CHAPTER 1

General introduction and thesis outline

1.1 Introduction

Tropical forest area is disappearing at the rate of 13.5 million hactares each year, mainly due to clearing for agriculture, shifting cultivation and timber harvesting. The latter results in more than 5 million hectares of tropical forest annually being transformed into degraded, poorly managed, logged-over forests that affect not only the sustainable production of timber but also the global environment (Kobayashi, 2004). Therefore, the use of small woodlots for timber production is becoming increasingly essential to supply the world market. *Tectona grandis* Linn.f. (teak) is also well known as the king of timbers because it is highly durable, easily worked, attractive, strong and relatively light. Teak plantations are being established throughout the tropics as growers are attracted by the high prices that natural teakwood can achieve in global markets (Sarre and Ma, 2004). *T. grandis* was the most important export timber in Thailand until all logging in natural forests was banned in 1989 (Kollert and Cherubini, 2012). *Aquilaria crassna* and *T. grandis* plantlets are required for domestic and foreign markets such as Australia, Guatemala, Indonesia, Jamaica, Laos, Malaysia, Mozambique, and Sri Lanka. *Aquilaria crassna* Pierre ex Lec. (agarwood) is one of the threatened tropical trees species included in Appendix II of the Convention on International Trade in Endangered Species of wild fauna and flora (CITES, 2004). It is of particular economic interest as it is the principal source of aromatic resin-infused wood that is used for incense, perfume, traditional medicines and other products. The increasing interest in establishing *Aquilaria* plantings worldwide is largely due to its high rate of decline in natural stands due to over-harvesting in tropical Asian and Pacific countries. Micropropagation has been recognized as a very successful technique for rapid production of several commercially important varieties of horticultural and

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forest tree species. However, the major impediment for the success of tissue culture is the high mortality rate of plantlets after transplantation because of declining photosynthetic rate and photoinhibitory impairment (Jeong-Hoon *et al*., 2000). These are the most common problems that impede its usage for commercial plant production and may lead to high use of fertilizers and expense in early growth of plantlets. Therefore, information on methods of growth enhancement for both species is highly important for managing and reforestation of these species.

Arbuscular mycorrhizal (AM) fungi are soil fungi that are associated as mutualists with plant roots in a wide spectrum of tropical and temperate tree species (Habte, 2000). AM fungi are obligate symbiotic fungi (Harley and Smith, 1983) which therefore propagate by growing with a living host plant. They improve the supply of water and nutrients, such as phosphate and nitrogen, to the host plant. In return, up to 20% of plant-fixed carbon is transferred to the fungus (Parniske, 2008). Nutrient transport occurs through symbiotic structures inside plant root cells known as arbuscules. AM fungal spore production is carried out by growing them in pot culture with growing plants or in aeroponic systems, hydroponic systems, and *in vitro* root organ culture. AM fungi can only be identified morphologically in the spore stage, but spore counts do not fully demonstrate changes in species composition or abundance in the soil (Morton *et al*., 1995). Moreover, spores cannot be related to individual colonies within roots in field soils, as they are formed on hyphae in the surrounding soil and are easily detached (Helgason *et al*., 1999). Therefore, Polymerase chain reaction-based methods have been widely used in AM fungal community studies to identify the vegetative phase of the fungi. Various studies have designed sets of specific primers for AM fungi (Helgason *et al*., 1998; Lee *et al*., 2008; Krüger *et al*., 2009) to facilitate rapid detection and identification directly from field-grown plant roots. It has been suggested that terminal restriction-fragment length polymorphism (T-RFLP) is a more sensitive technique for fungi than denaturing gradient gel electrophoresis (DGGE) (Brodie *et a*l., 2003; Singh *et al*., 2006). It is efficient to use T-RFLP to process large numbers of samples and then to make clone libraries from selected samples for sequencing to obtain identities of key species (Dickie and FitzJohn, 2007). Previously, T-RFLP was used to study the AM fungi community in roots of arable crops (Daniell *et al*., 2001), perennial herbs (Pietikäinen *et al*., 2007), grass species (Vandenkoornhuyse *et al*., 2003; Johnson

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et al., 2004), and grass species with herbaceous flowering plants (van der Heijden *et al*., 2003; Mummey *et al*., 2005). None of these studies included rhizosphere soils, so there is no information on comparing the AM fungal community in rhizosphere soils and roots. A number of these studies demonstrated that the AM fungal community differed among host species, suggesting that it may be important to take host preference into account when selecting AM fungi for inoculation. Since AM fungi can improve the survival rate and performance of nursery seedlings through (Habte *et al*., 2001; Turjaman *et al*., 2006), knowledge of the AM fungal diversity in *A. crassna* and *T. grandis* is important in order to develop inoculum production strategies for plant propagation and reforestation of these trees. Although information about AM fungal diversity in both plants has been reported, mostly for India (Thapar and Klan, 1988; Kanakdurga *et al*., 1990; Tamuli and Boruah, 2002; Singh *et al*., 2003), there are few reports of AM fungi in rhizosphere soil of both plants in Thailand (*T. grandis*: Ramanwong, 1998), especially *A. crassna*.

1.2 Thesis objectives

1.2.1 To study the AM fungal community in some woodlots of *A. crassna* and *T. grandis* in northern and eastern Thailand.

