ARBUSCULAR MYCORRHIZAL FUNGI INCREASED EARLY GROWTH OF GAHARU WOOD OF *Aquilaria malaccensis* and *A. crasna* UNDER GREENHOUSE CONDITIONS

Maman Turjaman, Erdy Santoso and Yana Sumarna

**ABSTRACT**

Gaharu wood stand has an important source of profits to the forest community in South and Southeast Asia tropical forest countries, but *Aquilaria* species have reduced in number and turn out to be endangered due to overexploitation. Today, the planting stocks of *Aquilaria* species are not sufficient to sustain the yield of gaharu wood and promote forest conservation. The objective of this study was to determine the effect of five arbuscular mycorrhizal (AM) fungi: *Entrophospora* sp., *Gigaspora decipiens*, *Glomus clarum*, *Glomus* sp. ZEA, and *Glomus* sp. ACA, on the early growth of *Aquilaria malaccensis* and *A. crasna* under greenhouse conditions. The seedlings of *Aquilaria* spp. were inoculated with *Entrophospora* sp., *Gi. decipiens*, *Glomus clarum*, *Glomus* sp. ZEA, *Glomus* sp. ACA and uninoculated (control) under greenhouse conditions. Then, percentage AM colonization, plant growth, survival rate and nitrogen (N) and phosphorus (P) content and mycorrhizal dependence (MD) were measured. The percentage AM colonization of *A. malaccensis* and *A. crasna* ranged from 83 to 97% and from 63 to 78%, respectively. Colonization by five AM fungi increased plant height, diameter, and shoot and root dry weights. N and P content of the seedlings were also increased by AM colonization. Survival rates were higher in the AM-colonized seedlings at 180 days after transplantation than those in the control seedlings. The MD of *Aquilaria* species was higher than 55%. The results suggested that AM fungi can be inoculated to *Aquilaria* species under nursery conditions to obtain vigorous seedlings, and the field experiment is underway to clarify the role of AM fungi under field conditions.

Keywords: AM fungi, tropical forest, gaharu wood, *Aquilaria* species, reforestation

**I. INTRODUCTION**

A tropical tree genus which currently has been an international attention focus is *Aquilaria* (Soehartono and Newton, 2001a). This genus is the main source of gaharu wood, a fragrant resinous wood, which rank among the most highly valuable non-timber products harvested from tropical forests. Gaharu wood is used in the manufacture of perfume, incense, traditional medicine, and other commercial products by Moslems and Asian buddhists. *Aquilaria* species are commonly found in primary and secondary lowland forests of Kalimantan, Sumatra, Sulawesi, Moluccas, West Papua, Papua New Guinea, Vietnam, India, Bangladesh, Bhutan, Myanmar, China, Cambodia, Thailand, Malaysia, Singapore, and the Philippines. Due to the high value of gaharu wood, *Aquilaria* has been severely overharvested throughout Asia during the last 20 years (Paoli et al., 2001). The species was
placed on appendix II of the Convention on the International Trade in Endangered Species (CITES) and considered to be threatened according to the IUCN Red List (CITES, 2005). Nevertheless, many *Aquilaria* spp. remain fallow, making it difficult not only to measure the protection status of *Aquilaria* spp., but also to sustain producing gaharu wood.

Many tropical soils are nutrient deficient or have substantial constraints to plant growth (Haselwandter and Bowen, 1996). Many of the soils, particularly in the tropical forests are very fragile, rapidly depleting their meagre soil resources on cultivation and becoming prone to wind or water erosion. It is required to improve the productivity of tropical forest lands and to enhance the commercial value of pulp, timber and non timber forest products. The fast production of high quality seedlings in nurseries is a vital stage for replenishing degraded tropical forest lands. Indigenous tree species, such as *Aquilaria* species, often grow slowly in the early growth stage compared with such fast growing species as *Paraserianthes falcataria*, *Leucaena leucocephala*, *Acacia mangium*, and *Calliandra calothyrsus*.

