

Efficacy of native arbuscular mycorrhizal fungi in promoting growth of Albizia saman in coal mine spoil-bank: a prospect in restoring mined tropical forest, Indonesia

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I. INTRODUCTION

The extensive opencast coal mining brings consequences in the large formation of coal mine spoil-bank (tailing) wide world in Czech Republic [1], Ohio [2], India [3], Spain [4], including in Kalimantan, Indonesia. Coal mine spoil-bank is diverse in soil characteristics and unsuitable for plant growth. It is reported that coal mine spoil-bank may have a very low pH [2], high temperature, tend to be droughty, highly erodible, content of very low fertility, and moderately slow permeability [5]. Therefore, establishment of spontaneous natural vegetation is difficult in open mine spoil-bank [2]. This adverse condition of coal mine spoil-bank needs to be handled immediately.

Restoration of coal mine spoil-bank becomes a serious concern as the new coal mine spoil-bank is still produced by continue operation of opencast coal mining in Kalimantan Indonesia. Though restoration by layering of fertile soil, organic substrate, or fertilizer over original barren spoil-bank is well-proven to establish plant and yield production [6], however, the use of soil amendments for restoration is very costly [7]. Furthermore, high nutrition in this soil amendment lead to the invasive grow of dominant plant that possible create a monoculture plant cover [6]. In many cases, high failure of restoration practice is increased due to high mortality of seedling which may be due to unsuitable soil acidity, nutrient deficiency such as N, P, and K, high salt content, soil compaction, and low soil microbial activity [8].

Clearly, restoration of degraded area in opencast coal mining using cheap and environmentally friendly method is very important. Few studies have investigated the use of native AM fungi in symbiosis with plant to restore opencast coal mining in Indonesia. Restoration of degraded coal mine spoil-bank can be facilitated by selection of tolerance plant as well as soil microorganism in improving the vegetation cover which can be fulfill the function of early stabilization in ecosystem [9]. Plant-microbe symbiosis in the rhizosphere, such as nitrogen fixation [10], rhizobacterium *Pseudomonas putida* –a heavy metal binder [9], and mycorrhizal symbiosis [11], has long been studied for its potential in facilitating the plant establishment in degraded area. Among symbiotic soil

Abstract—The extensive open cast coal mining under forest area brings consequences in the large environment degradation, induces soil degradation, forms a hug quantity of mined spoil, and may contributes on climate change in Kalimantan, Indonesia. Establishment of spontaneous natural vegetation is difficult in coal mine spoil-bank due to its diversity in soil characteristics. Reforestation by application of fertilizer is commonly used. However, the high cost fertilizer and its long term negative effect on environment may not be effective in economic perspective. Native tropical tree and fast growing species, *Albizia saman*, is potential to promote the first rotation of reforestation. Selected native arbuscular mycorrhizal (AM) fungi play an important role on plant growth in soil condition. This study aimed to clarify the effect of native and nonnative AM fungi in maintaining growth of *A. saman* in spoil-bank of open cast coal mining. Seedlings were inoculated with or without native AM fungi, *Acaulospora* sp., *Glomus* sp.1, and *Glomus* sp.2, and nonnative AM fungi, *Gigaspora decipiens*, in sterilized spoil-bank and grown in greenhouse for three months. Seedling height, diameter, leaf number, shoot dry weight, root fresh weight, shoot N, P, K, and Ca concentrations were measured two months after sowing. *Acaulospora* sp. colonized plant 83%, *Glomus* sp.1 90%, *Glomus* sp.2 93%, and *G. decipiens* 38%. Though all colonization of AM fungi increased shoot dry weight, colonization by native AM fungi tended to have higher shoot dry weight than nonnative AM fungi. All native AM fungi increased root fresh weight, shoot P concentration, shoot P, K, and Mg content. All colonization of AM fungi increased shoot N uptake. This result indicates that application of native AM fungi is prospective for tropical reforestation in open cast coal mining area by improving nutrient uptake and accelerating plant growth.

microorganism, arbuscular mycorrhizal fungi provide more plant nutrition and growth [12], increase drought tolerance of plant [13] and consequently increase survival rate of plant [14] in harsh adverse condition.

