CHAPTER 3

Diversity of arbuscular mycorrhizal fungi and glomalin in rhizosphere soils of *Aquilaria crassna* and *Tectona grandis*

3.1 Introduction

Timber harvesting results in more than 5 million hectares of tropical forest annually being transformed into degraded, poorly managed forests and affects not only the sustainable production of timber but also the global environment (Kobayashi, 2004). At present, woodlot growing is required for timber and non-timber products. *Aquilaria crassna* Pierre ex Lec. (agarwood) is a threatened tropical forest tree species included in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, 2004). This species is native to Thailand and of particular economic interest as it is the principal source of aromatic, resin-infused wood used for incense, perfume, traditional medicines and other goods (Chen *et al*., 2013). The increasing interest in establishing *Aquilaria* plantings worldwide is largely due to its high rate of decline in natural stands owing to over-harvesting in tropical Asian and Pacific countries. *Tectona grandis* Linn.f. (teak) is well known for its high quality timber, and has also been used for traditional medicine in Southeast Asia. It was the most important export timber in Thailand until all logging in natural forests was banned in 1989 (Kollert and Cherubini, 2012). At present, quality, uniform plantlets of *A. crassna* and *T. grandis* are produced in large scale via tissue culture.

Arbuscular mycorrhizal (AM) fungi are the most important symbionts of terrestrial plants; more than 85% of those plants form this root/fungus association (Harley and Smith, 1983). AM fungi have major effects on plant growth, the best known is increasing phosphorus uptake. In addition, AM fungi can improve soil aggregate stability through the action of mycelia and glomalin (Wright and Upadhyaya,

1998; Rillig, 2004). Glomalin is a glycoprotein produced by AM fungi which acts as a soil particle-binding agent. Glomalin in soils, typically quantified as glomalin-related soil protein (GRSP), is an alkaline-soluble protein material with a low turnover (Rillig, 2004), and as such contributes to the soil carbon pool in native grasslands (Purin *et al*., 2006).

Previously, AM fungi were used as inocula for enhance growth of *Aquilaria* sp. and *T. grandis*. Colonization of *A. filaria* (Turjaman *et al*., 2006) and *T. grandis* (Rajan *et al*., 2000; Swaminathan and Srinivasan, 2006) by AM fungus increased plant height, diameter, and shoot and root dry weights, shoot N and P concentrations of the seedlings and survival rates after transplantation. Moreover, Tabin *et al*. (2009) proposed that mycorrhizal inoculation could reduce the percentage of damping-off disease of *A. agallocha* seedlings caused by the pathogenic fungus (*Pythium aphanidermatum*). Arbuscular mycorrhizal fungi have been applied to plantlets and have benefited early tree establishment in the field (Habte *et al*., 2001; Urgiles *et al*., 2009). Information about AM fungal diversity in *T. grandis* and *A. crassna* has been reported mostly from natural habitats in India (Thapar and Klan, 1988; Kanakdurga *et al*., 1990; Tamuli and Boruah, 2002; Singh *et al*., 2003; Dhar and Mridha, 2012). There are few reports of AM fungi in rhizosphere soil of both trees in Thailand (Ramanwong, 1998), especially in *A. crassna*.

Given the importance of these tree species and the potential economic and biological impact of the AM symbiosis, a study of the naturally-occurring symbionts in natural and older managed stands of these trees is important for future management decisions regarding inoculation and inoculum production. The aim of the study reported here was to conduct a detail examination of the AMF status of *A. crassna* and *T. grandis* cultivated fields, the relationship between edaphic factors, AMF spore density, root colonization and soil glomalin concentrations in different regions of Thailand.

3.2 Materials and methods

3.2.1 Study sites and sampling

Most *T. grandis* plantations in Thailand are in the north, e.g. Chiang Mai, Chaing Rai and Phetchabun provinces, while *A. crassna* plantations are mostly in the east, e.g. Chanthaburi, Rayong and Trat provinces and central, Nakhon Nayok and Prachin Buri provinces. Rhizosphere soils were sampled from plantations of *T. grandis* and *A. crassna* in eight study plots located in five provinces of Thailand (Figure 3.1 and 3.2; Table 3.1): Doi Saket, Chiang Mai province (DCM); Wiang Papao, Chiang Rai province (WCR); Khao cha-ngok (KCP) and Chondan (CDP), Phetchabun province; Banna (BNN) and Thai Orchids Lab Ltd. (TOL), Nakhon Nayok province; and Meung (MRY) and Glang (GRY), Rayong province. DCM and WCR sites were monocultures of *T. grandis*, planted 2 m apart and left to grow naturally with accumulated leaf litter and some understory perennial plants. *Tectona grandis* and *A. crassna* were planted alternately at KCP, CDP and TOL sites, but only *T. grandis* was sampled at CDP. Weed control at those three sites was by plowing or herbicide treatment, and they were fertilized with organic fertilizer (chicken or cow dung). MRY, GRY and BNN sites were monocultures of *A. crassna*. Trees were left to grow naturally at MRY and BNN, but organic fertilizer (chicken dung) and weed control (plowing) were used at GRY.

Figure 3.1 Map of Thailand showing five provinces of study sites: (A) Chiang Rai province; (B) Chiang Mai province; (C) Phetchabun province; (D) Nakhon Nayok province; (E) Rayong province.