It has been reported that arbuscular mycorrhizal (AM) fungi increased growth of some tropical trees. AM fungi increased seedling growth of *Dicorynia guianensis* (Caesalpiniaceae) from a tropical rain forest in French Guiana under nursery conditions (Béreau et al., 2000). Muthukumar et al. (2001) reported that the inoculation of *Agadirachta indica* (Meliaceae) with AM fungi improved plant growth compared with that of control seedlings. The AM fungi improved growth of the Brazilian pine *Araucaria angustifolia* (Araucariaceae) (Zandavalli et al., 2004). There are no reports on the improved growth of Thymelaeaceae tree species following AM fungal inoculation. Tawaraya et al. (2003) found the natural AM colonization of *Gonystylus bancanus* (Thymelaeaceae) in the peat swamp forests of Kalimantan. However, no information is available regarding the effect of AM fungi on the early growth of *Aquilaria* species. The objective of this study was to determine the effect of five AM fungi, *Entrophospora* sp. Ames and Schneider, *Gigaspora decipiens* Hall and Abbott, *Glomus clarum* Nicholson and Schenk, *Glomus sp.* ZEA Tulasne and Tulasne and *Glomus* sp. ACA Tulasne and Tulasne, on the early growth of gaharu wood species *A. malaccensis* Lamk., and *Aquilaria crasna* Pierre ex Lecomte under greenhouse conditions.

## II. MATERIALS AND METHODS

Seeds of *Aquilaria malaccensis* (provenance Bangka) were collected from a lowland forest in Bangka island and seeds of *A. crasna* (provenance Bogor) were collected from a forest of Salak mountain in Bogor, West Java. The seeds were soaked in water for about 2 hours and then surface-sterilized by shaking in 5% NaClO for 5 min. They were thoroughly rinsed twice in sterile distilled water. The seeds were sown in a plastic flat contained autoclave-sterilized zeolite and grown under a 55% shading intensity net to control solar radiation because these species require shady conditions. The seeds of *Aquilaria* species were allowed to germinate for 21 days after sowing. Ultisol was collected from Haurbentes Experimental Forest, Jasinga, West Java and stored in a greenhouse. The soil was passed through a 5 mm sieve and then mixed with river sand (3:1, v/v) to improve drainage. The pH (H₂O) of the mixed soil was 4.7, available P (Bray-1) was 0.17 mg kg⁻¹ and total N (Kjeldahl) was 0.17 %. The mixed soil was sterilized at 121 °C for 30 minutes. Arbuscular mycorrhizal (AM) fungi of *Entrophospora* sp, *Gi. decipiens*, *G. clarum*, *Glomus sp.* ZEA and *Glomus* sp. ACA were isolated.
from peat soil of Kalampangan, Palangkaraya, Central Kalimantan, by trap culture. The pot cultures began as single spore cultures. They were propagated in pot cultures of *Pueraria javanica*. Plastic pots were filled with 175 g of sterilized zeolite and 5 g of AM fungal inoculum in the planting hole. Then, two of 6-day-old *P. javanica* seedlings were transplanted into the pots and placed under natural light greenhouse conditions with no temperature and humidity control. After 90 days in that condition, spores, external hyphae and colonized roots of *Entrophospora* sp, *Gi. decipiens*, *G. clarum*, *Gloous* sp. ZEA and *Glomus* sp. ACA were observed in the zeolite. Polyethylene pots (size 15 x 10 cm) were filled with 500 g of sterilized mixed soil. AM inoculation was achieved by placing 5 g of AM inoculum of each species 1-3 cm under seedling. One 21-day-old *A. malaccensis* or *A. crasna* seedling was transplanted into the pots. Seedlings were watered daily with tap water to field capacity. Weed and pest controls were carried out manually. The seedlings were grown for 180 days in a greenhouse of the Forest and Nature Conservation Research and Development Center (FNCRDC), Bogor, West Java.

