Effect of Arbuscular Mycorrhiza Fungal Inoculation on Growth of Tropical Tree Species under Nursery and Post-Opencast Bauxite Mining Field in Bintan Island, Indonesia

Ricksy Prematuri[1], Maman Turjaman[2] and Keitaro Tawaraya[3*]

[1]*Research Centre for Bio-resource and Biotechnology, IPB University, Kampus Dramaga, Bogor 16680, Indonesia.*
[2]*Forest Research and Development Centre, the Environment and Forestry Research and Development Agency (FORDA), Jalan Gunung Batu No.5, Bogor 16680, Indonesia.*
[3]*Faculty of Agriculture, Yamagata University, Tsuruoka 997-8555, Japan.*

Authors' contributions

This work was carried out in collaboration among all authors. Authors RP, MT and KT designed the study, performed the experiment and wrote the protocol. Authors RP and KT drafted and edited the manuscript. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

The purpose of this investigation was to examine the effects of native AM fungal inoculation on the growth of *Gmelina arborea, Samanea saman, Falcataria moluccana*, and *Enterolobium cyclocarpum* under nursery and post-opencast bauxite mining field conditions. Two native AM fungi, *Rhizophagus clarus* and *Gigaspora decipiens*, were inoculated into seeds of *G. arborea*, *S. saman*, *F. moluccana*, and *E. cyclocarpum. The seeds were* sown in post-bauxite mining soil and grown in the nursery for three months. Seeds without AM inoculation were used as the control treatment. The seedlings were transplanted into a post-opencast bauxite mining field and grown for 12 months. Arbuscular mycorrhizal fungal colonization and shoot and root dry weights were measured. Under nursery conditions, *G. arborea* inoculated with *G. decipiens* increased shoot and root dry weights by 1,431 and 359 %, respectively, while shoot dry weight of *E. cyclorapum*

---

*Corresponding author: E-mail: tawaraya@tds1.tr.yamagata-u.ac.jp;

inoculated with *R. clarus* and *G. decipiens* increased by 510 and 220%, respectively, in comparison with control seedlings. Root dry weight of *E. cyclorapum* inoculated with *R. clarus* increased by 224%, in comparison with control seedlings. Shoot dry weight of *E. cyclorapum* inoculated with *R. clarus* increased by 90%, in comparison with seedlings inoculated by *G. decipiens.* Twelve months after transplanting into post-opencast field conditions, the shoot dry weight of *F. moluccana* inoculated with *G. decipiens* was higher than that of the control seedlings by 188%. Shoot dry weight of *E. cyclorapum* inoculated with *R. clarus* and *G. decipiens* increased by 198% and 149%, respectively, in comparison with control seedlings. Shoot dry weight of *E. cyclorapum* seedlings inoculated with *R. clarus* was higher by 20% than that of seedlings inoculated with *G. decipiens.* These results show that AM fungal inoculation promotes the growth of tropical tree species on post-opencast bauxite mining land.

1. INTRODUCTION

Indonesia is a fast-growing, emerging economic country in Southeast Asia [1]. Energy resources and mineral mining provide the largest contribution to the country's foreign exchange. Major mining commodities produced in Indonesia's tropical rainforests are coal, nickel, bauxite, gold, copper, and tin, which are extracted by opencast mining. Forest exploitation by opencast mining causes erosion, flooding, loss of the top layer of soil, reduction of soil pH, lowering of soil organic matter content, and a reduction in soil fertility [2]. Opencast mining damages the environment [3] and destroys ecological functions and services [4]. Opencast mining operations have effects on nearby landscapes and influence soil quality due to the removal of the vegetation and soil surface [5].

A mining company in Indonesia must rehabilitate its post-mining land. However, the result of rehabilitation activities in opencast mining in most companies can be regarded as unsuccessful. Many transplanted tree seedlings die in the first year. Moreover, the seedlings have poor growth performance and become stunted in the field because of the low quality of transplanted seedlings. Soil degradation makes the rehabilitation of degraded post-mining land extremely challenging [6]. Soils in post-opencast mining land have low fertility, low organic matter, and poor soil properties that limit their ability to sustain vegetation growth and development [7].

The application of chemical fertilizers is required to promote the success of post-mining land rehabilitation. Fertilizer utilization is important for silvicultural management to maintain forest seedling growth and health [8]. However, inappropriate fertilization can also lead to unexpected effects, such as inhibiting forest-tree seedling growth, contaminating the environment,

and enhancing production costs. Application of chemical fertilizers in industrial plantations or mining companies is carried out several times a year, and fertilization is carried out until the trees are three years old in the field. Utilization of symbiotic microorganisms such as nitrogen-fixing bacteria, ectomycorrhizal fungi, arbuscular mycorrhizal fungi, endophytic fungi, and plant growth-promoting rhizobacteria can increase tree growth under pot culture conditions [9,10,11].

Arbuscular mycorrhizal (AM) fungi are important components of soil microorganisms that contribute to the stability and heterogeneity of natural ecosystems [12]. Arbuscular mycorrhizal fungi provide a direct biological and physical link between the host plant root and the soil. These fungi can increase production and plant growth under degraded post-mining land, despite low pH, water stress, nutrient deficiency, and soil toxicity [13]. Moreover, the domination of AM fungi in tropical forests demonstrates that AM fungi play an important role in these forests [14]. There are several efficient and effective ways to promote the early growth of tree seedlings in post-mining sites [15,16,17]. A symbiosis between host tree seedlings and AM fungi has a real influence on the success of rehabilitation activities in the damaged areas.

A proven strategy to enhance the success of revegetation in degraded post-opencast bauxite mining fields is to select fast-growing tropical tree species. *Gmelina arborea* (Linn.) Roxb., *Samanea saman* (Jacq.) Merr. *Falcataria moluccana* (Miq.) Barneby &J. W. Grimes, and *Enterolobium cyclocarpum* (Jacq.) Griseb are four tropical forest tree species that are grown to support sustainable forest rehabilitation programs in Indonesia. They have a variety of uses as industrial wood. *Gmelina arborea* is used as a raw material for matches, charcoal, light construction, plywood, particleboard, pulp, and

paper [18]. *Falcataria moluccana* is a multipurpose tree widely planted in forest community gardens on Java Island. This species can be used for packing boxes, housing construction, furniture, pulp, paper, and other purposes [19]. *Enterolobium cyclocarpum* is used for forage trees, fuelwood, and shade trees [20]. *Samanea saman* is usually planted as an urban forestry species to provide shade and minimize air pollution on roadsides, around office buildings, and in parks and schoolyards in urban areas [21].

