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

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The dependence of physic nut (*Jatropha curcas* L.) on beneficial soil fungi for growth is not known. Therefore, the spore density and species diversity of arbuscular mycorrhizal fungal (AMF) associated with physic nut was assessed by extracting spores from physic nut plantings from 10 sites across 6 provinces in northern and north-eastern Thailand. Approximately 700 AMF spores, obtained using the wet sieving and sucrose gradient centrifugation methods, were identified into morphospecies. Colonization by AMF was assessed under a compound microscope using root samples stained with trypan blue. 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 ranged from 3 to 11 (average 6.2). Roots of physic nut were colonized by AMF at all sites sampled and infection levels ranged from 38 to 94% of root length. The presence of mycorrhizas in soils varying in pH from acidic to calcareous, of low to moderate organic matter and of low to high available P suggests that physic nut may be highly dependent on AMF.

Key words – ecology – species diversity – soil chemistry – spore density

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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 species is introduced 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. The optimistic yield estimate for physic nut is 1,300 litre of oil per hectare, lower than oil palm but higher than canola (Anonymous 2007). As diesel fuel prices continue to escalate, opportunities will open for conversion from crude-oil into bio-diesel based

fuel consumption. Several countries as well as domestic and international organizations have proposed greatly increasing the area under physic nut cultivation for oil production.

Arbuscular mycorrhizal fungi (AMF) are abundant and ubiquitous in almost all natural terrestrial communities and form obligate symbiotic associations with some 80% of vascular plants (Harley & Smith 1983, Smith & Read 1997). It is apparent that these fungal symbionts are 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). A number of authors have documented that associations between agronomic plant 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 some members of the Euphobiaceae 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 Thailand. We hypothesized that the mycorrhizal status and species richness differ between sites. Furthermore, soil conditions and plant age are likely to play a key role for determining species diversity on root tips of physic nut.

Methods

Sample collection

Ten 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) provinces were selected as study sites 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 (5 cm diameter). Approximately 1 kg total of rhizosphere soil from each site was collected. Soil samples were mixed into composite samples and root samples were removed from the soil and preserved in 70% ethanol for each tree. Each composite sample representing one plot was a mixture of four soil core samples. The samples were kept in an ice-box and transported by car to a laboratory. All soil samples were kept in a cold room and processed within one month The analysis of soil samples included AMF spore isolation and enumeration, identification of species; and determination of the following soil parameters: soil moisture content (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.

AMF spore isolation and identification

AMF spores occurring in the rhizosphere soil samples were extracted by wet sieving and sucrose density gradient centrifugation methods (Brundrett et al. 1996). 100 g of each soil sample was suspended in 500 ml of water and stirred for 10 mins. Sieve sizes of 250, 106 and 45 μm, were used for spore collection. The spores retained on each sieve were transferred to 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]).

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.

Table 1. Geographic location and number of soil samples in each site.

Geography	Site *									
	CR1	**CR2**	**CM1**	**CM2**	**CM3**	**CM4**	**LO1**	**LP1**	**KK1**	**NK1**
Latitude	E99º48′	E99º26′	E98º55′	E98º30′	E98º55′	E98º54′	E101º21′	E99º07′	E102º53′	E102º43′
Longitude	N19º54′	N19º52′	N18º45′	N18º09′	N18º45′	N18º44′	N17º27′	N18º34′	N16º23′	N17º51′
MSL**(m)	398	399	340	1,137	340	360	800	337	150	163
Sample 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

Spore density (SD) is the number of spores in 100 g soil. Relative abundance (RA) was defined as the percentage of spore numbers of a species divided by the total spores observed (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 was observed. The dominant AMF species according to relative abundance (RA > 6%) and spore density in 100 g soil (spore density higher than 40 spores) and species richness were determined for 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 data were arc sin transformed prior to analysis. One-way analysis of variance (ANOVA) was carried out for root colonization and spore density. Statistical analyses were performed with the Statistical Package for Social Sciences version 11.5 (SPSS Inc., Wacker Drive, Chicago, IL). All factors were analyzed at $\alpha = 0.05$.

