The Occurence of Arbuscular Mycorrhizal Fungi and Bacteria at Primary Degraded Forest, Secondary Forest and Degraded Forest Land of Grand Forest Park Sultan Thaha Syaifuddin, Jambi

Technical Report No. 7

Sri Wilarso Budi R, Iskandar Z. Siregar and Ulfah J. Siregar
Silviculture Laboratory, Department of Silviculture, Faculty of Forestry-IPB

Abstract

Arbuscular Mycorrhiza Fungi (AMF) is one fungi that exhibits symbiosis with most plants, either forestry or agricultural plants. The presence of such symbiosis has been proven to be able to improve the growth and health of the host plants. The role of bacteria on enhancement of mycorrhizae growth was well documented. The objective of the present work was to know the diversity of AMF, plant mycorrhizal status and viable bacteria population in relation to forest type, which classified as primary degraded forest, secondary forest and degraded forest land. Spore densities of AM fungi in the secondary forest were highest, whereas no spore was observed in degraded forest land . Based on the morphology of AMF spores found in the three ecosystem types, three genera of arbuscular mycorrhizae were identified as Glomus sp, Gigaspora sp and Acaulospora sp. The highest arbuscular mycorrhizal diversity was found in secondary forest. Among 7 species of plants found in degraded primary forest, 6 species were mycorrhizal and 1 species were not colonized by mycorrhiza as indicated by root staining. In the secondary forest there were 5 species, which all have mycorrhizae, whereas in the degraded forest land there were 2 species, which were not colonized by mycorrhizae. The mycorrhiza colonization ranged between 25 - 40 % in the degraded primary forest and 40 - 60 % in the secondary forest. The presence of bacteria in the degraded forest land was lower than two other ecosystem types.

Key words: AM Fungi, Bacteria, Degraded Forest

Introduction

Grand Forest Park Sultan Thaha Syaifuddin, Jambi, known as Tahura Senami was selected for demonstration site of ITTO Project PD 210/03. Rev 3 (F). The current forest condition is very critical due to deforestation that has taken place since the past years. The deforestation has negative impact, such as increased soil erosion, loss of biological diversity, damage of wildlife habitats, degradation of watershed areas and deterioration in the quality of life (UNCED, 1992). To rehabilitate the deforested area is not an easy task due to several major constraints, which related to unfavorable environmental conditions existing at the reforestation sites. Edaphic factors (low nutrients, acidic soils), climatic factors (high level of solar radiation and high temperatures) and biological factors (aggressive competition from the predominant grass *Imperata cylindrica*, high chance of fire and low soil microbial activities) are unfavorable to plants and will reduce the performance of planted tree.

The application of beneficial microbial technology such as mycorrhizal fungi as alternative strategy should be attempted and developed in order to increase the survival, quality and growth rate of seedling in the field.

Arbuscular mycorrhizal fungi (AMF) that belongs to the Glomales (Zygomycetes), and consists of about 130 species in the genera of Glomus, Sclerocystis, Gigaspora, Enthtropospora, Scutellospora and Acaulospora have long been recognized as forming symbiotic associations with plants (Schenk and Perez, 1990). These fungi are extremely widespread; they are not limited to only one plant family and have an exceptionally wide range of host and habitat (Smith and Read, 1997). The novel function of these fungi as biological agent for bioremediation of heavy metal contaminated soil (Setiadi, 1998, Hashem, 1995) and as helping agent in early seedling establishment on degraded sites are recognized. Arbuscular mycorrhizal fungi (AMF) has also been employed to increase resistance to plant pathogens (Liu, 1995) and Salinity (Azcon and El-Atrash, 1997).

The role of bacteria on enhancement of mycorrhizae growth was well documented (Garbaye, 1994, Budi et al, 1998)

To assess whether introduction of arbuscular mycorrhizal fungi (AMF) into a given ecosystem will be beneficial, it is necessary to evaluate the extent of indigenous mycorrhizal population and bacteria as well.

The objective of the present work was to know the diversity of AMF, plant mycorrhizal status and viable bacteria population in Tahura Senami, which can be utilized further to help the restoration and rehabilitation process.

Materials and Methods

Study sites

Grand Forest Park Sultan Thaha Syaifuddin or Tahura Senamis lies geographically between south latitudes of 1° 45′ 55″ and 2°14′ 30″, and between east longitudes of 103° 12′ 30″ and 104°47′ 30″, with an annual mean temperature range of 23°C – 33°C and annual rain fall of 2472 mm The soils are red yellow podzolic (70%) followed by Alluvial (18%), Granosol (3.24%) and other soils (8.58%).