Figure 3.2 Study sites in 8 study plots, (A) Meung (MRY), Rayong province; (B) Thai Orchids Lab Co., Ltd. (TOL), Nakhon Nayok province; (C) Khao cha-ngok (KCP); (D) Chondan (CDP), Phetchabun province; (E) Glang (GRY), Rayong province; (F) Banna (BNN), Nakhon Nayok province; (G) Wiang Papao (WCR), Chiang Rai province and (H) Doi Saket (DCM), Chiang Mai province.

At each site, approximately 500 g of soil was randomly sampled to a depth of 15 cm at three replicate locations within 50 m^2. Only roots attached to the main roots of both plants were sampled. All collections were carried out during the June–July, 2009 rainy season. Soil samples were air dried and passed through a 2 mm mesh to remove stones, fine roots and other debris before processing.

Table 3.1 Site chemical properties and agricultural management of soils collected from various difference of *A. crassna* and *T. grandis* cultivation sites during wet season (June–July).

Study plot	Location	Plant species	Soil texture	Soil pH[a]	Soil organic carbon (g/100g)[a]	Agricultural management
Khao cha–ngok (KCP)	100º 47´ E / 16º 2´ N	*A. crassna* and *T. grandis*	Loamy soil	5.6 cd	2.96 a	Plowing, organic fertilizer, herbicide
Chondan (CDP)	100º 58´ E / 16º 12´ N	*T. grandis*	Loamy soil	6.4 ab	2.82 a	Plowing, organic fertilizer, herbicide
Thai Orchids Lab (TOL)	101º 7´ E / 14º 16´ N	*A. crassna* and *T. grandis*	Silty soil	6.7 a	3.53 a	Plowing, organic fertilizer, herbicide
Banna (BNN)	101º 16´ E / 14º 9´ N	*A. crassna*	Loamy soil	6.3 ab	2.56 a	No management
Glang (GRY)	101º 42´ E / 12º 47´ N	*A. crassna*	Loamy soil	5.7 bcd	1.78 a	Plowing, organic fertilizer
Meung (MRY)	101º 29´ E / 12º 40´ N	*A. crassna*	Sandy soil	6.2 abc	2.86 a	No management
Doi Saket (DCM)	99º 15´ E / 18º 58´ N	*T. grandis*	Peaty soil	5.7 bcd	3.96 a	No management
Wiang Papao (WCR)	99º 29´ E / 19º 14´ N	*T. grandis*	Loamy soil	5.2 d	2.98 a	No management

[a] Means of three observations. Means in the same column followed by the same letter are not significant different at $P<0.05$ (ANOVA and Tukey's pairwise comparisons).

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3.2.2 Determination of root length colonization

Three replicates of fine roots from each site were carefully removed and thoroughly washed in tap water. Root colonization percentage was evaluated after treating a root sample with 10% (w/v) KOH solution and staining with 0.05% trypan blue in lactoglycerol (Phillips and Hayman, 1970). Fungal colonization was quantified by the glass slide method, in which 30 randomly selected 1 cm root segments were examined microscopically (Giovannetti and Mosse, 1980). Roots were mounted on slides with polyvinyl alcohol lacto-glycerol (PVLG) and screened for AM fungus colonization based on morphological features such as vesicles, arbuscules, hyphal coils, intracellular hyphae, and intercellular hyphae.

3.2.3 AM fungal spore assessment

Spores were isolated from soil samples (100 cm^3) by wet sieving and sucrose density gradient centrifugation (Brundrett *et al*., 1996). Isolated spores were observed with a stereoscopic microscope, their viability was estimated, considering their turgidity, color and damage, counting was carried out taking into account alive, dead and total spores (Stürmer *et al*., 2006). Spores were considered viable if they had clear content under the light microscope with intact spore wall and non-viable if they were either empty or showing evidence of parasitism on the spore wall (Guadarrama *et al*., 2008). Three replicates of soil samples from each site were wet sieved separately. AM fungal spores were counted, characterized and identified under a stereomicroscope and compound microscope. Permanent slides were prepared by placing the spores in PVLG and PVLG mixed with Melzer's reagent (1:1, v/v). Different spore morphological types were identified by comparing spore morphological characterization with original published species descriptions from the available website (http://schuessler. userweb.mwn.de/amphylo/amphylo_species.html). We determined species richness for field samples based on identification data. Relative abundance for a given species was calculated as the number of spores of those species divided by the total number of spores. The Shannon-Wiener diversity index (H') were also calculated: $H' = \Sigma p_i \ln (p_i)$ where pi= the proportion of total number of species of each study site.

3.2.4 Soil analysis

Glomalin in soil samples was quantified as glomalin-related soil protein using the Bradford assay (Bradford reactive soil protein; BRSP). Two pools of BRSP, easily extractable (EE-BRSPs) and total (T-BRSPs) were quantified in aggregates (Wright and Upadhyaya, 1996). Three replicates per each sample, each of 2 g of dry-sieved 0–2 mm aggregates, were extracted with 2 ml of extractant. EE-BRSPs were extracted once with 20 mM citrate pH 7.0 at 121ºC for 30 min in an autoclave. Soil samples were treated repeatedly with 50 mM citrate, pH 8.0 at 121ºC until the supernatant showed none of the red-brown color typical of glomalin for extraction of T-BRSP. Extracts from each replicate were pooled and centrifuged to remove the soil particles (10,000 xg for 5 min). The glomalin concentration in the extracts was determined by Bradford assay using bovine serum albumin as a standard.