The absence of AM fungi population leads to the problem of establishment and survival of plant population [15]. Although AM fungi are ubiquitous in terrestrial ecosystem, soil disturbance such as opencast mining has negative effect on soil microorganism both fungi and bacteria [16] including AM fungi [17, 18], however native AM fungi adapted to soil condition have better mutualism symbiosis than nonnative AM fungi in enhancing plant growth [19,20]. Isolation and selection of indigenous AM fungi that is assumed stress adapted is potential for restoration in degraded ecosystem [21]. However, the native AM fungi isolated from degraded habitats are not always more effective than those nonnative AM fungi [22]. This information leads us to evaluate the efficacy between native and nonnative AM fungi in forming symbiosis to accelerate plant growth under adverse condition of coal mine spoil-bank.

In this study, we focused on the effect of different AM fungi in improving growth of *Albizia saman* grown in coal mine spoil-bank. We compared response of plant growth inoculated with native and nonnative AM fungi using coal mine spoil-bank as the substrate. We used three native AM fungi, *Acaulospora* sp., *Glomus* sp.1., *Glomus* sp. 2., which was isolated from opencast coal mining in PT Berau Coal, Tanjung Redeb Berau Regency, East Kalimantan Indonesia. The nonnative AM fungus that we used was *Gigaspora decipiens*, which was isolated from peat swamp Central Kalimantan, Indonesia. This *G. decipiens* was used due to their efficacy in promoting nutrient uptake and growth of tropical peat-swamp plants [23]. *A. saman* was selected as the host plant as this plant adaptive in wide range of soil type and soil pH [24], adaptive in dry site [25], and has a consistently high survival rate [26], making it potential for restoration in degraded area. We investigated the native and nonnative AM fungi in their ability to form symbiosis relating to nutrient uptake and plant growth, in particular we address the following question: (1) Does inoculation of both native and nonnative AM fungi in adverse condition of coal mine spoil-bank will give different capability in forming symbiosis to plant? (2) Which AM fungi give better symbiosis in improving nutrient uptake and accelerating plant growth?

II. MATERIALS AND METHODS

A. Coal mine spoil collection

Coal mine spoil-bank were collected on 5 – 9 March 2009 from post coal mining in Binungan (02°02'N, 117°27'E), Tanjung Redeb Berau Regency, East Kalimantan, Indonesia. There was no vegetation grow in this post coal mining site. Soils were air dried and passed through < 2 mm fraction and steam-sterilized at 80°C for 45 minute. Superphosphate, ammonium sulfate, and potassium sulfate were applied at the rate 1.3 g P kg⁻¹, 1 g N kg⁻¹, and 1 g K kg⁻¹, respectively. The original soil chemical characteristics before fertilizer addition were pH H₂O 4.5 and pH KCl 3.96; total carbon concentration was 6.9 mg g⁻¹; the total nitrogen concentration was 0.7 mg g⁻¹ (Sumigraph N-C 220 F); the C:N ratio was 9.86; the available P

concentration was 1.3 mg P₂O₅.kg⁻¹ [27]; the potassium concentration was 0.45 (cmol_c.kg⁻¹); the sodium concentration was 0.06 (cmol_c.kg⁻¹); the calcium concentration was 0.31 (cmol_c.kg⁻¹); the magnesium concentration was 0.51 (cmol_c.kg⁻¹); the cation exchange capacity was 12.17 (cmol_c.kg⁻¹); the base saturation was 10.9%; the exchangeable cadmium concentration was 0.69 mg kg⁻¹; the exchangeable copper concentration was 2.4 mg kg⁻¹; the exchangeable nickel concentration was 9.10 mg kg⁻¹; and the exchangeable zinc concentration was 0.60 mg kg⁻¹.

