

Utilization of mycorrhizal fungi for rehabilitation of degraded forest in Indonesia

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The rate of reforestation has increased all countries in the Southeast Asia region during the last 15 years. Simultaneously, the rate of forest destruction, forest exploitation, illegal logging, clear-cut forest areas, amended adverse sites, old agricultural lands, mine lands and post-wildfire areas, conversion of natural forests into plantations, resettlement areas, and other uses have also been increasing. There is also increasing concern that the planting stocks of reforestation program are not sufficient to sustain the production of timber and non-timber forest products (NTFPs) and promote forest conservation. Mycorrhizas play an important a role to increase plant growth, nutrient content and survival rates of forest tree species in temperate and sub-tropical region. However no information is available regarding the effect of mycorrhizas on growth of tree species in tropical rain forest forests. The objective of this thesis was to utilize mycorrhizal fungi for rehabilitation of degraded tropical rain forest in Indonesia. Several experiments were carried out to determine whether ectomycorrhizas (ECM) and arbuscular mycorrhizal (AM) fungi enhance mycorrhizal colonization, nutrient content, plant growth and survival rates of some tropical rain forest tree species in Indonesia under greenhouse, nursery and field conditions.

The families of tropical tree species used in the experiment were Dipterocarpaceae, Guttiferae, Thymelaeaceae, Apocynaceae, and Verbenaceae and these families are important as they provide timber and NTFPs. The tree species were *Shorea pinanga*, *S. seminis*, *S. balangeran* (Dipterocarpaceae), *Dyera polyphylla*, *Alstonia scholaris* (Apocynaceae), *Calophyllum hosei*, *Ploiarium alternifolium* (Guttiferae), *Aquilaria malaccensis*, *A. filaria*, *A. crasna*, *A. microcarpa* (Thymelaeaceae) and *Tectona grandis* (Verbenaceae). ECM species were *Pisolithus arhizus* and *Scleroderma columnare* for *S. pinanga* and *S. seminis*. Four ECM species were *Calvatia* sp., *Boletus* sp., *Scleroderma* sp., and *Strobilomyces* sp for *S. balangeran*. *P. arhizus* and *S. columnare* were crushed and pelletized. The mycelium of ECM inoculum was grown in Pachlewski medium and mixed in sodium alginate. The inoculum paste was in calcium chloride solution to obtain a granular alginate inoculum. Inoculation of seedlings was carried out after germination. One tablet or two alginate beads was applied to a seedling of *S.*

pinanga or *S. seminis*. For *S. balangeran* experiment, each of ECM fungal species was blended in distilled water. The spore suspension was applied to *S. balangeran* seedling. For AM inoculum, *Glomus clarum*, *Gigaspora decipiens*, *Glomus* sp. ACA, *Entrophospora* sp. and *Glomus* sp. ZEA were propagated in pot cultures. Plastic pots were filled with zeolite and AM isolates. *Pueraria javanica* were transplanted into the pots. All propagules of five AM isolates were observed after 3 months. AM inoculation was placed five g of inoculum below seedlings. For ECM experiments, peat soil was sterilized by heating over a wood fire. Seeds of *Shorea* species were sown in peat soils and grown under shading net. In the field experiment, The *S. balangeran* seedlings were planted at a line spacing was 1 x 1 m. For AM experiments, an ultisol used was mixed with river sand and sterilized an autoclave. The seedlings were grown in a greenhouse. Shoot height, stem diameter, leaf number, shoot fresh and dry weight, nutrient content, total root length, mycorrhizal colonization and survival rates were measured 4-7 months after transplanting. After harvest, shoots and roots were separated. They were oven-dried and weighed. Ground shoots were digested with H₂SO₄ and H₂O₂ solution. N (Nitrogen) and P (phosphorus) concentration in the digested solution were determined by the semi-micro Kjeldahl method and vanadomolybdate-yellow assay, respectively. Survival rates (%) = number of viable seedlings / number of initial seedlings x 100. To calculate ECM colonization (%), the total number of root tips and the number of ECM short roots were counted under a dissecting microscope. 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.

