MYCORRHIZAL SEEDLING PRODUCTION FOR ENHANCING REHABILITATION OF DEGRADED FOREST IN INDONESIA

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ABSTRACT

Tropical deforestation has become an extremely important global environmental issue over the past five years. The range of the annual deforestation rate in Indonesia is between 0.9-1.2 million hectare and about 13.2 million hectares of degraded tropical rain forest in Indonesia are classified as critical sites, urgently needing re-vegetated. The Indonesia government has an ambitious programme to accelerate efforts to re-vegetate deforested areas. Reforestation however is not an easy task. Adverse edaphic and climatic conditions and low activity of soil microbes are major constraints limiting success of this activity. Transplanted seedlings often have poor growth and low survival rates. To ensure successful tree establishment under such conditions, producing more planting stock of improved quality and introducing effective mycorrhizal fungi onto the seedlings in the nursery are required. Improvement of planting stock quality by manipulation of seedling containers, potting media, inoculation with selected and effective mycorrhizal fungi and nursery management are discussed. This paper provides an overview of techniques to produce mycorrhizal planting stock for enhancing rehabilitation of degraded forest in Indonesia.

Key words: Degraded tropical lands, forest restoration, nursery techniques, seedling quality.

INTRODUCTION

Tropical deforestation has become an extremely important global environmental issue over the past five years. The causes of deforestation are varied, for example population pressure, shifting cultivation, agricultural development, transmigration, forest fires and unsupervised, poor logging practices (WORLD RESOURCES INSTITUTE, 1990; BARROW, 1991). By the end of the 1980s, the annual deforestation rate in Indonesia was between 0.9-1.2 million hectares or about 0.8% of the total forested area (FAO, 1990; FRIENDS OF THE EARTH, 1991).

The negative impact of deforestation leads to increased soil erosion, loss of biological diversity, damage of wildlife habitats, degradation of watershed areas and deterioration in the quality of life (UNCED, 1992). Actions that can help to reduce the rate of deforestation of the tropical Indonesian forest and help to support preservation and restoration of existing

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different forest ecosystems are urgently needed. Concern about the negative effects of deforestation has lead to a reforestation programme becoming a top priority of the Indonesian government and of private companies involved in the forest industry. The government has stepped up efforts to re-vegetate about 13.2 million hectares of critically deforested areas. However, the proposed target will be difficult to achieve due to several major constraints, which are related to the unfavourable environmental conditions existing at the chosen reforestation sites. Edaphic factors (low nutrients, acidic soils), climatic factors (high levels of solar radiation and high temperatures) and biological factors (aggressive competition from the predominant grass *Imperata cylindrica*, a high chance of fire and low soil microbial activities) combine to reduce performance of planted trees.

Edaphic factors

Most reforestation zones and industrial plantations are located in Sumatra and Kalimantan, both dominated by Oxisol and Ultisol soils. About 15 million hectares of potential reforestation sites in Indonesia are acidic, supporting *Imperata cylindrica* grassland (FA0, 1990). These soils exhibit an imbalance in nutrient availability. While they are generally low in nitrogen, phosphorus and bases, such as calcium, magnesium and potassium, they often contain appreciable amounts of exchangeable aluminium, manganese and iron, which may reach toxic levels. Due to this imbalance in nutrient supply, the establishment and growth of tree seedlings is often problematic.

Climatic factors

Climatic factors include a high level of solar radiation, temperature extremes and periods of high wind velocity. These factors promote a fast transpiration rate in newly planted seedlings in reforestation sites. The relative inability of newly planted seedlings to absorb enough water during early establishment, especially during dry periods, can often result in drought stress and increased seedling mortality.

Biological factors

The most significant biotic factor inhibiting the growth of tree seedlings in reforestation sites in the tropics is dominance of the invasive grass *Imperata cylindrica*. This weed has a high competitive ability for water, nutrients and space and also releases allelopathic substances into the rhizosphere. It also has an ability to thrive either in fertile or infertile soils. The inability of tree seedlings to compete with this weed during their establishment has a significant effect on their growth. As a result, seedlings are often stunted and show symptoms of nutrient deficiency.

