

Research Article

Arbuscular Mycorrhizal Colonization Enhanced Early Growth of *Mallotus paniculatus* and *Albizia saman* under Nursery Conditions in East Kalimantan, Indonesia

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Forest over logging, forest fire, forest conversion, and opencast mining have promoted deforestation in Indonesia, and reforestation is needed immediately. However, reforestation is limited by low seedling quality and production, and slow seedling growth in nurseries. Native tropical tree and fast-growing species, *Mallotus paniculatus* and *Albizia saman*, are potential to promote the first rotation of reforestation. Arbuscular mycorrhizal (AM) fungi are known to promote nutrient uptake and plant growth. We examined the effects of two native AM fungi, *Gigaspora decipiens* and *Glomus clarum*, on the growth of *M. paniculatus* and *A. saman* seedlings under nursery conditions. At harvest, after six months, we determined AM colonization, shoot dry weight, and shoot N and P concentration. Approximately 90% and 50% of *M. paniculatus* and *A. saman* roots, respectively, were colonized by AM fungi, without any difference between the inoculation treatments. *G. decipiens* and *G. clarum* increased shoot height, leaf number, shoot dry weight, and shoot N and P uptake of both species. A positive correlation was observed between N and P uptake and shoot dry weight. These results suggest that AM fungi are effective in accelerating nutrient uptake and plant growth, which will, in turn, promote reforestation and sustainable forest timber production.

1. Introduction

In Indonesia, deforestation is occurring rapidly owing to over logging, forest fire, forest conversion into agricultural land or oil rubber plantation [1], and opencast mining [2], and, therefore, immediate restoration is required by applying a comprehensive and systematic reforestation method. Natural forest recovery, particularly in forestland used for bare opencast mining, requires several hundred years and consists of the initial, middle, and climax stages [3]. Pioneering and light-requiring species, such as leguminous trees, grasses, and shrubs, are established first [4] in the initial stage of forest succession, followed by gap-opportunistic species (Meliaceae, Dipterocarpaceae, and *Flindersia* spp.) in the middle stage, and finally shade-tolerant species in the mature or climax stage [5]. The preparation of seedlings of native tree species [6] and the selection of fast-growing leguminous

species [7] with improved nitrogen (N), phosphorus (P), and potassium (K) uptake and biomass production are vital for the initial stage of reforestation.

Mallotus paniculatus (Euphorbiaceae) is distributed throughout the Malesian region, including Indonesia [8]. As an evergreen timber tree [9], this plant is an important pioneer species in Kalimantan, Indonesia, because it contributes to the aboveground biomass in secondary forests [10].

Albizia saman (Fabaceae) is native to Northern South America and has become naturalized in the tropics [11]. *A. saman* is usually planted for agroforestry [11] and timber purposes [12]. As a moderately fast growing species [11], *A. saman* has a high survival rate [13] and grows in a wide range of climatic conditions [11], making it potentially useful for reforestation.

The rapid production of *M. paniculatus* and *A. saman* seedlings in nurseries is important for successful reforestation.

However, the initial growth of *A. saman* is slow [11]. Furthermore, poor nutrient uptake due to the low fertility and the high acidity of the tropical soil in Indonesia has made it difficult to improve the seedling growth.

Manure or green compost is a substrate usually used together with the amendment of other organic nutrients or fertilizers in nurseries to meet the nutritional requirements for plant growth. As fertilizer application is costly, it is important to adopt an inexpensive and environmentally friendly method to meet the nutritional requirements for plant growth improvement. As for the plants grown in pots in nursery, the possible contact between plants with soil microorganisms in the ground including AM fungi is very low. The application of symbiotic soil microorganisms that may facilitate nutrient transfer from substrate to plant may enhance nutrient uptake efficiency. It is well documented that arbuscular mycorrhizal (AM) fungi can improve seedling growth, tree height, and plant yield [14] by increasing nutrients [15]. Considering these abilities, AM fungi can assist plant nutrient uptake and therefore promote seedling growth under nursery conditions.