The percentage of ECM colonization by *S. pinanga* and *S. seminis* was 35-86%. The colonization increased N and P content in both *Shorea* species. There was no difference in the percentage colonization between spore and mycelium inoculum nor between *P. arhizus* and *S. columnare*. Survival rates of *S. pinanga* and *S. seminis* were greater for inoculated seedlings than in control seedlings. A positive relationship was found between N or P content and shoot dry weight of *S. seminis* inoculated with both ECM fungi. The percentage of ECM colonization by *S. balangeran* was 59-67%. Colonization of *S. balangeran* increased shoot height, fresh and dry weight 6 months after transplanting under nursery conditions. The percentage AM colonization of *D. polyphylla*, *A. filaria*, *C. hosei*, *P. alternifolium*, *A. crasna*, *A. malaccensis*, *A. microcarpa* (WK), *A. microcarpa* (EK), *A. scholaris* and *T. grandis* ranged from 87-93%, 22-39%, 27-32%, 18-19%, 63-78%, 83-97%, 83-97%, 63-94% and 50-90%, respectively. Colonization by *G. clarum* and *G. decipiens* increased shoot height, stem diameter, and shoot and root dry weights of *D. polyphylla* and *A. filaria* seedlings. Shoot N and P concentrations of *D. polyphylla* and *A. filaria* seedlings were increased by AM colonization by as much as 70-153% and 135-360%, respectively. Colonization by *G. clarum* and *G.*

aggregatum increased shoot height, stem diameter, leaf number, shoot and root dry weights of *P. alternifolium* and *C. hosei*, with the propagation cutting system. Shoot P content of *P. alternifolium* and *C. hosei* seedling were increased by AM colonization. Colonization by *Entrophospora* sp., *G. decipiens*, *G. clarum*, *Glomus* sp. ZEA and *Glomus* sp. ACA increased shoot height, stem diameter, fresh weight, dry weight, N and P content of *A. crasna*, *A. malaccensis*, *A. microcarpa* (WK) and *A. microcarpa* (EK) seedlings. *Entrophospora* sp. was more effective in improving N and P content, shoot height, stem diameter, fresh weight, dry weight of *Aquilaria* species than *G. decipiens*, *G. clarum*, *Glomus* sp. ZEA and *Glomus* sp. ACA. Moreover, AM colonization also increased total root length of *A. scholaris* seedlings 5 months under greenhouse conditions. Inoculation of seven AM fungal isolates increased shoot height, stem diameter, dry weight of two seedlots of *T. grandis*. *G. manihotis* (Indo-1) showed better compatibility to perform symbiosis with *T. grandis* seedlings, than those other isolates. Survival rates were higher in the AM colonized seedlings at 4-6 months after transplantation than in the control seedlings. The survival rates of *D. polyphylla*, *A. filaria*, *C. hosei*, *P. alternifolium*, *A. crasna*, *A. malaccensis*, *A. microcarpa* (WK), *A. microcarpa* (EK), *A. scholaris* and *T. grandis* ranged from 92-100%, 100%, 100%, 100%, 87-100%, 93-100%, 87-100%, 87-100%, 93-100% and 90-98%, respectively. In the field experiment, colonization by four ECM fungi increased shoot height and stem diameter of *S. balangeran* 40 months after transplanting in the degraded field. To summarize, the results of this study confirm that utilization of mycorrhizal fungi can increase plant growth, nutrient content and survival rates of some tropical tree members of the family Dipterocarpaceae, Apocynaceae, Guttiferae, Thymelaeaceae, and



Verbenaceae under greenhouse, 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. *P. arhizus*, *S. columnare*, *Boletus* sp. (ECM), *G. clarum*, *G. decipiens*, and *Entrophospora* sp. (AM). Inoculation is to be a promising way to optimize the production of vigorous seedling stocks species which are economically and ecologically important in the rehabilitation of degraded tropical forest lands in Indonesia.