Collection of soil and root samples

Three conditions of sites were chosen; they were degraded primary forest, secondary forest and degraded forest land. The criteria of each site are in line with ITTO Policy Development Series No. 13 for the restoration, management and rehabilitation of degraded and secondary tropical forest. Three samples from each site (approximately 500 g) were collected. Surface soil (approximately 1-2 mm) was removed at 0-20 cm and 20-40 cm soil depth, and were collected including fine roots and rhizosphere soil of the host plant. A sub sample of approximately 100 g was taken for extraction of AM fungal spores and viable bacteria.

Isolation and counting of AM fungal spores

Spores or sporocarps were extracted from 50 g air-dried soil samples from each landuse type in triplicate by wet sieving and decanting according to the method of Nicholson and Gardeman (1963) followed by flotation-centrifugation in 50 % sucrose (Dalpe, 1993). The finest sieve used was 45 um. The spores were collected on Petri dish (10 x 10 cm) and counted using dissecting microscope at 40x magnifications. Spores were identified according the method of Schenk and Perez (1990).

Assessment of AM colonization

Fresh root sample were processed according to the method of Phylip and Hayman (1970). The soil was removed from the root by washing and clearing it in 10 % (w/v) KOH at 90 0C for 30-60 min. The KOH were then removed and they were soaked in HCL 2 M for 10 min. The root sample was stained with 0.5 % (w/v) trypan blue. The percentage of root length colonized by AM fungi was determined by grid line intersect method of Brundet et al (1996).

Numbers and distribution of AM fungal spores

The number of AM fungal spores was determined by the method of Shi et al (2004). Spore density (SD) is expressed as number of fungal spores in 50 g dry soil.

Isolation of viable bacteria

The viable bacteria were isolated from soil according to the method of Zuberer (1994). The isolating medium for bacteria was Nutrient Agar (Oxoid). Dilution series were made and spread on the Petri dish containing nutrient agar medium. The colonies which appeared on the plates were counted in correspond to the dilution series. The number of viable bacteria was expressed as cfu/g dry soil.

Statistical analysis

The data were subjected to analysis of variance and Duncan's test for multiple range tests using SPSS statistical programme version 1.4.

Results & Discussion

Spore density of AM fungi

The spore densities in degraded primary forest, secondary forest and degraded forest land are presented in **Figure 1**.

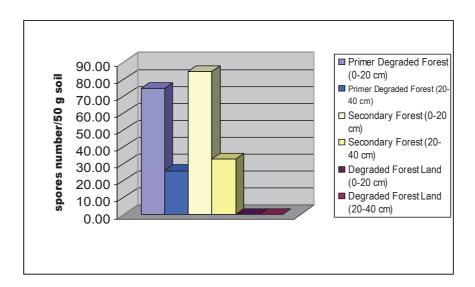


Figure 1. Spore numbers isolated from three different sites at 0-20 and 20-40 soil depth.

Spore densities of AM fungi in the secondary forest were highest, whereas no spore was observed in the degraded forest land. _Spores' numbers were higher in the upper part of soil layer and getting lower with increasing soil depth. Significant differences were observed among venue and soil depth respectively.

The data presented in Figure 1 show that the number of spore is varied depending on the type of forest ecosystems and soil depth. The highest spores number were found in the secondary forest, whereas in the degraded forest land the spores were not found, This fact indicated that those areas do not have symbiotic microorganism.

The richness of arbuscular mycorrhizal fungi (AMF) spore found in the secondary forest might be due to the favorable sites for sporulation. According to Abbott and Gazey (1994), there are many factors that affect the sporulation of arbuscular mycorrhizal fungi (AMF) i.e.: the host, edaphic, climate and biological factor. It is also possible that the change in host due to the invasion of new host from adjacent area may have a greater influence on AMF sporulation.

The absence of spore in degraded forest land might due to lack of AM Fungi host, as shown in Table 1, that there is no mycorrhizal plant found in that site.