Results

Soil characteristics

Soil pH ranged from 5.3 to 8.0, OM 0.63 to 7.22%, N 0.02 to 0.44%, P 11.5 to 175.5 mg/kg, K 22.2-1058.0 mg/kg and soil moisture 7-23% across the sites (Table 2).

AMF status

In total, 699 AMF spores and sporocarps were derived using wet sieving and sucrose gradient centrifugation methods from 95 rhizosphere soil samples of physic nut. Spore density in samples 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 many of the sites (Table 3).

Thirty four morphospecies of AMF were identified using spore characteristics. Species richness of AMF ranged from 3 to 11 (average 6.1). Species were distributed as follows: 3 species from CR1, 4 from CR1, 6 from CM1, 7 from CM2, 5 from CM3, 11 from CM4, 7 from LO1, 6 from LP1, 4 from KK1 and 8 species from KK1 (Table 4). *Acaulospora* and *Glomus* occurred most frequently and, overall, were the most prevalent, containing 16 and 10 species, respectively. There were 5 species in *Scutellospora*, 2 species in *Gigaspora* and 1 species in *Entrophospora* across the 10 sampling sites (Table 4).

In this study, *Acaulospora scrobiculata* was the most widely distributed species. It was found in 7 out of 10 sampling sites, namely CR1, CR2, CM1, CM2, CM3, CM4 and NK1 (70% IF). The next most widely distributed taxon was *A. excavate* which appeared in 5 sites, CM1, CM3, CM4, LO1 and LP1 (50% IF). Some species were only found at one site (10% IF), for example, *Acaulospora* sp.2 and *Acaulospora* sp.3 in CR2, *A. denticulata, Glomus* sp.5 and *Scutellospora* sp.1 in CM4, *A. spinosa* in KK1, *Acaulospora* sp.1, *Acaulospora* sp.4 and *G. etunicatum* in NK1, *G. clavisporum* and *Scutellospora* sp.2 in CM2, *E. colombiana* and *Gi. rosea* in CM3, *G. fulvum* in CR1 and *Glomus* sp.1, *Glomus* sp.2 and *Scutellospora* sp.3 in LP1 (Table 4).

Table 2. Soil characteristics of 10 physic nut plantings in northern and north-eastern Thailand.

Site	Plantation age (year)	pH_{H2O}	Moisture (%)	OM (%)	N (%)	P (mg/kg)	K (mg/kg)
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<10 mg/kg is Low (L), P ranging between 11 and 25 mg/kg is Medium (M), P ranging between 26 and 45 mg/kg is High (H), P>45 mg/kg is Very High (VH).

Table 3. Spore density (SD), Shannon-Weiner index (*H´*), Simpson's index (*D*) and root colonization by AMF at 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

Based on spore density and relative abundance, seven 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.1 (53 spores, 7.6%) and *Gi. rosea* (51 spores, 7.3%) (Table 5). The morphological characteristics of some dominant AMF are illustrated in Fig. 2.

Species diversity was calculated using two indices. The Shannon–Weiner diversity index ranged from 0.28 to 0.83. The highest occurred in CM4 (*H´*=0.83) and the lowest in CR2 (*H´*=0.28). Similarly, the Simpson's index ranged from 0.32 to 0.86, the highest was in CM1 and the lowest was 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 exceeded 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).

Table 4. Spore density (S) and relative abundance (RA) of AMF at each sample site.

