THE EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI AND SLOW RELEASE FERTILIZER ON THE GROWTH OF *Alstonia scholaris* (L.) Br. SEEDLINGS IN THE NURSERY

Ragil S.B. Irianto¹

ABSTRACT

Alstonia scholaris (L.) Br. is one of fast growing species found growing in industrial forest plantation or natural stands, mainly in Sumatra and Kalimantan. Soil of degraded land is generally infertile, having limited macro and micro nutrients, and is acidic with thin topsoil. A potential solution to improve plant growth in this soil condition is inoculation of seedling with arbuscular mycorrhizal fungi (AMF) and fertilization with slow release fertilizer (SRF) in the nursery. This research was conducted in the nursery of Forest and Nature Conservation Research and Development Centre from April until August 2007. Two factors experiments in randomized completely block design was applied with four replications, six treatments and two factors of AMF types (*Glomus* sp. and *G. aggregatum*) and SRF dosage. The results showed that combinations of AMF and SRF could accelerate height, diameter and dry mass of seedlings by 95-521%, 43-237%, and 571-2,735%, respectively, compared to control (non-inoculated seedlings). Arbuscular mycorrhizal fungal inoculation with *G. aggregatum* and SRF fertilization with 0.4 g gave the highest growth of *A. scholaris* seedlings. This research should be followed by field trials to know whether inoculation of the AMF and application of SRF seedlings effectiveness continues in the fields. This finding can contribute to the implementation of Gerhan (*Gerakan Rehabilitasi Hutan dan Laban* or forest and land rehabilitation movement) in degraded lands by preparing vigorous AMF and SRF seedlings.

Keywords: *Glomus aggregatum*, inoculation, dryweight, land rehabilitation

I. INTRODUCTION

Degraded secondary forests in seven main islands in Indonesia from 2000-2005 have reached 5,447,800 ha and annual deforestation rate is very high of 1,089,560 ha (Forestry Planning Agency, 2000). Commonly, the degraded lands are located outside of Java Island, such as Sumatra and Kalimantan. Soils in degraded land are generally infertile, having limited macro and micro nutrients (low nitrogen (N), phosphorus (P), and potassium (K)), and acidic with thin topsoil (Setiadi, 2000).

¹ Forest and Nature Conservation Research and Development Center, Forestry Research and Development Agency, Jl. Gunung Batu No. 5, Bogor 16610, West Java, Indonesia. E-mail: ragilirianto@yahoo.com

Therefore rehabilitations in Kalimantan and Sumatra are often unsuccessful in these degraded lands.

One approach to solve the problems is by inoculation of seedling with arbuscular mycorrhizal fungi (AMF) and fertilization with slow release fertilizer (SRF). A symbiotic of AMF with higher plants plays an important role in plant nutrition cycle (Harley and Smith, 1983). The AMF stimulate the growth of many tropical trees and shrubs by enhancing the uptake of nutrient elements, such as N, P, and K and by increasing the resistance of plants to drought stress and root pathogens (Janos, 1988; Michelsen and Rosendahl, 1990).

Alstonia scholaris is a minor commercial timber in Indonesia. The distribution of this tree species is found up to 900 m above sea level and in almost islands of Indonesia (Heyne, 1987). Recently, wood of this plant interests some countries to be utilized for pencil and picture frame.

The aim of this study was to determine the effect of AMF and slow release fertilizer on the growth of *A. scholaris* seedlings in nursery. This research finding would be useful to prepare robust seedlings to support one of the priorities of the Ministry of Forestry (MoF) programs, named Gerhan (*Gerakan Rehabilitasi Hutan dan Laban* or forest and land rehabilitation movement) and HTI (*Hutan Tanaman Industri* or Industrial Forest Plantation) to rehabilitate degraded forest lands.

II. MATERIALS AND METHODS

A. Seedling Preparation

Alstonia scholaris seeds were collected from arboretum of Forest and Nature Conservation Research and Development Centre (FNCRDC) at 06°35'854" S, 106°46'900" E. The seeds were surface sterilized for 5 minutes in 5% sodium hypochlorite (NaOCl) solution and germinated for 2 weeks in a 17 cm x 43 cm x 24 cm container filled with sterilized zeolyte as growth media. The moisture was maintained by sprinkling with water. Seedlings of about the same height and diameter were transplanted into a 250-ml polyethylene bag filled with 1 kg sub soil sterilized with dazomet 98% at a dose of 200 g/m³ soil. This research was conducted in the nursery of FNCRDC from April until August 2007.

Two-factor experiment in a randomized completely block design (Mattjik and Sumertajaya, 2000) was applied with four replicates, six treatments and two factors of AMF types and SRF doses. Three levels of AMF include: non-inoculated control, *Glomus* sp., and *Glomus aggregatum*; and two level doses of slow release fertilizer (SRF) include: 0 g (control) and 0.4 g per pot containing 500 g of sterilized subsoil. At the same time with seedling transplantation to the pot, 5 g of AMF inoculants were applied in the root zone, while 0.4 g of SRF were given 5 days after transplantation, in a hole with 1 cm in distance from seedling and 1 cm in depth.

