Science Meso 2006 (งานประชุมสัมมนาและเสนอผลงานเมธีวิจัยอาวุโส สกว. สาขาพืชไร่ ประจำปี 2549), 3-4 December 2006, Faculty of Agroindustry, Chiang Mai University, Chiang Mai, Thailand
3. Charoenpakdee, S., Chunhaluechanon, S., Dell, B., Bussaban, B., Lumyong, P. and Lumyong, S. 2007. Arbuscular mycorrhizal fungi associated with the biofuel plant Physic Nut (*Jatropha carcass* L.) in Thailand. The 5th International Symposium on Biocontrol and Biotechnology, November 1-3. Khon Kaen University, Nong Kai Campus, Nong Khai, Thailand.
4. Chanthiboon, P., Lumyong, S. Biodiversity of Arbuscular Mycorrhizal Fungi in arabica coffee's rhizosphere soil. การประชุม "วันวิชาการ ครั้งที่ 2 วิถีวิจัย: ตามรอยพระยุคลบาท" ณ หอประชุมมหาวิทยาลัยเชียงใหม่ จังหวัดเชียงใหม่ ระหว่างวันที่ 8-10 ธันวาคม 2549

5. Chanthiboon, P., Yimyam, N., Lumyong, P., Lumyong, S. Diversity and propagation of Arbuscular mycorrhiza fungi associated wiyh coffee (*Coffea arabica* L.) seedling in Chiang mai and Chiang rai, Thailand. The 5th International Symposium on Biocontrol and Biotechnology, November 1-3. Khon Kaen University, Nong Kai Campus, Nong Khai, Thailand.
6. Jaiyasen, A., Lumyong, S. การเพิ่มจำนวนสปอร์เอนโดมายคอร์ไรซาและผลของเชื้อราต่อพืชอาศัย. ในงานประชุม “เดินตามรอยเบื้องพระยุคลบาท” IRPUS 51 ระหว่างวันที่ 28-30 มีนาคม 2551 ณ ศูนย์การค้าสยามพารากอน กรุงเทพฯ
7. เจนใจ สุธีพรวิโรจน์ และ ศ.ดร. สายสมร ลำยอง “การเพิ่มจำนวนสปอร์อาร์บัสคูลาร์ไมคอร์ไรซาในพืชตระกูลหญ้า” นิทรรศการแสดงผลงานพัฒนาเทคโนโลยีทุนปริญญาตรี สกว. ครั้งที่ 7 IRPUS 52 ระหว่าง 27-29 มีนาคม 2552 ณ ศูนย์การค้าสยามพารากอน กรุงเทพฯ

เอกสารตีพิมพ์

1. Nandakwang, P., S. Elliott, S Youpensuk, B. Dell, N. Teaumroong and S. Lumyong. 2008. Arbuscular mycorrhizal status of indigenous tree species used to restore seasonally dry tropical forest in northern Thailand. *Research Journal of Microbiology* 3: 51-61.
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ภาคผนวก

เอกสารตีพิมพ์

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Arbuscular Mycorrhizal Status of Indigenous Tree Species Used to Restore Seasonally Dry Tropical Forest in Northern Thailand

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Abstract: Arbuscular Mycorrhizal (AM) status of native plants in the tropical forest of northern Thailand was surveyed. Twenty four framework tree species, used to forest restoration were examined at 3 sites: FORRU's research tree Nursery (FN), Forest Restoration plot (FR) and Natural Forest (NF). Eleven dominant herb species were examined at 2 sites: Degraded Watershed (DW) and Forest Soil extraction area (FS). Rhizosphere soil samples were collected and AM fungal spores were counted and identified morphologically. Most plant species were intensively colonized by AM fungi except *Cyperus cyperoides*. Twenty four AM species were identified: *Glomus* (15 species), *Acaulospora* (6 species) and *Scutellospora* (3 species). *Glomus rubiforme* was the dominant species. Spore density varied from 16.1 to 97.4 per 100 g soil (averaged 59.7). Spore number at DW and FS were 129 and 479 spores, respectively, with species richness of 6 and 8, respectively. Spore number at FN, FR and NF were 1,152, 2,337 and 1,376 spores, respectively, with species richness of 17, 21 and 15, respectively. The AM diversity was lower in the sites dominated by herbs than in sites examined for trees. In the deforested sites, reduced plant diversity was related with reduced mycorrhizal diversity. In contrast, the trial plot had the highest AM fungal community. Therefore, the forest restoration techniques allow tree species grown in nursery to become AM associated. The association is still maintained after planting out trees in restored area.

Key words: Arbuscular mycorrhizal fungi, framework tree species, herb species, tropical forest restoration

INTRODUCTION

Tropical deforestation causes forest fragmentation and permits the extensive areas of degraded land. This leads to losses of biodiversity, a decline in soil fertility and deterioration of soil physical and biological properties. The seasonally dry tropical forests of Doi Suthep-Pui National Park in northern Thailand support more than 480 indigenous tree species. Deforestation within the park has had adverse consequences for biodiversity and environmental quality. Previous reforestation programs, to counteract this problem, used mainly exotic tree species. This was partly due to lack of knowledge about the environmental requirements of indigenous trees. One method of forest restoration that have proved very successful in Queensland, Australia, is the so-called framework species method (Goosem and Tucker, 1995; FORRU, 2006), which involves planting mixtures of several indigenous tree species in a single step. Therefore, the Forest Restoration Research Unit (FORRU) at Chiang Mai University (CMU) began assessing the suitability of a wide range of indigenous trees to restore evergreen forest

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on degraded sites within the national park. The unit looked for trees likely to act as framework species i.e., those that would out-compete weeds and attract seed-dispersing animals. Candidate framework species were selected, grown in a nursery and tested in trial plot, established in 1998 in a degraded watershed area in the north of national park (Elliott *et al.*, 2003).

Most sites requiring forest restoration have soil with low-fertility and a low inoculum potential of microorganisms beneficial to plants, such as Arbuscular Mycorrhizal (AM) fungi (Setiadi, 2000; Korb *et al.*, 2003). AM fungi are only one of numerable microorganisms in soil that provide a direct link between plant roots and the soil matrix. AM fungi are widespread in natural ecosystems; play a crucial role in the mineral nutrition of forest trees and provide important nutrient-acquiring mechanisms (Koide and Mosse, 2004). Inoculation with effective AM fungi, combined with early intense colonization of seedlings in tree nurseries, stimulates plant growth and establishment in the field under nutrient-deficient and drought-stressed conditions (Setiadi, 2000). Thus, the capacity of potential framework species to associate with indigenous AM fungi is a very important strategy for forest restoration. The purposes of the present study were to: (1) obtain information on the AM status of 24 framework trees and 11 weedy herbs in Doi Suthep-Pui National Park (2) assess the diversity and distribution of AM fungi associated with plant species in natural forest, degraded area and restoration area. In addition this study was carried out to select AM fungi to produce inoculum for improving nursery tree seedling performance and promoting sapling growth in reforestation areas.

MATERIALS AND METHODS

Study Sites

This study was carried out at 5 sites within Doi Suthep-Pui National Park, Chiang Mai, Thailand. There are two kinds of forest, including deciduous forest (from the lowlands up to about 950 m a.s.l) and Evergreen Forest (EGF) (from about 950 m a.s.l to the summit, 1,685 m a.s.l). Average annual rainfall was 2094.9 mm. Temperatures range from 4.5°C in December to 35.5°C in March (Elliott *et al.*, 2003).

FORRU's research tree Nursery (FN) is situated near the accommodation center of national Park (18°50'N, 98°50'E) at about 1,000 m a.s.l. FORRU screened indigenous tree species to select potential framework species for field trials. Saplings were grown in black plastic bags containing a nursery potting medium of primary evergreen forest soil, coconut husk and peanut husk (2:1:1).

Forest Restoration plot (FR) was established in a degraded watershed area (18°52'N, 98°51'E). Trial plot was positioned along the ridges of a degraded watershed area at 1,207-1,310 m a.s.l. At least 48 nursery saplings of each of 24 framework species were planted out randomly in the field plot.

The Natural evergreen Forest (NF) of Doi Suthep-Pui is one of the most luxuriant in northern Thailand. The forest supports many indigenous tree species, but the framework species in this sampling are sparsely scattered across the national park at 1,050-1,475 m a.s.l.

The Degraded Watershed area (DW) had originally been covered in EGF, but the forest was cleared for cultivation about 20 years previously. Degraded areas at about 1,207-1,310 m a.s.l, near the field plot still supported a few remnant forest trees and were dominated by herbaceous weeds.

The Forest Soil extraction area (FS) was in disturbed EGF at 1,685 m a.s.l. The area, surrounded by natural forest was covered with a mature tree canopy and herbaceous weeds. The forest soil is rich in organic matter with high moisture-holding capacity.

Study Plants

Among the plants of the evergreen forest zone of national park, 24 potential framework species (representing 19 families) were selected for study. All tree species are reported to be multipurpose and suitable for acceleration forest regeneration. Root and soil samples from saplings at the FN, planted trees at the FR and mature trees at the NF of each species were collected. At the other study sites, dominant herb species (representing 5 families) were chosen as study plants; 8 species were sampled at the DW and 6 species at the FS.

Root and Soil Sampling

Sampling of plant roots and their rhizosphere soil took place at 5 study sites between February and April 2005 (dry season). Soil and root samples (about 500 g) were collected to a depth of 10 cm of three individual plants of each species and stored at 4°C until analyzed.

Estimation of Mycorrhizal Colonization

Roots of study plants were separated from each soil sample. The root samples were cleared in 10% (w/v) KOH at 121°C for 15 min and rinsed with water on a 90 μm sieve. Cleared roots were stained with 0.5% acid fuchsin (Brundrett *et al*., 1996). Thirty stained root segments from each plant (about 1 cm long) were taken at random and mounted on microscopic slides to assess mycorrhizal colonization (McGonigle *et al*., 1990).

Spore Extraction and Counting

Spores were isolated from 100 g air-dried soil taken from each field soil sample using the wet-sieving method as described by An *et al*. (1990). Spores were recovered by filtering through a 53 μm sieve onto filter paper. The intact spores on filter paper were counted under a dissecting microscope. A sporocarp was counted as one unit.

Identification of AM Fungi

Spores of AM fungi isolated from the field soils were mounted on glass slides in polyvinyl lactic acid (PVA) or PVA+Melzers reagent (Morton, 1988). The spores were identified according to morphological characteristics of original published species descriptions and using the Internet information from the INVAM website (http://invam.caf.wvu.edu).

Data Analysis

Species richness, spore density, spore number, frequency and relative abundance of AM fungi were expressed as follows: species richness = species observed per sample; spore density = No. of spores per 100 g dried field soil; spore number = No. of spores observed per total samples; frequency = (No. of the samples in which the species or genus observed per total samples) × 100%; relative abundance = (No. of spores of a species or a genus per total spores) × 100%. The data were subjected to one-way ANOVA. Duncans Multiple Range Test ($p<0.05$) was used to compare means.

RESULTS

Arbuscular Mycorrhizal Status of Plant Species and Extent of Mycorrhizal Colonization

All plants studied formed AM symbioses. The extent of AM colonization (Table 1 and 2) was uneven among the different growth stages of individual tree species (saplings and mature) and ground herbs. All the typical AM features, such as arbuscules, vesicles, intracellular hyphal coils, extra and intraradical hyphae, were observed in the samples. Most plant species were usually densely colonized by intraradical hyphae, followed by arbuscules and vesicles in the root cortical tissues. Colonization percentages in tree roots across all sites, ranged from 56.0% for *Ficus glaberrima* to 98.3% for *Macaranga denticulata*. Seven species had colonization percentages higher than 90%: *M. denticulata*,

Table 1: Mean arbuscular mycorrhizal colonization percentages±S.E. (per 30 root pieces of plant species) (n = 2) and spore density±SE (per 100 g dry wt soil) (n = 3) associated with different herb species at two study sites

	Study site			
	Degraded watershed area		Forest soil extraction area	
Herb species	Colonization (%)	Spore density	Colonization (%)	Spore density
Ageratum conyzoides L. (Compositae)	95.10±0.36ns	12.67±1.20b	81.71±8.03ns	84.66±14.33a
Anaphalis margaritacea (L.) Bth. and Hk.f. (Compositae)	65.80±12.97ns	7.67±1.20b	99.09±0.91ns	52.67±6.06a
Conyza sumatrensis (Retz.) Walk. (Compositae)	47.76±9.50	8.00±1.53		
Crassocephalum crepidioides (Bth.) S. Moore (Compositae)	83.22±7.52	15.67±2.60		
Cyperus cyperoides (L.) O.K. (Cyperaceae)	2.44±2.44	32.67±2.40		
Eupatorium adenophorum Spreng. (Compositae)			84.01±1.84	64.33±8.51
Microstegium vagans (Nees ex Steud.) A. Camus (Gramineae)			73.46±6.80	112.67±11.46
Mitracarpus villosus (SW.) DC. (Rubiaceae)	81.31±8.41	28.00±6.08		
Pteridium aquilinum (L.) Kuhn ssp. (Dennstaedtiaceae)	82.26±0.16b	7.67±1.86b	100.00±0.00a	36.33±6.12a
Spilanthes paniculata Wall. ex DC. (Compositae)			91.58±8.42	127.67±20.10
Thysanolaena latifolia (Roxb. ex Horn.) Honda (Gramineae)	89.84±4.94	16.67±3.71		
Average spore density*		16.13±3.37d		79.72±14.44b
Spore number		129.00		479.00

Letter(s) indicate significant differences within each row at $p<0.05$ as determined by ANOVA and Duncans Multiple Range Test; ns, not significant, *: Values were analyzed to compare statistically across all five study sites in Table 1 and 2

Nyssa javanica, *Melia toosendan*, *Hovenia dulcis*, *Heynea trifuga*, *Erythrina subumbrans* and *Rhus rhetsoides*. *Cyperus cyperoides*, which is considered to be non-mycorrhizal or rarely forming mycorrhizas, was also found to be colonized by AM fungi in this study, but had a low (<2.4%) colonization percentage.