Opencast bauxite mining reduces soil fertility; total carbon (C), nitrogen (N), available phosphorus (P) concentrations, and exchangeable sodium (Na), calcium (Ca) and magnesium (Mg) concentrations were reduced by 75.7%, 75%, 15.7%, 52%, 92%, and 100%, respectively, compared to the forest soils [22]. The use of beneficial soil microorganisms has been recommended to promote the successful restoration of the post-mining sites [23,24]. Inoculation of mycorrhizal fungi and nitrogen-fixing bacteria can increase nutrient absorption by plants [23]. Application of AM fungi to post-bauxite mining land is a biological approach that ensures good practices and is effective in enhancing plant growth [25].

To increase the growth of *G. arborea, S. saman, F. moluccana*, and *E. cyclocarpum,* two AM fungal species, *Rhizophagus clarus* and *Gigaspora decipiens* were inoculated into seeds. These AM fungal species were adopted because they are native to Indonesia and previous studies have shown that they have the capacity to promote plant growth in Indonesia's tropical peat-swamp forests [26], coal mining [27,16]. This technique, combining fast-growing tree species and native AM fungi, should be relatively easy for staff in the environmental divisions of bauxite mining companies to apply. The objective of the current investigation was to determine the effect of inoculation with two indigenous AM fungi on the growth of *G. arborea, S. saman, F. moluccana*, and *E. cyclocarpum* in the nursery and post-opencast bauxite mining field conditions.

2. MATERIALS AND METHODS

2.1 Nursery Site and Soil Substrate Preparation

The experiment was conducted in the nursery at the Agriculture, Forestry, and Livestock Office of Riau Archipelago Province, Bintan Island, Indonesia. The site was a post-opencast bauxite mining area managed by a national mining company. Ultisol soil was collected near the nursery area and stored in a nursery. The soil was air-dried and sieved through a < 5 mm sieve. The soil chemical characteristics were as follows: pH (H_2O), 4.96; total carbon, 7.00 g kg^{-1}; total N, 0.4 g kg^{-1}; available P, 11.30 mg P_2O_5 kg^{-1} [22]. The soil substrate was prepared by mixing river sand with the soil (1:3, v/v) to increase drainage and porosity.

2.2 Inoculum Propagation and Inoculation of Arbuscular Mycorrhizal Fungi

Two AM fungi, namely, *Rhizophagus clarus* Nicholson & Schenk and *Gigaspora decipiens* Hall & Abbott, were isolated from peat soil in Kalampangan, Palangkaraya, Central Kalimantan, Indonesia [26]. *Rhizophagus clarus* and *G. decipiens* were propagated in pot cultures of *Pueraria javanica* Benth. Plastic pots (7.5 cm height × 4 cm diameter) were filled with 175 g of sterilized zeolite. Two 7-day-old *P. javanica* were placed in pots, and 5 g of AM fungal inoculum was inoculated surrounding the roots of *P. javanica*. The inoculum of arbuscular mycorrhizal fungi contained a substrate of zeolite with external hyphae, spores, and mycorrhizal roots from a pot culture of *P. javanica* grown in a greenhouse with no humidity and temperature control in the Forest Research and Development Centre (FRDC), FORDA, The Ministry of Environment and Forestry, Bogor, West Java, Indonesia. Pot cultures were irrigated daily to field capacity using sterilized water to maintain the moisture content. After 120 days, an AM fungal inoculum with colonized roots, spores, and hyphae of *R. clarus* and *G. decipiens* was observed under a microscope in the zeolite media. Ten grams of inoculum was then mixed with 500 g of soil in a polyethylene bag (15 cm height × 10 cm diameter), and 10 g of zeolite was placed into no-inoculated pots as a control treatment.

2.3 Seed Germination

Four tropical forest trees, *Gmelina arborea*, (Linn.) Roxb., *Falcataria moluccana* (Miq.) Barneby &J. W. Grimes, *E. cyclocarpum* (Jacq.) Griseb, and *Samanea saman* (Jacq.) Merr. were selected for this investigation. Tropical forest-tree seeds were purchased from a local seed company in Solo, Central Java, Indonesia. The seeds were soaked in hot water at 85 °C for 2 min. Five-hundred grams of soil substrate was

poured into a polyethylene bag (15 cm height×10 cm diameter). Three seeds were sown and, after germination, one forest-tree seedling was allowed to grow in the polyethylene bag. The pots were placed in a randomized block design on the bench in a nursery. Tap water was applied two times a day. The source of tap water from groundwater, with water quality index (WQI) analysis, shows lightly polluted (WQI = 0.59) [28]. There was no application of chemical fertilizer or pesticides. Because these tree species require shade conditions, the seedlings were grown under 55% shading intensity net to control solar radiation for four months in the nursery of Agriculture, Forestry, and Livestock Office of Riau Archipelago Province, Bintan Island, Indonesia. The tree seedlings were then transplanted into the field. The experiment consisted of three treatments for *G. arborea, S. saman, F. moluccana,* and *E. cyclocarpum* seedlings at nursery (a) control, (b) *R. clarus, and (c) G. decipiens*. There were 20 replicates of four tree species per treatment.

2.4 Field Plantation and Growth Parameters

The field experiment was conducted on post-opencast bauxite mining land at the nursery of the Agriculture, Forestry, and Livestock Office of Riau Archipelago Province, Bintan Island, Indonesia. A planting field experiment without vegetation was covered with disposal waste and overburden from bauxite mining activity. Commercial organic compost was obtained from PT Green Planet Indonesia at the local market on Bintan Island. The organic compost contained: N, 1–3%; P_2O_5, 2–5%; K_2O, 1–3%; water content, 9–11%; and C-organic, 15-17%. A complete randomized block design with three treatments and six replications per treatment was used in this experiment. The field experiment consisted of three treatments: (1) control, (2) *R. clarus* AM fungal inoculation, and *(3) G. decipiens* AM fungal inoculation. On flat areas with similar soil conditions, six (24 m × 6 m) blocks with a distance between blocks of 5 m were prepared in the post-opencast bauxite mining field. Planting holes (30 cm × 30 cm × 30 cm) with 2 m distance between holes were laid out in each block. Five hundred grams of organic compost was then applied to the planting hole. Each block contained a treatment area (6 m × 6 m) with a distance between the treatment areas of 3 m. Three-month-old *G. arborea*, *S. saman*, *F. moluccana*, and *E. cyclocarpum* were transplanted into the holes for each treatment.