The experiment consisted of six treatments of *A. malaccensis* and *A. crasna* seedlings:

(a) control (no inoculum); (b) *Entrophospora* sp.; (c) *Gi. decipiens*; (d) *G. clarum*; and (e) *Glomus* sp. ZEA; (f) *Glomus* sp. ACA. Each treatment was replicated five times. After harvest, shoots and roots were separated. They were oven-dried at 70°C for 72 h before weighing. Ground shoots were digested with H$_2$SO$_4$ and H$_2$O$_2$ solution (3:1, v/v). Nitrogen (N) and phosphorus (P) contents in the digested solution were determined by the semi-micro Kjeldahl method and molybdovanado-phosphoric acid method (Olsen and Sommers, 1982) respectively. An additional 30 seedlings each of *A. malaccensis* and *A. crasna* with *Entrophospora* sp., *Gi. decipiens*, *G. clarum*, *Glomus* sp. ZEA, *Glomus* sp. ACA or uninoculated were grown under the same conditions as those of the seedlings in the above experiment as controls. Numbers of viable seedlings were counted 180 days after transplanting. Survival rate was calculated as follows:

Survival rate (%) = (number of viable seedlings / number of initial seedlings 30) x 100

The roots were cleared in 100 g l$^{-1}$ KOH for 1 h, acidified with diluted HCl and stained with 500 mg l$^{-1}$ tryphan blue in lactoglycerol (Brundrett et al., 1996). The roots were then destained in 50% glycerol. 1-cm segments were viewed under a compound microscope at x200 magnification. The percentage root length colonized by AM fungi was estimated by scoring the presence or absence of AM fungal structures (McGonigle et al., 1990). Mycorrhizal dependency was calculated according to Plenchette et al. (1983): MD (%) = (dry weight of mycorrhizal plant-dry weight of non mycorrhizal plant) / (dry weight of mycorrhizal plant) x 100. Data were statistically analyzed using the analysis of variance (ANOVA) with the statistical software StatView 5.0 (Abacus Concepts). Comparison of means was done using the least significant difference (LSD) method and showed significant differences at 5% probability level. Interaction between nutrient content (N and P) and shoot growth were tested with a linear regression (fitted line plot) and determined correlation coefficients.
III. RESULTS AND DISCUSSION

A. AM Colonization and Shoot Nutrient Concentrations

*A. crasna* roots were more highly colonized by *Entrophospora* sp., *Gi. decipiens*, *G. clarum*, *Glomus* sp. ZEA, and *Glomus* sp. ACA than roots of *A. malaccensis* at 6 months after transplantation under greenhouse conditions (Table 1). There was significant in percentage colonization among treatments, except *Glomus* sp. ACA in weight, steam diameter and *G. clarum* in shoot DW and root DW. The control seedlings of both *Aquilaria* species were colonized by indigenous AM fungi.

Table 1. Arbuscular mycorrhizal (AM) colonization, plant growth and survival rates of *A. malaccensis* and *A. crasna* inoculated with or without AM fungi under greenhouse conditions

| Treatment       | AM colonization (%) | Height (cm) | Stem diameter (mm) | Fresh weight (FW) | Dry weight (DW) | Survival rates (%) |
|-----------------|---------------------|-------------|--------------------|-------------------|-----------------|--------------------|
| *A. malaccensis*|                     |             |                    |                   |                 |                    |
| Control         | 1 a                 | 16.43 a     | 2.28 a             | 1.46 a            | 0.52 a          | 0.41 a             | 0.18 a             | 73                |
| Entrophospora sp.| 97 b               | 25.97 c     | 3.88 e             | 4.68 e            | 2.24 c          | 1.44 c             | 0.48 c             | 100               |
| Gigaspora decipiens | 88 b           | 21.91 b     | 3.02 b             | 2.92 b            | 1.20 b          | 0.88 b             | 0.27 b             | 100               |
| Glomus clarum   | 83 b               | 19.96 b     | 2.94 b             | 2.90 b            | 1.28 b          | 1.95 c             | 0.78 c             | 97                |
| Glomus sp. ZEA  | 84 b               | 22.33 b     | 3.26 b             | 2.62 b            | 1.38 b          | 0.79 b             | 0.27 b             | 90                |
| Glomus sp. ACA  | 86 b               | 21.30 b     | 3.12 b             | 2.74 b            | 1.22 b          | 0.89 b             | 0.26 b             | 93                |
| *A. crasna*      |                     |             |                    |                   |                 |                    |                    |                   |
| Control         | 4 a*               | 20.90 a     | 2.90 a             | 0.68 a            | 1.06 a          | 0.33 a             | 0.13 a             | 70                |
| Entrophospora sp.| 73 b               | 46.14 c     | 5.40 c             | 12.58 b           | 5.72 b          | 3.82 b             | 1.35 b             | 100               |
| Gigaspora decipiens | 63 b           | 29.58 b     | 4.10 b             | 11.64 b           | 7.36 b          | 3.26 b             | 1.56 b             | 100               |
| Glomus clarum   | 78 b               | 32.43 b     | 4.40 b             | 8.82 b            | 4.30 b          | 0.86 a             | 0.27 a             | 100               |
| Glomus sp. ZEA  | 78 b               | 38.94 c     | 4.70 b             | 9.92 b            | 4.54 b          | 2.99 b             | 1.01 b             | 87                |
| Glomus sp. ACA  | 59 b               | 24.60 a     | 3.70 a             | 13.46 b           | 6.94 b          | 4.19 b             | 1.52 b             | 100               |

