# MYCORRHIZAL FUNGI INCREASED EARLY GROWTH OF TROPICAL TREE SEEDLINGS IN ADVERSE SOIL

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## ABSTRACT

The rate of reforestation has increased throughout the countries in Southeast Asia region during the last 20 years. At the same time, inconvenient situations such as forest destruction, forest exploitation, illegal logging, clear-cut forest areas, old agricultural lands, post-wildfire areas, conversion of natural forests into plantations, resettlement areas, mine lands, and amended adverse soils have also been increasing significantly. Mycorrhizas, hovewer, play important role to increase plant growth, enrich nutrient content and enhance survival rates of forest tree species in temperate and sub-tropical regions. Unfortunately, a little information so far is available regarding the effect of mycorrhizas on growth of tree species growing in tropical forests. In relevant, several experiments were carried out to determine whether ectomycorrhizal (ECM) fungi and arbuscular mycorrhizal (AM) fungi can enhance mycorrhizal colonization, nutrient content, and plant growth of some tropical rain forest tree species in Indonesia under nursery and field conditions. The families of tropical tree species used in the experiment were Thymelaeaceae (*Aquilaria crassna*), Leguminosae (*Sesbania grandifolia)*, Guttiferae (*Ploiarium alternifolium* and *Calophyllum hosei*), Apocynaceae (*Dyera polyphylla* and *Alstonia scholaris*), and Dipterocarpaceae (*Shorea belangeran*). These families are important as they provide timber and non-timber forest products (NTFPs). This paper discusses the role of mycorrhizal fungi in increasing early growth of tropical tree seedlings in adverse soil.

Keywords: Deforestation, Ectomycorrhiza, arbuscular mycorrhiza, colorization, nutrient content

## I. INTRODUCTION

Tropical forests do far more than sustain biodiversity (Laurance, 1999). They are homes to indigenous people, shelter pharmacopeias of natural products, and

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provide vital ecosystem services, such as flood amelioration and soil conservation. At regional and global scales, tropical forests also have a major influence on climate and carbon storage. However, tropical forests have been disappearing at the alarming rate of approximately 13.5 million ha each year, due largely to logging, burning and clearing for agriculture and shifting cultivation (Kobayashi, 2004). Timber harvesting has resulted in the transformation of more than 5 million ha of tropical forest annually into over-logged, poorly managed and degraded forests. Degraded tropical forests require wide-scale rehabilitation. It is necessary to improve the biological diversity of tropical forests and concurently to enhance the commercial value of timber, pulp and non timber forest products (NTFPs).

Today, degraded tropical forests require extensive rehabilitation and an annual supply of high-quality seedling stocks. The major obstacle in the rehabilitation of tropical forests is slow growth and high mortality of seedlings in the nursery for reforestation. Poor plant growth in highly acidic soil can be due to a combination of acidity, toxicities and nutrient deficiencies and lack of beneficial microbes, such as ectomycorrhizal (ECM) and arbuscular mycorrhizal (AM) fungi. Acid soils in tropical peat land and mine land may physically and chemically damage root system, thus reducing the ability of roots to penetrate the soil and gain access to water and nutrients. In addition, plants weakened by growing in acidic soils are more vulnerable to microbial attack and disease (Jeffries *et al.*, 2003). Furthermore, the acidic soils may become compacted to the extent of restricting root growth, which, in turn, reduce root exploration of the soil for nutrients and water (Augé, 2001). Today, overburdened mining area and degraded peat-swamp forests require extensive rehabilitation and an annual supply of high-quality seedling stocks. The major obstacles in the rehabilitation of overburdened mining area and peat-swamp forests are slow growth and high mortality of seedlings in the nursery for reforestation. ECM and AM fungi play an important role to increase plant growth, enrich nutrient content and enhance survival rates of forest tree species in temperate and sub-tropical region. However, a little information is available regarding the effect of ECM and AM fungi on growth of tree species in tropical forests.

In consequence, the objective of this study was to utilize mycorrhizal fungi for rehabilitation of degraded tropical forest in Indonesia. Several experiments were carried out to determine whether or part ectomycorrhizas (ECM) and arbuscular mycorrhizal (AM) fungi improve early growth of some tropical rain forest tree species in Indonesia under nursery and field conditions.