The replicate soil samples from each plot were separately analyzed for soil chemical properties. Soil pH in a 1:1 soil: water slurry was determined by direct measurement with pH-meter (Waterproof EC Testr, EUTECH instruments). Available phosphorus was measured employing the Bray II method (Houba *et al*., 1988). Total inorganic nitrogen, exchangeable potassium and soil organic carbon were quantified following the methods of soil analysis outlined in Sparks *et al*. (1996).

3.2.5 Statistical analysis

Statistical procedures were carried out with the software package SPSS 11.5 for Windows. The effect of main effects (e.g. plant species, location) on measured variables was tested by one–way analysis of variance (ANOVA) and Tukey's pairwise comparisons. Pearson's correlation coefficients were calculated to determine the relationships among the soil factors, glomalin and AM fungi variables; a multivariate approach based on principal component analysis (PCA) was performed and considered significant at 0.05 level using XLSTAT (Addinsoft).

3.3 Results

3.3.1 Soil chemical analysis

Most soil types in all study areas were loamy except for TOL, MRY and DCM which had silty, sandy and peaty soils, respectively (Table 3.1). Soil pH values of studied sites ranged from 5.2 at WCR to 6.7 at TOL. Chemical characteristics of soil were highly variable among sites. Soil organic carbon ranged from 1.78 to 3.96%, though differences were not statistically significant. Total inorganic N in soils tended to be highest in TOL site (201 g kg^{-1} soil) and lowest in MRY site (105 g kg^{-1} soil). Available phosphorus was highest in TOL site (369 mg kg^{-1} soil) and differed significantly from DCM, WCR, KCP and CDP sites, and was lowest in WCR (49 mg kg^{-1} soil) (Figure 3.3). Exchangeable potassium tended to be highest in KCP site (359 mg kg^{-1} soil) and differed significantly from GRY, MRY and WCR sites.

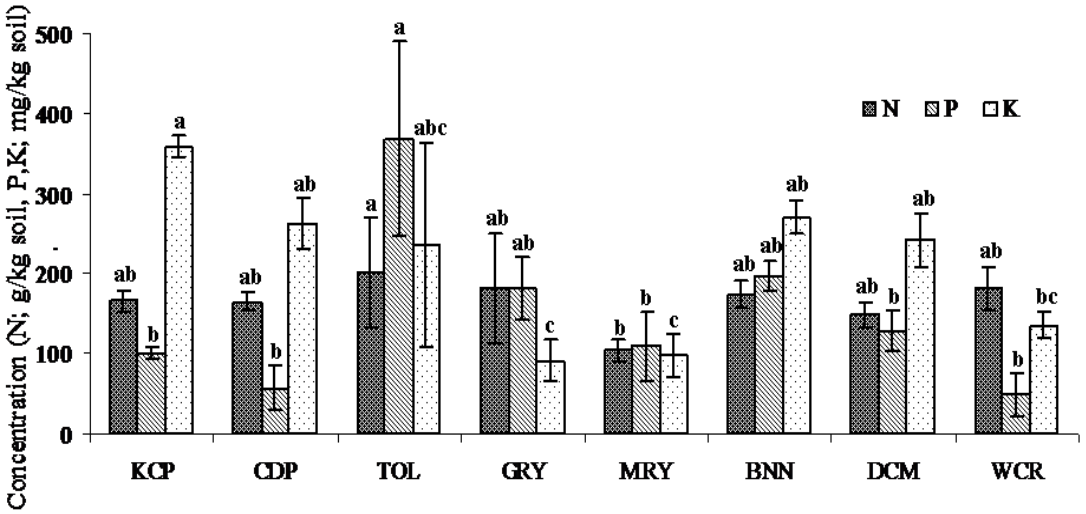

Figure 3.3 Total inorganic N, available P (Bray II), and exchangeable K at the eight sampling sites. Means of three observations ± SEM; for each variable, values across sites with the same letter are not significantly different at $P<0.05$ level (ANOVA and Tukey's pairwise comparisons). See Table 3.1 for abbreviations of sample sites.

3.3.2 Soil glomalin analysis

Total BRSP content ranged from 7.10 to 7.59 mg g^{-1} (Figure 3.4). The highest content was at DCM, where T-BPSP differed significantly from the TOL, MRY, KCP, CDP, BNN and WCR sites. Easily extractable BRSP in MRY site differed significantly from DCM site, and this trend was similar to the pattern of T-BRSP content. The ratio of T-BRSP and EE-BRSP to soil organic carbon (SOC) ranged from 4.07 to 1.95 mg g^{-1}, and 1.36 to 0.65 mg g^{-1}, respectively. Both T-BRSP and EE-BRSP to soil organic carbon ratios were highest in MRY site (4.07 and 1.36 mg g^{-1}, respectively), and markedly differed from that in DCM site (1.95 and 0.65 mg g^{-1}, respectively), which showed the lowest ratios.