Arbuscular Mycorrhizal Species and Frequency of Occurrence

Five thousand four hundred and seventy three AM fungal spores (including sporocarps) were retrieved from the 86 composite soil samples from all 5 study sites, representing 24 AM species, identified according to published descriptions. The frequency (F%) and relative abundance (RA%) of the genera and species of AM fungi are presented in Table 3. Most of the isolated species belonged to the family *Glomaceae*, all of which were *Glomus* (15 species). The most abundant species present was *G. rubiforme*. Four species had formerly been assigned to *Sclerocystis* (*G. clavisporum*, *G. coremioides*, *G. rubiforme* and *G. sinuosum*). Six species were in the family *Acaulosporaceae*, all of which were in the genus *Acaulospora*. The most abundant species present was *A. scrobiculata*. Three species were members of the family *Gigasporaceae* and belonged to the genus *Scutellospora*. Species in the genera *Archaeospora*, *Paraglomus*, *Entrophospora* and *Gigaspora* were not found.

Some species of AM fungi appeared to be generalists since they were found in the rhizospheres of study plant species at virtually all study sites: *A. elegans*, *A. scrobiculata*, *G. ambisporum*, *G. microcarpum*, *G. rubiforme* and *G. sinuosum*. Of these, *A. scrobiculata*, *G. microcarpum* and *G. rubiforme* were dominant at the FS, FN, RF and NF, but they were also abundant at the DW. Some species (*G. microaggregatum* and *S. heterogama*) were found in the soils of disturbed areas both at the DW and FR, but were absent from natural evergreen forest soils at the FS, FN and NF. Some AM species (*A. bireticulata* and *S. pellucida*) were found in the soils of the FN and FR but were absent from the soils at the DW, FS and NF. In the rhizosphere of herb species, some species (*G. microaggregatum*, *G. sinuosum* and *S. heterogama*) were found in the DW but were absent from the FS.

Table 2: Mean arbuscular mycorrhizal colonization percentages ±SE (per 30 root pieces of plant species) (n = 2) and spore density ±SE (per 100 g dry wt. soil) (n = 3) associated with different tree species at three study sites

	Study site					
	FORRU's research nursery		Forest restoration plots		Natural evergreen forests	
Tree species	Colonization (%)	Spore density	Colonization (%)	Spore density	Colonization (%)	Spore density
Acrocarpus fraxinifolius Wight ex Arn. (Caesalpinioideae)	65.56±4.44b	120.67±4.33a	94.77±0.83a	60.67±6.36b	74.38±4.20b	12.00±1.73c
Balakata baccata (Roxb.) Ess. (Euphorbiaceae)	97.22±2.78a	4.00±2.08b	100.00±0.00a	216.67±32.69a	26.14±1.14b	8.67±2.33b
Castanopsis acuminatissima (Bl.) A. DC. (Fagaceae)	11.46±1.16b	4.33±2.19b	83.42±14.20a	16.00±2.08a	82.12±0.64a	8.00±2.65ab
Erythrina subumbrans (Hassk.) Merr. (Papilionoideae)	83.22±9.43ns	8.33±1.45b	98.34±1.66ns	75.67±4.63a	94.54±5.46ns	4.33±2.33b
Ficus altissima Bl. (Moraceae)	95.28±2.96a	32.67±5.17b	94.76±5.24a	59.67±8.29a	51.94±5.94b	4.33±0.88c
Ficus benjamina L. var. *benjamina* (Moraceae)	75.66±19.34ns	32.67±2.85b	75.18±2.90ns	23.67±2.96b	87.05±1.63ns	119.67±6.33a
Ficus glaberrima Bl. var. *glaberrima* (Moraceae)	69.64±17.46a	16.67±3.18a	39.68±3.18a	4.00±2.31b	8.61±3.25b	8.33±2.03ab
Ficus hispida L. f. var. *hispida* (Moraceae)	70.92±4.64ns	29.00±2.08b	49.99±0.86ns	60.00±7.81b	55.99±8.92ns	251.33±34.12a
Ficus racemosa L. var. *racemosa* (Moraceae)	88.14±9.92a	68.67±12.35b	98.41±1.59a	204.33±22.82a	32.46±7.69b	4.00±1.00c
Ficus subulata Bl. var. *subulata* (Moraceae)	39.75±4.37b	35.67±3.48b	69.69±20.32ab	56.67±3.38b	98.86±1.14a	124.66±20.50a
Glochidion kerrii Craib (Euphorbiaceae)	83.35±3.66a	36.67±1.76b	59.06±0.17a	23.67±3.53b	64.00±4.62b	64.67±6.94a
Gmelina arborea Roxb. (Verbenaceae)	98.00±2.00ns	32.33±7.68ab	100.00±0.00ns	48.33±4.51a	67.30±14.52ns	23.67±5.24b
Heynea trijuga Roxb. ex Sims (Meliaceae)	84.48±7.38ns	19.67±4.33b	99.28±0.72ns	595.67±58.89a	94.09±0.65ns	3.33±1.76b
Hovenia dulcis Thunb. (Rhamnaceae)	100.00±0.00a	28.00±2.00c	84.35±2.53b	292.00±12.17a	93.73±4.60ab	64.67±6.89b
Macaranga denticulata (Bl.) M.-A. (Euphorbiaceae)	100.00±0.00ns	56.67±13.30b	96.22±2.28ns	128.33±2.33a	96.68±1.32ns	64.00±19.55b
Machilus bombycina King ex Hk. f. (Lauraceae)	75.43±2.35ns	51.67±2.90a	49.45±25.06ns	55.33±9.24a	96.34±0.90ns	15.67±6.69b
Melia toosendan Sieb. and Zuc. (Meliaceae)	92.08±6.39ns	20.33±2.96b	96.94±3.06ns	104.33±21.49a	93.02±5.30ns	8.00±2.65b
Michelia baillonii Pierre (Magnoliaceae)	100.00±0.00a	40.00±5.03a	97.66±2.34a	15.67±0.33b	68.61±6.39b	34.00±6.03b
Nyssa javanica (Bl.) Wang. (Nyssaceae)	92.19±7.81ns	108.33±4.63b	100.00±0.00ns	172.00±22.37ab	98.16±1.92ns	200.67±23.69a
Prunus cerasoides D. Don (Rosaceae)	76.85±6.48a	32.00±12.06b	21.24±5.60b	43.33±2.73b	87.72±7.72a	112.67±9.26a
Rhus rhetsoides Craib (Anacardiaceae)	100.00±0.00ns	204.33±12.84a	91.26±7.26ns	40.33±3.28b	84.74±1.80ns	28.00±4.58b
Sapindus rarak DC. (Sapindaceae)	98.87±2.20a	28.67±2.60b	92.10±0.16ab	12.33±2.40b	62.67±9.55b	196.33±25.64a
Sarcosperma arboreum Bth. (Sapotaceae)	40.90±23.25ns	8.67±2.91b	92.38±0.48ns	24.33±0.88a	55.67±8.61ns	12.33±3.71b
Spondias axillaris Roxb. (Anacardiaceae)	93.34±3.48ns	128.33±17.84a	72.20±13.50ns	4.00±1.53b	51.24±9.94ns	8.00±0.58b
Average spore density*		48.01±9.72c		97.38±26.55a		57.35±14.80c
Spore number	1152.00		2337.00		1376.00	

Letter(s) indicate significant differences within each row at p<0.05 as determined by ANOVA and Duncan's Multiple Range Test, ns: Not significant. *: Values were analyzed to compare statistically across all five study sites in Table 1 and 2

Fourteen of the 15 AM species recorded in natural evergreen forest were maintained in the forest restoration plot (all except *G. fulvum*). Furthermore, the restoration plot supported 7 additional recruit species that were not recorded in the rhizospheres of forest trees (Table 3). Even in the nursery, only 4 of the AM species recorded in natural evergreen forest were absent. It should be noted that forest soil, incorporated into the nursery potting medium carried only 5 of the 15 forest AM species. Therefore, at least 9 species must have colonized the nursery potting medium as the trees were growing in their containers on the ground.

Spore Abundance and Species Richness of Arbuscular Mycorrhizal Fungi

From all composite soil samples, spore density and species richness of AM fungi differed substantially. Average spore densities in the soils at 5 study sites ranged from 16.1 to 97.4 spores per

Table 3: Frequency (F%) and relative abundance (RA%) of genera and species of arbuscular mycorrhizal fungi in the rhizosphere of different plant species for each study site

	Study site			
	Degraded watershed area		Forest soil extraction area	
AM fungal species	F%	RA%	F%	RA%
Acaulospora	75.00	52.71	66.67	29.48
1. *A. bireticulata* Rothwell and Trappe				
2. *A. elegans* Trappe and Gerd.			33.33	9.27
3. *A. foveata* Trappe and Janos				
4. *A. laevis* Gerd. and Trappe				
5. *A. mellea* Spain and Schenck			33.33	5.02
6. *A. scrobiculata* Trappe	75.00	52.71	50.00	15.19
Glomus	87.50	44.19	100.00	68.78
7. *G. aggregatum* Schenck and Smith				
8. *G. ambisporum* Smith and Schenck			33.33	12.54
9. *G. clavisporum* Trappe				
10. *G. coremioides* Berk. and Broome				
11. *G. fulvum* (Berke. and Broome) Trappe and Gerd.				
12. *G. intraradices* Schenck and Smith				
13. *G. microaggregatum* Koske, Gemma and Olexia	12.50	3.62		
14. *G. microcarpum* Iqbal and Bushra	25.00	6.71	33.33	22.58
15. *G. mosseae* (Nicol. and Gerd.) Gerd. and Trappe				
16. *G. multicaule* Gerd. and Bakshi				
17. *G. rubiforme* Gerd. and Trappe	50.00	25.07	100.00	31.22
18. *G. scintillans* Rose and Trappe				
19. *G. sinuosum* Gerd. and Bakshi	25.00	8.79		
20. *G. tortuosum* Schenck and Smith				
21. *G. viscosum* Nicol.			16.67	2.44
Scutellospora	12.50	3.10	16.67	1.74
22. *S. gregaria* (Schenck and Nicol.) Walker and Sanders				
23. *S. heterogama* Walker and Sanders	12.50	3.10		
24. *S. pellucida* (Nicol. and Schenck) Walker and Sanders			16.67	1.74
Species richness	6.00		8.00	

	Study site					
	FORRU's research nursery		Forest restoration plots		Natural evergreen forests	
AM fungal species	F%	RA%	F%	RA%	F%	RA%
Acaulospora	66.67	40.50	66.67	43.63	33.33	5.91
1. *A. bireticulata* Rothwell and Trappe	4.17	0.66	4.17	0.33		
2. *A. elegans* Trappe and Gerd.	16.67	2.37	50.00	39.17	16.67	4.38
3. *A. foveata* Trappe and Janos	4.17	0.69	8.33	1.36	4.17	0.31
4. *A. laevis* Gerd. and Trappe	4.17	0.40	4.17	0.19	4.17	0.31
5. *A. mellea* Spain and Schenck	20.83	6.31	4.17	0.36		
6. *A. scrobiculata* Trappe	58.33	30.06	20.83	2.24	12.50	0.90
Glomus	91.67	56.03	87.50	49.32	91.67	94.09
7. *G. aggregatum* Schenck and Smith			8.33	0.38	4.17	0.29
8. *G. ambisporum* Smith and Schenck	54.17	14.23	12.50	3.05	58.33	31.19
9. *G. clavisporum* Trappe			8.33	0.33	4.17	0.61
10. *G. coremioides* Berk. and Broome			25.00	2.03	4.17	4.65
11. *G. fulvum* (Berke. and Broome) Trappe and Gerd.					8.33	7.85
12. *G. intraradices* Schenck and Smith	4.17	3.53	4.17	0.70	8.33	0.61
13. *G. microaggregatum* Koske, Gemma and Olexia			4.17	0.36		
14. *G. microcarpum* Iqbal and Bushra	16.67	4.57	16.67	9.94	25.00	7.22
15. *G. mosseae* (Nicol. and Gerd.) Gerd. and Trappe	12.50	3.24	8.33	5.43	12.50	16.88
16. *G. multicaule* Gerd. and Bakshi	33.33	12.76	29.17	14.23	29.17	15.19
17. *G. rubiforme* Gerd. and Trappe	58.33	10.38	41.67	12.02	29.17	6.73
18. *G. scintillans* Rose and Trappe	4.17	2.78				