Species	Code	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**
A. colossica	CMU04	-	-	-	-	-	-	-	-	-	-	-	-	3	7.1	7	6.2	-	-	-	-
A. denticulata	CMU08	-	-	-	-	-	-	-	-	-	-	2	2.3	-	-	-	-	-	-	-	-
A. dilatata	CMU09	-	-	-	-	-	-	-	-	-	-	11	12.8	-	-	-	-	-	-	40	24.5
A. excavata	CMU12	-	-	-	-	4	21.0	-	-	12	8.0	13	15.1	6	14.3	7	6.2	-	-	-	-
A. foveata	CMU02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	4.8	73	44.8
A. lacunosa	CMU14	-	-	37	82.2	-	-	-	-	-	-	31	36.0	-	-	6	5.4	-	-	-	-
A. morrowiae	CMU16	-	-	-	-	-	-	-	-	-	-	12	14.0	-	-	7	6.2	-	-	-	-
A. nicolsonii	CMU11	-	-	-	-	-	-	3	8.8	-	-	-	-	1	2.4	-	-	-	-	2	1.2
A. rehmii	CMU10	-	-	-	-	2	10.5	2	5.9	-	-	-	-	-	-	-	-	-	-	-	-
A. scrobiculata	CMU06	5	18.5	3	6.7	2	10.5	5	14.7	7	4.7	5	5.8	-	-	-	-	-	-	2	1.2
A. spinosa	CMU01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	4.8	-	-
A. tuberculata	CMU03	-	-	-	-	-	-	3	8.8	-	-	-	-	-	-	31	27.7	-	-	-	-
A. sp.1	CMU07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	1.2
A. sp.2	CMU13	-	-	2	4.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. sp.3	CMU15	-	-	3	6.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. sp.4	CMU26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	37	22.7
Entrophospora		**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**74**	**43.3**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
E. colombiana	CMU05	-	-	-	-	-	-	-	-	74	49.3	-	-	-	-	-	-	-	-	-	-
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**
G. clavisporum	CMU21	-	-	-	-	-	-	10	29.4	-	-	-	-	-	-	-	-	-	-	-	-
G. etunicatum	CMU18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	1.8
G. fulvum	CMU27	15	55.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G. sinuosum	CMU22	-	-	-	-	5	26.3	4	11.8	-	-	-	-	-	-	-	-	-	-	-	-
G. sp.1	CMU17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	2.7	2	9.5	-	-
G. sp.2	CMU19	-	-	-	-	-	-	-	-	-	-	-	-	6	14.3	-	-	-	-	-	-
G. sp.3	CMU20	-	-	-	-	-	-	-	-	-	-	-	-	16	38.1	-	-	-	-	-	-
G. sp.4	CMU23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	17	80.9	-	-
G. sp.5	CMU24	-	-	-	-	-	-	-	-	-	-	1	1.2	-	-	-	-	-	-	-	-
G. sp.6	CMU25	-	-	-	-	-	-	-	-	6	4.0	2	2.3	-	-	-	-	-	-	-	-
Gigaspora		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**51**	**34**	**2**	**2.3**	**0**	**0**	**51**	**45.5**	**0**	**0**	**0**	**0**

Table 4 (Continued). Spore density (S) and relative abundance (RA) of AMF at each sample site.

Species	**Code**	**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**
Gi. rosea	CMU29	-	-	-	-	-	-	-	-	51	34.0	-	-	-	-	-	-	-	-	-	-
Gi. sp.	CMU28	-	-	-	-	-	-	-	-	-	-	2	2.3	-	-	51	45.5	-	-	-	-
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**
S. pellucida	CMU31	7	25.9	-	-	3	15.8	-	-	-	-	3	3.5	-	-	-	-	-	-	4	2.4
S. heterogama	CMU33	-	-	-	-	3	15.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. sp.1	CMU30	-	-	-	-	-	-	-	-	-	-	4	4.7	-	-	-	-	-	-	-	-
S. sp.2	CMU32	-	-	-	-	-	-	7	20.6	-	-	-	-	-	-	-	-	-	-	-	-
S. sp.3	CMU34	-	-	-	-	-	-	-	-	-	-	-	-	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**	