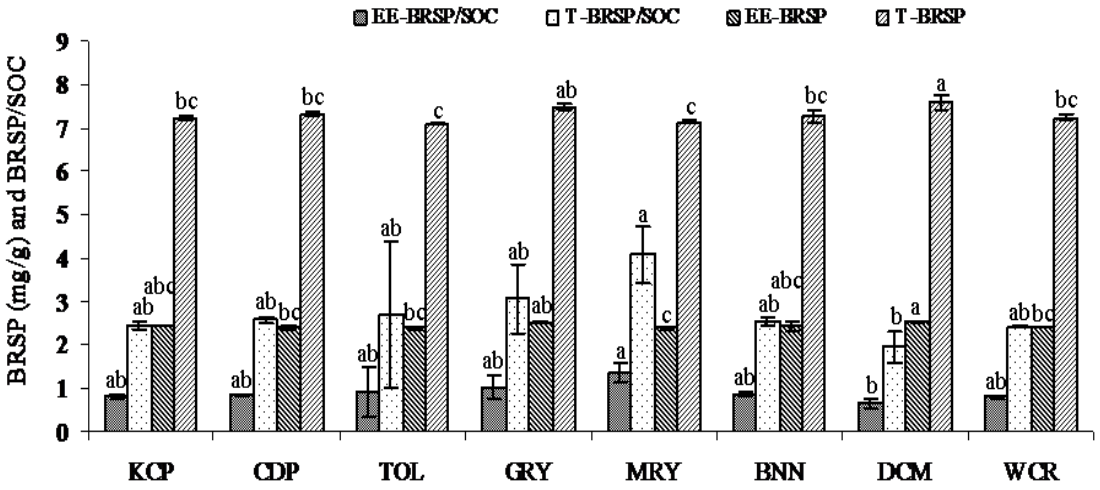

Figure 3.4 Total- and easily-Bradford reactive soil proteins (T-BRSP and EE-BRSP) and the ratio between the BRSPs to soil organic carbon (SOC) in the eight sampling sites**.** Bars ± SEM, n=3; for each column, values with the same letter are not significantly different at $P<0.05$ level (ANOVA and Tukey's pairwise comparisons). See Table 3.1 for abbreviations of sample sites.

3.3.3 AM fungal spore diversity and morphological colonization

AM fungal spore population density tended to be larger in rhizosphere soils of *T. grandis* than in *A. crassna* except for TOL and DCM sites (Table 3.2). Morphological characterization of AM fungal spores based on the new classification of Schüßler and Walker (2010) revealed a total 29 of AM fungal morphotypes, belonging to four dominate families: *Glomeraceae* (46.82%), *Acaulosporaceae* (36.38%), *Claroideoglomeraceae* (10.70%), and *Gigasporaceae* (6.10%). Among the 29 morphotypes, 21 were identified to species and only 16 occurred in rhizosphere soils of both tree species (Table 3.3). Some identified spores were showed in Figure 3.5. The most frequently-occurring genera in *T. grandis* soils belonged to the order *Glomerales* (*Claroideoglomus etunicatum*, *Funneliformis mosseae*, *G. delhiense* and *G. macrocarpum*), while in *A. crassna* soils, the most common genera belonged to the Diversisporales (*A. morrowiae*, *A. rugosa*, and *A. scrobiculata*) (Table 3.3). The Shannon-Wiener diversity index was highest at the WCR site (host = *T. grandis* (H' = 2.034) and lowest at MRY (host = *A. crassna* (H' = 1.216)).

Table 3.2 Root length colonization, spore density and distribution frequency of AM fungi in study sites.

Study site	Plant species	Colonization (% root length)[a]	Spore density (spore/ 100 g soil)[b]	Acaulo (%)[c]	Glom (%)[c]	Claroid (%)[c]	Gigas (%)[c]
KCP	*Aquilaria crassna*	52 bc (cd)	394 bcd	25.38	74.62	-	-
TOL	*A. crassna*	75 a (ab)	422 bcd	72.52	20.38	-	7.10
GRY	*A. crassna*	70 ab (bc)	814 ab	15.12	81.64	3.24	-
MRY	*A. crassna*	44 c (d)	329 bcd	76.26	18.39	-	5.35
BNN	*A. crassna*	79 a (ab)	311 cd	50.16	23.80	-	26.04
KCP	*Tectona grandis*	79 a (ab)	799 abc	35.50	3.06	60.84	0.60
TOL	*T. grandis*	84 a (ab)	170 d	28.24	28.82	42.94	-
CDP	*T. grandis*	91 a (a)	1,081 a	22.44	76.24	-	1.32
DCM	*T. grandis*	77 a (ab)	85 d	-	78.67	-	21.33
WCR	*T. grandis*	90 a (ab)	1,253 a	38.15	61.29	-	0.56

[a] Means of percentage root length colonized followed by the same letter outside of parentheses for comparison within tree species, indicate no significant different at $P<0.05$, n=3 (ANOVA and Tukey's pairwise comparisons). Letters within parentheses indicate significance for comparison among all values within a column.

[b]Means in the same column followed by the same letter are not significant different at $P<0.05$, n=3 (ANOVA and Tukey's pairwise comparisons).

[c]AM fungal family; Acaulo: *Acaulosporaceae*, Claroid: *Claroideoglomeraceae*, Gigas: *Gigasporaceae*, and Glom: *Glomeraceae*.

Table 3.3 Relative abundance, Shannon- Wiener diversity index and species richness of AMF spores found in rhizosphere soils of *Aquilaria crassna* and *Tectona grandis* at 8 different woodlots.