The seedlings were irrigated with tap water once a day for two weeks. The source of tap water from groundwater, with water quality, shows lightly polluted (WQI = 0.59) [28]. There was no weeding or fertilizer application to the seedlings after transplanting. The seedlings were grown for 12 months in the mining field.

2.5 Data Collection and Arbuscular Mycorrhizal Fungal Colonization

Three months after sowing in the nursery, each treatment consisting of six replicates of seedlings was harvested. Twelve months after transplanting into the mining field, the seedlings from each treatment with six replications were also harvested. After separation, shoots and roots were oven-dried at 70 °C for 72 h. The dry shoots and roots were then weighed. After harvest in the nursery, roots of *G. arborea*, *S. saman*, *F. moluccana, and E. cyclocarpum* were washed gently under running tap water over a 2-mm sieve to separate them from soil particle debris. Using the methods of [29], the roots were cleared with KOH (100 g l-1) for 1 h, acidified with dilute HCl, and stained with 500 mg l-1 trypan blue in lactoglycerol. The roots were de-stained in 50% glycerol, and 30 1-cm segments were viewed under a compound microscope at 200 × magnification. The percentage of colonization by the AM fungi was calculated using the gridline intersect method [30].

2.6 Statistical Analysis

The Minitab package (Minitab, USA) was used to analyze all collected data from the nursery and mining fields. The least significant difference (LSD) test was used to compare the significant differences between the means of the treatments when F showed a significant value.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Arbuscular mycorrhizal colonization of seedlings under nursery conditions

Arbuscular mycorrhizal fungi colonized all tree seedlings of *G. arborea, S. saman,* and *E. cyclocarpum*, and there was no significant difference in AM fungal colonization among the treatments in the nursery (Table 1). Colonization of *F. moluccana* inoculated with *R. clarus* was lower than that of control seedlings and the seedlings inoculated with *G. decipiens.*

3.1.2 Shoot and root dry weights of seedlings under nursery conditions

Shoot and root dry weights of *G. arborea* inoculated with *G. decipiens* were higher than those of the control seedlings (Table 1). There was no significant difference in shoot and root dry weights between seedlings inoculated with *R. clarus* and control seedlings. Shoot dry weight of *E. cyclorapum* inoculated with *R. clarus* and *G. decipiens* was higher than that of control seedlings. In comparison with control forest-tree seedlings, the root dry weight of *E. cyclocarpum* inoculated with *R. clarus* was higher than that of control seedlings. Shoot dry weight of seedlings inoculated with *R. clarus* was higher than that of seedlings inoculated with *G. decipiens*. Shoot and root dry weights of *S. saman* and *F. moluccana* inoculated with AM fungi were not different from that of control seedlings.

3.1.3 Arbuscular mycorrhizal colonization of seedlings under field conditions

Arbuscular mycorrhizal fungi colonized all *G. arborea* seedlings (Fig. 1A), *S. saman* (Fig. 2A), *F. moluccana* (Fig. 3A), *E. cyclocarpum* (Fig. 4A), and control seedlings in the post-opencast bauxite mining field. Arbuscular mycorrhizal fungal colonization did not differ among treatments in any species.

3.1.4 Shoot dry weight of seedlings under field conditions

There was no significant difference in *G. arborea* and *S. saman* among treatments (Fig. 1B, Fig. 2B). Shoot dry weight of *F. moluccana* inoculated with *G. decipiens* was higher than that of the control seedlings (Fig. 3B). There was no significant difference in shoot dry weight between seedlings inoculated with *R. clarus* and control seedlings. Shoot dry weight of *E. cyclocarpum* inoculated with *R. clarus* and *G. decipiens* was higher than that of control seedlings (Fig. 4B). Shoot dry weight of seedlings inoculated with *R. clarus* was higher than that of seedlings inoculated with *G. decipiens*.

3.2 Discussion

3.2.1 Growth of colonized forest tree seedlings in the nursery

Successful post-bauxite mine land rehabilitation requires a large number of forest tree seedlings with high-quality performance. For this purpose,

Table 1. Mycorrhizal colonization, shoot and root dry weight of *Gmelina arborea*, *Samanea saman*, *Falcataria moluccana* and *Enterolobium cyclocarpum* grown with or without mycorrhizal fungi under nursery conditions adjacent to a bauxite mine in Bintan Riau Islands, Indonesia, three months after sowing

Plant Treatment	Mycorrhizal Colonization (%)				Shoot Dry Weight (g/plant)				Root Dry Weight (g/plant)			
Gmelina arborea												
Control	92.77	±	5.33	a	0.48	±	0.45	b	0.32	±	0.13	b
Rhizophagus clarus	89.93	±	12.22	a	4.47	±	3.86	ab	0.88	±	0.61	ab
Gigaspora decipiens	74.90	±	22.17	a	7.35	±	3.45	a	1.47	±	0.23	a
Samanea saman												
Control	10.13	±	15.35	a	0.84	±	0.61	a	0.50	±	0.20	a
Rhizophagus clarus	24.03	±	28.77	a	1.09	±	0.47	a	0.42	±	0.23	a
Gigaspora decipiens	2.63	±	3.55	a	0.44	±	0.07	a	0.35	±	0.12	a
Falcataria moluccana												
Control	82.08	±	14.86	a	1.08	±	0.57	a	0.27	±	0.13	a
Rhizophagus clarus	25.80	±	15.50	b	1.93	±	0.74	a	0.41	±	0.18	a
Gigaspora decipiens	63.13	±	6.88	a	1.22	±	0.42	a	0.21	±	0.03	a
Enterolobium cyclocarpum												
Control	26.50	±	24.44	a	2.29	±	1.06	c	1.11	±	0.49	b
Rhizophagus clarus	3.05	±	3.54	a	13.97	±	3.22	a	3.60	±	1.09	a
Gigaspora decipiens	5.65	±	7.06	a	7.34	±	0.88	b	2.38	±	0.28	ab