Remarks: * values with the same letter are not significantly different (P<0.05)

N and P concentrations in shoot of *A. malaccensis* were higher in AM-colonized seedlings than that in control seedlings (Table 2) and there were differences in shoot N and P concentrations of *A. malaccensis* among five AM fungi. The AM colonization by *Entrophospora* sp., *Gi. decipiens*, *G. clarum*, *Glomus* sp. ZEA, and *Glomus* sp. ACA increased shoot N and P contents of *A. malaccensis*. *A. malaccensis* inoculated with five AM fungi was difference in shoot N and P contents. The N and P concentrations were significantly different in shoots of *A. crasna* seedling inoculated with *Entrophospora* sp, *G. clarum*, *Glomus* sp., ZEA and *Glomus* sp. ACA from those of control seedlings (Table 2), except N concentrations of *A. crasna* inoculated with *Gi. decipiens* were not different from control seedlings. There were differences in shoot N and P contents of *A. crasna* among five AM fungi.
Table 2. Nutrient concentrations and contents of _A. malaccensis_ and _A. crasna_ inoculated with or without AM fungi under greenhouse conditions

| Treatment            | N concentrations (mg/g) | N contents (mg/plant) | P concentrations (mg/g) | P contents (mg/plant) |
|----------------------|-------------------------|-----------------------|-------------------------|------------------------|
| **A. malaccensis**   |                         |                       |                         |                        |
| Control              | 8.6 ± 0.2 a             | 3.49 ± 0.5 a          | 0.65 ± 0.02 a           | 0.26 ± 0.04 a          |
| Entrosphora sp.      | 12.1 ± 0.1 d            | 17.28 ± 2.09 c        | 0.73 ± 0.01 b           | 1.06 ± 0.15 b          |
| **Gigaspora decipiens** | 10.7 ± 0.1 c          | 9.02 ± 0.7 b          | 0.85 ± 0.01 c           | 0.75 ± 0.07 b          |
| Glomus clarum        | 10.4 ± 0.1 b            | 20.5 ± 3.3 c          | 0.72 ± 0.02 b           | 1.60 ± 0.20 c          |
| Glomus sp. ZEA       | 11.1 ± 0.2 c            | 8.8 ± 0.9 b           | 0.77 ± 0.03 b           | 0.60 ± 0.07 b          |
| Glomus sp. ACA       | 10.9 ± 0.2 c            | 9.7 ± 1.8 b           | 1.04 ± 0.03 d           | 0.92 ± 0.17 b          |
| **A. crasna**        |                         |                       |                         |                        |
| Control              | 7.9 ± 0.1 a*            | 2.6 ± 0.6 a           | 0.78 ± 0.02 a           | 0.26 ± 0.06 a          |
| Entrosphora sp.      | 9.8 ± 0.1 c             | 37.7 ± 4.3 d          | 1.42 ± 0.03 c           | 5.40 ± 0.60 c          |
| **Gigaspora decipiens** | 8.2 ± 0.2 a            | 26.7 ± 4.1 c          | 0.85 ± 0.02 b           | 2.80 ± 0.50 b          |
| Glomus clarum        | 8.7 ± 0.2 b             | 7.46 ± 1.05 b         | 0.95 ± 0.02 c           | 0.82 ± 0.14 b          |
| Glomus sp. ZEA       | 8.7 ± 0.1 b             | 25.87 ± 3.67 c        | 0.96 ± 0.03 c           | 2.85 ± 0.41 b          |
| Glomus sp. ACA       | 10.8 ± 0.2 d            | 45.9 ± 9.6 e          | 1.22 ± 0.02 d           | 5.14 ± 1.00 c          |