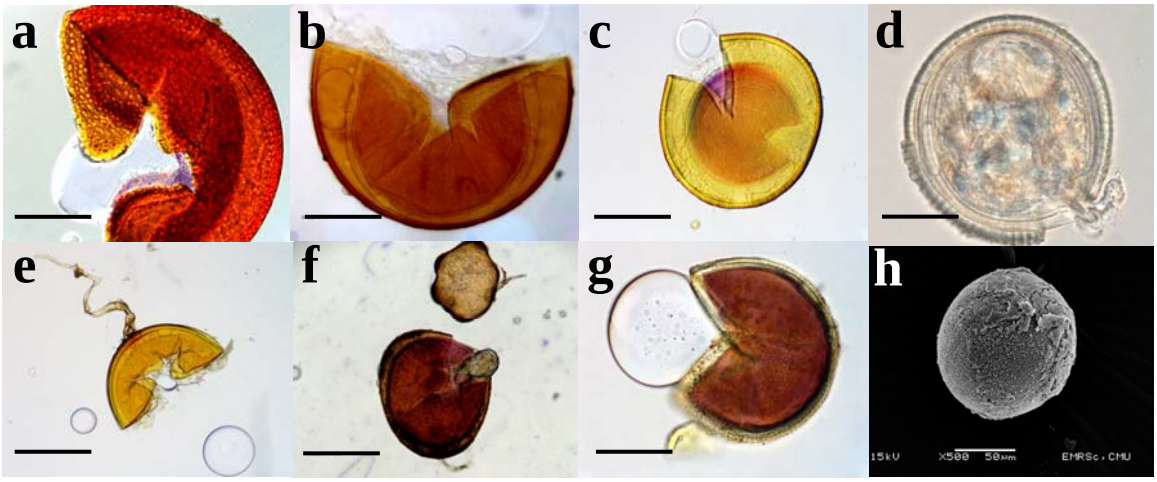

Fig. 2 – Spore characteristics of the dominant AMF collected from physic nut rhizosphere under light (a-g) and scanning electron (h) microscopes. *Acaulospora foveata* (CMU02) [a], *Entrophospora colombiana* (CMU05) [b, h], *A. dilatata* (CMU09) [c], *A. lacunosa* (CMU14) [d], *Gigaspora rosea* (CMU29) [e], *Gigaspora* sp.1 (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.