For each plant species, different letters within the column indicate significant difference (P = .05) by Tukey HSD test. Means ± standard error are shown (n = 6)

several bauxite mining companies in Indonesia have built nurseries to produce seedlings that can be used for post-mining land rehabilitation activities. Production of forest tree seedlings in nurseries, however, is not easy. Slow growth limits the quantity and quality of seedling production. In addition to seed quality, some factors required to produce high-quality seedlings for mine site rehabilitation, are knowledge of the breakdown of seed dormancy, seed germination, and seed storage tolerance [31,32], plant growth, and nutrient requirements [33]. It is also important to select a suitable medium to support seed growth and produce seedlings in the pots. In Indonesia, compost is one of the most frequently used pot-substrates for growing forest-tree seedlings in the nursery. However, not all mining sites can provide good quality compost because mining companies in Indonesia are scattered in remote areas.

To save costs, the fresh soil near the bauxite mining site could be used as an alternative growing medium to produce forest tree seedlings in the nursery. However, due to the opencast mining system, the fresh soil near bauxite mining is often limited and categorized as post-mine soil with low fertility. The process of opencast mining, including surface soil stripping, excavation, transportation, and dumping, causes physical, chemical, and biological damage to the forest surface. Furthermore, the re-established landscape generates small-scale spatial heterogeneity of soils after mining [3].

It has been demonstrated that colonization by AM fungi enhances the growth of forest tree seedlings in the nursery. Inoculation with AM fungi is highly recommended to promote the growth of mycorrhizal tree seedlings in the nursery before transplantation into the degraded land of the mining field. Arbuscular mycorrhizal fungi improved the early growth of *Mallotus paniculatus* in the nursery [27]. These fungi also enhance leguminous seedling growth and P uptake of *P. falcataria*, *Calliandra calothyrsus*, *Cassia siamea*, *and Sesbania grandifolia* [34]. Arbuscular mycorrhizal fungal inoculation with *Funneliformis mossease* (syn. *Glomus mossease*), *Rhizophagus intraradices* (syn. *Glomus intraradices*), and *Claroideoglomus etunicatum* (syn. Glomus etunicatum) increased the growth and drought tolerance of *Acacia seyal* Del. seedlings [35]. The inoculated seedlings of *Eucalyptus tereticornis* with various bioinoculants, Azospirillum+Phosphobacterium (PGPR), *Glomus fasciculatum* (AM fungi), and

pink-pigmented facultative methylotrophic bacteria (PPFM), have shown improved performance in terms of seedling survival, shoot length, and collar diameter in the nursery [36].

Bauxite mining soil contains pollution of heavy metals. The elements, such as iron (194,912 ± 30,229 ppm), mercury (2.63 ± 0.40 ppm), arsenic (25.17 ± 37.49 ppm), lead (108.06 ± 78.88 ppm), copper (100.09 ± 32.79 ppm) were detected in bauxite mining soil in Kuantan, Pahang, Malaysia [37]. Aluminum was determined in the formation of High-Grade Al Deposits of the Dopolan Karst Type Bauxite, Iran [38] while pollutant of arsenic, lead, and copper were also detected in bauxite mining soil from Bintan Island, Indonesia [39]. AM fungi ameliorate metal toxicity as they intensify the plant's ability to tolerate metal stress [40]. Agus et al. [41] reported that fast-growing legume species of *Pongamia pinnata* and AM fungi application can not only increase nutrient contents of post-coal mining soil but also increases iron absorption, which is mostly accumulated in the root system. Arbuscular mycorrhizal fungi *Gigaspora margarita* and *E. cyclocarpum* seedlings can tolerate up to 375 µM Hg supply [42]. Root colonization by symbiotic AM fungi enhances plant resistance to acidity and phytotoxic levels of aluminum in the soil environment [43]. Arbuscular mycorrhizal fungal treatment could reduce the toxic effects of arsenic on the growth of *G. arborea* in degraded soil during the nursery stage [44], on phyto-extraction by Corn (*Zea mays*) of lead-contaminated soil [45]. Research has established the potential of carbonized rice hull (CRH) and AM fungi inoculation to improve the health of *Paraserianthes falcataria*, grown under the Cu-stressed soil in the nursery [46].

Our study showed that using the Ultisol soil near the bauxite mining site, mixing with river sand as a growing medium for the seedlings combined with AM fungal inoculation, irrigating with lightly polluted tap water (WQI = 0.59) [28], enhanced tree seedling growth of *G. arborea* and *E. cyclocarpum* at the nursery stage, three months after planting. In *G. arborea* seedlings, inoculation with *G. decipiens* increased both shoot and root growth. *Rhizophagus clarus* and *G. decipiens* showed similar responses by enhancing the shoot and root growth of *S. saman* and *F. moluccana*. In *E. cyclocarpum* seedlings, both *R. clarus* and *G. decipiens* improved shoot growth. In comparison with the previous investigation, this result showed that *R. clarus* and *G. decipiens* enhanced shoot growth of *S.*

saman and *M. paniculatus* [27], *A. saman*, and *P. falcataria* [16]. This result confirms that AM inoculation could promote the growth of seedlings on low-soil-fertility media in the nursery.

3.2.2 Growth of colonized seedlings under post-opencast bauxite mining conditions

Information about the use of AM fungal colonization to increase plant growth in post-bauxite mining fields is still limited. The success of rehabilitation in the post-bauxite mining field is dependent on the plant's growth; however, there are no studies to clarify the effect and utilization of AM fungal inoculation to improve the growth of *G. arborea, S. saman, F. moluccana*, and *E. cyclocarpum* in post-bauxite mining fields in Indonesia. This study showed the importance of AM fungal inoculation in promoting the growth of tropical tree seedling species in the post-bauxite mining field in Bintan Island, Riau Archipelago, Indonesia.