Remarks: * values with the same letter are not significantly different (P<0.05)

Almost all parameters of plant growth of _Aquilaria_ species were correlated with N and P content. Total fresh weight and dry weight of _A. malaccensis_ or _A. crasna_ were significantly correlated with N and P content (Table 3 and Table 4). Stem diameter of _A. malaccensis_ or _A. crasna_ was not significantly correlated with N and P contents. Only shoot height of _A. malaccensis_ or _A. crasna_ has significant relationship (P < 0.05) with P content and N content. Correlation analyses revealed a positive relationship between shoot dry weight of _A. malaccensis_ or _A. crasna_ and N content (Figure 1). Similarly, P content was positively correlated with shoot dry weight of _A. malaccensis_ or _A. crasna_ (Figure 2).

Table 3. Correlation coefficient (r) between shoot growth and nutrient content of _A. malaccensis_ seedlings inoculated with or without AM fungi

| Parameter      | Height | Stem diameter | Fresh weight | Dry weight | N content | P content |
|----------------|--------|---------------|--------------|------------|-----------|-----------|
| **Height**     | *****  | *****         | *****        | NS         | *         | NS        |
| **Stem diameter** | *****  | *****         | *****        | NS         | NS        | NS        |
| **Fresh weight**   | *****  | *****         | **NS**       | **NS**     | **NS**    | **NS**    |
| **Dry weight**    | NS     | NS            | NS           | ***        | ***       | ***       |
| **N content**     | *      | NS            | *            | ***        | **NS**    | **NS**    |
| **P content**     | NS     | NS            | *            | ***        | **NS**    | **NS**    |

Remarks: ****: P < 0.01
****: P < 0.001
*: P < 0.05
NS : non-significant
Table 4. Correlation coefficient (r) between shoot growth and nutrient content of *A. crasna* seedlings inoculated with or without AM fungi

| Parameter  | Height | Stem diameter | Fresh weight | Dry weight | N content | P content |
|------------|--------|---------------|--------------|------------|-----------|-----------|
| Height     | ***    | ***           | *            | NS         | NS        | *         |
| Stem diameter | ***    | ***          | *            | NS         | NS        | NS        |
| fresh weight | *      | *            | ***          | ***        | ***       | ***       |
| Dry weight | NS     | NS           | ***          | ***        | ***       | ***       |
| N content  | NS     | NS           | ***          | ***        | ***       | ***       |
| P content  | *      | NS           | ***          | ***        | ***       | ***       |

Remarks: ***: P < 0.001  
**: P < 0.01  
*: P < 0.05  
NS: non-significant

Table 5. Mycorrhizal dependency values (Plenchette *et al.*, 1983) calculated from shoot dry weights of 6-month-old seedlings of *Aquilaria* species, inoculated or not with five different AM fungi

| Aquilaria species | Mycorrhizal Dependency (MD, %) |
|------------------|--------------------------------|
|                  | Control | Entrophospora sp. | *G. decipiens* | *G. clarum* | Glomus sp.ZEA | Glomus sp.ACA |
| *A. malaccensis* | -       | 72              | 54           | 79          | 49          | 55          |
| *A. crasna*      | -       | 91              | 89           | 62          | 89          | 92          |

B. Mycorrhizal Dependency (MD)

For a given fungus, MD values varied depending on the host species (Table 5). MD values obtained in this study with *Aquilaria* exceeded 55%. The MD of *A. malaccensis* and *A. crasna* ranged from 49 to 79% and from 62 to 92%, respectively. *A. crasna* had the highest MD values.