## II. MATERIALS AND METHODS

### A. Seed and Soil Preparation

Cutting materials of *P. alternifolium* and *C. hosei* were collected from wildlings at Nyaru Menteng arboretum near Palangkaraya in Central Kalimantan. The wildlings were 1-2 years old and stood approximately 0.5 to 1.5 m in height. The wildlings

with orthotropic position were cut to a length of approximately 10 cm leaving with two leaves. The leaf area was reduced to approximately half its original size. Cutting medium containing of crushed coconut fiber mixed with rice husks (2:1, v/v) was sterilized in an autoclave at 121 °C for 50 minutes. Seeds of *A. scholaris*, *A. crassna*, and *S. grandifolia* were collected from Bogor, West Java. Meanwhile, seeds of *S. belangeran* and *D. polyphylla* were brought in from Palangkaraya in Central Kalimantan. These seeds were soaked in water for about 2 hours and then surface-sterilized by shaking in 5% NaClO solution for 5 minutes. They were thoroughly rinsed twice in sterile distilled water. The seeds were sown in a plastic flat containing autoclave-sterilized zeolite and grown into seedlings under the net with 55% shading intensity to control incoming solar radiation. For *A. scholaris* seeds, the soil used in the experiment was an ultisol type collected from Haurbentes, Bogor. For *A. crassna* and *S. grandifolia* seeds, the soil used was an ultisol collected from overburdened mining area, Tanjung and stored in a nursery. The soil particles were passed through a 5 mm sieve and then mixed with river sand (3:1, v/v) to improve drainage. For *S. belangeran* seeds, peat type soil as used for the pot experiment was collected from a peat swamp forest in Kalampangan, Palangkaraya, Central Kalimantan and then sterilized by heating over a wood fire.

**B. Mycorrhizal Inoculation**

Fungi of *Glomus clarum*, *Gigaspora decipiens*, *Glomus* sp. ACA, *Entrophospora* sp. and *Glomus* sp. ZEA were propagated in plastic pot cultures. *Glomus aggregatum* was obtained from Osaka Gaz Ltd. (Japan). The plastic pots were then filled with zeolite and sometime later grown with AM isolates. *Pueraria javanica* were transplanted into the pots. All propagules of the AM isolates were observed three months afterwards. AM inoculation was required by placing 5 g of inoculums below seedlings. For *A. crassna* and *S. grandifolia* seedlings, the following treatments were used: (1) control (no inoculation), (2) AM inoculum of *G. clarum.* There were 50 replications for each treatment. For *P. alternifolium* and *C. hosei* cuttings, the experiment consisted of three treatments: (1) control (no inoculation); (2) inoculation with *G. aggregatum*; and (3) inoculation with *G. clarum.* There were 15 replications for each treatment. For *D. polyphylla*, the experiment consisted of three treatments: (1) control; (2) inoculation with *G. clarum*; (3) inoculation with *G. decipiens*. There were 30 replications for each treatment. For *A. scholaris*, the experiment consisted of six treatments: (1) control; (2) inoculation with *G. clarum*; (3) inoculation with *G. decipiens*; (4) inoculation with *Glomus* sp. ACA; (5) inoculation with *Entrophospora* sp.; (6) inoculation with *Glomus* sp. ZEA. There were 30 replications for each treatment. Four ectomycorrhizal (ECM) fungi species were *Calvatia* sp., *Boletus* sp., *Scleroderma* sp., and *Strobilomyces* sp for *S. belangeran*. Each fruit body of ECM species was blended in distilled water (1:10, v/v) for 60 seconds using a blender on low speed. A drop of Tween 80 was added to the blender to assist suspension. There were 100 replication for each treatment.

### C. Growth Parameters

The seedlings were grown under nursery conditions. Shoot height, stem diameter, biomass, nutrient content, and mycorrhizal colonization were measured 4-6 months under nursery conditions. After harvest, shoots and roots were separated. They were oven-dried and weighed. Ground shoots were digested with $H_2SO_4$ and $H_2O_2$ solution. N (nitrogen) and P (phosphorus) concentration in the digested solution were determined by the semi-micro Kjeldahl method and vanadomolybdate-yellow assay (Olsen and Sommers, 1982), respectively. C (carbon) concentration was analyzed by gas chromatography. To calculate ECM colonization (%), the total number of root tips and the number of ECM short roots were counted under a dissecting microscope (Brundrett *et al*., 1996). To calculate AM colonization (%), the roots were stained and observed under a compound microscope. The percentage of AM fungi was estimated by scoring the presence or absence of AM structures (Giovannetti and Mosse, 1980).