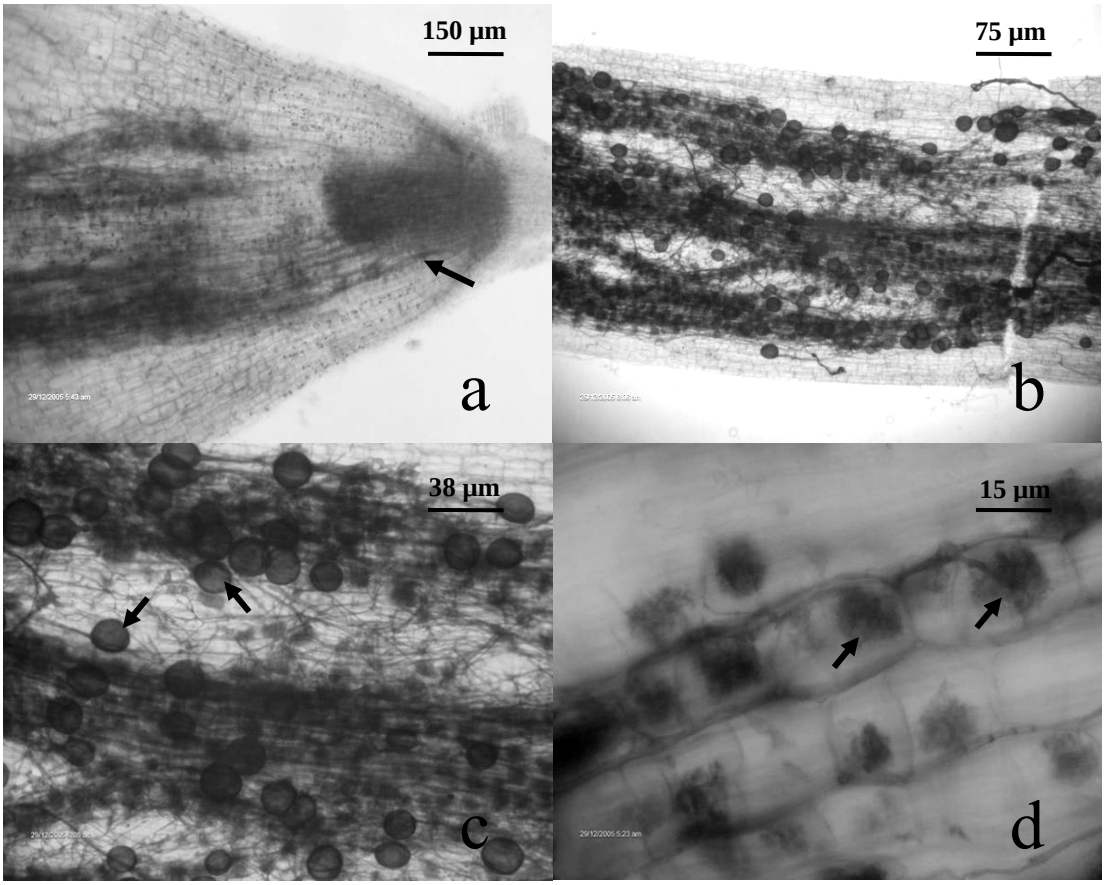

Fig. 3 – AMF colonization in physic nut roots collected from the field. (a) AMF structures near the root tip (arrow), (b) a highly infected root, (c) vesicles (arrows), and (d) arbuscules (arrows).

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

Code	Species	S	IF	RA
CMU04	*Acaulospora colossica* P.A. Schultz, Bever & J.B. Morton	10	10	1.4
CMU08	*Acaulospora denticulata* Sieverd. & S. Toro	2	10	0.3
CMU09	***Acaulospora dilatata*** J.B. Morton	**51**	**20**	**7.3**
CMU12	***Acaulospora excavata*** 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.1	2	10	0.3
CMU13	*Acaulospora* sp.2	2	10	0.3
CMU15	*Acaulospora* sp.3	3	10	0.4
CMU26	*Acaulospora* sp.4	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.1**	**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.1	5	20	0.7
CMU19	*Glomus* sp.2	6	10	0.9
CMU20	*Glomus* sp.3	16	10	2.3
CMU23	*Glomus* sp.4	17	10	2.4
CMU24	*Glomus* sp.5	1	10	0.1
CMU25	*Glomus* sp.6	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.1	4	10	0.6
CMU32	*Scutellospora* sp.2	7	10	1.0
CMU33	*Scutellospora heterogama* (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders	3	10	0.4
CMU34	*Scutellospora* sp.3	10	10	1.4
Total: AMF 34 species		**699**		**100**

Discussion

Members of the plant 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 report for the first time the population density, composition of AMF and root colonisation in selected physic nut plantings in Thailand. AMF were present in all the study sites both in physic nut roots and in soil with moderate to high levels of colonisation regardless of plant age as grapevine (Schreiner & Mihara 2009). In contrast Narendra et al. (2009) observed higher colonisation in older plants in India. Nevertheless, this suggests that the plant is strongly mycorrhizal dependent.

The number of AMF species obtained in the present study (34 species) is similar to other studies for a range of hosts in Thailand (Table 6). Weravart (2003) identified 27 AMF species from *Acacia mangium* in the North and North-eastern part of Thailand whilst Nandakwamg et al. (2008) described 24 AMF species from indigeneous forest trees in the North of Thailand. Furthermore, Youpensuk et al. (2004) reported 29 AMF species associated with *Macaranga denticulata* in upland shifting agriculture in the North of Thailand. The number of AMF species in the present study, however, were double the number of AMF species detected under continuous maize cropping in central Thailand where high P and N inputs were applied in long-term fertilization trials

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	
Macaranga denticulata (Youpensuk et al. 2004)	6	1	-	2	17	1	2	-	29
Acacia mangium (Weravart 2003)	6	-	-	7	7	-	6	1	27
Zea mays (Nabhadalung et al. 2005)	2	-	2	-	9	-	1	2	16
Indigenous trees (Nandakwang et al. 2008)	6	-	-	-	15	-	3	-	24
Jatropha 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.

(Nabhadaluang et al. 2005).