Table 3: Continued

	Study site					
	FORRU's research nursery		Forest restoration plots		Natural evergreen forests	
AM fungal species	F%	RA%	F%	RA%	F%	RA%
19. *G. sinuosum* Gerd. and Bakshi	4.17	0.35	4.17	0.33	16.67	2.88
20. *G. tortuosum* Schenck and Smith	4.17	1.42				
21. *G. viscosum* Nicol.	8.33	2.78	8.33	0.51		
Scutellospora	12.50	3.47	50.00	7.05		
22. *S. gregaria* (Schenck and Nicol.) Walker and Sanders			4.17	1.01		
23. *S. heterogama* Walker and Sanders			8.33	0.50		
24. *S. pellucida* (Nicol. and Schenck) Walker and Sanders	12.50	3.47	41.67	5.53		
Species richness	17.00		21.00		15.00	

Table 4: Arbuscular mycorrhizal species and species richness associated with different herb species at two study sites

	Study sites	
	Degraded watershed area	Forest soil extraction area
Herb species	Species^a; (Species richness)^b	Species^a; (Species richness)^b
A. conyzoides	6, 17; (2)	2, 6, 17; (3)
A. margaritacea	18; (1)	6, 17; (2)
C. sumatrensis	6, 18; (2)	
C. crepidioides	6; (1)	
C. cyperoides	6, 14, 17, 22; (4)	
E. adenophorum		5, 6, 7, 17; (4)
M. vagans		2, 5, 7, 14, 17; (5)
M. villosus	13, 6; (2)	
P. aquilinum	6, 17; (2)	17, 20, 23; (3)
S. paniculata		14, 17; (2)
T. latifolia	14, 17; (2)	
Species richness	6	8
Average species richness	2.00	3.17

^{a}: No. of column refer to the codes of AM fungal species in Table 3, ^{b}: No. of brackets refer to the number of AM fungal species observed per rhizosphere of each herb species

100 g dry soil, with an average of 59.7 (Table 1 and 2). Average spore density at the FS, FN, FR and NF were high 79.7, 48.0, 97.4 and 57.4, respectively while the lowest spore density at the DW was 16.1. At the degraded watershed area, average spore density was significantly the lowest while it was highest at the forest restoration plot. Spore density in the rhizosphere of each plant species was highly variable, ranging from 4.0 to 595.7 spores per 100 g dry soil. The spore numbers of AM fungi at the study sites varied from 129 to 2,337 spores (Table 1 and 2). The spore numbers in the soils at the FN, FR and NF were high (1,152, 2,337 and 1,376, respectively). Much lower numbers were found at the DW and FS; 129 and 479, respectively.

Similarly, species richness of AM fungi at the study sites varied from 6 to 21 species per soil sample (Table 3). Species richness in the soils at the FN, FR and NF were also high (17, 21 and 15, respectively) and were lower at the DW and FS (6 and 8, respectively) showing trends with positively related to spore numbers. Species richness of AM fungi in the rhizosphere of each plant species was highly variable, ranging from 1 to 8 species per soil samples (Table 4 and 5). Average species richness of AM fungi in the soils ranged from 2.0 to 3.2 per soil samples, with an average of 2.8. In the rhizosphere of indigenous trees at the FN, RF and NF, species richness and spore number were higher than for ground herbs at the DW and FS.

Table 5: Arbuscular mycorrhizal species and species richness associated with different tree species at three study sites

	Study site		
	FORRU's research nursery	Forest restoration plots	Natural evergreen forests
Tree species	Species[a]; (Species richness)[b]	Species[a]; (Species richness)[b]	Species[a]; (Species richness)[b]
A. fraxinifolius	2, 6, 7, 16, 17, 23; (6)	3, 4, 16, 17; (4)	12, 14; (2)
B. baccata	5; (1)	14, 23; (2)	2, 12; (2)
C. acuminatissima	7; (1)	1, 16; (2)	8, 17; (2)
E. subumbrans	15, 17; (2)	2, 7, 13, 22; (4)	4; (1)
F. altissima	5, 6; (2)	2, 6, 10; (3)	17; (1)
F. benjamina	7, 16, 17; (3)	6, 22, 23; (3)	7, 14, 16, 18; (4)
F. glaberrima	5, 6, 7, 16; (4)	17; (1)	7; (1)
F. hispida	6, 14, 19; (3)	2, 5, 17, 20; (4)	7, 10, 15; (3)
F. racemosa	6, 7, 14, 17, 20; (5)	2, 8, 15, 17, 18; (5)	7; (1)
F. subulata	1, 6, 7, 16, 17; (5)	2, 15, 17, 23; (4)	7, 14, 16, 17; (4)
G. kerrii	7, 14, 16; (3)	24; (1)	15, 16; (2)
G. arborea	6, 17; (2)	2, 6, 14, 16; (4)	7, 17, 18; (3)
H. trijuga	7, 17; (2)	2, 3, 8, 10, 23; (5)	11; (1)
H. dulcis	2, 3, 6,15; (4)	2, 7, 16; (3)	2, 15, 18; (3)
M. denticulata	7, 17, 21; (3)	9, 10, 17, 23; (4)	7; (1)
M. bombycina	2, 6, 7, 16, 18; (5)	2, 10, 17, 23; (4)	6, 7, 14, 17; (4)
M. toosendan	4, 17; (2)	2, 9, 23; (3)	2; (1)
M. baillonii	6, 17; (2)	17, 20; (2)	3, 7, 18; (3)
N. javanica	5, 6, 17, 20, 23; (5)	6, 16, 23; (3)	7, 16, 17; (3)
P. cerasoides	7, 16, 17; (3)	7, 10, 14, 16, 17; (5)	7, 9, 14, 17; (4)
R. rhetsoides	2, 5, 6, 7, 14, 16, 17, 23; (8)	2, 6, 14, 17; (4)	6, 7, 14, 16; (4)
S. rarak	15; (1)	2, 23; (2)	2, 6, 11, 16; (4)
S. arboreum	6, 7; (2)	10, 12, 23; (3)	7, 16; (2)
S. axillaris	6, 12, 17; (3)	16; (1)	7; (1)
Species richness	17	21	15
Average species richness	3.21	3.17	2.38

[a]: No. of column refer to the codes of AM fungal species in Table 3, [b]: No. of brackets refer to the number of AM fungal species observed per rhizosphere of each tree species

DISCUSSION

The results of our study on the 24 framework tree species and 11 dominant herb species in the seasonally dry tropical forests of northern Thailand showed that most plant species are highly colonized hosts of AM fungi. This reflects the mycotrophic nature of the plant species studied, the age of the sites and the ability of AM fungi in soils to colonize a wide range of host species. It has been reported that many tree species are highly dependent on AM fungi (Janos, 1980; Onguene and Kuyper, 2001) and most herbaceous weeds and grasses are associated with AM fungi (Murakoshi *et al*., 1998). There were many intraradical hyphae, arbuscules and vesicles in the fine roots. Mycorrhizal colonization percentages differed among plant species and among sites. Colonization percentages were uneven in framework tree roots belonging to all growth stages. Some tree species had high colonization percentages at all sites: *M. denticulata*, *N. javanica*, *M. toosendan*, *H. dulcis*, *H. trijuga*, *E. subumbrans* and *R. rhetsoides*. This showed that indigenous forest trees may have a strong dependency on AM, as all surveyed plants formed AM and their roots were intensively colonized. The only exception was *C. cyperoides*, which is considered to be non-mycorrhizal or rarely mycorrhizal (Muthukumar and Udaiyan, 2000). In this study, it was found that this species commonly formed AM, with low colonization percentages (<2.4%).

Twenty four AM species were found in the rhizosphere of the plant species at surveyed study sites. AM fungi belonging 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). *Acaulospora* species are often associated with acidic soils. Most of the soils in our study sites were acidic and this could explain our frequent detection of *Acaulospora*. Among these, *A. elegans*, *A. scrobiculata*, *G. ambisporum*, *G. microcarpum*, *G. rubiforme* and *G. sinuosum* were the most commonly encountered species. This suggests that these species have a widespread and broad host range.

Spore densities of AM fungi vary greatly in different ecosystem. Here, the average density varied from 16.1 to 97.4 spores per 100 g soil and the species richness ranged from 6 to 21. The results of the current work showed that spore density was not related to colonization levels and species richness when all the study sites were considered together. Of the 24 tree species studied, 16 species had higher colonization percentage, 10 species were colonized by more AM species and 13 species supported higher spore densities in the forest restoration plot than in the natural evergreen forest. This clearly demonstrates than the forest restoration techniques used by FORRU maintains or increases AM fungal communities in the rhizosphere of most tree species planted. This may also help to account for the very high growth rates recorded for these tree species after planting them out in degraded areas (Elliott *et al.*, 2003). This indicates that local environmental conditions and host plant species in each study site override AM fungal colonization, diversity and spore production.

The Degraded Watershed area (DW) had the lowest species richness (6 species) and average spore density (16.1 spores per 100g soil) in the rhizosphere of 8 herb species. These results indicate the influence of disturbance on mycorrhizal fungi in this site. It is well-known that land disturbance reduce below-ground AM fungal communities, depending on the disturbance intensity (Allen *et al.*, 1998; Korb *et al.*, 2003). In the Forest Soil extraction area (FS), the rhizosphere of 6 herb species supported higher species richness and average spore density (8 species and 79.7 spores 100 g soil, respectively) than the DW, even though the number of herb species examined from the FS was less than at the DW. Some species, such as *M. vagans* and *S. paniculata*, had high spore densities of AM fungi. This was probably because the FS was surrounded by an undisturbed area that still supported some favourable host plants. These plants may directly influence the below-ground AM fungal community composition, because different plant species exhibit varied abilities to establish mycorrhizal associations and to benefit from them (Lovelock *et al.*, 2003).

The diversity of the AM fungi and the abundance of each species in the rhizosphere of 11 herbs at the DW and FS were low; very low compared to the rhizosphere of 24 indigenous trees at the other three sites. In FORRU's research Nursery (FN), the rhizosphere of selected tree saplings supported high AM diversity (17 species) and average spore density (48.0 spores per 100 g soil), but only a few AM species (5 species) were probably derived from the forest soil that is included as a component of the nursery potting medium. Furthermore, the growing of many tree species in close proximity in the nursery, the practice of raising plants on the ground or the location of the nursery within Evergreen Forest (EGF) all potentially contributed to high AM diversity and spore density in the nursery.

Highest AM diversity (21 species) and average spore density (97.4 spores per 100 g soil) were found in the rhizosphere of planted trees in the Forest Restoration plot (FR). This may be the result of the plant growth stage and plant diversity in the plot. This contrasts markedly with nearby degraded areas which support few AM fungal spores. After planting with framework trees however the AM fungal community was re-established. Forest restoration by planting 24 framework species therefore clearly promoted re-establishment of below-ground AM fungi that were still present or provided host trees for spores dispersed into the plot from nearby forest and enhanced establishment of new residual AM fungi that were inside the potting medium of transplanted saplings.

A denser plant community helps the colonizing obligate AM fungi to spread extensively, with less propagules being lost to passive stochastic dispersal. Dense plant cover also produces a high litterfall and great amount of root biomass for maintaining a diverse AM fungal community (Friberg, 2001). Similarly, the rhizosphere of mature trees showed high AM diversity (15 species) and average spore density (57.4 spores per 100 g soil) in the Natural evergreen Forest (NF). The density and diversity of AM fungi increased with increasing tree canopy cover especially in deciduous forest, because plants in this habitat may more effectively convert the higher interception of light into photosynthate which can then be directed to the roots, providing a food source for AM fungi (Koske, 1987). In contrast, almost all samples in this study site were collected in the EGF with much higher and denser tree canopy than that of the deciduous forest. Fewer AM fungal spores were observed with increasing canopy cover, because the trees grew close to each other, strongly limiting light penetration to the soil, reducing soil temperature and possibly limiting sporulation and colonizaion of AM fungi.