Inoculation with *R. clarus* and *G. decipiens* demonstrated the positive effects of increasing the growth of *G. arborea*, *S. saman*, *F. moluccana*, and *E. cyclocarpum*, by using low soil fertility as a growth medium under nursery conditions. The effect of these AM fungi was also examined under field conditions to ensure consistency. Twelve months after transplanting into the field, *R. clarus* and *G. decipiens* tended to increase the shoot growth of *G. arborea* (Fig. 1B). In *S. saman* seedlings, both *R. clarus* and *G. decipiens* seem to have a beneficial effect on shoot growth (Fig. 2B). Inoculation with *G. decipiens* increased the shoot growth of *F. moluccana* seedlings (Fig. 3B). Both *R. clarus* and *G. decipiens* enhanced shoot growth in *E. cyclocarpum* seedlings (Fig. 4B).

Arbuscular mycorrhizal fungi colonization is important for promoting the growth of mycotrophic plant species in the field. Arbuscular mycorrhizal fungal inoculation has been reported to enhance early growth and nutrient absorption by some tropical forest-tree species in a nursery and in the field [47], *P. falcataria* and *A. saman* growth in a post-opencast coal mining field in Kalimantan, Indonesia [16]. *Enterolobium cyclocarpum* was classified as a highly mycorrhizal-dependent plant species in response to low soil P concentration [48]. A combination of AM fungi and *Rhizobium* sp. increased the growth of *A. saman* in degraded gold-mining land

in Pongkor, West Java, Indonesia [15]. Some studies on the application of AM fungi in poor soil and post-mining have shown that this treatment was very effective in increasing the growth of *G. arborea* under salt stress [49]. Arbuscular mycorrhizal fungi association also influence soil fertility through the enhancement of chemical, biological, and physical properties. Arbuscular mycorrhizal fungi have a positive correlation with organic carbon, organic matter, total phosphorus, cation exchange capacity, water level, soil fungi, and soil bacteria [50]. There is a plentiful scientific confirmation to indicate that AM fungi significantly promote soil attributes, improve above and belowground biodiversity, significantly enhance tree seedlings survival, growth, and establishment on moisture and nutrient stressed soils after the restoration of degraded lands [25]. It could be expected that, after rehabilitation, trees affected on soil conditions. Rehabilitation through forest vegetation is one of the efficient means of restoring soil fertility through improved soil organic matter content, available nutrients, cation exchange capacity, increase biological activities as well as improvement in physical conditions of the soil [51]. A study in the jarrah forest for the rehabilitation of bauxite mines in south-west Australia shows that level of total nitrogen of soil in rehabilitated lands enhanced from around 0.04 – 0.05% after 8.5 years, while soil pH decreased after rehabilitation [52]. After rehabilitation, using *Eucalyptus camaldulensis* and *Brachiaria decumbens*, in a soil contaminated with Zn, Cu, Cd, and Pb can enhance the soil pH, phosphorus (P) concentration, and exchangeable K by 31%, 40%, and 98%, respectively while decreased Ca, Mg, Al, and Mn by 97%, 96%, 93% and 96%, respectively [53].

The inoculation of *R. clarus* and *G. decipiens* enhanced growth of tropical tree species in post-opencast bauxite mining. *Gmelina arborea* inoculated with *G. decipiens* increased shoot and root dry weights by 1,431 and 359 %, respectively, while shoot dry weight of *E. cyclorapum* inoculated with *R. clarus* and *G. decipiens* increased by 510 and 220%, respectively, in comparison with control seedlings, under nursery conditions. Root dry weight of *E. cyclorapum* inoculated with *R. clarus* increased by 224%, in comparison with control seedlings. Shoot dry weight of *E. cyclorapum* inoculated with *R. clarus* increased by 90%, in comparison with seedlings inoculated by *G. decipiens.* Under field conditions, the shoot dry weight of *F. moluccana* inoculated with *G.*

decipiens was higher than that of the control seedlings by 188%. Shoot dry weight of *E. cyclorapum* inoculated with *R. clarus* and *G. decipiens* increased by 198% and 149%, respectively, in comparison with control seedlings. Shoot dry weight of *E. cyclorapum* seedlings inoculated with *R. clarus* was higher by 20% than that of seedlings inoculated with *G. decipiens.* Our study showed that regarding the enhancement of plant growth, *R. clarus* was found to be superior to *G. decipiens*. Growth of *E. cyclorapum* seedlings inoculated with *R. clarus* was higher than that of seedlings inoculated with *G. decipiens* under both nursery and post-opencast bauxite mining field. In contrast to our results, growth of seedlings, *Mallotus paniculatus* and *Albiziz saman* inoculated with *G. decipiens* was found to be superior to *R. clarus* [27]. It is well known that AM fungi can exhibit a considerable level of selectivity in their association with different plants

species or plant ecological groups [54]. Moreover, different AMF strains displayed different colonization rates, which suggest that AMF strain has certain selectively to their host plants [55].

This study demonstrated the consistent effect of *R. clarus* and *G. decipiens*, which are AM species indigenous to Indonesia, in increasing plant growth, not only in post-bauxite mining but also in tropical peat-swamp forests [26], post-coal mining in nursery conditions [27], and post-coal mining in both nursery and field conditions [16]. It appears that native AM inoculation increases plant growth thus saving time and increasing cost efficiency during the rehabilitation of post-bauxite mining land. Further research should be conducted to determine the capability of native AM species to increase plant growth on other post-mining land in Indonesia.
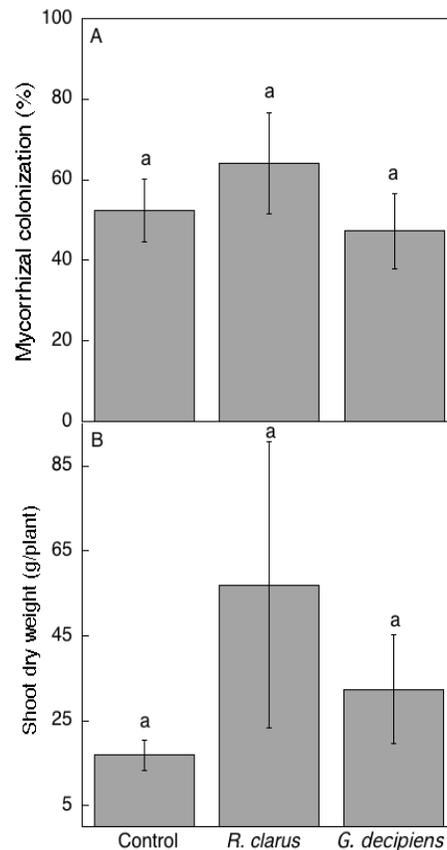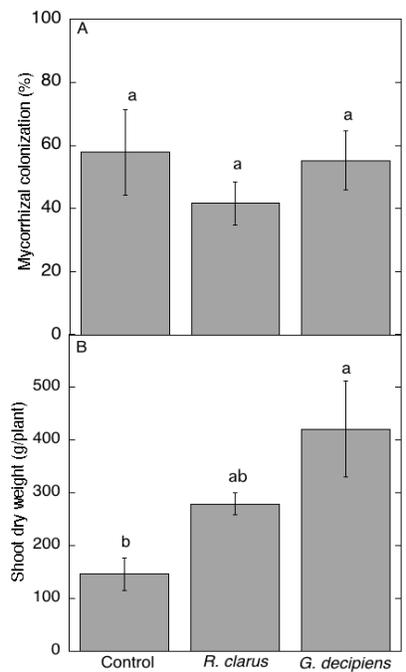