AM colonization was observed in *M. paniculatus* [16] and *A. saman* [17]. Inoculation with AM fungi increased the height and shoot dry weight of *Macaranga denticulata* (Euphorbiaceae) [18]. Inoculation with AM fungi also changed the chlorophyll, carotenoid, sugar, and protein contents of *A. saman* in nurseries in India [19]. However, they did not measure the AM fungi colonization and its effect on nutrient uptake and growth. Therefore, to the best of our knowledge there is no information about the effect of AM fungal colonization on the N and P uptake and growth of *M. paniculatus* and *A. saman*. We hypothesize that the AM fungal inoculation of *M. paniculatus* and *A. saman* leads to AM colonization, thereby improving N and P uptake and growth of these plant species.

In order to enhance the growth of *M. paniculatus* and *A. saman* in nurseries, two AM fungal species, *Gigaspora decipiens* Hall & Abbott and *Glomus clarum* Nicholson & Schenck, were inoculated. Those two AM fungal species were used because they are indigenous to Kalimantan and they have the ability to improve nutrient uptake and growth of tropical peat-swamp plants [20]. The objective of this research was to determine whether inoculation with the two native AM fungal species could improve N and P uptake and growth of native *M. paniculatus* and *A. saman* under nursery conditions in Binungan, Tanjung Redeb, Berau Regency, East Kalimantan, Indonesia.

2. Materials and Methods

2.1. Soil Preparation. Compost is one of the most common substrates used to grow seedling in pot in nursery in Indonesia. Compost is used to reduce chemical fertilizer application and to prepare good healthy seedling before transplanting it to the field which is usually covered by compost. Compost was collected from a local area in Binungan (N02° 02', E117° 27'), Tanjung Redeb, Berau Regency, East Kalimantan, Indonesia. Examination of the compost substrate used in the nursery revealed no spores of AM fungi, indicating that the compost substrate possibly did not support spontaneous AM

colonization. To distinguish other soil fungi or soil microorganisms, the compost was sterilized in a drum by heating over wood fire for 3 hours and further stored at room temperature. The compost chemical characteristics before sterilization showed that the available P [21] was 622 mg P₂O₅ kg⁻¹, total N concentration was 26.5 g kg⁻¹, and C concentration was 372.1 g kg⁻¹ (Sumigraph N-C 220 F). The C : N ratio was 14.04, pH H₂O was 5.25, and pH KCl was 4.82.

2.2. Inoculum Preparation and AM Fungi Inoculation. *Glomus clarum* Nicholson & Schenck and *Gigaspora decipiens* Hall & Abbott were isolated from peat soil in Kalamangan (S2° 13', E113° 56'), Palangkaraya, Central Kalimantan, Indonesia [20]. *Pueraria javanica* Benth was cultivated in zeolite to propagate those two AM fungal species under greenhouse conditions for 90 days. The roots, zeolite, and spores were used as the AM fungal inoculum. Inoculation with AM fungi was accomplished by mixing 20 g of the inoculum with 800 g of the sterilized compost in a polyethylene bag (10 cm diameter × 15 cm height). Noninoculated compost was prepared by adding more 20-gram sterilized compost into 800-gram sterilized compost as control.

2.3. Seed Germination. Seeds of *M. paniculatus* (Lamk.) Müll. Arg. were collected from natural forests in Binungan. Seeds of *A. saman* (Jacq.) Merr. were purchased from a local seed company, Bogor, West Java, Indonesia. The *A. saman* seeds were soaked in water at 80°C for 1-2 minutes [11]. No scarification was applied to the seeds of *M. paniculatus*. Approximately five to seven seeds were sown in the inoculated and noninoculated composts at a depth of 1 cm on 29 September 2011. One seedling per polyethylene bag was grown after germination. Due to the large size of the *A. saman* seedlings, the seedlings were transferred to larger polyethylene bag (15 cm diameter × 20 cm height) four months after sowing on 30 January 2012 by using the same sterilized compost. No fertilizer was applied. The experimental design used was completely randomized design (CRD) where the seedlings in the polyethylene bag were arranged randomly in the nursery. Tap water was applied once every two days. The seedlings were grown under 50% shade from 29 September 2011 to 8 March 2012 in the nursery in Binungan. This plant growth period was determined to get appropriate size of seedling for transplanting to the field.