In addition, the knowledge provided here has practical implications to forest management and regeneration technologies as follows (1). Including forest soil with indigenous AM fungi in the potting medium mix, allows most framework tree species grown in nurseries to become AM associated. The association is maintained after planting out trees in deforested sites (2). Tree nurseries should be located within forest areas and deforested landscapes should retain at least some natural forest to provide a continuous supply of AM fungal spores (3). Using forest soil with indigenous AM fungi as inoculum is preferable to the introduction of commercial innoculant products containing exotic AM fungi for growing framework tree saplings. Based on these studies, forest soil with indigenous AM fungi in the potting medium mix are important for the establishment, growth and survival of framework tree saplings at trial plot that might possibly accelerate natural regeneration of forest ecosystems and encourage biodiversity recovery in a degraded watershed area in Doi Suthep-Pui National Park.

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เอกสารตีพิมพ์

Title:	Effects of arbuscular mycorrhizal inoculation and fertilizer on production of *Castanopsis acuminatissima* saplings for forest restoration in northern Thailand
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Effects of Arbuscular Mycorrhizal Inoculation and Fertilizer on Production of *Castanopsis acuminatissima* Saplings for Forest Restoration in Northern Thailand

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Abstract: *Castanopsis acuminatissima* is a native tree used to restore forest in Thailand. To accelerate seedling growth experiments were carried out to determine the efficacy of applying to *C. acuminatissima*. Arbuscular Mycorrhizal (AM) fungi, produced on sorghum, were used as inoculum to investigate the symbiosis on seedlings. The effects of AM inoculation (*Acaulospora elegans, Glomus etunicatum, Glomus mosseae*) together with phosphate fertilization (KH_2PO_4) on seedlings in a P-deficient soil were studied under greenhouse conditions. Increasing P-application rates greatly enhanced seedling growth (maximum at 250 mg kg^{-1} soil). Growth was most rapid with *G. etunicatum*-colonized plants with P application (40.8 cm), whereas much lower height was found with non-AM plants without P added (14.4 cm). The mycorrhizal effective for *C. acuminatissima* in previous experiments were confirmed by growing seedlings in a forest soil with slow-release fertilizer (NPK) and combined with AM species under nursery performance conditions. Plant height was significantly enhanced by fertilizer but not by fungi. The greatest height was found in non-AM plants with fertilization (14.5 cm), whereas lower height was found for non-AM plants with no fertilizer added (10.9 cm). AM inoculation greatly enhanced seedling growth in P-deficient soil more than in forest soil due to differences in abilities of AM species to establish a symbiosis. Therefore, in sapling production, the soil properties and level of fertilization should be evaluated keeping secondary effects caused by changed mycorrhizal association.

Key words: Arbuscular mycorrhizal fungi, framework tree species, forest restoration, mycorrhizal seedling production, phosphorus fertilizer

INTRODUCTION

Forest restoration means the re-establishment of the original forest ecosystem that was present before deforestation occurred. The goals of forest restoration are environmental protection and wildlife conservation (Anonymous, 2006). In 1994, the Forest Restoration Research Unit at Chiang Mai University (FORRU), started to investigate the possibility of restoring forests on degraded sites in northern Thailand by adapting the framework species method (first developed in Queensland, Australia) (Goosem and Tucker, 1995; Anonymous, 2006) to local conditions. FORRU screened indigenous forest tree species to select potential candidate framework species for field trials. In the FORRU's research tree nursery, experiments were designed to develop seedling production for high quality planting stock. *Castanopsis acuminatissima* (Bl.) A. DC. (Fagaceae), was confirmed as a potential framework species that could be used to restore seasonally dry tropical forest in northern Thailand but this species grow relatively slowly and difficult to raise in nursery. To solve such problems, studies have been made on modified potting media and fertilizer application (Anonymous, 2006). Arbuscular Mycorrhizal (AM) fungi have form symbiosis with a wide range of forest tree

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Corresponding Author: S. Lumyong, Department of Biology, Chiang Mai University, 50200, Chiang Mai, Thailand species (Gai *et al.*, 2006). Such symbioses provide many benefits to host trees and are especially important in development of seedlings grown in nurseries and establishment of saplings planted in deforested sites.

AM symbioses result in increased growth of plants depending on the fungal strains (Pattinson *et al.*, 2004; Youpensuk *et al.*, 2005). AM fungi also protect plants against root pathogens, confer resistance to drought and increase soil aggregation (Dubský *et al.*, 2002; Rilling *et al.*, 2005; Wu *et al.*, 2006). Lack of nutrient availability in tropical soils often limits plant growth. The ability of AM fungi to enhance nutrient absorption, (particularly phosphorus) by hyphal uptake and translocation towards the plant, is an important advantage (Koide and Mosse, 2004). The possibility of using beneficial attributes of AM fungi in planting stock will depend on preliminary assessments of whether inoculation is a suitable management option. AM fungi are obligate symbionts, usually propagated by growing them with living host plants in pot cultures. For starting pot cultures of AM fungi, the combination of appropriate host plant and substrate media for production of mycorrhizal inoculum is crucial (Setiadi, 2000). Pot cultures, which consist of soil, spores and mycorrhizal roots etc., can be used as inoculum for experiments or applied to seedling grown in a nursery or broadcast in the field (Brundrett *et al.*, 1996; Setiadi, 2000; Klironomos and Hart, 2002). Knowledge about the ability of plant species to form symbiosis with AM fungi is very important for restoration success and indicates the need for inoculum in plants cultivated in forest nurseries (Wubet *et al.*, 2003). The purposes of present experiment were to: (1) select appropriate host plants under pot culture conditions for production of indigenous AM fungal inoculum in the nursery, (2) examine the effects of 3 AM species with 6 rates of P application on plant development in P-deficient soil medium under greenhouse conditions and (3) examine the effects of AM fungal inoculation and conventional fertilization on growth of seedlings in forest soil under nursery performance conditions.

MATERIALS AND METHODS

Inoculum Production

The first experiment consisted of 30 treatments with 6 indigenous AM species [*Acaulospora elegans* Trappe and Gerd., *A. mellea* Spain and Schenck, *A. scrobiculata* Trappe, *Glomus etunicatum* Becker and Gerd, *G. mosseae* (Nicol. and Gerd.) Gerd. and Trappe and *Scutellospora heterogama* Walker and Sanders] and 5 host plants [maize (*Zea mays* L.), marigold (*Tegetes erecta* L.), soybean (*Glycine max* (L.) Merr.), sorghum (*Sorghum vulgare* Pers.) and upland rice (*Oryza sativa* L. cv. Bue Bang)] with 3 replications. The experiment was undertaken in clay pots (22 cm top diameter, 18 cm bottom diameter and 19 cm depth) with drainage hole containing 3 kg P-deficient soil medium (P-deficient soil and coarse sand ratio 2:1), autoclaved twice at 121°C for 30 min with a 2 day interval. The soil pH (H_2O) was 5.98 and contained 0.041% total N (Kjeldahl method), 1.4 mg kg^{-1} available P (Bray II method) and 44.0 mg kg^{-1} extractable K (1 M NH_4OAc, pH 7). Seeds were surface-sterilized with 10% sodium hypochlorite for 5 min, rinsed with sterile water and sown in Petri dishes, containing the moist tissue paper for 1 week. Seedlings were transplanted 3 seedlings per pot. AM spores were extracted from the soil samples by wet-sieving and 50% sucrose centrifugation (Brundrett *et al.*, 1996) and collected on a 53 μm sieve. Fifty spores were inoculated into each pot. Seedlings were grown in a greenhouse at the Chiang Mai University (CMU) for 4 months between November 2005 and February 2006. Seedlings were watered once every 2 days with 500 mL of tap water. Twice a month, 80 mL of 4 full strength Hoagland' solution (Hoagland and Arnon, 1950), without P was added to each pot. At harvest, root samples were separated from soil and cleaned with tap water. The root samples were cleared in 10% KOH at 121°C for 15 min and stained with 0.05% trypan blue in lactoglycerol (Brundrett *et al.*, 1996). Thirty stained root segments from each plant (1 cm long) were taken at random and mounted on microscopic slides to assess mycorrhizal colonization (McGonigle *et al.*, 1990). One hundred gram dried soil of all different treatments were used to determine spore density.

Effects of AM fungi with Phosphorus Fertilizer on Seedling Growth in Greenhouse Experiment

The second experiment consisted of 90 pots with 5 inoculation treatments (no inoculation and AM inoculation: *A. elegans*, *G. etunicatum*, *G. mosseae* and mixed AM species) and 6 levels of P application (KH_2PO_4) (at the rates of 0, 50, 100, 150, 200 and 250 mg P kg^{-1} medium) with 3 replications. Spores of AM species were produced on sorghum pot cultures in P-deficient soil medium as shown above and used for inoculation. The experiment was undertaken in clay pots containing 3 kg autoclaved P-deficient soil medium. Seeds of *C. acuminatissima* were surface-sterilized with 10% sodium hypochlorite for 10 min, rinsed with sterile water and sown in a plastic tray containing the autoclaved forest soil medium (primary evergreen forest soil, coconut husk and peanut husk ratio 2:1:1). The medium pH (H_2O) was 5.60 and contained 0.628% total N, 15.8 mg kg^{-1} available P and 132.0 mg kg extractable K. Three month-old seedlings (5-6 cm tall) were transplanted one seedling per pot. In each AM treatment, 150 spores were inoculated into each pot. Seedlings were grown in a greenhouse at the CMU for 6 months between December 2005 and March 2006. Seedlings were watered once every 2 days with 500 mL of tap water. Two weeks after transplanting, 6 levels of KH_2PO_4 were added to each treatment. Twice a month, 80 mL of 4 full strength Hoagland' solution without P was added to each pot. At harvest, height and stem diameter of seedlings were measured. Roots were divided into 2 random sub-samples. Shoot samples and one root sub-sample were oven dried at 60°C for 48 h. Dry samples were analyzed for P content by the dry ashing and molybdovanado-phosphoric acid method. The second root sub-sample was used to determine AM colonization and soil sub-samples were assessed for spore density.

Effects of AM fungi with Slow-Release Fertilizer on Seedling Growth in Nursery Sapling Production

The third experiment consisted of 10 treatments with 5 inoculation treatments (no inoculation and AM inoculation: *A. elegans*, *G. etunicatum*, *G. mosseae* and mixed AM species) and 2 levels of slow-release fertilizer (NPK 14-14-14) (at the rates of 0 and 375 mg kg medium) with 28 replications. Slow-release fertilizer has been used successfully at FORRU for many framework species (Anonymous, 2006). The experiment was undertaken in plastic bags (23×6 cm) with drainage holes, containing 800 g autoclaved forest soil medium. Three month-old seedlings of *C. acuminatissima* were transplanted one seedling per bag. In each AM treatment, 150 spores were inoculated into each pot. Slow-release fertilizer was applied in fertilization treatments at the start of the experimental period. Seedlings were grown at the FORRU's nursery for 6 months between April and September 2006. Seedlings were watered once a day with tap water. Every 3 months, slow-release fertilizer was applied to each treatment. At harvest, height and stem diameter of seedlings were measured. Fresh shoot and root samples were treated in the same way as described previously. Dry plant samples were analyzed for P content. Root sub-samples were used to determine colonization percentage and soil sub-samples were assessed for spore density.

Data Analysis

All data were subjected to analysis of variance (ANOVA) for a completely randomized design. Residuals were normally distributed with constant variance. SPSS software version 12.0 was used to conduct the ANOVA. Duncan's Multiple Range Test ($p<0.05$) was used to compare treatment means.