Fig. 1. Mycorrhizal colonization (A) and shoot dry weight (B) of *Gmelina arborea* grown on post opencast bauxite mining 12 months after transplanting into the field
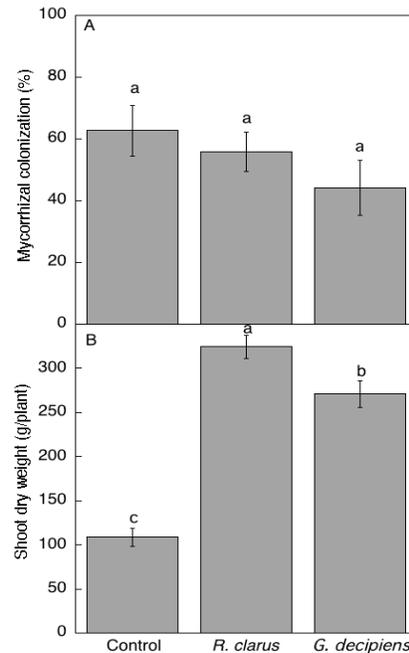*On each column, different letters indicate a significant difference (P = .05) by t test. Data are shown as mean ± standard error (n = 6)*
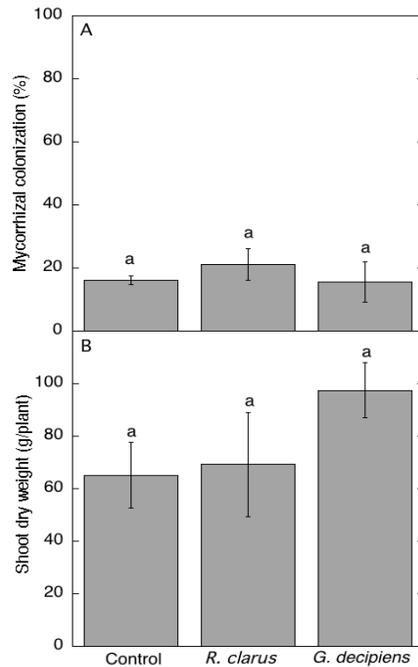
Fig. 2. **Mycorrhizal colonization (A) and shoot dry weight (B) of *Samanea saman* grown on post opencast bauxite mining 12 months after transplanting into the field**
On each column, different letters indicate a significant difference (P = .05) by t test. Data are shown as mean ± standard error (n = 6)

Fig. 3. Mycorrhizal colonization (A) and shoot dry weight (B) of *Falcataria moluccana* grown on post opencast bauxite mining 12 months after transplanting into the field
On each column, different letters indicate a significant difference (P = .05) by t test. Data are shown as mean ± standard error (n = 6)

Fig. 4. Mycorrhizal colonization (A) and shoot dry weight (B) of *Enterolobium cyclocarpum* grown on post opencast bauxite mining 12 months after transplanting into the field
On each column, different letters indicate a significant difference (P = .05) by t test. Data are shown as mean ± standard error (n = 6)

4. CONCLUSIONS

Arbuscular mycorrhizal fungal inoculation of *R. clarus* and *G. decipiens,* which are native to Indonesia, have demonstrated positive effects in enhancing the forest tree growth of *G. arborea* and *E. cyclocarpum* under nursery condition while *F moluccana* and *E. cyclocarpum,* in post-opencast bauxite mining field conditions. Regarding the enhancement of plant growth, *R. clarus* was found to be superior to *G. decipiens*. Growth of *E. cyclorapum* seedlings inoculated with *R. clarus* was consistently higher than that of seedlings inoculated with *G. decipiens* under both nursery and post-opencast bauxite mining field. These AM fungi promoted tropical tree species growth on low fertility soil in the post-opencast bauxite mining field. This method, therefore, can be potentially used to enhance the success of forest rehabilitation and ensure the sustainability of tropical forests and environmental services.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Tiess G, Mujiyanto S. Mineral resources policies and governances in Indonesia: Conference in Mineral Resources Policies and Governance in Indonesia. 2019;1-18. Available:https://www.researchgate.net/publication/334785537_Mineral_Resources_Policies_and_Governance_in_Indonesia.

2. Ghose MK. Effect of opencast mining on soil fertility. Journal of Scientific and Industrial Research. 2004; 63:1006–1009.

3. Feng Y, Wang J, Bai Z, Reading L. Effects of surface coal mining and land reclamation on soil properties: A Review. Earth Science Review. 2019;191:12–25.

4. Mentis M. Environmental rehabilitation of damaged land. Forest Ecosystems. 2020;7(19):1-16.

5. Paramasivam CR, Anbazhagan S. Soil fertility analysis in and around magnesite mines Salem, India. Geology, Ecology and Landscapes. 2020;4(2):140-150 DOI: 10.1080/24749508.2019.1608407.

6. Parrotta JA. Restoration and management of degraded tropical forest landscapes. In: R.S. Ambasht & N.K. Ambasht (Eds.), Modern Trends in Applied Terrestrial

Ecology, Kluwer Academic/Plenum Press, New York. 2002;135-148.

7. Mushia NM, Ramoelo A, Ayisi KK. The Impact of the Quality of Coal Mine Stockpile Soils on Sustainable Vegetation Growth and Productivity. Sustainability. 2016;8(546).
DOI:10.3390/su8060546.