C. Plant Growth and Survival Rate

The AM colonization by five AM fungi increased shoot height of *A. malaccensis* (Table 1). *Entrophospora* sp., *G. decipiens*, *G. clarum*, Glomus sp. ZEA, and Glomus sp. ACA also increased the stem diameter of *A. malaccensis*. In addition, inoculation with five AM fungi increased shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight of *A. malaccensis*. There were differences in plant growth among *A. malaccensis* seedlings inoculated with five AM fungi. The AM colonization by *Entrophospora* sp., *G. decipiens*, *G. clarum*, Glomus sp. ZEA increased shoot height and stem diameter of *A. crasna* at 6 months after transplantation (Table 1). Furthermore, inoculation of *Entrophospora* sp., *G. decipiens*, *G. clarum*, Glomus sp. ZEA and Glomus sp. ACA increased shoot fresh weight, root fresh weight, shoot dry weight and root dry weight. There were differences in plant growth among *A. crasna* seedlings inoculated with five AM fungi. The AM colonization by Glomus sp. ACA did not increase the shoot height and the stem diameter of *A. crasna*.

The AM colonization by *Entrophospora* sp., *G. decipiens*, *G. clarum*, Glomus sp. ZEA, and Glomus sp. ACA increased the survival rates of *A. malaccensis* at 6 months after transplantation under greenhouse conditions (Table 1). The survival rates of *A. crasna* inoculated with five AM fungi were also higher than those of *A. crasna* with control.
Arbuscular mycorrhizal fungi increased shoot P content and shoot dry weight of *A. malaccensis* (A) and *A. crasna* (B) inoculated with or without AM fungi under greenhouse conditions. ( ○ ) Control; ( □ ) *Entrophospora* sp.; ( ■ ) *G. decipiens*; ( △ ) *G. clarum*; ( △ ) *Glomus* sp. ZEA; ( □ ) *Glomus* sp. ACA. ***: *P* < 0.001; **: *P* < 0.01; *: *P* < 0.05; ns : non-significant.

The present study shows the importance of AM fungi for the early growth and mineral nutrition of three species of gaharu woods. It demonstrates for the first time the effect of AM fungi on *A. malaccensis* and *A. crasna* seedlings under greenhouse conditions at 6 months after transplantation. Our results provide evidence that not only AM fungi improve plant growth and nutrient content, but also gaharu wood trees differ in their response when inoculated with selected AM fungi during the early growth. Our finding agrees with this previous study which showed that AM fungi increased early growth of 11 species of *Eucalyptus*. (Adjoud *et al.*, 1996), *Sesbania aegyptiaca* and *S. grandiflora* (Giri and Mukerji, 2004).

Shoot N or P concentration of *Aquilaria* species were more higher in the AM seedlings than in the control ones, demonstrating that in the absence of AM associations, *Aquilaria* species were not capable to harvest enough N or P from the soil and keep adequate levels in their tissues, despite the major reduction in plant growth. The level of AM effectiveness of all the plant-fungus combination examined was also influenced by functional compatibility, measured as P concentrations. The P concentration had the most consistent effect on shoot biomass production in tropical fruit trees (Guissou *et al.*, 1998). This has already been well-documented in studies on other mycotrophic plants' production in fruit trees. Guissou *et al.* (1998) observed that *Parkia biglobosa*, *Tamarindus indica* and *Zizyphus mauritiana* responded to
three isolate of *Acaulospora spinosa*, *Glomus aggregatum* and *G. manihotis* better than to *G. mosseae* and *G. intraradices*. They suggested that enhanced P nutrition is the most likely cause of an increase in yield of these fruit tree seedlings.