AM fungal species	Relative abundance (%)									
	A. crassna site					*T. grandis* site				
	KCP	BNN	TOL	MRY	GRY	KCP	CDP	TOL	DCM	WCR
Acaulosporaceae										
Acaulospora brasiliensis Walker, Krüger & Schüßler	6.85	-	-	-	-	-	-	-	-	-
A. foveata Trappe & Janos	-	-	-	-	15.12	-	3.96	-	-	7.82
A. mellea Spain & Schenck	18.53	-	-	-	-	18.9	9.93	-	-	13.01
A. morrowiae Spain & Schenck	-	-	22.75	**57.53**	-	3.89	5.28	-	-	6.07
A. rugosa Morton	-	-	**38.63**	-	-	-	-	-	-	-
A. scrobiculata Trappe	-	**50.16**	-	-	-	-	3.27	18.24	-	2.55
Acaulospora sp.1	-	-	11.14	-	-	12.71	-	-	-	1.12
Acaulospora sp.2	-	-	-	18.73	-	-	-	-	-	7.58
Acaulospora sp.3	-	-	-	-	-	-	-	10.00	-	-
Claroideoglomeraceae										
Claroideoglomus etunicatum Becker & Gerd.	-	-	-	-	3.24	**60.84**	-	**42.94**	-	-
Glomeraceae										
Funneliformis mosseae (Gerd. & Trappe) Walker & Schuessler	-	-	-	12.37	7.10	-	**43.86**	15.29	-	-
Glomus aggregatum Schenck & Smith	-	-	-	-	-	-	-	-	12.00	1.36
G. delhiense Mukerji, Bhattacharjee & J.P. Tewari (1983)	**50.25**	-	12.32	-	**56.02**	-	15.67	-	**42.67**	3.11
G. macrocarpum Tul. & Tul.	-	-	-	-	-	-	-	-	-	**43.17**
G. microaggregatum Koske, Gemma & Olexia	-	-	-	-	-	-	-	-	-	0.24
G. multicaule Gerd. & Bakshi	-	-	-	6.02	1.23	-	-	-	-	1.68
G. multisubstensum Mukerji, Bhattacharjee & Tewari	7.87	-	-	-	-	2.09	2.76	-	-	-
G. tortuosum Schenck & Smith	-	-	8.06	-	-	-	-	13.53	-	-
Glomus sp.1	-	12.22	-	-	-	-	7.75	-	-	3.67
Glomus sp.2	-	11.58	-	-	-	-	-	-	24.00	-
Glomus sp.3	16.50	-	-	-	17.28	-	6.20	-	-	4.07
Sclerocystis clavisporum (Almeida & Schenck) Trappe	-	-	-	-	-	0.97	1.03	-	-	0.48
Scl. rubiformis (Gerd. & Trappe) Almeida & Schenck	-	-	-	-	-	-	-	-	-	1.44
Scl. sinuosa (Gerd. & Bakshi) Almeida & Schenck	-	-	-	-	-	-	0.29	-	-	2.07
Gigasporaceae										
Gigaspora sp.1	-	-	4.97	-	-	-	-	-	-	-
Racocetra gregaria Oehl, Souza & Sieverd	-	-	-	-	-	0.60	-	-	-	-
Scutellospora pellucida (Nicolson & Schenck) Walker & Sanders	-	16.72	-	5.35	-	-	-	-	21.33	0.56
Scutellospora scutata Walker & Dieder	-	-	2.13	-	-	-	-	-	-	-
Scutellospora sp.1	-	9.32	-	-	-	-	-	-	-	-
Species richness (total number of species)	5	5	7	5	6	8	11	5	4	17
Shannon- Wiener Diversity Index (H´)	1.339	1.373	1.641	1.216	1.267	1.411	1.810	1.461	1.290	2.034

Note: Values in bold indicate dominant abundance of AM fungal species in each study sites.