### D. Statistical Analysis

Data were analyzed statistically using analysis of variance with the statistical software StatView 5.0 (Abacus Concepts). Comparison of means was done using the least significant difference (LSD) method at the 5% probability level where the F-value was significant. Data of AM and ECM colonization were transformed into arcsin (square root of percentage) before statistical analysis.

## III. RESULTS AND DISCUSSION

### A. Adverse Soil: Overburdened Coal Mining Area

AM colonization by *G. clarum* increased shoot height and stem diameter of *A. crassna* after four month exposure under nursery conditions (Figure 1). Present study shows the importance of AM fungi for the early growth and mineral nutrient of gaharu wood of *A. crassna species*. It demonstrates for the first time the positive effect of AM fungi on *A. crasna* seedlings under nursery conditions.

AM colonization by *G. clarum,* increased shoot height of *S. grandifolia* after four month exposure in overburderned coal mining area (Figure 2.). AM fungi also increased the stem diameter of *S. grandifolia*. Our finding agree with this early study which showed that AM fungi increased early growth of 11 *Eucalyptus* spp. (Adjoud *et al*., 1996), 17 leguminous plants (Duponnois *et al*., 2005), *S. aegyptiaca* and *S. grandiflora* (Giri and Mukerji, 2004).

This demonstrates for the first time the positive effect of AM fungi on growth of plants in the families Thymelaeaceae and Leguminosae in overburdened coal mining area. Shoot height and stem diameter were increased by inoculation of AM fungi. These parameters can determine the yield of these species on the timber and NTFPs. Enhancement of plant growth of these species would increase production of

the timber and NTFPs. Furthermore, application of AM fungi of these tree seedling species would be helpful for nature conservation of *A. crassna* as these species listed in Appendices II of the CITES.

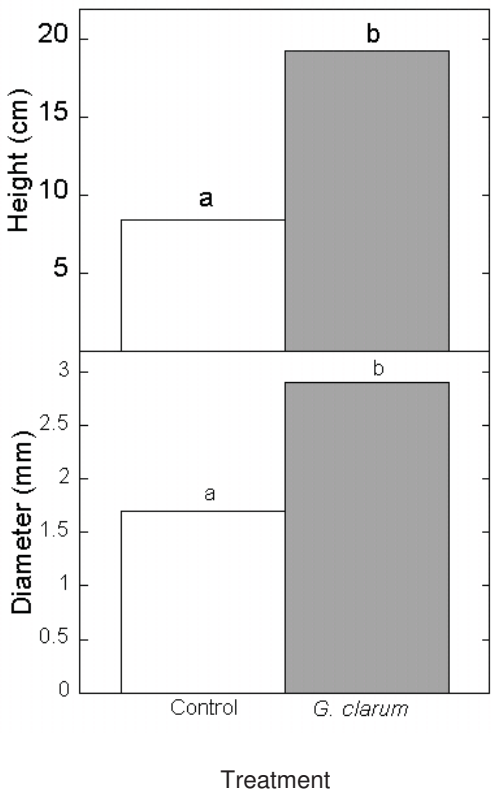


Figure 1. Shoot height and diameter of *A. crassna* inoculated with AM fungi *G. clarum* four months under nursery conditions in post mining area. Values with the same letter are not significantly different ($P < 0.05$). Control (□); *G. clarum* (■).