B. Measurement and Data Analysis

Seedlings growth was evaluated by measuring dry mass of shoot and root, diameter, and height at 4 months after transplanting. Data were statistically analyzed by JMP Start Statistics software (Sall *et al.*, 2005) and Tukey test at $p = 0.05$ was used to group non-significantly different treatments.

III. RESULTS AND DISCUSSION

A. Plant Height

Combination of AMF and SRF treatments increased height growth of *A. scholaris* seedlings in the nursery (Table 1). Seedlings inoculated with AMF (*Glomus* sp. and *G. aggregatum*) + SRF 0.4 g accelerated plant growth by 509% and 521%, respectively, compared to those non-inoculated seedlings (control). Non-AMF seedlings fertilized with 0.4 g SRF accelerated 95% of plant growth compared to non AMF/SRF control seedlings.

Table 1. Effects of combinations between AMF and SRF on height (cm) of *A. scholaris* seedlings at 4 months after transplanting in the nursery

AMF	Height (cm)	
	SRF 0 g	SRF 0.4 g
Non-inoculated (control)	4,09 c (0)	8,01 c (95)
<i>Glomus</i> sp.	14,81 b (261)	24,96 a (509)
<i>G. aggregatum</i>	17,69 b (332)	25,44 a (521)

Notes: AMF: arbuscular mycorrhizal fungi; SRF: slow release fertilizer; means followed by the same letters are not significantly different at $p < 0.05$ according to the Tukey test; numbers in the parentheses denote percentage (%) of growth increase over control

B. Plant Diameter

Inoculation of AMF and fertilization of SRF increased diameter of seedlings in the nursery (Table 2). Fertilization with 0.4 g SRF accelerated 43% of seedling growth compared to non-inoculated (control), but inoculation of AMF (*Glomus* sp. and *G. aggregatum*) + application of 0.4 g SRF accelerated seedling growth by 237% and 222%, respectively, compared to control.

Table 2. Effects of combinations between AMF and SRF on diameter (mm) of *A. scholaris* seedlings at 4 months after transplanting in the nursery

AMF	Diameter (mm)	
	SRF 0 g	SRF 0.4 g
Non-inoculated (control)	1,10 c (0)	1,57 c (43)
<i>Glomus</i> sp.	2,84 b (157)	3,71 a (237)
<i>G. aggregatum</i>	3,38 a (207)	3,55 a (222)

Notes: AMF: arbuscular mycorrhizal fungi; SRF: slow release fertilizer; means followed by the same letters are not significantly different at $p < 0.05$ according to the Tukey test; numbers in the parentheses denote percentage (%) of growth increase over control

C. Dry Mass of Seedlings

Application of AMF and SRF on *A. scholaris* seedlings increased dry mass of seedlings in the nursery (Table 3). Seedlings only fertilized with 0.4 g SRF accelerated 571% of dry mass compared to control, but seedlings inoculated with AMF (*Glomus* sp. and *G. aggregatum*) + application of 0.4 g SRF accelerated the seedling dry mass by 2.7% and 2.7%, respectively, compared to non-AMF.

Table 3. Effects of combinations between AMF and SRF on dry weight/mass (g) of *A. scholaris* seedlings at 4 months after transplanting in the nursery

AMF	Dry weight (g)	
	SRF 0 g	SRF 0.4 g
Non-inoculated (control)	0,046 c (0)	0,309 c (571)
<i>Glomus</i> sp.	0,649 b (1,310)	1.289 a (2,701)
<i>G. aggregatum</i>	0,923 b (1,904)	1.305 a (2,735)

Notes: AMF: arbuscular mycorrhizal fungi; SRF: slow release fertilizer; means followed by the same letters are not significantly different at $p < 0.05$ according to the Tukey test; numbers in the parentheses denote percentage (%) of growth increase over control

D. Mycorrhizal Infection

All seedlings inoculated with AMF had better percentage of mycorrhizal infection compared to those non-inoculated seedlings (control). However, the non-inoculated seedlings still had mycorrhizal infection at low values (Table 4).

Table 4. Effects of combinations between AMF and SRF on mycorrhizal infection (%) of *A. scholaris* seedlings at 4 months after transplanting in the nursery

AMF	Mycorrhizal infection (%)	
	SRF 0 g	SRF 0.4 g
Non-inoculated (control)	2.6 c (0)	33.79 b (231)
<i>Glomus</i> sp.	96.92 a (327)	97.92 a (328)
<i>G. aggregatum</i>	95.42 a (326)	98.25 a (328)

Notes: AMF: arbuscular mycorrhizal fungi; SRF: slow release fertilizer; means followed by the same letters are not significantly different at $p < 0.05$ according to the Tukey test; numbers in the parentheses denote percentage (%) of growth increase over control

E. Nutrient Uptake

Inoculation of AMF and fertilization of SRF increased N, P, and K uptake of seedlings in the nursery (Table 5). Fertilization with 0.4 g SRF and/or inoculation of *Glomus* sp. and *G. aggregatum* + SRF 0.4 g accelerated N, P, and K uptake of seedlings compared to control (Table 5).