To improve chances of tree establishment in such adverse sites, production of high quality seedlings and application of mycorrhizal fungi have been tried. The beneficial effects of mycorrhizae in improving seedling performance are widely recognised. This paper reviews techniques of mycorrhizal seedling production for enhancing rehabilitation of degraded land in Indonesia and suggests some directions that it might take in the future.

PLANTING STOCK PRODUCTION

Species selection

To increase reforestation success, planting stock should comprise seed or clonal plant material able to tolerate prevailing site conditions. However, the adaptability and growth of the majority of native tree species in new sites in Indonesia are not well known. Consequently, tree species should be selected for drought resistance, an ability to recover after fire, strong competitive ability against invasive grasses, relatively fast growth rate, allelopathic and acid tolerance, ability to absorb nutrients from infertile soil (nitrogen-fixing and form mycorrhizae) and pest tolerance.

Attempts to evaluate species adaptability on degraded forest land have been carried out, for example in a semi-arid area, with a mean annual rainfall of 370 mm; altitude 49 m asl; sandy-clay soil and pH 6.8-7.2. Among the species tested, highest survival after 16 months was achieved by *Cassia siamea* (89%), followed by *Acacia arabica* (77%), *Eucalyptus* spp. (69%) and *Aleurites mollucana* (59%). Other species (*Sesbania grandiflora*, *Paraserianthes falcataria*, *Artocarpus heterophylla* and *Gmelina arborea*) had a low survival rate (below 40%) and the growth of surviving plants was poor (SETIADI, 1991). Experiments in humid tropical forest (mean annual rainfall 3,900 mm, altitude 60 m asl, poor nutrients, pH 4.6-5.2, vegetation cover dominated by *Imperata* grass) evaluated the survival of 8-month-old trees of five species. Highest survival was achieved by *Vitex pubescens* (88%), followed by *Gmelina arborea* (72%), *Peronema canescens* (68%) and *Swietenia macrophyla* (63%). *Ochroma bicolor* exhibited a low survival (below 40 %) and poor growth. Similar studies of nickel mining sites (SETIADI, 1992) identified *Paraserianthes falcataria*, *Acacia mangium*, *Eucalyptus* spp, *Trichospermum burretii* and *Pinus merkusii* as the most adaptable of 17 species tested after 22 months.

Mycorrhizal seedling production

An increase in denuded areas, which need to be re-vegetated, has increased the need to produce high quality planting stock. Sites in Indonesia targeted for reforestation include those with P-deficient acid soil, where seedling growth is limited. In addition, seedlings may be exposed to adverse environmental conditions and may be susceptible to damage from drought, fire, competition and insects. Therefore, it might be expected that production of high quality seedlings combined with the application of mycorrhizal technology might improve the success of reforestation.

BIODIVERSITY AND PLANT STATUS OF MYCORRHIZAL FUNGI

The novel functions of arbuscular mycorrhizal fungi (AMF), as biological agents for improving growth and health of plants; bioremediation of soil contaminated with heavy-metals and seedling establishment on degraded sites are well recognised (SETIADI, 1995a). Most tree species in tropical regions are colonised by AMF (LEAKEY & NEWTON, 1994).

However, little attention has been paid to the potential application of AMF in tropical silviculture (MICHELSEN, 1992).

A survey of local AMF's and plant mycorrhizal status in disturbed ecosystems such as disturbed forest, grass land, secondary forest, spoil mine sites, tailings, peat forest and semi arid forest has been conducted (SETIADI, 1998a). An initial assessment of mycorrhizal-root interaction of different plants growing in different disturbed ecosystems demonstrated that of 112 trees species assessed, almost 87% of them had mycorrhizal associations. Most pioneer plants establishing on adverse sites are colonised by arbuscular mycorrhizal fungi (SETIADI, 1998b). This may indicate that mycorrhizae are required for the establishment of plants on degraded sites. Spores of AMF have been found in all forest ecosystems studied, except in swamp and mangrove forest. Among the forest ecosystems studied, the number of AMF spores found in converted peat forest was the highest, whilst the number of AMF spores found in Alpine/sub-alpine forest (3,200-4,255 m asl.) was the lowest. Over 23 different spore types were found in a range of Indonesian forests and the AMF species richness (based on spore types) varied among the ecosystems studied. Species richness of AMF was higher in re-colonised tailings and *Imperata* grassland ecosystems, compared to other environments. The genera of *Glomus, Sclelocystis, Acaulospora, Scutelospora* and *Gigaspora,* and AMF species of *Glomus oculltum* and *Acaulospora morrowaiae* were commonly found in all ecosystems. Information regarding diversity of indigenous AMF, their distribution and the frequency of occurrence of specific AMF in different tropical soils in Indonesia is scarce. Further investigation into the biodiversity of these fungi is urgently required.