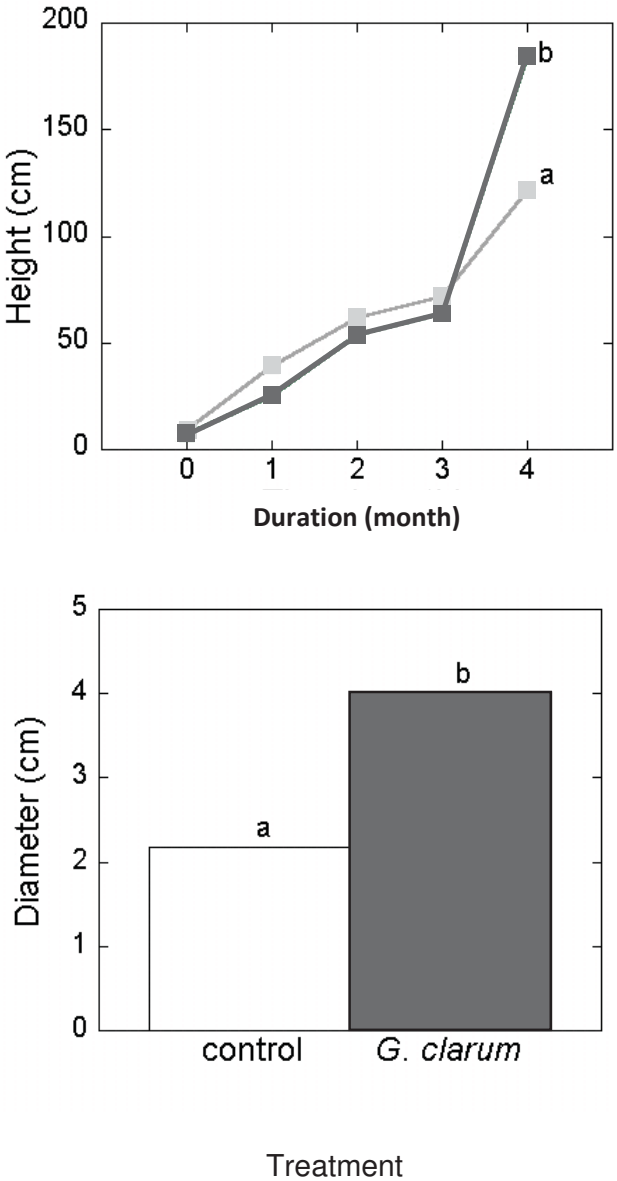


Figure 2. Shoot height (above) and diameter (below) of *S. grandifolia* inoculated with AM fungi *G. clarum* four months after transplanting under field conditions in post mining area. Values with the same letter are not significantly different ($P < 0.05$). Control (□); *G. clarum* (■).

## B. Adverse Soil: Peat Swamp Forest

Inoculation with mycorrhizal fungi increased the plant growth of A. *Scholaris*, *C. hosei*, *D. polyphylla, P. alternifolium*, *and S. belangeran* under nursery conditions or green house conditions (Table 1.). In this study, the inoculation with mycorrhizal fungi significantly increased nutrient content of the tree seedling species compared to the species without inoculation (control). This demonstrates for the first time the positive effect of mycorrhizal fungi on growth of plants in the families Apocynaceae, Guttiferae, and Dipterocarpaceae in peat swamp forests. Shoot height, stem diameter, and biomass of the seedlings were increased by inoculation of mycorrhizal fungi. These parameters can determine the yield of these species on the timber and non-timber forest products (NTFPs). Enhancement of plant growth of these species would increase production of the timber and NTFPs.

Table 1. Mycorrhizal colonization, height, diameter, biomass, and nutrient content of *A. scholaris*, *C. hosei, D. polyphylla*, *P. alternifolium*, *S. belangeran* with or without mycorrhizal fungi inoculation under nursery condition.

| No | Tree species | Family | Treatment | Mycorrhizal coloniz.(%) | Height (cm) | Diameter (mm) | Biomass (g) | Nutrient content N (mg/plt) | P(mg/plt) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | *A. scholaris* | Apocynaceae | control | 5 | 16 | 3.9 | 1.1 | 4.5 | 1 |
| | | | five AM | 64-91 | 32-36 | 6.5-6.9 | 5.0-5.6 | 24-32 | 5.3-7.4 |
| 2 | *C. hosei* | Guttiferae | control | 3 | 5.9 | 1.3 | 1.3 | _ | 2.2 |
| | | | two AM | 18-19 | 8.5-8.9 | 1.9 | 2.7-3.1 | _ | 5.7-5.9 |
| 3 | *D. polyphylla* | Apocynaceae | control | 10 | 11.2 | 2.9 | 0.79 | 12 | 0.34 |
| | | | two AM | 87-93 | 14.1-16.7 | 3.4-3.6 | 1.53-1.67 | 20-25 | 0.8-1.58 |
| 4 | *P. alternifolium* | Guttiferae | control | 3 | 7.2 | 3.3 | 0.21 | _ | 2.17 |
| | | | two AM | 27-32 | 9.5-11.5 | 5.1-5.3 | 0.49-0.53 | _ | 5.74-5.89 |
| 5 | *S. belangeran* | Dipterocarpaceae | control | 12 | 23 | 2.3 | 0.68 | _ | _ |
| | | | four ECM | 59-67 | 27-31 | 2.4 | 0.74-1.12 | _ | _ |