RESULTS

Inoculum Production of Arbuscular Mycorrhizal Fungi

Four months after starting the pot culture, all 5 host plant species inoculated with 6 AM species had developed mycorrhizas. Spore density and colonization percentage varied greatly among the

Table 1: Spore density of AM fungi (per 100 g dry wt. soil) (n = 3) in pot cultures with 5 host plants inoculated with spores of 6 AM fungal isolates

	Spore density of AM fungi (spores/100 g soil)					
Host plant	*A. elegans*	*A. mellea*	*A. scrobiculata*	*G. etunicatum*	*G. mosseae*	*S. heterogama*
G. max	426.33dB	13.33 nsC	14.67abC	598.00cdA	40.00dC	9.33bC
O. sativa	1683.33bcA	14.67 nsD	13.33abD	396.00dB	190.00bcC	6.67bD
S. vulgare	2713.33aB	13.33 nsC	16.67abC	6148.67aA	642.67aC	22.67aC
T. erecta	967.67cdB	9.33 nsC	6.00bC	1457.33cA	102.00cdC	11.33abC
Z. mays	1867.33bB	10.00 nsC	19.33aC	3933.33bA	231.33bC	4.67bC
Analysis of variance						
AM species			***			
Host plant species			***			
AM species × Host plant species			***			

Means followed by the same letter (s) (lower case within columns and capitals within rows) are not significantly different by Duncan's Multiple Range Test; ns: not significant. ***: significant at $p<0.001$

Table 2: Root colonization (per 30 root pieces of plant species) (n=3) in pot cultures with 5 host plants inoculated with spores of 6 AM fungal isolates

	AM colonization (%)					
Host plant	*A. elegans*	*A. mellea*	*A. scrobiculata*	*G. etunicatum*	*G. mosseae*	*S. heterogama*
G. max	87.95aB	20.00nsC	14.81abC	90.00bB	100.00aA	5.00cD
O. sativa	73.00bB	11.11nsC	3.70abC	100.00aA	95.00bA	3.33cC
S. vulgare	100.00aA	51.85nsC	13.33abD	100.00aA	100.00aA	78.33aB
T. erecta	92.16aAB	73.33nsB	16.66bC	93.33abAB	100.00aA	25.00bC
Z. mays	93.79aA	55.00nsB	56.67aB	100.00aA	100.00aA	11.66cC
Analysis of variance						
AM species			***			
Host plant species			***			
AM species × Host plant species			***			

Means followed by the same letter (s) (lower case within columns and capitals within rows) are not significantly different by Duncan,s Multiple Range Test; ns: not significant. ***: significant at $p<0.001$

different host species. Spore density and mycorrhizal colonization were increased by the host species and AM species which interacted (Table 1 and 2). Three fungal species: *A. elegans*, *G. etunicatum* and *G. mosseae* produced significantly the highest spore densities and colonization abilities on *S. vulgare* and *Z. mays*. The spore density of these AM species on sorghum was significantly higher than on maize, whereas mycorrhizal colonization on both plants did not differ from each other (93.8-100.0%). The highest spore number were found on sorghum inoculated with *G. etunicatum* (6148.7 spores/100 g soil), *A. elegans* (2713.3 spores/100 g soil) and *G. mosseae* (642.7 spores/100 g soil), respectively. Whilst much lower densities and root colonization of other 3 AM species were found on all host species (Table 1 and 2).

Effects of AM Inoculation with P Application on Growth of *C. acuminatissima* in P-Deficient Soil Medium

Growth of *C. acuminatissima* seedlings in the P-deficient soil experiment was highly influenced by both AM inoculation and P-application rates (Table 3). Six months after transplant, plant height, shoot and root dry weights as well as shoot P content were all significantly increased by both factors which interacted, whereas root to shoot ratio and root P content were also increased by both factors but not by their interaction. The only exception was stem diameter, which was only increased by P rates. AM colonization was only increased by fungal inoculation, whereas spore density on the other hand, was increased by both P rates and fungus which interacted (Table 3). Root colonization ranged in the P applied treatments from 36.7-45.4%, which did not differ with P rates (Table 3). In the AM treatments, colonization percentages ranged from 40.1-55.3%. Root colonization in the plants

Table 3: Effects of AM inoculation and P application (KH_2PO_4) on growth of *C. acuminatissima* seedlings grown in P-deficient soil medium and root colonization and spore density of AM fungi in plant rhizoshere (n = 3)

Treatments	Plant height (cm)	Stem diameter (cm)	Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})
AM inoculation				
Uninoculation	17.89c	0.23ns	0.66c	0.89d
A. elegans	24.25b	0.24ns	1.40ab	2.59a
G. etunicatum	27.36a	0.23ns	1.60a	2.08bc
G. mosseae	22.44b	0.24ns	1.17b	2.04c
Mixed species	19.85c	0.22ns	0.84c	2.04a
P applied (mg P kg^{-1}medium)				
0	15.29d	0.21c	0.54c	1.36c
50	16.30d	0.21c	0.58c	1.51c
100	19.87c	0.22bc	0.77c	1.67c
150	25.80b	0.23bc	1.49b	2.33b
200	32.11a	0.24b	1.36b	2.39b
250	17.86a	0.27a	2.07a	2.77a
Analysis of variance				
AM inoculation	***	ns	***	***
P applied	***	***	***	***
AM inoculation × P applied	***	ns	**	*

Treatments	Root to shoot ratio (dry weight)	Shoot P content (mg plant^{-1})	Root P content (mg plant^{-1})	Root colonization (%)	Spore density (spores/100g soil)
AM inoculation					
Uninoculation	1.44c	0.20c	0.22d	0.00c	0.00d
A. elegans	2.26b	0.64a	0.76a	53.72a	23.11c
G. etunicatum	1.86bc	0.74a	0.67ab	55.33a	31.83b
G. mosseae	2.28b	0.42b	0.50bc	55.26a	33.50ab
Mixed species	2.83a	0.40b	0.45c	40.10b	38.33a
P applied (mg P kg^{-1}medium)					
0	2.68a	0.15c	0.25c	43.92ns	24.20abc
50	2.73a	0.18c	0.29c	45.39ns	28.07a
100	2.37ab	0.23c	0.32c	38.14ns	27.00ab
150	1.81bc	0.61b	0.62b	41.47ns	30.07a
200	1.89bc	0.52b	0.63b	39.64ns	21.93bc
250	1.35c	1.21a	1.02a	36.71ns	20.87c
Analysis of variance					
AM inoculation	***	***	***	***	***
P applied	**	***	***	ns	**
AM inoculation × P applied	ns	***	0.074	ns	*

Means in the same column followed by different letter(s) are significantly different by ANOVA and Duncan, s Multiple Range test. *, **, ***: Significant at $p<0.05$, 0.01, 0.001, respectively; ns: not significant

inoculated with the single species of AM fungi was significantly higher than for the mixed species inoculum. Spore density was significantly increased by P rates and AM fungi which interacted. Spore density in the P applied treatments ranged from 20.9-30.1 spores/100 g soil which reached maximum at 150 mg kg^{-1}soil (30.1 spores/100 g soil) and continued to decrease with the lowest at 250 mg kg^{-} soil (20.9 spores/100 g soil). Spore density ranged from 23.1-38.3 spores/100 g soil and the highest density was found in plants inoculated with mixed AM species.

P applications were highly beneficial for growth parameters of plants as measure by height, stem diameter (Table 4), dry weights (Table 5) and P contents (Table 6) and AM inoculations also had significant effects on plant growth. Six months after transplant, non-AM plants grown in P-deficient soil exhibited increasing either height or shoot dry weight to increasing P rates, whereas the other growth parameters showed no such differences. Contrast with all growth parameters of AM plants, tended to increase with increasing P rates (maximum at 250 mg P kg^{-1}) and mycorrhizal enhancement varied with the different kinds of AM species. In P applied treatments, seedlings generally grew very little and no significant differences between AM plants and non-AM plants were observed for plant height, stem diameter and P contents, whereas dry weights of AM plants significantly trended to be higher than non-AM plants.

Table 4: Height and diameter (n = 3) of *C. acuminatissima* seedlings grown in P-deficient soil medium containing increasing P application with AM inoculation

Plant height (cm)

Treatments	Uninoculation	*A. elegans*	*G. etunicatum*	*G. mosseae*	Mixed species
P applied (mg P kg^{-1}medium)					
0	14.44bNS	16.56cNS	15.59bNS	14.84cNS	15.02cNS
50	16.19bNS	18.02cNS	15.97bNS	16.05cNS	15.26cNS
100	18.13bNS	22.12bcNS	18.56bNS	22.19bNS	18.35bcNS
150	17.76bB	27.05bB	39.19aA	25.69bB	19.28bcB
200	17.89bC	26.81bAB	33.97acA	24.03bB	21.21bBC
250	22.92aC	34.95aAB	40.85aA	31.81aABC	30.00aBC

Stem diameter (cm)

Treatments	Uninoculation	*A. elegans*	*G. etunicatum*	*G. mosseae*	Mixed species
P applied (mg P kg^{-1}medium)					
0	0.22nsNS	0.21bNS	0.22nsNS	0.19bNS	0.21nsNS
50	0.22nsNS	0.21bNS	0.219nsNS	0.21bNS	0.21nsNS
100	0.22nsNS	0.20bNS	0.23nsNS	0.24abNS	0.22nsNS
150	0.22nsNS	0.24bNS	0.25nsNS	0.25abNS	0.20nsNS
200	0.23nsNS	0.23bNS	0.25nsNS	0.24abNS	0.25nsNS
250	0.24nsB	0.32aA	0.24nsB	0.29aA	0.24nsB

Means followed by the same letter (lower case within columns and capitals within rows) are not significantly different by Duncan's Multiple Range Test; ns: not significant

Table 5: Shoot and root dry weights (n = 3) of *C. acuminatissima* seedlings grown in P-deficient soil medium containing increasing P application with AM inoculation

Shoot dry weight (g plant^{-1})

Treatments	Uninoculation	*A. elegans*	*G. etunicatum*	*G. mosseae*	Mixed species
P applied (mg kg^{-1} KH_2PO_4)					
0	0.45bB	0.76bA	0.67bAB	0.42dB	0.42cB
50	0.54bAB	0.83bA	0.51bAB	0.53cdAB	0.46bcB
100	0.65bNS	0.82bNS	0.91bNS	0.96cNS	0.51bcNS
150	0.62bB	1.81abAB	2.63aA	1.45bAB	0.96bB
200	0.62bD	1.64abAB	2.136aA	1.46bBC	0.94bCD
250	1.07aC	2.58aAB	2.74aA	2.18aAB	1.77aBC

Root dry weigh (g plant^{-1})

Treatments	Uninoculation	*A. elegans*	*G. etunicatum*	*G. mosseae*	Mixed species
P applied (mg kg^{-1}KH_2PO_4)					
0	0.96nsB	1.77nsA	1.54bAB	1.48cAB	1.05cB
50	0.78nsC	2.28nsA	1.63bB	1.53cB	1.35bcB
100	0.91nsB	1.93nsA	1.42bAB	2.15bA	1.96bA
150	0.76nsC	3.13nsAB	3.51aA	2.34bAB	1.89bBC
200	0.82nsC	3.05nsA	3.11aA	2.13bB	2.83aAB
250	1.11nsB	3.37nsA	3.39aA	2.87aA	3.13aA

Means followed by the same letter (lower case within columns and capitals within rows) are not significantly different by Duncan's Multiple Range Test; ns: Not significant

AM inoculation significantly increased plant height over non-inoculated controls were found in *A. elegans* at 200-250 mg P kg^{-1}, *G. etunicatum* at 150-250 mg P kg and *G. mosseae* at 200 mg P kg^{-1}. The maximum height was 1.52 and 1.78 fold higher than the control in *A. elegans* and *G. etunicatum* at 250 mg P kg^{-1}, respectively, whereas, height of *G. etunicatum* plants was the highest (40.8 cm) (Table 4). Stem diameter was only influenced by P rates. Stem diameter had a 1.45 and 1.32 fold increase over control with no P added in *A. elegans* and *G. mosseae* at 250 mg P kg^{-1}, respectively, which did not differ from one another (0.3 cm) (Table 4).

Only *A. elegans* plants at the lowest P rate significantly had a higher shoot dry weight than non-AM plants and other AM plants. Whilst at high P rates, AM inoculation significantly increased over

Table 6: Shoot and root P contents (n = 3) of *C. acuminatissima* seedlings grown in P-deficient soil medium containing increasing P application with AM inoculation

	Shoot P content (mg plant^{-1})				
Treatments	Uninoculation	*A. elegans*	*G. etunicatum*	*G. mosseae*	Mixed species
P applied (mg kg^{-1} KH_2PO_4)					
0	0.12nsNS	0.21cNS	0.15bNS	0.11bNS	0.14cNS
50	0.13nsB	0.25cA	0.19bAB	0.15bAB	0.16cAB
100	0.20nsNS	0.20cNS	0.33bNS	0.24bNS	0.17cNS
150	0.19nsB	0.76bAB	1.23aA	0.31bB	0.57bB
200	0.16nsC	0.71bB	1.02aA	0.30bC	0.42bBC
250	0.40nsB	1.70aA	1.55aA	1.44aA	0.96aAB

	Root P content (mg plant^{-1})				
Treatments	Uninoculation	*A. elegans*	*G. etunicatum*	*G. mosseae*	Mixed species
P applied (mg kg^{-1} KH_2PO_4)					
0	0.21nsNS	0.29bNS	0.30bNS	0.23bNS	0.20dNS
50	0.21nsC	0.40bA	0.35bAB	0.26bBC	0.21dC
100	0.22nsNS	0.34bNS	0.35bNS	0.38bNS	0.31cdNS
150	0.23nsB	0.94abA	0.98abA	0.54bAB	0.43cAB
200	0.20nsB	1.05abA	0.85abAB	0.40bAB	0.64bAB
250	0.26nsB	1.54aA	1.18aA	1.22aA	0.89aAB

Means followed by the same letter (s) (lower case within columns and capitals within rows) are not significantly different by Duncan's Multiple Range Test; ns: not significant

non-AM plants were found in most fungus treatments, except for mixed fungal species. At 250 mg P kg^{-1}, the maximal shoot biomass exhibited 2.41, 2.56 and 2.34 fold increase over control in *A. elegans, G. etunicatum* and *G. mosseae*, respectively, whereas *G. etunicatum* plants gave the highest shoot biomass (2.7 g plant^{-1}) (Table 5). *A. elegans* plants with no P added also had a significantly higher root dry weight than non-AM plants and other AM plants. AM inoculation significantly increased over non-AM plants were found in all fungus treatments at the first 50 mg P kg^{-1} and continued to increase with further increase in the level of P rates. Root biomass reached their maximum at 250 mg P kg^{-1} and exhibited 3.04, 3.05, 2.58 and 2.82 fold increase over non-inoculated controls in *A. elegans* and *G. etunicatum G. mosseae* and mixed AM species, respectively, which did not differ from one another (2.8-3.4 g plant^{-1}) (Table 5).