8. Deng S, Shi K, Ma J, Zhang L, Ma L, Jia Z. Effects of Fertilization Ratios and Frequencies on the Growth and Nutrient Uptake of Magnolia wufengensis (Magnoliaceae). Forests. 2019;10(65):1-29.
DOI : 10.3390/f10010065.

9. Maulana AF, Turjaman M, Sato T, Hashimoto Y, Cheng W, Tawaraya K. Isolation of Endophytic Fungi from Tropical Forest in Indonesia. Symbiosis. 2018;76(2):151-162.

10. Singh A, Vaish B, Singh RP. Eco-restoration of degraded lands through microbial biomass: An ecological engineer. Acta Biomedica Scientia. 2016;3(1):133-135.

11. Wildman H. Improving mine rehabilitation success through microbial management. Microbial Management System. NSW 2773 Australia. 2014;2(1):1-15.
DOI: 10.3992/1573-2377-374X-1.1.32.

12. Van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, et al. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature. 1998;396:69–72.

13. Smith SE, Read DJ. Mycorrhizal symbiosis, 3rd eds. Academic Press. London; 2008.

14. Tresseder KK, Cross A. Global distributions of arbuscular mycorrhizal fungi. Ecosystems. 2006;9:305-316.

15. Setyaningsih L, Dikdayatama FA, Wulandari AS. Arbuscular mycorrhizal fungi and Rhizobium enhance the growth of *Samanea saman* (trembesi) planted on gold-mine tailings in Pongkor, West Java, Indonesia. Biodiversitas. 2020;21(2):611-616.

16. Wulandari D, Saridi, Cheng W, Tawaraya K. Arbuscular mycorrhizal fungal inoculation improves *Albizia saman* and *Paraserianthes falcataria* growth in post-opencast coal mine field in East Kalimantan, Indonesia. Forest Ecology and Management. 2016;376:67-73.

17. Gardner JH, Malajczuk N. Recolonisation of rehabilitated bauxite mine sites in Western Australia by mycorrhizal fungi. Forest Ecology and Management. 1988;24:27-42.

18. Soerianegara I, Lemmens RHMJ (eds.). Plant resources of South-East Asia. No. 5(1): Timber trees: major commercial timbers, Backhus Publishers; 1993.

19. Krisnawati H, Varis E, Kallio M, Kanninen M. *Paraserianthes Falcataria (L.) Nielsen* Ecology, Silviculture and Productivity, Center for International Forestry Research, Bogor, Indonesia; 2011.

20. Akkasaeng R, Gutteridge RC, Wanapat M. Evaluation of trees and shrubs for forage and fuel wood in Northeast Thailand. The International Tree Crops Journal. 1989;5:209-220.

21. Kabir M, Iqbal MZ, Shafiq M. Traffic density, climatic conditions and seasonal growth of *Samanea saman* (Jacq.) Merr. on different polluted roads of Karachi City. Pakistan Journal of Botany. 2012;44(6):1881-1890.

22. Prematuri R, Turjaman M, Sato T, Tawaraya K. Post bauxite mining land soil characteristics and its effects on the growth of *Falcataria moluccana* (Miq.) Berneby & J.W. Grimes and *Albizia saman* (Jacq.) Merr. Applied and Environmental Soil Science. 2020.
Article ID 6764380, 8 pages.
DOI.ORG/10.1155/2020/6764380.

23. Jasper DA. Beneficial Soil Microorganisms of the Jarrah Forest and Their Recovery in Bauxite Mine Restoration in Southwestern Australia. Restoration Ecology. 2007;15(4):74–84.

24. Taheri IW, Bever JD. Adaptation of plants and arbuscular mycorrhizal fungi to coal tailings in Indiana. Application Soil Ecology. 2010;45:138-143.

25. Asmelash F, Bekele T, Birhane E. The potential role of mycorrhizal fungi in the restoration of degraded lands. Frontiers in Microbiology. 2016;7(1095).
DOI:10.3389/fmicb.2016.01095.

26. Turjaman M, Tamai Y, Santoso E, Osaki M, Tawaraya K. Arbuscular mycorrhizal fungi increased early growth of two nontimber forest product species *Dyera polyphylla* and *Aquilaria filaria* under greenhouse conditions. Mycorrhiza. 2006;16:459-464.

27. Wulandari D, Saridi, Cheng W, Tawaraya K. Arbuscular mycorrhizal colonization enhanced early growth of *Mallotus paniculatus* and *Albizia saman* under

nursery conditions in East Kalimantan, Indonesia. International Journal of Forestry Research. 2014;8.
Article ID 898494.
Available:http://dx.doi.org/10.1155/2014/898494

28. Marganingrum D, Sumawijaya N, Rachmat A. The feasibility of raw water on Bintan Island in quantity and quality. Jurnal Wilayah dan Lingkungan. 2020;8(1):15-35. DOI:10.14710/jwl.8.1.15-35.

29. Brundrett M, Bougher N, Dell B, Grove T, Malajcuk M. Working with mycorrhizas in Forestry and Agriculture. ACIAR Monograph 32, Canberra; 1996.

30. Giovannetti M, Mosse B. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol. 1984;84:489-500.

31. Salazar A, Hodges SR, Maschinski J. Chemical scarifcation improves seed germination of *Trema lamarckiana* (Cannabaceae), a potential tree species to restore South Florida endangered ecosystems. Seed Science Technology. 2015;43:291–296.

32. Ramos SJ, Caldeira CF, Gastauer M, Costa DLP, Neto AEF, Machado de Souza FB, Souza-Filho PWM, Siqueira JO. Native leguminous plants for mineland revegetation in the eastern Amazon: Seed characteristics and germination. New Forests. 2019.
DOI: org/10.1007/s11056-019-09704-1

33. Carvalho JM, Ramos SJ, Furtini Neto AE, Gastauer M, Caldeira CF, Siqueira JO, et al. Infuence of nutrient management on growth and nutrient use efciency of two plant species for mineland revegetation. Restoration Ecology. 2018;26:303–310.

34. Maulana AF, Turjaman M, Sato T, Hashimoto Y, Cheng W, Tawaraya K. Growth response of four leguminous trees to native arbuscular mycorrhizal fungi from tropical forest in Indonesia. International Journal of Plant and Soil Science. 2017;20(3):1-13.