In this study, we showed the importance of mycorrhizal fungi inoculation to *P. alternifolium* and *C. hosei* tropical tree seedlings species on cutting systems. The practical problem faced by reforestation program on disturbed tropical rain forests is a regular supply of planting stocks in nursery. However, this is the preliminary finding about the integration of the propagation cutting system with AM inoculation technology on tropical tree species in Indonesia. Rajan *et al.* (2000)

reported that inoculation of AM fungi using propagation stump system increased plant growth of *T. grandis* under nursery conditions.

Commercially available mycorrhizal inocula can be considered as a mycorrhiza inoculum source for implementation in the mass production process of tropical seedling stocks. The latter can be produced in the company-owned commercial scale nursery. Mycorrhizal inocula must be tested for effectiveness and could be used as single mycorrhiza species or mixed inoculum. The benefits of mycorrhizal inoculation are likely to extend beyond the nursery, as the tree seedlings survive and grow better after out planting. Additional studies are necessary to ensure that these beneficial mycorrhizal effects in the pot and nursery are maintained, and that root colonization by the introduced fungi continues after transferring to out planting under field conditions, i.e. degraded peat-swamp forest, degraded coal-mining area, degraded tin-mining area, etc.

## IV. CONCLUSION

The results of this study confirmed that application of mycorrhizal fungi could increase plant growth, and shoot nutrient content in some tropical tree members of the family Apocynaceae, Dipterocarpaceae, Guttiferae, Thymelaeaceae, and Leguminosae under nursery and field conditions. In this study, some effective and suitable isolates of mycorrhizal fungi were found for inoculating in tropical tree seedling species, i.e. *Scleroderma* sp., *Boletus* sp. (ECM), *Glomus clarum* and *Gigaspora decipiens*. Inoculation can be a promising way to optimize the production of vigorous seedling stocks species which are economically and ecologically important in the rehabilitation of adverse soils in Indonesia.

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## 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.

Augé, R.M. 2001. Water relations, drought and VA mycorrhizal symbiosis. Mycorrhiza 11:3-42.

Bundrett, M., B. Dell, T. Grove, and N. Malajczuk. 1996. Working with mycorrhizas in forestry and agriculture. Australian Centre for International Agricultural Research. Canberra. 374p.

Duponnois, R., H. Founoune, D. Masse, and R. Pontanier. 2005. Inoculation of *Acacia holosericea* with ectomycorrhizal fungi in a semiarid site in Senegal: growth response and influences on the mycorrhizal soil infectivity after 2 years plantation. Forest Ecology and Management 207: 351-362.

Giovannetti, M. and B. Mosse. 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytologist 84: 489-500.

Giri, B. and K.G. Mukerji. 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.

Jeffries, P., S. Gianinazzi, S. Perotto, K. Turnau, and J.M. Barea. 2003. The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biology and Fertility of Soils 37: 1-16.

Kobayashi, S. 2004. Landscape rehabilitation of degraded tropical forest ecosystems case study of the CIFOR/Japan project in Indonesia and Peru. Forest Ecology and Management 201: 13-22.

Laurance,W.F. 1999. Reflection on the tropical deforestation crisis. Biological Conservation 91: 109-117.

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. Pp. 403-430.

Rajan, S.K., B.J.D. Reddy, and D.J. Bagyaraj. 2000. Screening of arbuscular mycorrhizal fungi for their symbiotic efficiency with *Tectona grandis*. Forest Ecology and Management 126: 91-95.