Studies undertaken in China have provided the same order of AMF diversity as recorded so far in Thailand. For example, in tropical rain forests in south China, Zhao et al. (2003) identified 27 AMF species and Dandan & Zhiwei (2007) recorded 43 AMF morphospecies from a hot and dry valley in south-west China. By contrast, 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 there were 34 AMF species associated with physic nut roots but the species richness at a site level was low (average 3.4 AMF species per site).

Previous studies have shown that agricultural management practices, such as tillage, fertilization and cropping systems, have a negative impact on the AMF associated 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 an important abiotic factor influencing growth, colonisation, sporulation, composition and distribution of AMF (Johnson 1993, Egerton-Warburton & Allen 2000, Nabhadalung et al. 2005, Zhang et al. 2006, Wang et al. 2009). In general, high levels of P fertilizer can negate the mycorrhizal effect in the field, reducing infection of roots and sporulation (Douds & Schenck 1990, Tang et al. 2001, Rubio et al. 2003, Alguacil et al. 2010). Several authors have indicated that increasing P fertilization significantly reduced the species diversity of AMF and altered the species composition (Johnson 1993, Kahiluoto et al. 2001, Wang et al. 2009). Alguacil et al. (2010) clearly demonstrated that P fertilization affected AMF diversity and composition in a tropical savanna forage system when different sources and high doses of P were applied. Apart from their P contents, high soil N contents can also influence the species composition of AM fungi and colonisation (Blanke et al. 2005, Treseder & Allen 2002, Wang et al. 2009). In our case, most soil samples had medium to very high concentrations of available P. The high P contents may have contributed to the low species richness and diversity at the site level. 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) concluded that AMF diversity varies considerably depending on edapho-climatic conditions, including rainfall, soil texture and soil management practices. 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 showed that *Acaulospora* was the predominant genus in terms of spore density and species diversity (Table 4). A similar finding was obtained from rhizosphere soil under food crops planted into an upland swidden farm and in dry tropical forests in northern Thailand (Nandakwang et al. 2008, Wongmo 2008). Species of *Acaulospora* have been identified mainly in low input farming systems, forest and grassland soils. They are considered as facultative symbionts adapted to a wide array of soil and host species, appearing in soils 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).

Glomus species are considered as cosmopolitan fungi in many ecosystems (Sýkorová et al. 2007). They dominate habitats in cold, temperate and tropical regions. They usually occur in neutral and slightly alkaline soil (Mukerji et al. 2002). In particular, *G. etunicatum* has a worldwide distribution and can be found in many ecotypes (Becker & Gerdemann, 1977). In Thailand, *Glomus* was the most prevalent AMF genus under *Macaranga denticulata* (Youpensuk et al. 2004), an indigenous colonizing tree of North Thailand (Nandakwang et al. 2008). Most of the soils in our study sites were acidic. Therefore, this could explain our less frequent detection of *Glomus*.

Other genera were less common in the present study, with only a few examples of species, such as *Entrophospora colombiana, Gigaspora rosea, Scutellospora pellucida* and *S. heterogama*. There were only a small number of species present in the Gigasporaceae. Often, *Gigaspora* species predominate in sandy soils such as dunes (Lee & Koske 1994). Many soils in northern Thailand have a low sand content. *Scutellospora* is an 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 & Morton 1996). It also appears that *Scutellospora* might be a poor competitor in colonizing plant roots and that the host plants favours fungi from the Glomerales (Sýkorová et al. 2007). Species of *Gigaspora* and *Scutellospora* are much more frequently associated with wild plants than with field crops (Gai et al. 2006).

Physic nut was well colonized by AMF in all field locations studied. The ability of this crop to harbor AMF across a wide range of site conditions makes it a good potential crop for large scale plantations. To our knowledge, the establishment of physic nut plantations in Thailand is often directed towards land that previously supported crops such as cassava or was forested or has become degraded. With land degradation, topsoils become eroded and leached. The associated loss of indigenous AMF means that the land has a limited capability to support growth of either indigenous or agronomic plants species without addition of considerable amounts of fertilizer. Under these situations, application of suitable and effective AMF should benefit the establishment of physic nut plantations.

Further research is required to identify effective AMF for physic nut in Thailand that can be used in inoculation programs in order to restore AMF diversity to degraded lands.

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