Genera of AM Fungi

Based on the morphology of arbuscula mycorrhizal fungi (AMF) spores found in the ecosystem types, three genera of arbuscular mycorrhizae were identified as Glomus sp, Gigaspora sp and Acaulospora sp. The highest arbuscular mycorrhizal diversity was found in the secondary forest (Table 1)

Table 1. Spore Numbers and AMF diversity in sampling venue

Sampling	Depth (cm)	Spore Number/50 g soil		
Venue				
		Glomus sp	Gigaspora sp	Acaulospora
				sp
Degraded	0 – 20	65	-	10
Primer Forest	20 - 40	19	-	7
Secondary	0 - 20	54	11	20
Forest	20 - 40	20	7	6
Degraded	0 - 20	0	-	-
Forest Land	20 - 40	0	-	-

The data presented in Figure 1 and Table 1 indicates that there are particular patterns in the formation and sporulation of arbuscular mycorrhizal fungi (AMF) which depend largely on the characteristics of ecosystems. According to the hypothesis of Cornnell (1978), the diversity of species should be lower for minimal and maximal disturbance, for recent and distant disturbance and for minor and major levels of disturbance than that of intermediate levels of disturbance. Due to its obligate symbiotic, the abundance of arbuscular mycorrhizal fungi (AMF) in the ecosystem was certainly depend on the host in the site.

Colonization by AM Fungi

There were 7 species of plants found in the degraded primer forest, of which 6 species were mycorrhizal and 1 species were not colonized by mycorrhiza as indicated by root staining. In the secondary forest there were 5 species and all have mycorrhizae, whereas in the degraded forest land there were 2 species and were not colonized by mycorrhizae. The mycorrhiza colonization ranged between 25 - 40 % in the degraded primary forest, and 40 - 60 % in the secondary forest (Table 2).

The data in **Table 2** shows that percentage of root colonization by mycorrhizae varied in different sites for the same species of plant, e.g. Lisau, which was high in the secondary forest (55 %) and 25 % in the degraded primary forest. The two plant species sampled in the degraded forest land, and one species from the degraded primary forest were not colonized by mycorrhiza. More investigation is needed to verify this result, a clarification whether the plants species are categorized as natural non-mycorrhizal plants or specific for their mycorrhizal symbionts. Confirmation is necessary particularly for *Gironiera subaegualis* from the degraded primer forest, where other plants species from the same venue were colonized by mycorrhiza. For Mengkirai and Mahang it's reasonable that they are not colonized by mycorrhizae, since the environmental conditions in those site are very extreme especially the soil condition (see Technical report number 6)

Table. 2 Arbuscular Mycorrhizal (AM) status of roots plants found at sampling sites

Sampling Venue	Plant species	Percent root length colonized
Degraded Primer Forest	1. Scapium masubsidiary cropodium	35
	2. Sebekal	25
	3. Memecylon sp	40
	4. Cinnamomum	25
	parthenoxylon	-
	5. Gironiera subaegualis	
	6. Pithecellobium bubalianum	30
	7. Trioma malaccensis	25
Secondary Forest	1. Sarangbuaya	40
_	2. Ampelar	50
	3. Bambosa sp	60
	4. Rumput Pahitan	60
	5. Lisau	55
Degraded Forest Land	1. Mengkirai	0
-	2. Mahang (<i>Macaranga sp</i>)	0

Colony Forming Unit of Viable Bacteria

The viable bacteria densities in degraded primary forest, secondary forest and degraded forest land are presented in **Figure 2**.

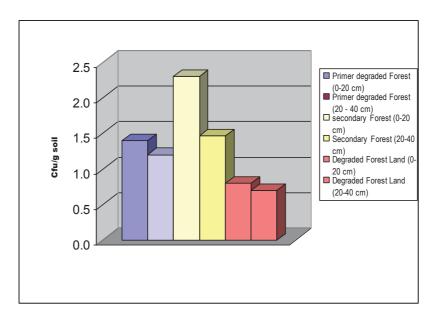


Figure 2. Number of viable bacteria (x 10^4) isolated from three different site at 0-20 cm and 20-40 cm soil depth

Viable bacteria in the secondary forest were highest, whereas in degraded primary forest was lowest. Bacteria numbers were higher in the upper part of soil layer and getting

lower with increasing soil depth in all sites. The significant differences were observed among the sites but not in soil depth respectively.

The presence of bacteria in degraded forest land was lower than two other ecosystem types. One possible explanation is that the limitation of organic matter and exudates from plants in degraded forest land may inhibit the growth of bacteria. In addition same literature showed that the presence of mycorrhizae in the rhizosphere might increase the population of bacteria (Bagyaraj, 1984).