CULTURING INDIGENOUS TROPICAL ARBUSCULAR MYCORRHIZAL FUNGI

Procedures for establishing pure pot cultures of AMF have mainly been developed for temperate species, collected from soils of neutral pH with moderate to high fertility. The adoption of similar procedures for culturing AMF, collected from tropical conditions (especially from acidic poor soils), is not always satisfactory. Using a modified test tube culture technique, more than 14 indigenous AMF isolates were successfully obtained in pure cultures (SETIADI, 1995a; 1996). These AMF cultures were routinely maintained on the host of *Pueraria phaseoloides,* grown in a soil-less medium of zeolite. Presently more than 50 species of AMF are maintained at the BTIG (Bank of Tropical Indigenous Glomales) of IUC-Biotech-IPB as a germplasm collection.

Inoculant production of indigenous arbuscular mycorrhizal fungi

Arbuscular mycorrhizal fungi are obligate biotrophs. Although several attempts at axenic culturing of these fungi have been made, sustained axenic culture has not yet been achieved. Thus, they cannot be propagated on artificial media without a living host (SIEVERDING, 1991). SETIADI (1995a) compared nine host-substrate treatments on spore production of *Glomus manihotis* (INDO-1), *Acaulospora tuberculata* (INDO-2), *Acaulospora delicata* (EJ-01) and *Glomus mossea* (PAL-03). The highest spore densities of isolate INDO-1 (81 spores g^{-1} of substrate) were produced with a combination of sand and sorghum, whilst lowest spore densities (24 spores g^{-1} of substrate) were produced in sand-bahia grass. Highest spore production of INDO-2 was achieved either in sand-kudzu (83 spores g^{-1} of substrate) or in zeolite-kudzu (61 spores g^{-1} of substrate) combinations. Kudzu-inolite was significantly the best combination for producing spores of both *Acaulospora. delicata* and *Glomus mosseae* (216 spores g^{-1} of substrate and 62 spores g^{-1} of substrate respectively). Combinations of sand and bahia grass had been used previously as a substrate medium and plant host respectively for establishing open pot cultures of AMF at IUC-IPB in Indonesia. This finding suggested that selection of the best combination of host and media for producing mycorrhizal inoculum is crucial, because this will determine the quality and potential of the inoculum.

Further work was carried out with The International Institute of Biotechnology, Kent University UK. Different physical and chemical conditions (types of soil-less media, particle size, pH, nutrient composition) and appropriate hosts were studied, to improve the inoculum quality of selected AMF for reforestation. Using this information, selected AMF were successfully bulked-up in open pot culture using *Sorghum bicolor* as a host, grown in soil-less medium of zeolite, and the mycorrhizal inoculum produced was registered as BIOGROW.