Studies on *Aquilaria* species and other tropical tree species (Muthukumar *et al.*, 2001) show that inoculation with AM fungi can reduce fertilizer requirement in plant production during nursery stage. Although such a benefit cost ratio was not tested in this study, the results undoubtedly indicate that AM inoculations can substantially reduce chemical fertilizer requirement in *Aquilaria* species seedling stocks production. Whatever may be the mechanism of action, inoculation with AM fungi, helps the early growth of *Aquilaria* species in acid soils having suboptimal AM fungi populations, regardless of the presence of native symbionts.

Mycorrhizal dependency (MD) in acid soil was higher in *A. crasna* than that in *A. malaccensis*. Based on these data, we propose a ranking of *Aquilaria* species according to the MD categories defined by Habte and Manajunath (1991): *A. crasna* were considered very highly dependent (MD > 75%) and *A. malaccensis* were highly dependent (25-50% MD). The MD is frequently related to the morphological properties of the root of different plant species and also root systems with only a few, short root hairs are indicative of a high MD of the plant species concerned (Baylis, 1970). However, these *Aquilaria* species had a considerable degree of dependence on *Entrophospora* sp., *Gi. decipiens*, *Glomus* sp. ACA, *G. clarum*, and *Glomus* sp. ZEA. A similar effectiveness of AM fungi for different plant species was reported by Adjoud *et al.* (1996) and Guissou *et al.* (1998). The growth of *A. crasna* is faster than that of *A. malaccensis* at 6 months after transplantation under greenhouse conditions. The AM *Entrophospora* sp. colonization showed higher compatibility for association with *A. crasna*. In addition, *A. crasna* seeds had the highest probability of germination success (92%) whereas those of *A. malaccensis*, *A. microcarpa* and *A. filaria* had the lowest (53%) (Soehartono and Newton, 2001b).

The survival rate of *Aquilaria* species seedlings is a criteria of success in reforestation programs. These results also have a number implication for management of *Aquilaria* species population. *Aquilaria* species may be mix planted in *Hevea brasiilensis* (rubber tree) plantation and *Elaeis guineensis* (oil palm) plantation with agroforestry system for accelerating the population because these estate crops have a wide spacing (6x6 m or 7x7 m). Alternatively, *Aquilaria* species may be planted in the border of each block of estate crops, because *Aquilaria* spp. are shade tolerant in the young seedling stage (Ding Hou, 1960). The estate crop company or farmers may get profit and help promoting nature conservation in the context of the CITES listing of *Aquilaria* spp.

**IV. CONCLUSION AND SUGGESTION**

It can be concluded that colonization by *Entrophospora* sp, *Gi. decipiens*, *G. clarum*, *Glomus* sp. ZEA and *Glomus* sp. ACA increased N and P content, plant growth and survival rates of *A. malaccensis* and *A. crasna* seedlings at 6 months after transplantation under greenhouse conditions. *Entrophospora* sp. showed higher compatibility for symbiosis with its host than *Gi. decipiens*, *G. clarum*, *Glomus* sp. ZEA and *Glomus* sp. ACA. *Entrophospora* sp. was more effective in improving nutrient content and plant growth of *Aquilaria* species than *Gi. decipiens*, *G. clarum*, *Glomus* sp. ZEA and *Glomus* sp. ACA. *Entrophospora* sp might be the best choice if these
Aquilaria species are selected for reforestation programmes. When Aquilaria seedlings are being grown in nurseries, the cultivation methods and the degree of AM symbiosis can affect post-planting success, particularly when the seedlings are destined for degraded lands or regeneration of tropical forest. Therefore, it is suggested Aquilaria should be adapted to P-limited environments, mainly when it is inoculated with specific AM fungi. In addition, AM fungi can help early growth of Aquilaria species and establish seedling stocks production in nursery scale.

REFERENCES

Adjoud D., C. Plenchette, R. Halli-Hargas and F. Lapeyrie. 1996. Response of 11 eucalyptus species to inoculation with three arbuscular mycorrhizal fungi. Mycorrhiza 6: 129-135.

Baylis, G.T.S. 1970. Root hairs and phycomycetous mycorrhizas in phosphorus-deficient soil. Plant Soil 33: 713716.