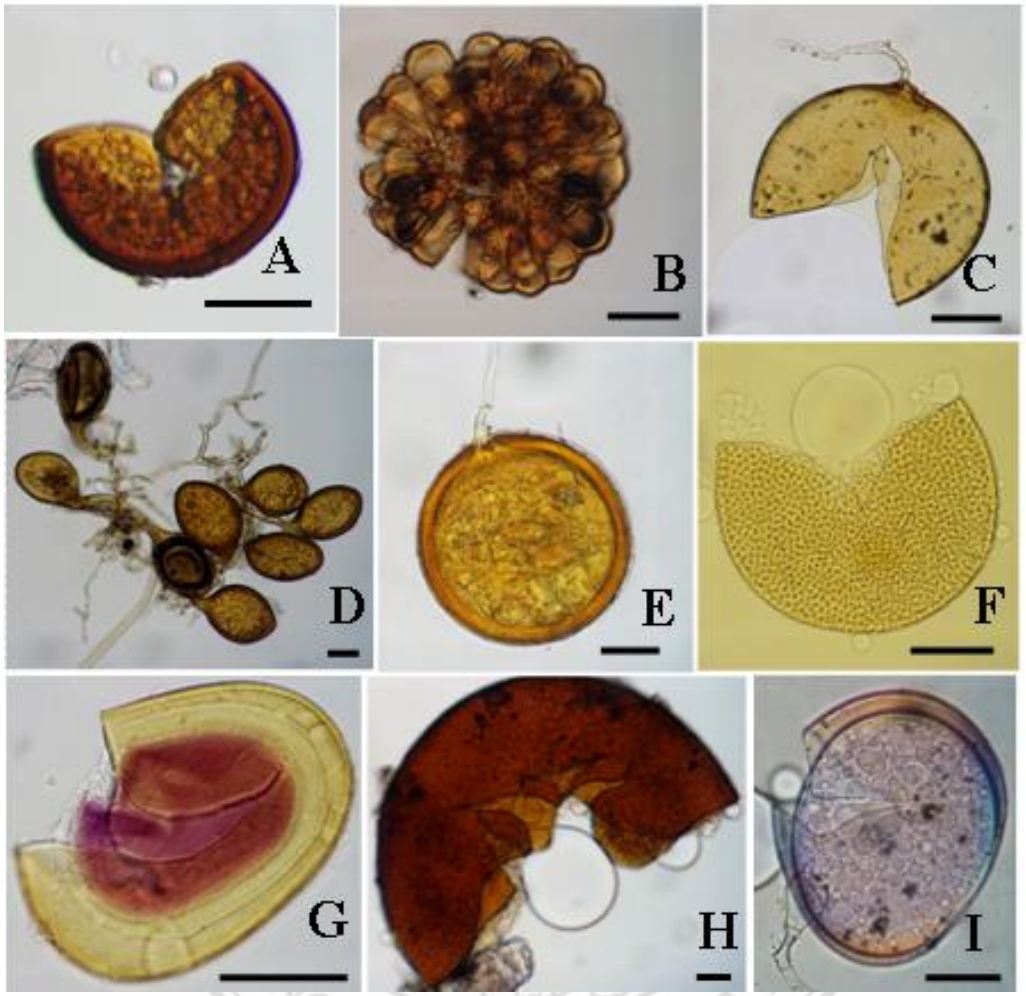

Figure 3.5 AM fungal spores morphology in rhizosphere soils of *T. grandis* and *A. crassna*: (A) *Glomus delhiense*, showing the thickness of the outer wall layer and granula cytoplasm; (B) *Sclerocystis clavispora*, clavate spores formed radially packed around a cetral plexus; (C) *Funneliformis mosseae*, showing funnel-shape base and septate hyphae spore; (D) *G. macrocapum*, a loose aggregate sporocarp with thick-walled subtending hyphae; (E) *Claroideoglomus etunicatum*; (F) *Acaulospora scrobiculata*, showing spore surface pitted with depressions; (G) *A. morrowiae*; a crushed spore in Melzer's reagent, showing multiple spore walls and the positive reaction of the inner membranous wall; (H) *A. foveata* with pitted spore surface; (I) *Scutellospora pellucida*, hyaline spore with brittle outer wall and pliable inner wall, bar = 50 µm.

Colonization of roots by AM fungi was observed in both *A. crassna* and *T. grandis* (Figure 3.6). Colonization was higher in *T. grandis* than in *A. crassna* (Main effect, $P<0.01$, F=19.664). The range of colonization in *A. crassna* varied 44–79%, while *T. grandis* varied 77–91% (Table 3.2).

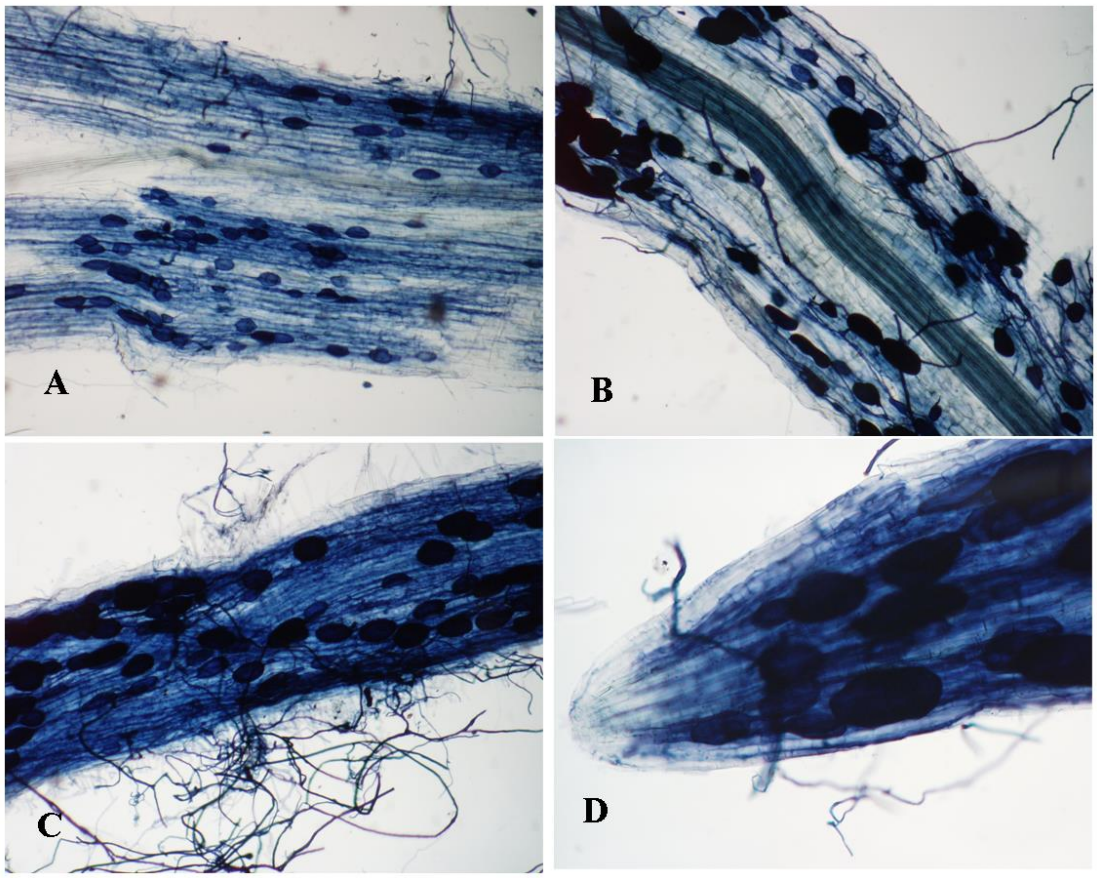

Figure 3.6 Colonized roots of A) *Aquilaria crassna* and B-D) *Tectona grandis*: show vesicles and hyphae.