2.4. Growth Parameters. *M. paniculatus* and *A. saman* seedlings were subjected to three treatments: (1) control, (2) inoculation with *G. clarum*, and (3) inoculation with *G. decipiens*. Each treatment had 20 replications and shoot height and leaf number were measured two, three, and four months after sowing. Six months after sowing, the seedlings were sampled and five replications were harvested for each treatment. The 15 remaining seedlings were left for transplanting to field. Shoots and roots were separately harvested. Shoots were oven dried at 70°C for 72 hours and weighed. Ground shoots were digested with HNO₃-HClO₄-H₂SO₄ solution. P concentration in the solution with the digested ground shoots was determined colorimetrically with

TABLE 1: Percentage arbuscular mycorrhizal colonization, shoot nutrient concentration, shoot nutrient content, and shoot dry weight of control seedlings or *Gigaspora decipiens* or *Glomus clarum* inoculated *Mallotus paniculatus* and *Albizia saman* seedlings six months after sowing under nursery conditions in Berau Regency, East Kalimantan, Indonesia.

Plant species	Treatment	AM colonization (%)	Shoot nutrient concentration		Shoot nutrient content		Shoot dry weight (g/plant)
			P (mg/g)	N (mg/g)	P (mg/plant)	N (mg/plant)	
<i>M. paniculatus</i>	Control	0 (0.00)*	2.49 (0.11) ^b	19.14 (0.27) ^a	1.09 (0.16) ^b	14.52 (0.69) ^b	0.8 (0.04) ^c
	<i>G. decipiens</i>	90 (4.85) ^a	4.18 (0.52) ^a	13.45 (1.21) ^b	6.89 (0.71) ^a	22.26 (1.58) ^a	1.7 (0.10) ^a
	<i>G. clarum</i>	89 (3.28) ^a	5.15 (0.69) ^a	10.60 (2.04) ^b	5.58 (0.99) ^a	11.76 (2.09) ^b	1.1 (0.05) ^b
<i>A. saman</i>	Control	0 (0.00)*	2.15 (0.19) ^a	39.97 (0.66) ^a	6.85 (0.76) ^c	130.23 (16.94) ^b	3.3 (0.40) ^b
	<i>G. decipiens</i>	53 (7.22) ^a	2.19 (0.03) ^a	39.11 (1.45) ^a	25.31 (2.65) ^a	451.12 (47.04) ^a	11.6 (1.24) ^a
	<i>G. clarum</i>	52 (7.62) ^a	1.79 (0.09) ^a	37.72 (2.00) ^a	14.67 (1.88) ^b	309.23 (42.99) ^a	8.2 (0.87) ^a

*Values in the parentheses are standard error (SE). Different letters within the same plant species indicate a statistically significant difference ($P < 0.05$) determined by the Tukey HSD test ($n = 5$).

the vanadomolybdate-yellow assay [22] using a spectrophotometer at 880 nm absorbance (Hitachi, U-2900). Shoot N concentration was determined using a Sumigraph N-C 220 F. Shoot P and N contents were calculated by multiplying the shoot nutrient concentration and the shoot dry weight.

2.5. Assessment of AM Colonization. AM colonization was observed by harvesting the roots of *M. paniculatus* and *A. saman*. The roots were cleared in KOH (100 g L⁻¹) at 80°C for 15 minutes, acidified with 1% HCl, and stained with 500 mg L⁻¹ aniline blue [23]. AM colonization was examined by the gridline intersect method [24] from the 100-point gridline intersection of a root under a compound microscope at 40 to 200x magnification.

2.6. Statistical Analysis. Statistical significance of inoculation and noninoculation treatments was analyzed using Kaleida Graph 4.1 software (Synergy Software 2012, USA) for analysis of variance (ANOVA). Post hoc analysis was performed using the Tukey HSD test ($P < 0.05$). The data of AM colonization were analyzed by ANOVA after arcsin transformation.

3. Results

3.1. AM Colonization. AM colonization was observed in all seedling species inoculated with *G. decipiens* and *G. clarum* six months after sowing under nursery conditions (Table 1). No AM colonization was observed in control seedlings of *M. paniculatus* and *A. saman*. No significant difference was noted in the percentage AM colonization between *G. decipiens* and *G. clarum* in the two seedling species.