Béreau M, T.S. Barigah, E. Louisanna and J. Garbaye. 2000. Effects of endomycorrhizal development and light regimes on the growth of Dicorynia guianensis Amshoff seedlings. Ann For Sci 57: 725-733.

Brundrett M, N. Bouger, B. Dell, T. Grove and N. Malajczuk. 1996. Working with mycorrhizas in Forestry and Agriculture. ACIAR Monograph 32, Canberra.

CITES. 2005. Convention on International Trade in Endangered Species of Wild Fauna and Flora. Appendices I, II and III of CITES. UNEP. 48 pp.

Ding Hou. 1960. Thymelaeaceae. In: C.G.G.J. Van Steenis (ed), Flora Malesiana, Series I, Vol. 6, Wolters-Noordhoff, Groningen, The Netherlands, pp. 1-15.

Giri B. and K.G. Mukerji KG. 2004. Mycorrhizal inoculant alleviates salt stress in Sesbania aegyptiaca and Sesbania grandiflora under field conditions: evidence for reduced sodium and improved magnesium uptake. Mycorrhiza 14: 307-312.

Guisso T, A.M. Bâ, J-M. Ouadba, S. Guinko and R. Duponnois. 1998. Responses of Parkia biglobosa (Jacq.) Benth, Tamarindus indica L. and Zizyphus mauritiana Lam. to arbuscular mycorrhizal fungi in a phosphorus-deficient sandy soil. Biol Fertil Soils 26: 194-198.

Habte M. and A. Manajunath. 1991. Categories of vesicular-arbuscular mycorrhizal dependency of host species. Mycorrhiza 1: 3-12.

Haselwandter K. and G.D. Bowen. 1996. Mycorrhizal relations in tree for agroforestry and land rehabilitation. For Ecol Manage 81:1-17.

McGonigle T.P., M.H. Miller, D.G. Evans, G.L. Fairchild and J.A. Swan. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol 115: 495-501.
Muthukumar T, K. Udaiy and V. Rajeshkannan. 2001. Response of neem (*Azadirachta indica* A. Juss) to indigenous arbuscular mycorrhizal fungi, phosphate-solubilizing and asymbiotic nitrogen-fixing bacteria under tropical nursery conditions. Biol Fertil Soils 34: 417-426.

Olsen S.R. and L.E. Sommers. 1982. Phosphorus. *In:* A.L. Page (ed), Methods of soil analysis, Part 2 Chemical and microbiological properties, American Society of Agronomy, Madison, p 403-430.

Paoli G.D., D.R. Peart, M. Leighton and I. Samsoedin. 2001. An ecological and economic assessment of the nontimber forest product gaharu wood in Gunung Palung National Park, West Kalimantan, Indonesia. Conservation Biology 15: 1721-1732.

Plenchette C., J.A. Fortin, and V. Furlan. 1983. Growth responses of several plant species to mycorrhizae in a soil of moderate P-fertility. I. Mycorrhizal dependency under field conditions. Plant Soil 70: 199209.

Soehartono T. and A.C. Newton. 2001a. Conservation and sustainable use of tropical trees in the genus *Aquilaria* II. Status and distribution in Indonesia. Biol Conservation 96: 83-94.

Soehartono T. and A.C. Newton. 2001b. Reproductive ecology of *Aquilaria* spp. in Indonesia. For Ecol Manage 152: 59-71.

Tawaraya K., Y. Takaya, M. Turjaman, S.J. Tuah, S.H. Limin, Y. Tamai, J.Y. Cha, T. Wagatsuma and M. Osaki. 2003. Arbuscular mycorrhizal colonization of tree species grown in peat swamp forests of Central Kalimantan, Indonesia. For Ecol Manage 182: 381-386.

Zandavalli R.B., L.R. Dillenburg, P.V.D. de Souza. 2004. Growth responses of *Araucaria angustifolia* (Araucariaceae) to inoculation with the mycorrhizal fungus *Glomus clarum*. App Soil Ecol 25: 245-255.