Colonization of *A. crassna* roots was positively correlated with pH and total inorganic nitrogen (Figure 3.7A), while *T. grandis* root length colonization was positively correlated with spore density and negatively correlated with EE-BRSP and soil organic carbon (Figure 3.7B). Spore density in rhizosphere of *A. crassna* showed a positive correlation with T-BRSP and EE-BRSP. EE-BRSP and T-BRSP were positively correlated in rhizosphere soils of both plants. Soil organic carbon in *A. crassna* sites was positively correlated with total inorganic nitrogen, whereas soil pH was positively correlated with exchangeable phosphorus.

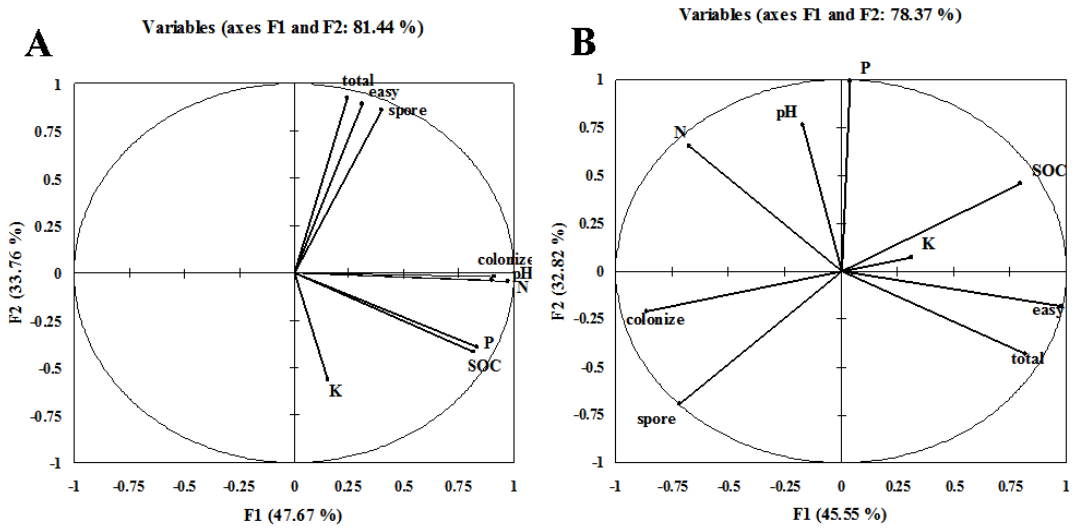

Figure 3.7 Principle component analysis (PCA) showing correlation between AM fungi; root colonization (colonize) and spore density (spore), glomalin content; easily extractable Bradford reactive proteins (easy) and total extractable Bradford reactive proteins (total), and soil chemical factors; pH, nitrogen (N), phosphorus (P), potassium (K), and soil organic carbon (SOC) in rhizosphere soils of *A. crassna* (A) and *T. grandis* (B).

3.4. Discussion

3.4.1 AM fungi and soil chemical factors

This study found that *A. crassna* and *T. grandis* formed symbiotic associations with AM fungi in agreement with previous studies (Thapar and Klan, 1988; Kanakdurga *et al*., 1990; Ramanwong, 1998; Tamuli and Boruah, 2002; Singh *et al*., 2003). For example, Dhar and Mridha (2012) found 77% of *T. grandis* root length colonized by AM fungi in Madhupur forest, Bangladesh. Our results showed that percentage root length of *A. crassna* colonized by AM fungi increased with increasing soil pH from 5.6 (KCP) to 6.7 (TOL), while colonization in *T. grandis* was not affected. This might indicate a soil pH preference for *A. crassna*. Osoguchi *et al*. (2002) reported that soils on the *A. crassna* research site in Trat province (the area of greatest cultivation of *A. crassna* in Thailand) were acidic with pH ranging from 5.4–5.8, while Kaosa-ard (1998) found the range of soil pH in *T. grandis* forests is wide (5.0–8.0). Many plant species are adapted to particular pH optima, and pH is one of the main

determinants of plant community composition (Helgason and Fitter, 2009). Moreover, the formation and development of arbuscules are favored by neutral or low acid but not alkaline conditions (Bai *et al*., 2009). Thus, it is not surprising that AM fungal communities also change with soil pH, as has been demonstrated (Wang *et al*., 1993; van Aarle *et al.*, 2002; Fitzsimons *et al*., 2008; Toljander *et al*., 2008). Therefore, soil pH preference of host plants and AM fungi might influence growth quality and root colonization rate, respectively. The variation of spore density in *T. grandis* in this study may result from the different soil type and edaphic factors. The spores and activities of AM fungi may not distribute equally in different soils (Bai *et al*., 2009). For example, DCM and TOL sites that had peaty and silty soil types with high soil organic carbon were found to have lowest spore density. While in other loamy soil sites (CDP, KCP, and WCR) had higher spore density. This may explain why spore density in rhizosphere of *T. grandis* decreased with increasing soil organic carbon in this study. We also found a positive correlation between soil pH and available phosphorus in studies sites. According to Helgason and Fitter (2009) indicated that soil pH also affects the nutrient availability in the soil by directly regulating their availability through ion exchange.