Table 5. Effects of combinations between AMF and SRF on nutrient uptake (mg/plant) of *A. scholaris* seedlings at 4 months after transplanting in the nursery

AMF	Nutrient uptake (mg/seedling)					
	SRF 0 g			SRF 0.4 g		
	N (mg/ plant)	P (mg/ plant)	K (mg/ plant)	N (mg/ plant)	P (mg/ plant)	K (mg/ plant)
Non-inoculated (control)	2.05 e (0)	0.04b (0)	1.66 d (0)	25.04 d (1,124)	0.9225 b (2,265)	21.08 c (1,168)
<i>Glomus</i> sp.	22.4 d (997)	0.77 b (1886)	17.73 cd (967)	96.89 a (4,638)	4.4843 a (11,398)	70.09 a (4,116)
<i>G. aggregatum</i>	43.12 c (2,008)	1.13 b (2,784)	42.52 b (2,458)	79.75 b (3,800)	3.6030 a (9,138)	73.55 a (4,324)

Notes: AMF: arbuscular mycorrhizal fungi; SRF: slow release fertilizer; means followed by the same letters are not significantly different at $p < 0.05$ according to the Tukey test; numbers in the parentheses denote percentage (%) of growth increase over control

F. Discussion

Alstonia scholaris seedlings showed high dependency towards AMF inoculation based on the parameters analyzed: seedling height, diameter, and dry mass. Based on seedling dry mass referred to formula of Plenchette *et al.* (1983), mycorrhizal dependency of these seedlings to treatment of two AMF (*Glomus* sp. and *G. aggregatum*) with 0 g and 4 g SRF ranged from 93.9 - 95.0% and 76.0 - 76.3%, respectively. Values of mycorrhizal dependency of these seedlings are very high based on formula of Cruz *et al.* (1999). The high mycorrhizal dependency value suggested that inoculation would be useful in the production of vigorous seedlings in the nursery which might establish better survival ability in the field toward drought, nutrient deficiency and pathogenic infection (Wilson *et al.*, 1991).

Arbuscular mycorrhizal fungi increased nutrient uptake and enhanced plant growth. The external hyphae of the symbiotic association grew into the soil beyond the phosphorus depletion zone around the root. Sieverding (1991) reported that the uptake of N, P, and K was higher on AMF plant than non-AMF plant. *Glomus aggregatum* used in this experiment has a broad host range that some researches have been conducted on other species. Utami (2001) found that *Paraserianthes falcataria* inoculated with *G. aggregatum* accelerated seedling growth and improved P and K. Irianto and Santoso (2005) stated that *G. aggregatum* inoculated on teak

(*Tectona grandis*) increased plant height by up to 61% and plant diameter by up to 47% compared to those of non-AMF teak.

Furthermore, higher cytokinin activity in the shoot of plant has been found in the AMF plants (Bass and Kuiper, 1989 in Edathil *et al.*, 1996) and cytokinin is known to promote leaf growth by cell division and cell expansion (Vanstanden and Davey, 1979 in Edathil *et al.*, 1996). Drüge and Schönbeck (1992) reported that AMF symbiosis raised internal cytokinin levels and this is a main factor causing improvement of plant growth.

The seedlings with non-inoculated mycorrhizal fungi (control) were also infected by contaminating mycorrhizal fungi (Table 4). Contamination of mycorrhizal fungi may be disseminated by several ways:

1. Water used to water the seedlings might bring along spores or other propagules, as the water was not sterilized.
2. Contaminating spore of mycorrhizal fungi from surrounding the nursery might have been dispersed by wind or rain. Marx (1980) and Allen (1988) reported that spores of mycorrhizal fungi could be disseminated in great distances by wind and rain.
3. Spore or other propagules of mycorrhizal fungi on the ground could have been dispersed by rain splash as the nursery where the trial was set up received high annual rainfall of around 2,500 mm per year and seedlings were placed on a bench of only 40 cm above the soil surface. Ashton and Macauley (1972) revealed that spores of pathogenic fungi *Ceuthospora innumerta* and *Piggotia substellata* could be dispersed by rain splash from both litter or soil on to seedlings up to 60 cm above the ground.

IV. CONCLUSION

Arbuscular mycorrhizal fungal inoculation of *Glomus* sp + 0.4 g SRF and *G. aggregatum* + 0.4 g SRF fertilization could accelerate height, diameter, dry weight, mycorrhizal infection, N uptake, P uptake, and K uptake of *A. scholaris* seedlings by 509 and 521%; 237 and 222%; 2.7 and 2.7%; and 4.6 and 3.8%; 11.4 and 9.1%; 4.1 and 4.3%, respectively, compared to non-inoculated control. This research should be followed by field trials to know whether the AMF-seedlings effectiveness continues in the fields. This finding can support the implementation of *Gerhan* in degraded lands by preparing vigorous AMF-seedlings. Preparation of AMF-seedlings in the nursery is cost efficient and many studies have revealed that AMF-inoculated seedlings have high survival rate in both nursery and field.

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