CHAPTER 8

General discussion and conclusion

This study provides the first molecular community analysis of AM fungi associated with field-collected roots and rhizosphere soils of *Tectona grandis* and *Aquilaria crassna* as part of a long term goal of finding effective AM fungus inoculation to enhance plantation and reforestation efforts with these trees. Inoculation methods both based on soil-based substrate and *in vitro* dual culture were carried out with *T. grandis* and *A. crassna* plantlets. In order to propagate selected AM fungal spores for large scale production, the pot culture with different diluents and host plants, and subsequent on-farm inoculum production by using leaf litter compost as a substrate component were performed and applied for tropical climates. Selected AM fungal colonization was also investigated in inoculated plant roots using denaturing gradient gel polymorphism.

8.1 Distribution of AM fungi in rhizosphere soils of *A. crassna* and *T. grandis*

Our study showed that all *T. grandis* and *A. crassna* root samples were colonized with AM fungi. *Tectona grandis* roots had greater AM fungal colonization than *A. crassna* (77–91% vs. 44–79%, respectively). Twenty-nine AM fungal morphotypes from 8 plantations of both plants were represented four families: *Acaulosporaceae, Claroideoglomeraceae, Glomeraceae,* and *Gigasporaceae*. The most frequently-occurring genera in both *T. grandis* and *A. crassna* soils belonged to the *Glomerales* (*Claroideoglomus etunicatum, Funneliformis mosseae, Glomus delhiense* and *G. macrocarpum*. Among AM fungal morphotypes, *F. mosseae* (previously *Glomus mosseae*) was found to be one of the dominant AM fungi in *T. grandis* soils. According to the previous studies on AM fungal diversity of *T. grandis* mostly in India (Chandra and Kehri, 2009), which found that *F. mosseae, G. multicaule, G. tortuosum* and *Sclerocystis rubiformis* was dominant (Kanakadurga *et al*., 1990). Singh *et al*. (2003)

also found those species in the Jhum fallow site at which dominanted with *T. grandis*. Verma and Jamaluddin (1995) found *C. etunicatum* and *A. scrobiculata* as most widely distributed species followed by *G. intraradices* and *G. mosseae* while *G. fasciculatum*, was found least frequent. In Thailand, Ramanwong (1998) also found 6 species of AM fungi include: *Acaulospora scrobiculata, G. aggregatum, G. deserticola, G. multicaule, G. microcapum* and unidentified species associated with *T. grandis* in Thailand plantations. Whereas, diversity of AM fungi associated with *A. crassna* have not been reported, only other *Aquilaria* sp. have been demonstrated. For example, Tamuli and Boruah (2002) reported that *Glomus* was a dominant species in rhizosphere soils of *A. malaccensis* and among that *Glomus* spp., *G. fasciculatum* were the most dominant followed by *G. aggregatum*. The studies on correlation between edaphic factor and AM fungi revealed that colonization percentage, spore density and glomalin content in both plants were correlated with various factors. For example soil pH in studied areas, nutrient content in soil especially phosphorus, and other soil factors have been reported to be correlate with AM fungi in many previous studies (Wang *et al*., 1993; van Aarle *et al*., 2002; Fitzsimons *et al*., 2008; Toljander *et al*., 2008; Bai *et al*., 2009). A positive correlation was observed between AM fungal spore density and total and easily extractable Bradford reactive soil proteins in rhizosphere soils of *A. crassna*, while in *T. grandis* spore density decreased when soil organic carbon and easily extractable Bradford reactive soil proteins increased. This variation may be resulted from the variation in spore density under *T. grandis* canopy in some study sites that had highest SOC and T-BRSP content but low spore density was found.

8.2 Community analysis of AM fungi in rhizisphere soils and roots of *A. crassna* and *T. grandis*

Terminal restriction fragment length polymorphism complemented with clone libraries revealed that AM fungal community composition in *A. crassna* and *T. grandis* from 5 study plots were similar. AM fungal communities in *T. grandis* samples from different sites were similar, as were those in *A. crassna*. Among a total of 38 distinct terminal restriction fragments (TRFs), 31 TRFs were shared between *A. crassna* and *T. grandis*. Virtual digestion of sequences using three restriction enzymes HinfI, Hsp92II and MboI yielded expected fragments that mostly matched

observed TRFs, linking possible AM fungal species to each TRF. The AM fungal sequences were represented *Claroideoglomeraceae, Diversisporaceae, Gigasporaceae* and *Glomeraceae*. Among those families, *Glomeraceae* were found to be common in all study sites and consistented with previously published phylogenies (Helgason *et al*., 1999; Mummey and Rillig, 2007; Pietikäinen *et al*., 2007). Specific AM taxa in roots and soils of *T. grandis* and *A. crassna* were not affected by host plant species and sample source (root vs. soil) but affected by collecting site. According to the conclusion of Öpik *et al*. (2006) that the number of AM fungal taxa per host plant species differed between habitat types and resulted in a high variability of taxon richness and composition between particular ecosystems. The T-RFLP technique can provide important information about the AM fungal diversity within the roots of plant species of interest, and DNA sequencing can reveal the main fungal species involved. However, trap cultures and cultured spores from the field site are still important in order to assess the ability of the AM fungi to enhance the growth of the plants, and to provide effective and stable inoculants.