At 50 mg P kg^{-1}, AM inoculation significantly increased shoot P content over non-AM plants was only found in *A. elegans*. Whilst at higher P rates, AM inoculation significantly increased shoot P content were found in *A. elegans* at 200-250 mg P kg^{-1}, *G. etunicatum* at 150-250 mg P kg and *G. mosseae* at 250 mg P kg^{-1}. At the highest rate, the maximal shoot P content exhibited 3.04, 3.05, 2.58 and 2.82 fold increase over non-AM plants in *A. elegans* and *G. etunicatum* and *G. mosseae*, respectively which did not differ from one another (1.4-1.7 mg plant^{-1}) (Table 6). Fungal inoculation significantly increased root P content over non-inoculated controls were found in *A. elegans* and *G. etunicatum* at first 50 mg P kg^{-1} and still increased at 150 mg P kg^{-1}. Whilst at 200 mg P kg^{-1}, only root P content of *A. elegans* plants were significant increased over controls. At 250 mg P kg^{-1}, root P content of most AM plants exhibited 5.92, 4.54 and 4.69 fold increase over non-AM plants in *A. elegans, G. etunicatum* and *G. mosseae*, respectively which did not differ from one another (1.2-1.5 mg plant^{-1}) (Table 6).

Effects of AM Inoculation with Slow-Release Fertilizer on Growth of *C. acuminatissima* in Forest Soil Medium

Growth of *C. acuminatissima* seedlings in the forest soil measured for 6 months showed consistent effects of slow-release fertilization throughout the experiment (Table 7). Plant height, dry weights and P contents were increased by fertilizer but not by AM fungi. However, fertilization effects

Table 7: Effects of AM inoculation and slow-release fertilizer application on growth of *C. acuminatissima* seedlings grown in forest soil medium and root colonization and spore density of AM fungi in plant rhizoshere (n = 28)

Treatments	Plant height (cm)	Stem diameter (cm)	Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})
Uninoculated	10.89cd	0.15cd	0.39bcd	0.62bcd
Uninoculated+Fertilizer	14.54a	0.16bc	0.58ab	0.78ab
A. elegans	10.89cd	0.15bcd	0.25d	0.51d
A. elegans+Fertilizer	11.41bcd	0.16bc	0.62a	0.61bcd
G. etunicatum	9.71d	0.14d	0.43abcd	0.64bcd
G. etunicatum+Fertilizer	12.73abc	0.17b	0.42abcd	0.76ab
G. mosseae	10.04d	0.15bcd	0.35cd	0.57cd
G. mosseae+Fertilizer	13.13ab	0.16bc	0.62a	0.83a
Mixed species	11.52bcd	0.16bc	0.39bcd	0.63bcd
Mixed species+Fertilizer	13.05ab	0.20a	0.52abc	0.70abc
Analysis of variance				
AM inoculation	0.075	*	ns	0.061
Fertilization	***	***	***	***
AM inoculation × Fertilization	ns	ns	0.053	ns

Treatments	Root to shoot ratio (dry weight)	Shoot P content (mg plant^{-1})	Root P content (mg plant^{-1})	Root colonization (%)	Spore density (spores 100 g^{-1} soil)
Uninoculated	1.83bc	0.05cd	0.04c	0.00f	0.00f
Uninoculated+Fertilizer	1.77cd	0.13b	0.10a	0.00f	0.00f
A. elegans	2.58a	0.03d	0.03c	52.56d	22.43e
A. elegans+Fertilizer	1.14d	0.19a	0.10a	79.52a	57.71ab
G. etunicatum	2.44ab	0.04d	0.03c	41.96e	20.86e
G. etunicatum+Fertilizer	1.95bc	0.10bc	0.09ab	65.30c	16.14e
G. mosseae	1.72cd	0.04d	0.03c	38.38e	42.28d
G. mosseae+Fertilizer	1.89bc	0.15ab	0.12a	77.58ab	49.14cd
Mixed species	1.65cd	0.07cd	0.05bc	58.87cd	65.14ab
Mixed species+Fertilizer	1.77cd	0.16ab	0.12a	68.32bc	74.57a
Analysis of variance					
AM inoculation	ns	ns	ns	***	***
Fertilization	*	***	***	***	***
AM inoculation × Fertilization	***	ns	ns	***	***

Means in the same column followed by different letter (s) are significantly different by ANOVA and Duncan, s Multiple Range Test. *, **, ***: Significant at $p<0.05$, 0.01, 0.001 respectively; ns: Not significant

on growth parameters were slightly higher it increased growth than non-AM plants without fertilization (controls). Plant growth was highest in all fertilization treatments for plant height (14.5 cm), shoot and root dry weights (0.6 and 0.8 g plant^{-1}, respectively) and shoot and root P contents (0.2 and 0.1 mg plant^{-1}, respectively). Whilst significant mycorrhizal effects were only found in stem diameter, which fungal-fertilizer interactions were not found (Table 7). Stem diameter of *G. etunicatum* and mixed AM species plants with fertilization were highly increased by AM fungi, whereas the highest diameter was found in AM plants with fertilization (0.2 cm). Root colonization and spore density were increased by both fungus species and fertilizer, which interacted (Table 7). Colonization percentages of all AM plants with fertilization were higher than without fertilization. The percentages were high, ranging from 38.4-79.5%, with the highest percentage found in *A. elegans* plants. Spores in fungal treatments with fertilization, generally recovered in higher number than without fertilization. The spore number ranged from 16.1-74.6 spores/100 g soil and the highest density was found in mixed AM species with fertilization.

DISCUSSION

Pot cultures, using host plants grown in soil diluted with sterile sand, are most commonly used to propagate AM fungi (Brundrett *et al.*, 1996). In our present study, all 6 indigenous AM fungi were recovered from all 5 host plant pot cultures. Variation in spore density and mycorrhizal colonization was increased by host plants and AM fungi. Results presented here for spore density and root colonization are in agreement with those found in the greenhouse and the field showing that AM species, host plant species and soil conditions have been reported to effect on mycorrhizal formation and sporulation in pot cultures (Brundrett *et al.*, 1996; Liu and Wang, 2003). From our observation, most plants inoculated with small to medium sized spores of *Glomus* and *Acaulospora* species was generally more successful than inoculation with the larger sized spores of *Scutellospora* species. The spore abundance must be related to their sporogenous characteristics. It has been reported that *Glomus* and *Acaulospora* species usually produce more spores than *Gigaspora* and *Scutellospora* species in the same environment conditions, because smaller spores require a short time to produce spores than large spores (Hepper, 1984; Bever *et al.*, 1996). The high success rates of spore density and colonization percentage of at least 3 of 6 AM species were observed on sorghum and maize suggested that these plants are favorable hosts especially for *A. elegans*, *G. etunicatum* and *G. mosseae* compared to other hosts tested. Thus, sorghum and maize pot culture-produced spores as inoculum in P-deficient soil medium are more suitable for large scale production as well as for research purposes and nursery practice.

In the greenhouse experiment, growth of non-AM plants was very stunted in P-deficient soil medium (available P 1.4 ppm). Applying additional P fertilizer to non-AM plants could enhance growth of plants. However, even with the different allotments of P fertilizer, all AM species greatly stimulated plant growth with bigger size than non-AM plants in this unsuitable soil condition. Enhancement effects on plant growth, tended to increase with increasing P rates and more efficiency varied with the different kinds of AM species. Phosphorus responses for the plant growth agreed with that reported for other AM plants grown in a controlled environment (Siqueira *et al.*, 1998a; Youpensuk *et al.*, 2005), thereby confirming mycorrhizal nutritional benefits and the strong interrelationship between P supply and mycorrhizal response under nutrient-stressed conditions. AM inoculation had slightly effects on seedling growth when plants received low P rates at planting, whereas strongly effects were found at higher P rates. The present results suggested that addition 250 mg P kg^{-1} to *C. acuminatissima* seedlings was suitable to produce either non-AM plants or AM plants in P-deficient soil. Although, non-AM plants at maximal P rate were higher than at minimal rate but still significantly lower than AM plants at the same P addition. The greatest height of AM plants was found in *G. etunicatum* plants with 250 mg P kg^{-1}, exhibited 1.8 fold over non-AM plants. Contrast with stem diameter of seedlings was not improved by the mycorrhizal symbiosis but diameter was also greatest with the highest P added, exhibited 1.5 fold increase over control with no P added. The Mycorrhizal Dependence (MD) of *C. acuminatissima* was high and trended to increase with applying fertilizer into P-deficient soil. In the absence of fertilizer, maximum MD exhibited 43.1% when fertilized with 150 mg P kg^{-1}, maximum MD exhibited 75.9% (data not shown). P-deficient plants lacking AM symbiosis tend to have a high root to shoot ratios usually associated with nutrient-stressed plants (Pacovsky *et al.*, 1986). Whilst, root to shoot ratios of AM plants in our study were higher than for non-AM plants, especially in the absence of P fertilizer or low P rates, the higher ratios probably resulted in mycorrhizal stimulation of root growth for improving P acquisition under limiting P condition. Plants characterized as inefficient at acquiring soil P, may substantially improve P acquisition by morphological and physical adaptations include changes in P and dry matter partitioning that favor growth of roots over shoots and the induction of a high-affinity P uptake and transport system in roots during the development (Cogliatti and Clarkson, 1983; Marschner *et al.*, 1996).

P contents in AM plants were significantly increased with levels of P application. AM fungi most likely increased nutrient uptake from the soil due to the external hyphae can exploring greater soil volume and delivering nutrients to the host plants (Joner and Jakobsen, 1995; Koide and Mosse, 2004). Root colonization of plant species in many greenhouse experiments is diminished by high soil

P availability and concomitant enhanced P concentration in plant tissues (Vaast *et al*., 1996; Youpensuk *et al*., 2005). Contrasting with this suppressive effect observed with AM colonization in *C. acuminatissima* was not differed by increasing P levels application while P status remained unaffected. This experiment showed that AM inoculation of *C. acuminatissima* seedlings produces large plants with improved P status, thus confirming the high AM-dependency of host plant. This study also indicates the tolerance abilities of selected AM species on P-application rates, resulting from their abilities to promote plant P accumulation.

In the nursery experiment, seedlings grown in forest soil medium with slow-release fertilizer applied were slightly bigger than controls. Most AM plants without fertilizer added grew poorly with growth parameters similar to those of non-AM plants. Plant height, shoot and root dry weights and shoot and root P contents were increased by fertilizer but not by mycorrhiza, whereas only stem diameter was increased by both factors. Higher stem diameter of *G. etunicatum* and mixed AM species plants with fertilization may result from direct fungal efficiency effects or fungal-fertilizer interactions in such soil condition. AM colonization and P concentrations of *C. acuminatissima* seedlings were quite high with slow-release fertilizer added. This may be fertilization effect on P accumulation through its influence on AM symbiosis. Heavy application of P fertilizer or sufficient P condition at planting may reduce mycorrhizal formation, sporulation and the MD of host and thus mycorrhizal effectiveness for the seedling growth (Siqueira *et al*., 1998b). From our observation, growth of AM plants grown in forest soil was less than grown in P-deficient soil. This may the result of plants responding well to mycorrhizas in low or moderate P soils are regarded as mycorrhizal dependent. That is, they depend on mycorrhiza to show their full potential (Haselwandter and Bowen, 1996). The components of forest soil medium (available P 15.8 ppm) were rich in both organic and inorganic nutrients. Thus, AM fungi may be loose their function on stimulating plant growth in this nutrient condition. High dissolved inorganic nutrients in tropical forest soil may make AM fungi unnecessary to meet nutrient (Maffia *et al*., 1993). The consistent effects of AM fungi on plant growth were diminished or disappeared with nutrient abundance in the soil. Strongly mycorrhizal effects on external P requirement for maximal growth of seedlings were high and consistent in nutrient poor soil but were diminished and varied unpredictably with levels of fertilizer and P requirement of individual plant species. Phosphorus is not only a suppressed factor on AM symbiosis. Further, Youpensuk *et al*. (2005) reports that application of high rates of P or N can depressed AM colonization and spore formation. *C. acuminatissima* seedling grown in forest soil medium with nutrient abundance did not respond to AM inoculation although root colonization was high. Without fertilizer added, all AM plants were still equal size with non-AM plants. It may be resulted in reduction of AM symbiosis caused decreasing nutrient uptake ability for growth of mycorrhizal plants.