Selection of indigenous AMF as potential inoculum

The possibility of using beneficial attributes of AMF in forestry to promote performance of transplanted seedlings and help re-establish mycotropic plant species will depend on preliminary assessments of whether inoculation is a suitable management option. The greatest immediate potential for AMF as inoculants is where indigenous populations of AMF have been eradicated or drastically reduced, such as in reforestation zones (JEFFRIES & DODD, 1991; SETIADI, 1998a). Reforestation sites in Indonesia are usually P-deficient acidic soils, where seedling growth is limited. Therefore, application of AMF technology might relieve problems of seedling establishment in such areas. All AMF species, however, are not equally effective at promoting seedling growth. Consequently pre-assessment of the effectiveness of AMF species in improving seedling performance in targeted soils is crucial before their use can be recommended. Studies on selecting the most effective indigenous AMF isolates for promoting plant growth in P-deficient acidic soils have been carried out (SETIADI, 1996). Among the AMF tested, *G. manihotis* (INDO-1) and *A. tuberculata* (INDO-2) were best at promoting early growth of seedlings of fast growing tree species. It was suggested that collection and selection of AMF isolates for use for inoculation in reforestation programmes should be made from the target areas. For a beneficial effect from AMF inoculation, selected AMF isolates should be applied in locations with edapho-climatic conditions that are similar to those where the isolates were first collected.

MYCORRHIZAL INOCULATION TECHNIQUES AT THE NURSERY STAGE

An effective method of raising colonised seedlings of trees for reforestation in the nursery has been developed (SETIADI, 1998b). There are four options for practising mycorrhizal inoculation in the nursery; layering pre-inoculation, mixed pre-inoculation, direct local inoculation and direct mixed inoculation. The two former techniques have been used successfully for small seeds that are pre-germinated prior transplanting into poly-bags; these techniques minimise the bulk quantity of inoculum needed for seedling inoculation. Two weeks after pre-germination, roots of 80-100% of seedlings are colonised with an intensity range of 40-60%. In contrast, only 10-15% root colonisation was obtained using the two latter techniques.

Until recently our inability to culture AMF in axenic conditions hampered the production of inoculum on a large scale. The total amount of inoculum required per hectare in forestry, however, is much smaller than in agriculture. The rates of application of inoculum reported in previous publications was 20-30 ton ha^{-1} (BAGYARAJ, 1992). In contrast, rates of only 15-30 kg ha^{-1} inoculum are required in forestry. This inoculum requirement can now be further reduced by up to 50-70% by improving the inoculum potential (high propagule density) and by adopting pre-inoculation techniques (SETIADI, 1996; SETIADI, 1998b). Thus, progress in forestry would not necessarily be hampered by a lack of sufficient inoculum. These studies have also shown that selection of the most appropriate combination of host and fungus, growth medium, appropriate pH and control of the nutrient regime (especially P) during open pot culture establishment can produce a high quality AMF inoculum. This approach, combined with the use of the pre-germination inoculation technique, can overcome many of the difficulties inherent in producing large quantities of inoculum. Thus, realistic large-scale application of AMF inoculation in the forestry sector should be feasible.

MYCORRHIZAL SEEDLING PERFORMANCE IN THE NURSERY

It was reported (SETIADI, 1996) that inoculation did not confer a beneficial growth response on *Eucalyptus* sp., *Melaleuca leucadendron*, *Ochroma bicolor* and *Pometia pinnata*. In contrast, inoculation with selective AMF isolates promoted growth of leguminous tree seedlings such as *Acacia mangium, Acacia crassicarpa, Paraserianthes falcataria, Enterolobium cyclocarpum, Pterocarpus viladianus, Prosopsis* sp., *Casia siamea* and *Sesbania.grandiflora*, and non-legumes such as *Duabanga moluccana*, *Gmelina arborea, Tectona grandis, Vitex* sp. *and Trichospermum burretii*. The responsiveness of these species to inoculation indicates that they would not grow well in degraded reforestation sites unless inoculated with effective AMF. Thus AMF inoculation technology can be chosen as a management practice during the nursery phase.

Liming the soil to raise the pH, combined with the application of commercial phosphate fertiliser are common practices used in reforestation sites in Indonesia to overcome P-deficiency. However, these practices do not always achieve their aim of stimulating early seedling growth, especially for highly mycotrophic plant species such as *Sesbania grandiflora*, *Pterocarpus* sp., *Duabanga moluccana*, *Gmelina arborea*, and *Prosopsis* sp. Non-inoculated seedlings, receiving fertiliser, remain small and stunted. Growth of these plants markedly increases when the soil is inoculated with selected and effective AMF.