8.3 Selection of AM fungi for growth enhancement of *A. crassna* and *T. grandis*

In preliminary experiment showed that *T. grandis* plantlets had the best growth responded to AM fungal spore inoculation than uninoculated plantlets. When tested with more replicates on soil-based substrate as well as *in vitro* inoculation, the result also confirmed that *T. grandis* plantlets had highest height and shoot biomass when inoculating with AM fungi compare to uninoculated plantlets. AM fungal inoculation resulted in higher plant height and stem diameter than uninoculate control. These results coincided with Ramanwong (1998) who inoculated 6 month-old *T. grandis* seedlings with 6 species of AM fungi and found the greater height, diameter at root collar, shoot dry weight and total dry weight in inoculated seedlings. Verma and Jamaluddin, (1995) also found that inoculating *T. grandis* seedlings with *G. fasciculatum* and mix AM fungi separately showed better plant height, biomass and percent root infection in nursery compare to uninoculated seedlings. Moreover, Rajan *et al*. (2000) studied the efficacy of nine arbuscular mycorrhizal (AM) fungi on *T. grandis* stumps and found the increasing of plant growth and plant nutritional status over those grown in uninoculated stumps. For *A. crassna*, inoculated plantlets tend to have greater height, number of leaf than uninoculated plantlets but there were no significantly differed in our study. This

may be resulted from the variation of plantlets during the maintenance. However, previous studies have showed that AM fungal inoculation could enhance growth of *Aquilaria* spp. For example, Turjaman *et al*. (2006) found the efficiency of *G. clarum* and *G. decipiens* in increasing plant height, diameter, shoot and root dry weights, shoot N and P concentrations and survival rate of *A. filarial* seedlings at 180 days after transplantation. Tabin *et al*. (2009) showed that AM fungus, *G. fasciculatum* had an ability to reduce the rotting incidence of *A. agallocha* seedlings causing by pathogenic fungus, *Pythium aphanidermatum* and significantly increased host plant height, total biomass and dry matter. Therefore, the AM fungal inoculation may be feasible for commercial propagation of *A. crassna* and *T. grandis*, the economically important tree species both by *ex vitro* or *in vitro* inoculation.

8.4 Propagation of AM fungal spore for large scale production

AM fungal spore propagation of *C. etunicatum* NNT10, *C. etunicatum* PBT03 and *F. mosseae* RYA08 using different culture materials and host plants was affected on root colonization and number of spores of each AM fungus isolate. The suitable host plant and diluent were *Zea mays* and vermiculite, respectively. Subsequent on-farm production of mycorrhizal fungus propagules with *Z. mays* in leaf litter compost mixed with vermiculite was considerably higher than that with other host plants. These results coincided with many previous studies (Millner and Kitt, 1992; Gaur and Adholeya, 2000; Douds *et al*., 2010) on AM fungal propagation the inert substrates as vermiculite have been used to dilute nutrient-rich compost for on-farm inoculum and serve an additional function such as enhance drainage, moisture retention, aeration, and having an influence on sporulation of AM fungi. Liu and Wang (2003) concluded that there is selectivity between host plants and AM fungi. Therefore, the number of species of AM fungi detected was influenced by host plants under certain conditions. For *in vitro* production, hyphal length of individual germinated spore and percentage of spore germination of *C. etunicatum* NNT10, *C. etunicatum* PBT03, *F. mosseae* RYA08, and *G. intraradices* were showed when inserted into both minimal (M) and modified Strullu Roman (MSR) medium but tend to be higher in MSR medium. Only *Glomus intraradices* and *F. mosseae* RYA08 hyphae were spreaded throughout the Petri plates and produced new spores successfully after 14 and 24 days of inoculation, respectively

while others produced only hyphae but not spread throughout the media. Douds (1997) demonstrated the 14% success rate of culture *F. mosseae* with carrot roots and no new spores were produced before termination after grew for 17–24 weeks. This *in vitro* production needs further research to complete the life cycle of *F. mosseae* as well as other strain that have not been reported. Therefore, we concluded that AM fungi and plant diversity was both enhanced and retarded each other. It should be taking into account when selecting AM fungal strains, host plants, and culture materials for AM fungal propagation.

8.5 Investigation of selected AM fungal spore colonization in target plant roots

The denaturing gradient gel electrophoresis experiment (DGGE) was used to investigate AM fungal colonization and community in target plant roots or soils for over a decade (Kowalchuk *et al*., 1997; Öpik *et al*., 2003; Hassan *et al*., 2011). In this study, DGGE was performed to investigate selected AM fungal spores colonization in inoculated *T. grandis* plantlets after one year of inoculation. The specificity of primers used in this experiment, confirmed by none observing of distinguishable difference in DGGE pattern between two replicates of polymerase chain reaction (PCR) production after nested PCR. Because of the lower resolving power of AM1/NS31GC amplification product on DGGE was also observed by Öpik *et al*. (2003) and Ma *et al*. (2005). Therefore, nested PCR with first pair of AMl1-AML2 primer should be used to resolution of that problem before using AM1/NS31GC. The contamination AM fungal species during the pot maintenance in the green house caused the complex of DGGE pattern in *T. grandis* roots. The result showed that restriction fragment length polymorphism (RFLP) pattern could not distinguish certain AM fungal species that have been inoculated into *T. grandis* roots. However, those patterns could estimate that the possible AM fungal species inside all roots from each treatment tend to be *C. etunicatum* when comparing RFLP patterns from both HinfI and Hsp92II digestions.