Conclusions

Over all data indicated that the abundance of AMF and bacteria can be found in Tahura Senami that can be subsequently used as a bank of glomalean fungi and bacteria. The large diversity of arbuscular mycorrhizal fungi (AMF) and bacteria found in those ecosystems, could be used as a base to select effective arbuscular mycorrhizal fungi (AMF) and bacteria to be utilized for reforestation and rehabilitation programmes.

ACKNOWLEDGMENTS

This Technical Report No. 7 on The Occurence of Arbuscular Mycorrhizal Fungi and Bacteria at Primary Degraded Forest, Secondary Forest and Degraded Forest Land of Grand Forest Park Sultan Thaha Syaifudin, Jambi has been prepared to fulfill **Objective 2 Point 2.4.** of the Workplan of ITTO Project PD 210/03. Rev 3 (F): Participatory Establishment of Collaborative Sustainable Forest Management in Dusun Aro, Jambi.

The author would like to thank ITTO, The Ministry of Forestry (GOI), Batang Hari Forest Distric Service, for their support. Appreciation also goes to the Project Steering Committee members for their suggestion

References

- Abbott, L.K., C. Gazey. 1994. An ecological view of the formation of VA mycorrhizas. In: Robson, A.D., C.K. Abbott., N. Malajczuk (eds). Management of mycorrhizas in agriculture, horticulture anf forestry. Kluwer Academic Publisher. London.
- Azcon, R., EL Atrash, F. 1997. Influence of arbuscular mycorrhizae and phosphorous fertilization on growth, nodulation and N₂ fixtation (N¹⁵) in *Medicago sativa* at four salinity levels. Biology and Fertility of Soils 24: 81-86
- Bagyaraj DJ .1984. Biological interactions with VA mycorrhizal fungi. In: Powel CL, Bagyaraj DJ (eds). VA mycorrhiza, CRC, Boca Raton, FL.
- Brundrett M, Bougher N, Dells B, Grove T and Malajczuk N. 1996. Working with Mycorrhizae in Forestry and Agriculture. ACIAR. Canberra.
- Budi S.W, J.P. Causannel dan S. Gianinazzi. 1998. The Biotechnology of Mycorrhizas. In "Microbiaal interaaction in Agriculture and Forestry" Vol I, N.S. Subba

- Rao and Y.R. Dommergues, eds, Oxford and IBH Publishing co Ltd., New Delhi, India. P 149-162
- Cornell, J.H. 1978. Diversity in tropical raiin forest and coral reefs. Science 199, 1302-1310
- Dalpe, Y. 1993. Vesicular-arbuscular mycorrhizae. In: Carter MR (ed) Soil sampling methods of analysis. Lewis Publishers, Boca Raton, FL.
- Garbaye, J. 1994. Helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytol, 128, 197-210
- Hashem, A.R. 1995. The role of mycorrhizal infection in the resistance of *Vaccinium macrocarpum* to manganese. Mycorrhiza 5: 289-292.
- Liu R-J. 1995. Effect of vesicular-arbuscular mycorrhizal fungi on *Verticillium* wilt of cotton. Mycorrhiza 5: 293-297.
- Nicholson, TH. and Gardemann JW 1963. Spores of mycorrhizal Endogones species extracted from soil by wet-sieving and decanting. Trans. Br. Mycol. Soc 46: 235-244
- Phillips, JM and Hayman, DS. 1970. Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55,158-161
- Setiadi, Y. 1998. Biodiversity of arbuscular mycorrhizal fungi at PT. Freepot Indonesia. Ecology Laboratory Report. (unpublished).
- Schenk, N.C., Y. Perez., 1990. Manual for the identification of VA mycorrhizal fungi. Gainseville, Florida.
- Shi, Z Y, YL Chen, G. Feng RJ Lin and P Christi. 2004. Arbuscular mycorrhizal fungi associated with the meliaceae on Hainan island, China. Mycorrhiza.
- Smith, S.E., D.J. Read. 1997. Mycorrhizal symbiosis. 2rd edition. Academic Press. London.
- UNCED. 1992. Combating deforestation. The Forest Chronicle, 68, 1-7.
- Zuberer, DA. 1994. Recovery and Enumeration of Viable Bacteria. In Methods of Soil Analysis, Part 2. Microbiology and biochemical Properties. Segoe Rd. Madison, USA.