3.2. Plant Growth. Shoot height and leaf number of *M. paniculatus* seedlings could not be measured two months after sowing due to the small size of the seedlings, that is, less than one cm in height, and the absence of any true leaves in their ontogenetic stage. Compared to the control seedlings, shoot height (Figure 1(a)) and leaf number (Figure 2(a)) of *M. paniculatus* seedlings increased after inoculation with *G. decipiens* and *G. clarum*; the increase was approximately 35% and 25% of control three and four months after sowing. There

was no significant difference in shoot height and leaf number between the seedlings inoculated with *G. decipiens* and those with *G. clarum*. Shoot dry weight of *M. paniculatus* seedlings inoculated with *G. decipiens* and *G. clarum* was 53% and 28% higher than that of control seedlings six months after sowing, respectively (Table 1). Seedlings inoculated with *G. decipiens* had higher shoot dry weight than those inoculated with *G. clarum*.

There was no nodulation observed in the root of *A. saman*. No significant difference in shoot height (Figure 1(b)) was observed between inoculated *A. saman* seedlings and control seedlings two months after sowing. Shoot height and leaf number of seedlings inoculated with *G. decipiens* were approximately 26% and 54% larger than those of control seedlings and seedlings inoculated with *G. clarum* three months after sowing, respectively. Four months after sowing, seedlings inoculated with *G. decipiens* and *G. clarum* showed 51% and 22% larger shoot height and 54% and 32% larger leaf number, respectively, than control seedlings. Seedlings inoculated with *G. decipiens* had larger shoot height and leaf number than those inoculated with *G. clarum*. *A. saman* seedlings inoculated with *G. decipiens* and *G. clarum* had 72% and 60% higher shoot dry weight, respectively, than control seedlings six months after sowing (Table 1). No significant difference in shoot dry weight was observed between *G. decipiens* and *G. clarum* inoculated seedlings.

3.3. Shoot Nutrient Concentration and Content. *M. paniculatus* seedlings inoculated with *G. decipiens* and *G. clarum* had 41% and 52% higher shoot P concentration and 84% and 81% higher shoot P content, respectively, than control seedlings (Table 1). There was no significant difference in shoot P concentration and content between *G. decipiens* and *G. clarum* inoculated seedlings. In contrast, shoot N concentration was higher in control seedlings than in seedlings inoculated with *G. decipiens* and *G. clarum*. Shoot N content was approximately 35% higher in *G. decipiens* inoculated seedlings than in *G. clarum* inoculated seedlings and control seedlings. No significant difference in shoot N content was found between *G. clarum* inoculated seedlings and control seedlings.