In addition, mycorrhizal inoculation with selected AM fungi and application of optimal P rate on early plant development are highly advantageous for high quality sapling production in forest tree nurseries and sapling establishment in low nutrient soils in forest restoration areas in Thailand. All AM species tested were greatly effective in promoting growth parameters of AM seedling in nutrient poor medium, but diminished their function in nutrient abundant medium. Differential responses of AM seedlings in these experiments appear to be related to nutrient available (inorganic and organic forms) in medium and form of fertilizer (easy soluble or slow-releasing) by plant and AM fungi. Therefore, the success of AM technology will depend upon dependent mycorrhizal host, optimal soil condition and well-adapted effective fungal strains.

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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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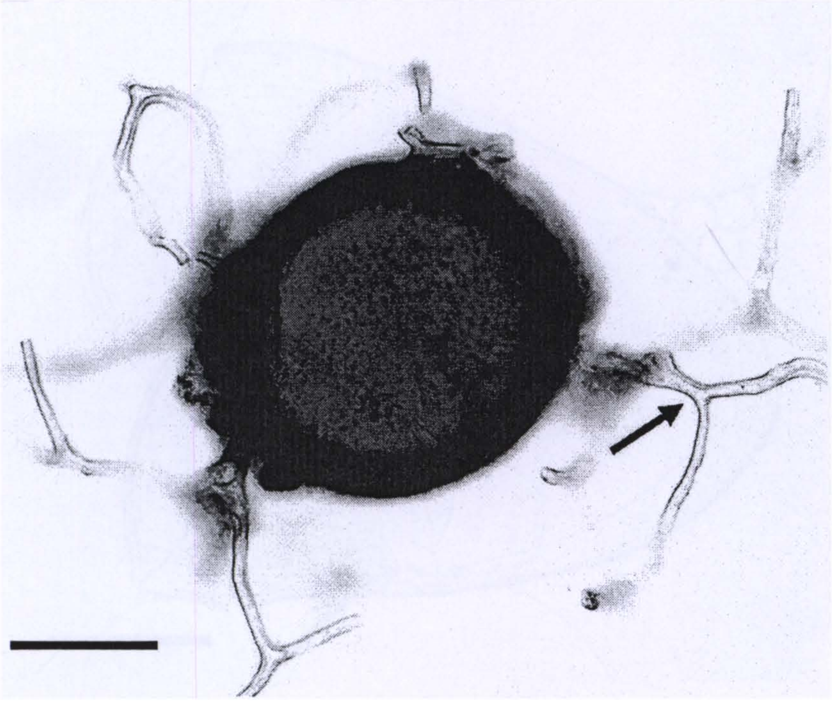

Fig. 1 Glomus multicaule: Spore with multiple subtending hypha (arrow) and rounded projections on the surface, bar = 50 μm.

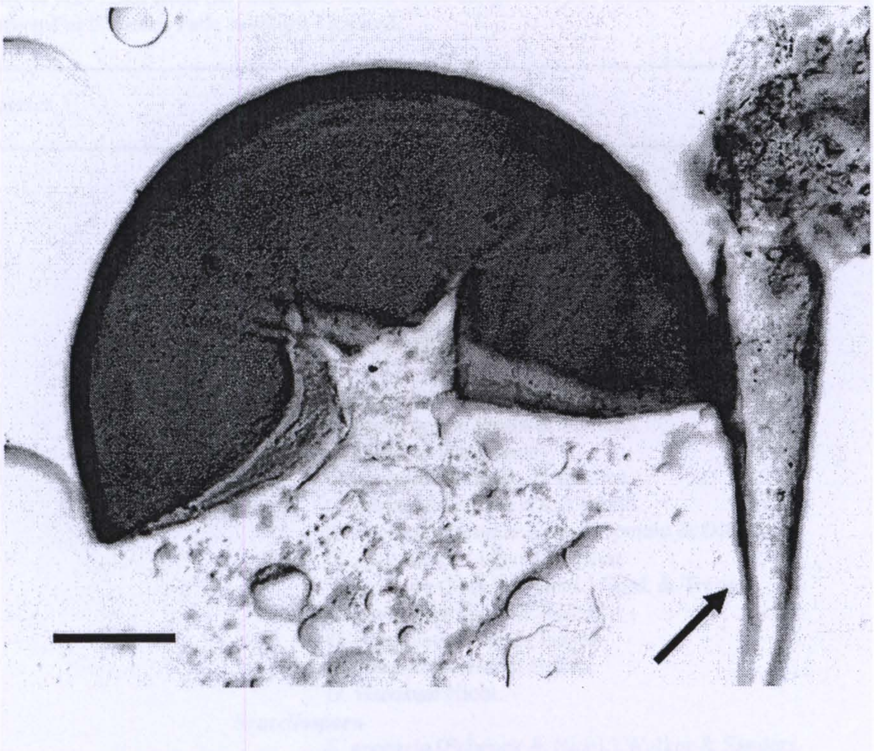

Fig. 2 Acaulospora elegans: Craked spore with sporiferous saccule (arrow) and crowded spines on the surface, bar = 50 μm.

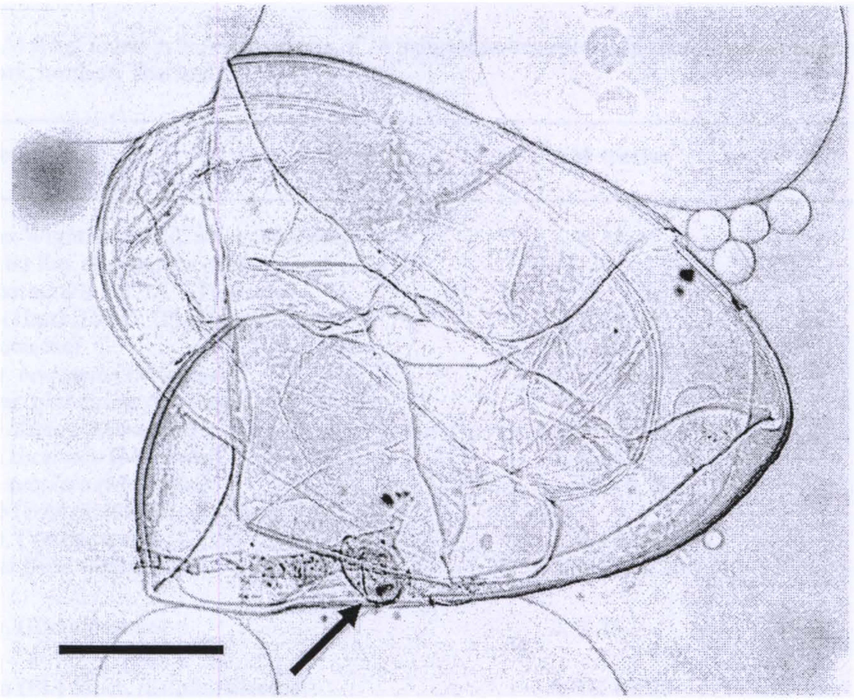

Fig. 3 Scutellospora pellucida: Cracked hyaline spore with hyaline bulbous subtending hypha, bar = 50 µm.

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
	Acaulospora	
1		*A. bireticulata* Rothwell & Trappe
2		*A. elegans* Trappe & Gerd.
3		*A. foveata* Trappe & Janos
4		*A. laevis* Gerd. & Trappe
5		*A. mellea* Spain & Schenck
6		*A. scrobiculata* Trappe
	Glomus	
7		*G. aggregatum* Schenck & Smith
8		*G. ambisporum* Smith & Schenck
9		*G. clavisporum* Trappe
10		*G. coremioides* Berk. & Broome
11		*G. intraradices* Schenck & Smith
12		*G. microaggregatum* Koske, Gemma & Olexia
13		*G. microcarpus* Iqbal & Bushra
14		*G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe
15		*G. multicaule* Gerd. & Bakshi
16		*G. rubiforme* Gerd. & Trappe
17		*G. sinuosum* Gerd. & Bakshi
18		*G. viscosum* Nicol.
	Scutellospora	
19		*S. gregaria* (Schenck & Nicol.) Walker & Sanders
20		*S. heterogama* Walker & Sanders
21		*S. pellucida* (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***
Acrocarpus fraxinifolius Wight ex Arn. (Caesalpinioideae)	3, 4, 14, 15
Balakata baccata (Roxb.) Ess. (Euphorbiaceae)	12, 20
Castanopsis acuminatissima (Bl.) A. DC. (Fagaceae)	1, 15
Erythrina subumbrans (Hassk.) Merr. (Papilionoideae)	2, 7, 12, 19
Ficus altissima Bl. (Moraceae)	2, 6, 10
Ficus benjamina L. var. *benjamina* (Moraceae)	6, 19, 20
Ficus glaberrima Bl. var. *glaberrima* (Moraceae)	16
Ficus hispida L. f. var. *hispida* (Moraceae)	2, 5, 16, 17
Ficus racemosa L. var. *racemosa* (Moraceae)	2, 8, 14, 16, 18
Ficus subulata Bl. var. *subulata* (Moraceae)	2, 14, 16, 20
Glochidion kerrii Craib (Euphorbiaceae)	21
Gmelina arborea Roxb. (Verbenaceae)	2, 6, 13, 15
Heynea trijuga Roxb. ex Sims (Meliaceae)	2, 3, 8, 10, 20
***Hovenia dulcis* Thunb. (Rhamnaceae)**	**2, 7, 15**
***Macaranga denticulata* (Bl.) M.-A. (Euphorbiaceae)**	**9, 10, 16, 20**
Machilus bombycina King ex Hk.f. (Lauraceae)	2, 10, 16, 20
Melia toosendan Sieb. and Zuc. (Meliaceae)	2, 9, 20
Michelia baillonii Pierre (Magnoliaceae)	16, 17
Nyssa javanica (Bl.) Wang. (Nyssaceae)	6, 15, 20
Prunus cerasoides D. Don (Rosaceae)	7, 10, 13, 15, 16
Rhus rhetsoides Craib (Anacardiaceae)	2, 6, 13, 16
Sapindus rarak DC. (Sapindaceae)	2, 20
Sarcosperma arboreum Bth. (Sapotaceae)	10, 11, 20
Spondias axillaris 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 ($\geq$ 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 occured 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.

Soil property [a]	
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

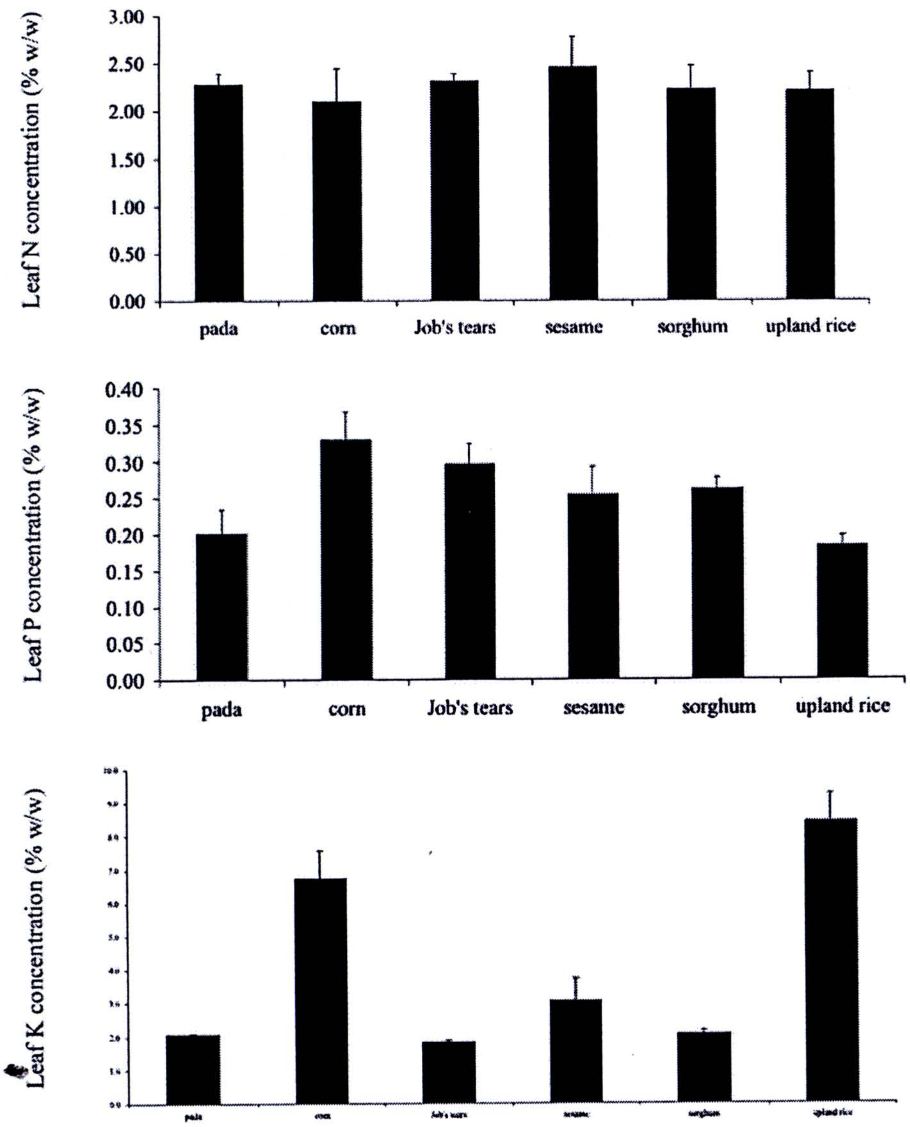

Figure 1. Foliar nutrient concentrations (N, P, K) in pada and swidden crops at Huai Tee Cha fields (vertical bar above each column represents one S.E.).