35. Abdelmalik AM, Alsharani TS, Al-Qarawi AA, Ahmed AI, Aref IM. Response of growth and drought tolerance of *Acacia seyal* Del. seedlings to arbuscular mycorrhizal fungi. Plant, Soil and Environment. 2020;6:264–271.

36. Murugesan S, Mohan V, Senthilkumar N, Lakshmidevi R, Suresh Babu D, Sumathi R. Effects of growing media with bioinoculants on quality seedlings production of *Eucalyptus tereticornis* in nursery conditions. European Journal of Experimental Biology. 2016;6(3):86-93.

37. Ismail SNS, Abidin EZ, Praveena SM, Rasdi I, Mohamad S, Ismail MIW. Heavy metals in soil of the tropical climate bauxite mining area in Malaysia. Journal of Physical Science. 2018;29(3):7–14.
Available:https://doi.org/10.21315/jps2018.29s3.2.

38. Ellahi SS, Taghipour B, Nejadhadad M. The Role of Organic Matter in the Formation of High-Grade Al Deposits of the Dopolan Karst Type Bauxite, Iran: Mineralogy, Geochemistry and Sulfur Isotope Data. Minerals. 2017;7(97). DOI:10.3390/min7060097.

39. Putra RD, Apriadi T, Suryanti A, Irawan H, Raja TS, Yulianto T, Atmadja et al. Preliminary study of heavy metal (Zn, Pb, Cr, As, Cu, Cd) contaminations on different soil level from post-mining bauxite production for aquaculture. E3S Web of Conferences 47, 02008, SCiFiMaS; 2018.
Available:
https://doi.org/10.1051/e3sconf/20184702008.

40. Dhalaria R, Kumar D, Kumar H, Nepovimova E, Kuˇca K, Islam MT, et al. Arbuscular Mycorrhizal Fungi as Potential Agents in Ameliorating Heavy Metal Stress in Plants. Agronomy. 2020;10(815). DOI:10.3390/agronomy10060815.

41. Agus C, Primananda E, Faridah E, Wulandari D, Lestari T. Role of arbuscular mycorrhizal fungi and *Pongamia pinnata* for revegetation of tropical open-pit coal mining soils. International Journal of Environmental Science and Technology. 2018;16(7):3365-3374.

42. Ekamawanti HA, Setiadi Y, Sopandie D, Santoso DA. The role of arbuscular mycorrhizal fungus (*Gigaspora margarita*) on mercury and nutrients accumulation by *Enterolobium cyclocarpum*. Microbiology Indonesia. 2013;7(4);167-176.

43. Seguel A, Cumming JR, Klugh-Stewart K, Cornejo P, Borie F. The role of arbuscular mycorrhizas in decreasing aluminium phytotoxicity in acidic soils: a review. Mycorrhiza. 2013;23:167–183.
DOI 10.1007/s00572-013-0479-x

44. Barua A, Gupta SD, Mridha MAU, Bhuiyan MK. Effect of arbuscular mycorrhizal fungi on growth of *Gmelina arborea* in arsenic-contaminated soil. Journal of Forestry Research. 2010;21(4);423-432.

45. Hovsepyan A, Greipsson S. Effect of arbuscular mycorrhizal fungi on phytoextraction by corn (*Zea mays*) of lead-contaminated soil. International Journal of Phytoremediation. 2004;6(4):305–321.
DOI: 10.1080/16226510490888820.

46. Rollon RJC, Galleros JEV, Galos GR, Villasica LJD, Carcia CM. Growth and nutrient uptake of *Paraserianthes falcataria* (L.) as affected by carbonized rice hull and arbuscular mycorrhizal fungi grown in an artificially copper contaminated soil. AAB Bioflux. 2017;9(2).
Available:http://www.aab.bioflux.com.ro.

47. Tawaraya K, Turjaman M. Use of arbuscular mycorrhizal fungi for reforestation of degraded tropical forests, In: Solaiman, Z., Abbott, L.K., Varma, A. (Eds.), Mycorrhizal Fungi: Use in Sustainable Agriculture and Land Restoration. Springer; 2014.

48. Habte M, Musoko M. Changes in the vesicular-arbuscular mycorrhizal dependency of *Albizia ferrugunia* and *Enterolobium cyclocarpum* in response to soil phosphorus concentration. Journal of Plant Nutrition. 1994;17(10);1769-1780.

49. Dudhane MP, Borde MY, Jite PK. Effect of arbuscular mycorrhizal fungi on growth and antioxidant activity in *Gmelina arborea* Roxb. under salt stress condition. Not. Sci. Biol. 2011;3(4);71-78.

50. Syib'li MA, Muhibuddin A, Djauhari S. Arbuscular mycorrhiza fungi as an indicator of soil fertility. Agrivita. 2013;35(1).
ISSN : 0126-0537.

51. Mensah AK. Role of revegetation in restoring fertility of degraded mined soils in Ghana: A review. International Journal of Biodiversity and Conservation. 2015;2(2);57-80.
DOI: 10.5897/IJBC2014.0775.

52. Ward SC. Soil development on rehabilitated bauxite mines in south-west Australia. Australian Journal of Soil Research. 2000; 38(2);453 – 464.

53. Leal PL, Varón-Lópezc M, Prado IGO, Santos JV, Soares CRFS, Siqueiraa JO, et al. Enrichment of arbuscular mycorrhizal fungi in a contaminated soil after rehabilitation. Brazilian Journal of Microbiology. 2016;47:853-862.

54. Varelo-Cevero S, Vasar M, Davison J, Barea JM, Opik M, Azcon-Aguilar C. The composition of arbuscular mycorrhizal fungal communities differ among the roots, spores and extraradical mycelia associated with five Mediterranean plant species. Environmental Microbiology. 2015;17:2882–2895.

55. Xiaoying C, Fengbin S, Fulai L, Chunjie T, Shengqun L, Hongwen X, et al. Effect of Different Arbuscular Mycorrhizal Fungi on Growth and Physiology of Maize at Ambient and Low Temperature Regimes. The Scientific World Journal. 2014;7.
Article ID 956141.
Available:http://dx.doi.org/10.1155/2014/956141.