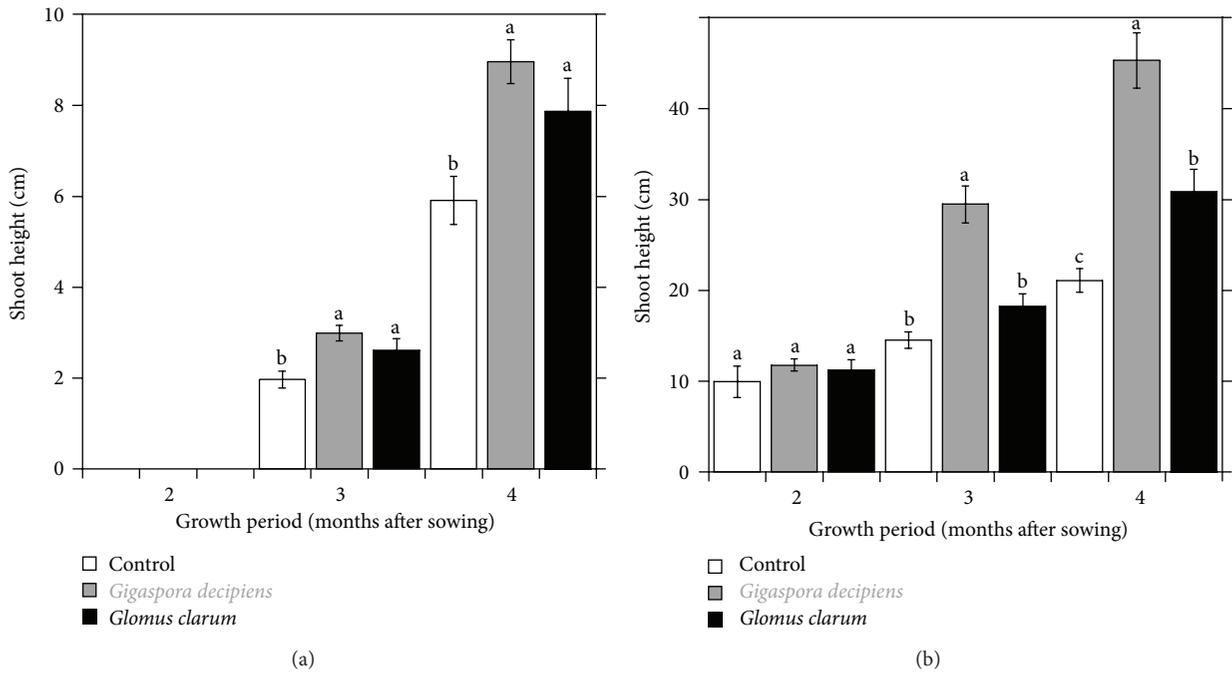


FIGURE 1: Shoot heights of *M. paniculatus* (a) and *A. saman* (b) inoculated with AM fungi two, three, and four months after sowing. Gray bar: *Gigaspora decipiens*; black bar: *Glomus clarum*; white bar: control. Different letters for the same month indicate a significant difference at $P < 0.05$ as determined by the Tukey HSD test ($n = 20$). Vertical bars represent standard errors of mean (SE).

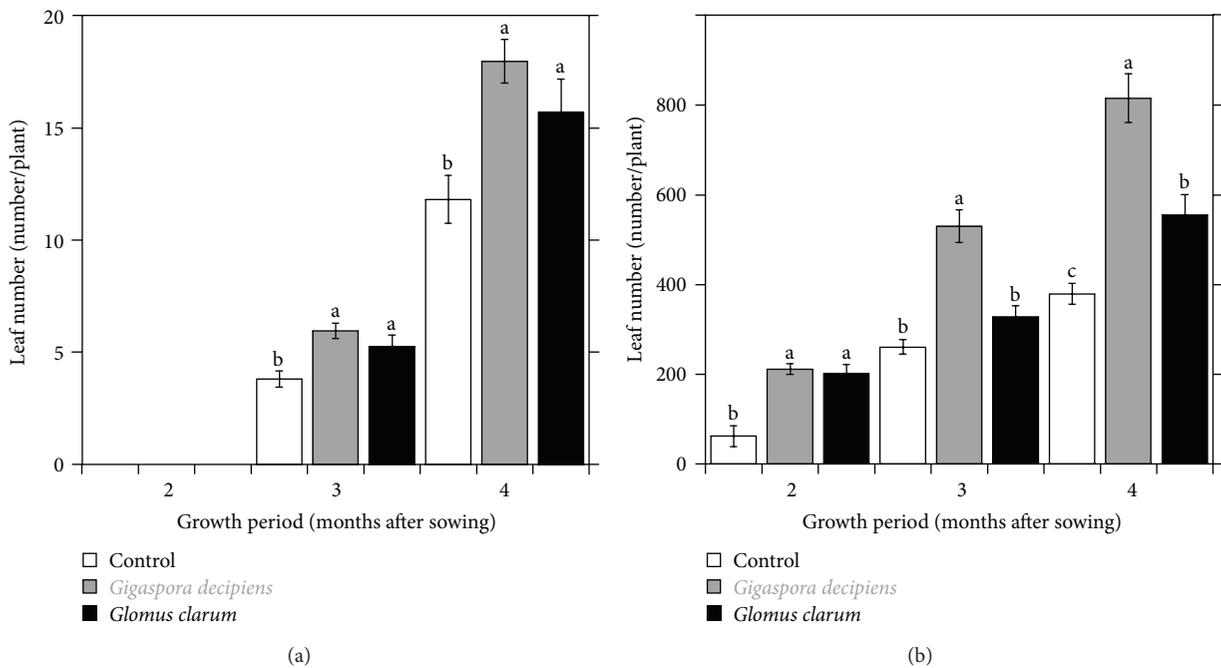


FIGURE 2: Leaf numbers of *M. paniculatus* (a) and *A. saman* (b) inoculated with AM fungi two, three, and four months after sowing. Gray bar: *Gigaspora decipiens*; black bar: *Glomus clarum*; white bar: control. Different letters for the same month indicate a significant difference at $P < 0.05$ as determined by the Tukey HSD test ($n = 20$). Vertical bars represent standard errors of mean (SE).