3.4.2 Glomalin–relate soil protein and soil organic carbon

The amount of T-BRSP was highest at DCM, a site that was undisturbed by plowing. MRY and TOL, where weeds were controlled by plowing and herbicide treatment, had the lowest concentrations of glomalin. This result agreed with previous research indicating that agricultural management such as tillage causes loss of soil organic carbon leading to low soil biological activity, including mycorrhizal fungal activity (Wright *et al*., 1999; Jansa *et al*., 2002; Rillig *et al*., 2003). Emran *et al*. (2012) also found that BRSP, SOC and structural stability of soil aggregates increased significantly along a transect from cultivated to an abandoned farm site and the values in cultivated soils were always lower than in soils under shrubs and pastures. In this study, the spore density in *A. crassna* (among different studied areas) was positively correlated to EE-BRSP and T-BRSP content. But spore density in *T. grandis* was not because there was a variation in spore density under *T. grandis* canopy. For example, DCM had highest amount of T-BRSP and percentage of root colonization, but lowest spore density was found. The spore density may not be a reliable indicator for

BRSP content. This may explain by Driver *et al*. (2005) that about 80% of glomalin (by weight) produced by the fungus was contained in hyphae and spores compared to that released into the culture medium, strongly suggesting that glomalin arrives mainly in soil via release from hyphae. This study also found that the peaty soil of the DCM site, which had the highest T-BRSP, had higher soil organic carbon, while the sandy soil of the MRY site had lowest soil organic carbon. Because as much as 4–5% of the total soil carbon and nitrogen in temperate and tropical soils can be conserved in glomalin-related soil protein, thereby making a significant contribution to soil carbon and nitrogen pools that can far exceed the contributions of soil microbial biomass (ranging from 0.08 to 0.2% of total C for the oldest soils) (Rillig *et al*., 2001). Therefore, it is not surprising that T-BRSP varied in the same way with EE-BRSP in every sampling site and was also positively correlated with soil organic carbon.

3.4.3 AM fungal spore diversity and colonization

This study showed that rhizosphere soils of *T. grandis* had greater AM fungus species richness and Shannon diversity index than *A. crassna* (9.0 ± 2.4 vs. 5.6 ± 0.4 for richness and H´= 1.601 ± 0.138 vs. 1.367 ± 0.074). Using similar methodologies and definitions, the AM fungal diversity in both plants was rather high compared with different tree species such as *Acacia auriculiformis*, *A. mangium*, *Artocarpus heterophyllus*, *Dalbergia sissoo*, *Eucalyptus camaldulensis*, *Hevea brasiliensis*, *Swietenia macrophylla* and *T. grandis* of Madhupur forest, Bangladesh that H´ ranged from 0.503−1.397 (Dhar and Mridha, 2012). Our research found that the most common genus of AM fungi in *T. grandis* rhizosphere soils was *Glomus*. Previous studies of the AM fungal diversity of *T. grandis*, mainly in Indian forests, found *Glomus mosseae, G. multicaule, G. tortuosum* and *Sclerocystis rubiformis* to be dominant (Kanakadurga *et al.*, 1990). Those species also found in the Jhum fallow site at which *T. grandis* was the dominant tree species (Singh *et al*., 2003). We also found *F. mosseae* (previously *G. mosseae*) to be one of the dominant AM fungi in *T. grandis* soils (Table 3).

Management practices such as plowing, cultivation for weed control, and herbicide usage, especially at Phetchabun (KCP and CDP), Nakhon Nayok (TOL) and Rayong (GRY) could influence AM fungal activity and diversity. These agricultural practices may be unfavorable for the survival of some AM fungi (Daniell *et al*.,

2001). *Glomeraceae* are capable of colonizing by mycelium fragments, colonized root pieces, and spores (INVAM, 1993), while *Gigasporaceae* are only capable of propagation/colonization by spores since they do not produce vesicles (Biermann and Linderman, 1983). This difference might explain the dominance of the *Glomeraceae* over *Gigasporaceae* in disturbed agricultural environments. *Claroideoglomus etunicatum* was dominant at 2 of these 4 managed sites, in agreement with previous work in other disturbed systems (Douds *et al*., 1995). In addition, host tree species, environment, and seasonal dynamics of AMF spore formation imply different life strategies of different AM fungal species (Lovelock *et al*., 2003; Oehl *et al*., 2009). Some AMF species are prolific spore producers while others are not, this fact may explain some of spore density differences (Clapp *et al*., 1995). Therefore, various AM fungal species may be affected by different soil chemical properties and host plant species, which should be taken into account when selecting AM fungi for inoculation.

This study showed that rhizosphere soils of *T. grandis* had greater AM fungus species richness than *A. crassna*. Four to 17 species were found per site, with the most dominant individual species typically a member of the *Acaulosporaceae* (6 of 10 sites). The measured variables, such as edaphic factors, spore density, species richness, distribution frequency, root length colonization and glomalin concentration, varied in the different plantations. Among soil chemical factors; pH, soil organic carbon and total inorganic nitrogen influenced AM fungal parameters including colonization, spore density and glomalin concentration in the rhizosphere soils of *T. grandis* and *A. crassna* plantations. This work serves as a starting point for the targeted use of the AM symbiosis in production of these two important tree species in Thailand. We now know which AM fungus species are associated with *T. grandis* and *A. crassna*. Future inoculum production efforts can be targeted to those that are found most effective in greenhouse growth studies.