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	*Coix lachryma-jobi* L.	*	*	*	
Glutinous corn	*Zea mays* L.	*	*		
Sorghum	*Sorghum bicolor* L.	*	*		
Rice	*Oryza sativa* L.	*			*
White/black seed sesame	*Sesame indicum* L.	*			*

Sources: household interview in 2005 (after crop harvests)
F1=Food, F2=Animal feed, Or=Ornamental, SC=Spirit ceremony

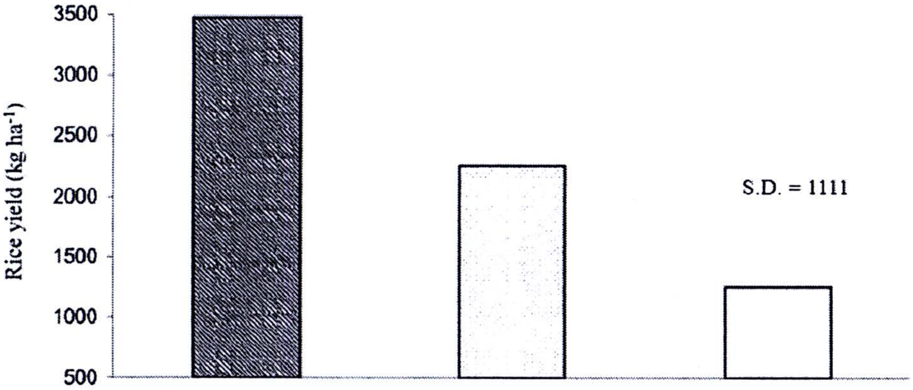

Figure 2. Rice yield (kg ha^{-1}) of Kayo, Murkur and Takae fields at Huai Tee Cha village in cropping year 2005.

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 identifed: *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 Kjehldahl 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 devided 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 (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 $H' = -\sum(n_i/N)\ \log2(n_i/N)$, 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 varience (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 Characteristisc 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 centrifigation 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 charateristics. 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 $\alpha = 0.05$

[1] mean±SD, n = 30

[2] mean±SD, n = 2

Table 4 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***
Acaulospora		**5**	**18.5**	**45**	**100**	**8**	**42.1**	**13**	**38.2**	**19**	**12.7**	**74**	**49.3**	**10**	**23.8**	**51**	**45.5**	**2**	**9.5**	**156**	***95.7***
CMU01	*Acaulospora spinosa*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	4.8	-	-
CMU02	*Acaulospora foveata*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	4.8	73	*44.8*
CMU03	*Acaulospora tuberculata*	-	-	-	-	-	-	3	8.8	-	-	-	-	-	-	31	27.7	-	-	-	-
CMU04	*Acaulospora colossica*	-	-	-	-	-	-	-	-	-	-	-	-	3	7.1	7	6.2	-	-	-	-
CMU06	*Acaulospora scrobiculata*	5	18.5	3	6.7	2	10.5	5	14.7	7	4.7	5	5.8	-	-	-	-	-	-	2	*1.2*
CMU07	*Acaulospora* sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	*1.2*
CMU08	*Acaulospora denticulata*	-	-	-	-	-	-	-	-	-	-	2	2.3	-	-	-	-	-	-	-	-
CMU09	*Acaulospora dilatata*	-	-	-	-	-	-	-	-	-	-	11	12.8	-	-	-	-	-	-	40	*24.5*
CMU10	*Acaulospora rehmii*	-	-	-	-	2	10.5	2	5.9	-	-	-	-	-	-	-	-	-	-	-	-
CMU11	*Acaulospora nicolsonii*	-	-	-	-	-	-	3	8.8	-	-	-	-	1	2.4	-	-	-	-	2	*1.2*
CMU12	*Acaulospora excavate*	-	-	-	-	4	21.0	-	-	12	8.0	13	15.1	6	14.3	7	6.2	-	-	-	-
CMU13	*Acaulospora* sp.	-	-	2	4.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CMU14	*Acaulospora lacunosa*	-	-	37	82.2	-	-	-	-	-	-	31	36.0	-	-	6	5.4	-	-	-	-
CMU15	*Acaulospora* sp.	-	-	3	6.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CMU16	*Acaulospora morrowiae*	-	-	-	-	-	-	-	-	-	-	12	14.0	-	-	7	6.2	-	-	-	-
CMU26	*Acaulospora* sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	37	*22.7*
Entrophospora		-	-	-	-	-	-	-	-	74	43.3	-	-	-	-	-	-	-	-	-	-
CMU05	*Entrophospora colombiana*	-	-	-	-	-	-	-	-	*74*	*49.3*	-	-	-	-	-	-	-	-	-	-

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 clavisporum*	-	-	-	-	-	-	10	29.4	-	-	-	-	-	-	-	-	-	-	-	-
CMU22	*Glomus sinuosum*	-	-	-	-	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 heterogama*	-	-	-	-	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. excavate* 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 ≥ 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 (Tawaraya 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 indigeneous 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 ecosites. 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 relativ abundances (RA) under physic nut rhizosphere (dominant species are written in bold).

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

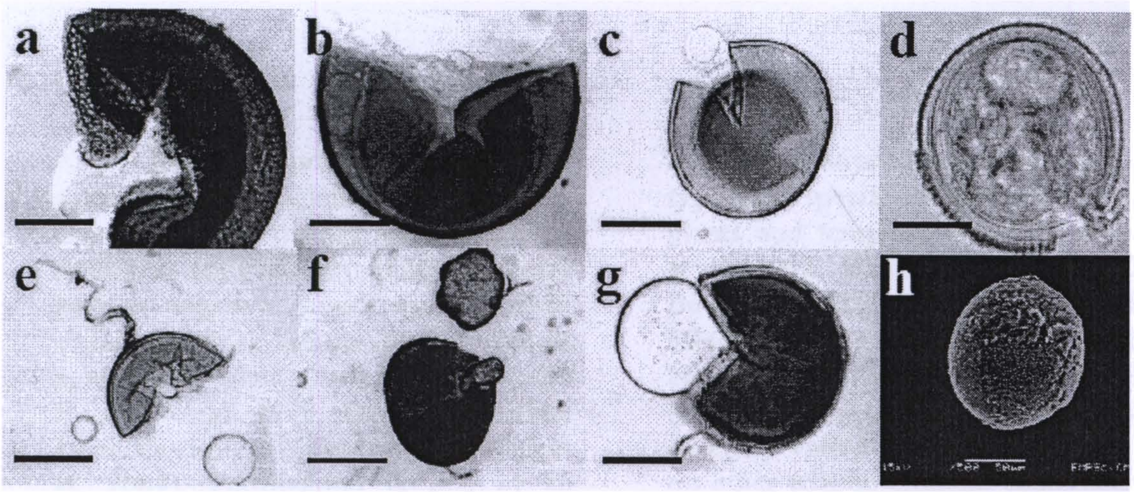

Fig.2 Spore characteristics of the dominant AMF collected from physic nut rhizosphere under light (a-g) and scanning electron (h) microscopes. *Acaulospora foveata* (CMU02) [a], *Entrophospora colombiana* (CMU05) [b, h], *A. dilatata* (CMU09) [c], *A. lacunosa* (CMU14) [d], *Gigaspora rosea* (CMU29)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

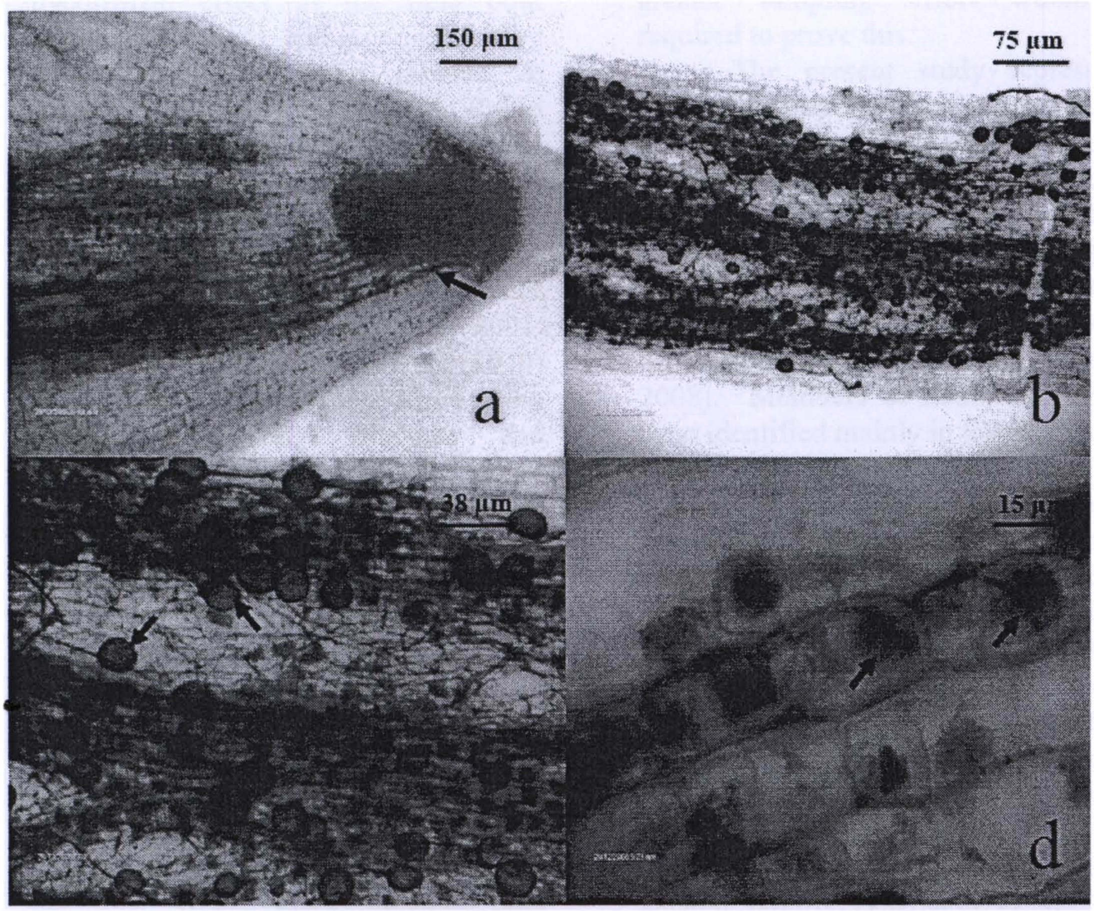

Fig.3. AMF colonization in physic nut roots collected from the field. (a) AMF structures near the root tip (arrow), (b) highly infected root, (c) vesicles (arrows), and (d) arbuscules (arrows).

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 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 samlings 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 soisl in our study sites were acidic. Therfore 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 planjt 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 haborred 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	
M. denticulata (Youpensuk et al. 2004)	6	1	-	2	17	1	2	-	29
A. mangium (Weravart 2003)	6	-	-	7	7	-	6	1	27
Z. mays (Nabhadalung et al. 2005)	2	-	2	-	9	-	1	2	16
Indigenous trees (Nandakwang et al. 2008)	6	-	-	-	15	-	3	-	24
J. curcas (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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