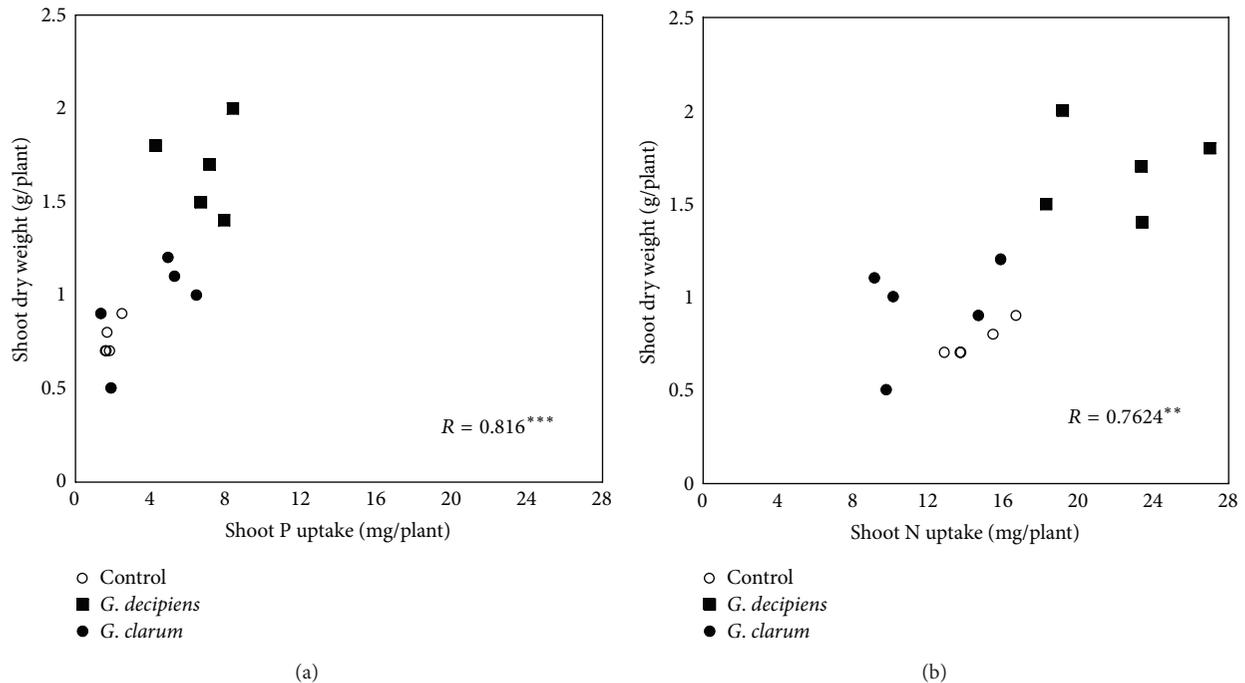


FIGURE 3: Correlation of shoot P uptake (a) and shoot N uptake (b) with shoot dry weight of *M. paniculatus* inoculated with AM fungi six months after sowing. Black squares: *G. decipiens*; black circles: *G. clarum*; white circles: control. *** $P < 0.001$.

No significant difference in shoot P and N concentration was observed between control seedlings and inoculated *A. saman* seedlings (Table 1). Shoot P and N content was approximately 70% higher in *G. decipiens* inoculated seedlings and 50% higher in *G. clarum* inoculated seedlings than in control seedlings. The inoculation with *G. decipiens* resulted in the highest shoot P content among the treatments. There was no significant difference in shoot N content between *G. decipiens* and *G. clarum* inoculated seedlings.

Positive correlation was observed between shoot P and N content and shoot dry weight in *M. paniculatus* seedlings (Figures 3(a) and 3(b)). Positive correlation was also observed between shoot P and N content and shoot dry weight in seedlings of *A. saman* (Figures 4(a) and 4(b)).

4. Discussion

It is a well-established fact that colonization by AM fungi increases shoot nutrient uptake and enhances plant growth. However, there is no study that clarifies the effects of AM fungal inoculation and colonization on the N and P uptake and growth of *M. paniculatus* and *A. saman* under tropical nursery conditions. In this study, we demonstrated the importance of AM fungi in promoting early growth and nutrient uptake of two native tropical tree species, *M. paniculatus* and *A. saman*.