Soil fertilisation is commonly used to stimulate plant growth in the nursery, as well as in field conditions. SETIADI (1995a) showed that inoculation with AMF alone in the nursery stimulates greater growth of mycotrophic tree seedlings than conventional phosphate fertiliser treatments. Thus, the costs required for use of commercial P-fertiliser can be reduced. In addition, promotion of growth of seedlings in the nursery resulting from inoculation with AMF can also reduce the time that seedlings need to stay in the nursery. Thus labour costs for maintenance of seedlings in nurseries can also be reduced.

MYCORRHIZAL SEEDLING PERFORMANCE AFTER TRANSPLANTING

It has been suggested that a much greater effort is required to investigate the effect of AMF under field conditions (ABBOTT & ROBSON, 1991) to accelerate tree establishment in disturbed ecosystems. The effectiveness of *G. manihotis* (INDO-1) and *A. tuberculata* (INDO-2) in improving the early growth of fast growing leguminous and non-leguminous trees in nurseries has already been shown (SETIADI, 1996). However, it was not known if this beneficial effect would also be found in field conditions. Consequently a study was designed to examine whether inoculation with *G. manihotis* (INDO-1) and/or *A. tuberculata* (INDO-2) could also increase performance of fast growing leguminous and non-leguminous trees after the transplantation of pre-inoculated seedlings into the field (acidic grassland, post-nickel-mine sites and degraded land after fire).

Initially, inoculation of nursery seedlings with AMF significantly increased stem diameter and height of 3 month-old, fast growing, leguminous trees i.e: *Acacia mangium, Acacia crassicarpa, Paraserianthes falcataria, Enterolobium* sp. and *Cyclocarpum* sp. Similar results were obtained with non-legumes i.e: *Gmelina arborea, Trichospermum burretii* and *Eucalyptus pelita.* No effect was noted with *Swietenia macrophylla* seedlings. The stem diameter and height of seedlings inoculated with a single inoculum of either (INDO-1) or (INDO-2) or with a mixed inoculum of both were significantly increased compared with the controls. The percentage of inoculated seedlings surviving 1 month after transplanting was relatively higher (91-95%) than for non-inoculated seedlings (80%). This trend continued 3 and 5 months after transplanting. However, the percentage of plants surviving after 8 months was variable. The survival rate of plants inoculated with the mixed inoculum was still high (88-90 %) for *Paraserianthes falcataria, Acacia crassicarpa* and *Swietenia macrophylla*, whilst 70-80% of plants inoculated with single fungal inoculum survived, and 60-68% of non-inoculated plants survived.

One month after transplanting on acidic grassland soil, the stem diameter and height of the seedlings inoculated with the mixed inoculum was significantly greater than all other inoculation treatments, including the non-inoculated controls. Eight months after transplanting, seedlings of *P. falcataria, A. mangium, E. cylocarpum, A. crassicarpa,* and *G. arborea* inoculated with the mixed inoculum still showed significantly greater stem diameters and greater height.

This study has shown that the beneficial effects of seedling inoculation with AMF in the nursery could not always be demonstrated after inoculated seedlings were transplanted into the field. However, growth of seedlings of certain species, inoculated with a mixed inoculum, was consistent and significantly better than that for non-inoculated plants, both in the nursery and in the field.

Field trials of inoculated seedlings of trees used for reforestation are still limited. To encourage a wider application of AMF in the forestry sector, large-scale inoculation trials are needed under a variety of field conditions, using different host-fungus combinations. In addition, the edapho-climatic conditions, the status of the indigenous AMF population and their effectiveness in targeted sites should also be determined.

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QUESTIONS AND COMMENTS

Kate Harwick

Do you think that mycorrhizal inoculation would increase seedling performance on less degraded sites, such as abandoned agricultural areas?

Yadi Setiadi

Mycorrhizae can be used on agricultural sites and may have a positive effect.

Ulfah Siregar

Not all species are compatible with mycorrhizae. Mycorrhizae provide most benefit in poor soil conditions. Consequently, positive growth benefits may be difficult to see where fertilisers have been applied. Furthermore, mycorrhizae can have negative effects in very dry conditions.