1.2.2 To isolate the effective AM fungi for growth enhancement of *A. crassna* and *T. grandis*.

1.2.3 To design an effective AM fungal propagation method for large scale production.

1.2.4 To improve AM fungal inocula for field application.

1.2.5 To monitor AM fungi colonization in selected plant roots by using molecular techniques.

1.3 Usefulness of the thesis

1.3.1 Efficient AMF to improve growth of *A. crassna* and *T. grandis* were applied in nursery.

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1.3.2 A method for propagating AMF spores on a large scale was selected to produce easy-to-use inocula.

1.3.3 AMF inocula were used to enhance growth rate of *A. crassna* and *T. grandis*; tissue culture seedlings.

1.3.4 Inoculated AMF behavior can be monitored from field condition.

1.4 Plan of the thesis

This thesis describes research on arbuscular mycorrhizal fungi of *A. crassna* and *T. grandis* in northern and eastern Thailand. It is organized in eight chapters (Figure 1.1).

Chapter 2 reviews our knowledge of arbuscular mycorrhiza and botanical description of the host plants.

Chapter 3 describes the diversity of AM fungi in rhizosphere soils of *A. crassna* and *T. grandis*.

Chapter 4 describes the community analysis of AM fungi in rhizosphere soils and roots of *A. crassna* and *T. grandis*.

Chapter 5 describes the selection of AM fungi for growth enhancement of *A. crassna* and *T. grandis*.

Chapter 6 describes the propagation of AM fungal spores for large-scale production.

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Chapter 7 describes the investigation of selected AM fungal spore colonization in target plant roots.

Chapter 8 summarizes and discusses the data in this thesis.

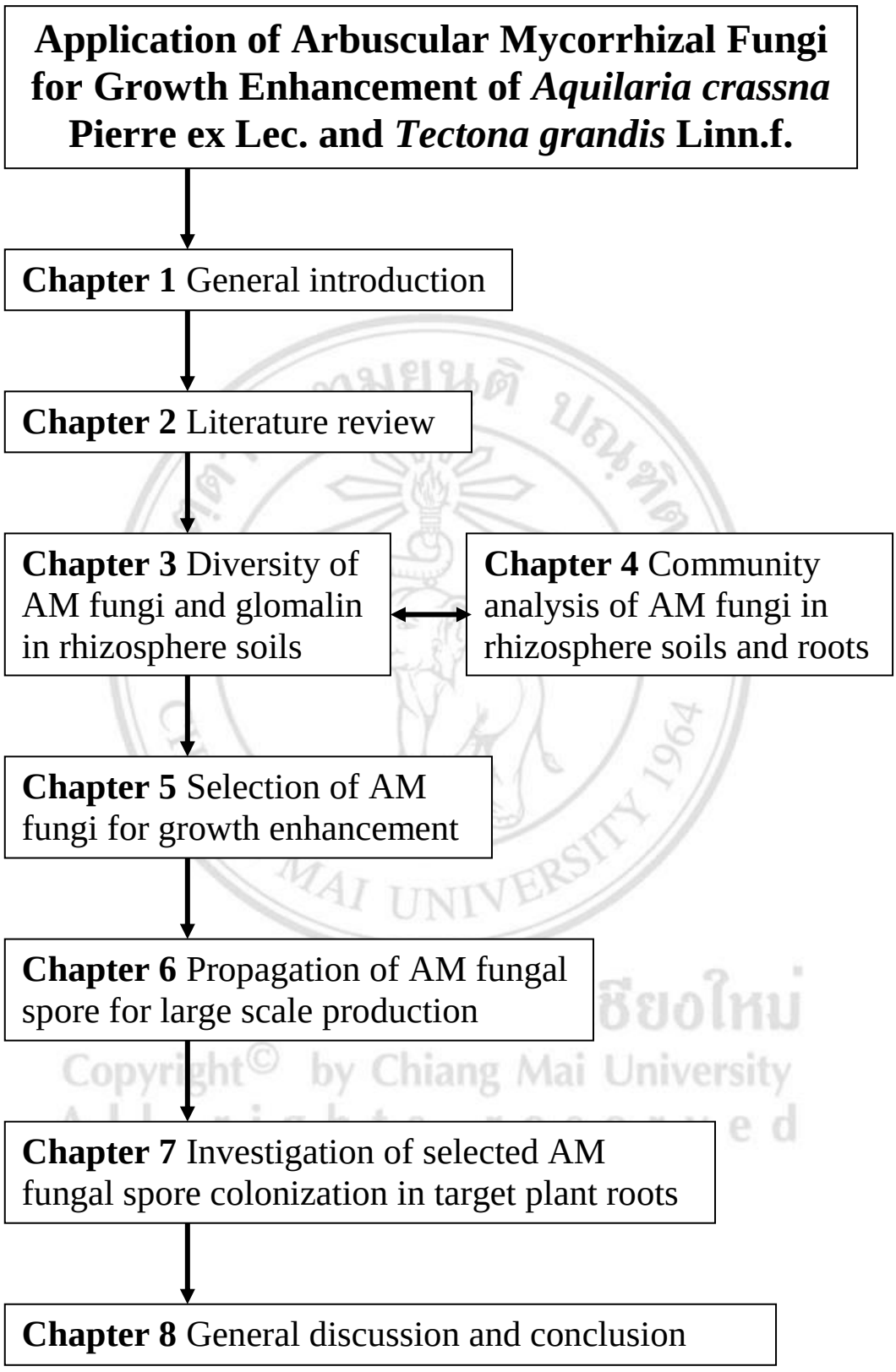

Figure 1.1 Schematic presentation of the relationships between chapters of the thesis.

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