In agreement with our result, no AM colonization was also observed in nonsterilized compost [25]. The high temperature of the composting process might have reduced mycorrhizal propagules. The inoculation with AM fungi is important to enhance growth of mycorrhizal plants in nursery conditions.

Our result on the increase of shoot N and P content by colonization of AM fungi is in agreement with a previous research that showed *Glomus fasciculatum*, *Sclerocystis dussi*, *Acaulospora laevis*, and *Gigaspora margarita* increased shoot P uptake in six-month-old *Phyllanthus emblica* (Euphorbiaceae) under greenhouse conditions [26]. *Glomus aggregatum* increased shoot N, P, K, and Mg concentrations in four-month-old *Tamarindus indica* (Fabaceae) under nursery conditions [27]. Our results suggest that AM fungi are crucial for promoting nutrient uptake in Euphorbiaceae and Fabaceae, particularly *M. paniculatus* and *A. saman*. No significant difference in shoot N and P concentration in *A. saman* inoculated seedling and the lower shoot N concentration in *M. paniculatus* inoculated seedling (Table 1) could be due to the dilution effect of higher shoot dry weight of plants with AM colonization.

Our study showed that *M. paniculatus* and *A. saman* exhibited positive growth response to *G. decipiens* and *G. clarum* inoculation. It was reported that shoot length, leaf number, and shoot dry weight were increased in *P. emblica* inoculated with *G. fasciculatum*, *S. dussi*, and *A. laevis* [26] and in *Senna spectabilis* (Fabaceae) inoculated with *G. etunicatum* and *Glomus macrocarpum* [28]. In contrast to our results, inoculation with a mixture of *Glomus claroideum*, *G. mosseae*, *G. intraradices*, and *G. geosporum* did not increase plant height, stem dry weight, and leaf dry weight of *Euphorbia pulcherrima* due to the late inoculation and poor colonization (34%) [29]. Similarly, poor colonization by commercial AM fungi (0.17%) did not increase plant height, collar root diameter, and biomass production of *A. saman* 30–170 days after transplanting [30]. Successful colonization by AM fungi is clearly a key factor for enhancing

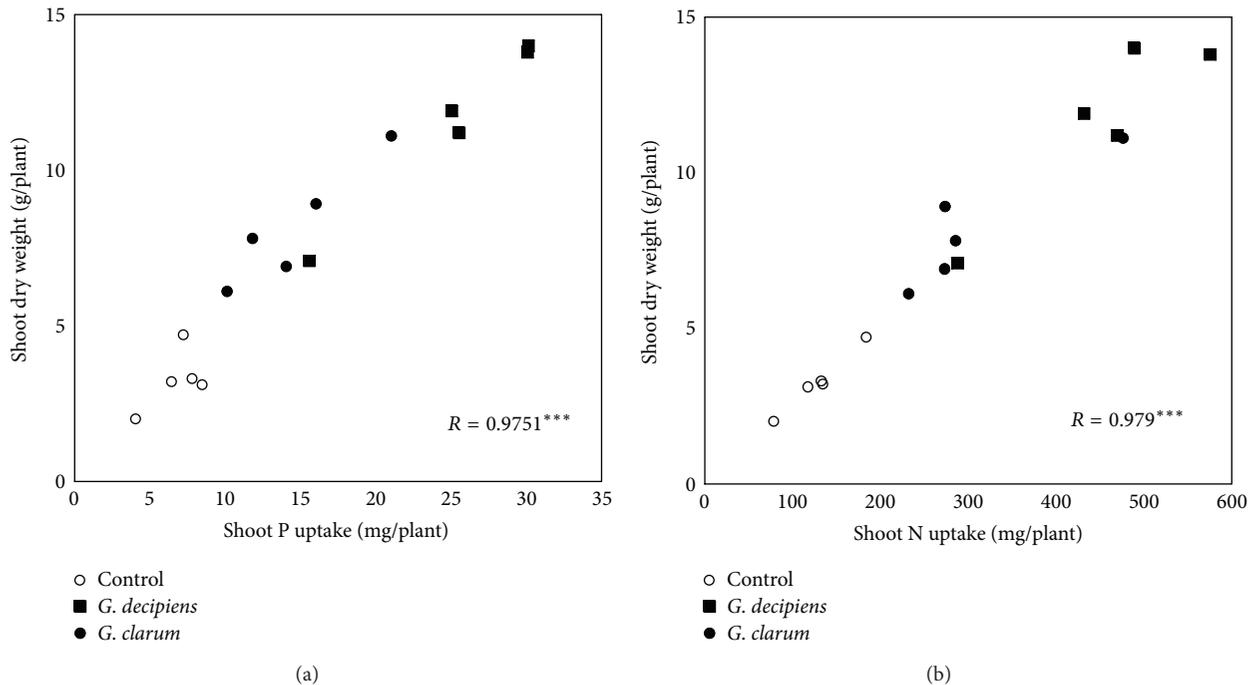


FIGURE 4: Correlation of shoot P uptake (a) and shoot N uptake (b) with shoot dry weight of *A. saman* inoculated with AM fungi six months after sowing. Black squares: *G. decipiens*; black circles: *G. clarum*; white circles: control. *** $P < 0.001$.

the growth of mycorrhizal plant species from Euphorbiaceae and Fabaceae. Although it was well documented that compost might suppress AM colonization and therefore the AM fungal activity [31] and that the inoculation with AM fungi did not increase shoot dry weight of pelargonium plants due to the high P concentration ($360 \text{ mg}\cdot\text{L}^{-1}$) in compost substrate [25], we successfully prepared AM fungal inoculum with a high percentage colonization of approximately 53% in *A. saman* seedlings and 90% in *M. paniculatus* seedlings, thereby enhancing the growth of both seedling species. The results suggest that inoculation with AM fungal symbionts with high mycorrhizal host-symbiont efficiency has the greatest potential for enhancing the early-stage establishment of tree seedlings in nurseries. Therefore, a good inoculum preparation of appropriate AM fungi, such as *G. decipiens* and *G. clarum*, is effective in promoting the growth of *A. saman*.

The gradual increase in shoot height or leaf number of both seedling species started two to three months after inoculation with AM fungi. It was reported that the inoculation with AM fungi resulted in 60% to 81% AM colonization in *P. emblica* seedlings 60 days after sowing [26]. Our results indicated that, even without fertilizer application, seedling growth could be accelerated two to three months after inoculation with AM fungi. The lack of a significant difference in shoot height between *A. saman* control seedlings and colonized seedlings at the early growth stage is consistent with the report of Koide [32], who explained the low nutrient utilization efficiency of mycorrhizal plants and the poor accumulation of nutrients during the early stage of plant growth.

A significant finding of our study for nursery practice was the increased nutrient uptake and plant growth by AM

colonization even without fertilizer application. The efficient transfer of nutrients, such as N and P, from the compost substrate by AM fungi may result in the optimal supply of nutrients to plants. A positive correlation was observed between shoot N and P content and shoot dry weight in both *M. paniculatus* and *A. saman* seedlings (Figures 3(a), 3(b), 4(a), and 4(b)).

The seedling growth parameters in this study are important for selecting the appropriate fungus in terms of symbiotic efficiency. Furthermore, these growth parameters are necessary for determining the potential of these timber species for timber production and reforestation purposes. Our findings on *M. paniculatus* and *A. saman* should be extended to other important forest timber species. The high demand for timber has resulted in intensive intervention of the natural forests in Indonesia, leading to the high rate of deforestation. From a long-term perspective, the improvement of seedling performance by inoculation with AM fungi may counteract this decline in timber resource supply and increase reforestation rate.

5. Conclusions

Colonization by *G. decipiens* and *G. clarum* increased shoot P and N uptake, shoot height, shoot dry weight, and leaf number of *M. paniculatus* and *A. saman* seedlings. Regarding the enhancement of plant growth and shoot nutrient uptake, *G. decipiens* was found to be superior to *G. clarum*. As the present results were obtained under nursery conditions, field trials are necessary to evaluate the growth and survival rates of these two plant species colonized by AM fungi. We conclude that the inoculation with AM fungi had a strong impact

on planting success particularly for *M. paniculatus* and *A. saman*. Therefore, this method can be adopted for ecological conservation and sustainable reforestation in Indonesia.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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