B. Inoculum preparation and inoculation of AM fungi

AM fungi were collected from post coal mining soil in PT Berau Coal, Tanjung Redeb Berau Regency East Kalimantan, Indonesia. AM fungi were collected by trap culture method. Selection of AM fungi is based on the screening of trapped AM fungi for their ability in nutrient uptake and plant growth. Screened AM fungi then propagated in sterilized sand by growing *Sorghum bicolor* (CV) New sorgo No.2 for three months in greenhouse conditions at Yamagata University, Tsuruoka, Japan (38°44'N, 139°50'E). Propagated AM fungi were identified by morphology and molecular method. There are three AM fungi are identified and used: *Acaulospora* sp, *Glomus* sp.1, and *Glomus* sp.2. *Gigaspora decipiens* Hall & Abbott was also propagated by *S. bicolor* in sterilized sand in greenhouse condition. *G. decipiens* were isolated from peat soil in Kalamangan (2° 13'S, 113° 56'E), Palangkaraya, Central Kalimantan, Indonesia [23]. This *G. decipiens* was used because its ability in promoting plant growth has been proven [23]. The roots, sand, and spore were used as inoculums AM fungi. Inoculation of AM fungi was prepared by mixing 20 gram of inoculums with 500 gram sterilized post coal mining soil into 500 ml pot. Non-inoculated soil was also prepared as the control.

C. Seed germination and seedling preparation

Seeds of *A. saman* (Jacq.) Merr. were immersed in water at 80°C for 1-2 minutes [24] and sown in sterilized sand in about 1 cm depth. About 14 days old germinated seedlings were transferred into 500 ml pot contain 500 gram coal mine spoil-bank with or without inoculation of AM fungi. Seedlings in pots were placed randomly and watered every two days by 100 ml tab water. One seedling per pot was allowed to grow for ten weeks in 3 August – 12 October 2012 in greenhouse conditions at Yamagata University, Tsuruoka, Japan (38°44'N, 139°50'E).

D. Growth parameter and plant analysis

Five treatments were applied to *A. saman*: (1) control, (2) inoculated with *Acaulospora* sp., (3) inoculated with *Glomus* sp.1., (4) inoculated with *Glomus* sp.2., (5) inoculated with *G. decipiens*. Each treatment has five replications. Nine week old *A. saman* seedlings were harvested and washed by tap water to remove soil particles. Root and shoot then separated and continue weighing for root fresh weight. Shoot were then oven-dried at 70°C for 72 hours, weighed, ground, and analyze for P, K, Ca, and Mg concentration. Analysis of P concentration was performed by determining calorimetrically with vanadomolybdate-yellow assay [28] using a spectrophotometer at 880 nm absorbance (Hitachi, U-2900) after digested ground

shoot in a $\text{HNO}_3\text{-HClO}_4\text{-H}_2\text{SO}_4$ solution. Concentration of K, Ca, and Mg in the digested solution was measured by atomic absorption spectrophotometer. Shoot N concentration was determined by a C:N analyzer (Sumigraph NC-220F, Tokyo).

Mycorrhizal dependency of nutrient uptake and growth of plants were calculated based on [29]:

Dependency of nutrient uptake:

$$= \frac{X1 - X2}{X1} \times 100$$

where X1 is the amount of nutrient content absorbed by mycorrhizal plant in milligram per plant; X2 is the amount of nutrient content absorbed by non-mycorrhizal plant in milligram per plant.

Dependency of growth

$$= \frac{Y1 - Y2}{Y1} \times 100$$

where Y1 is the biomass of mycorrhizal plant in milligram per plant; Y2 is the biomass of non-mycorrhizal plant in milligram per plant.

E. Assessment of AM colonization

The harvested roots were cleared by 10% potassium hydroxide (KOH), acidified with 1% HCl, and stained with 500 mg l⁻¹ aniline blue [30]. The percentage of root length colonized by AM fungi was calculated by the gridline intersect method [31] from the 100-point gridline intersection of a root under a compound microscope at 40 – 200 X magnification. Positive counts for AM fungi colonization included the presence of arbuscules, vesicle, and internal hyphae within the root.

F. Statistical analysis

Statistical significance of inoculated and non-inoculated treatments data were analyzed using KaleidaGraph 4.1 software (Synergy Software 2012, USA) for analysis of variance (ANOVA) test. Post-hoc analysis was performed using Tukey HSD test ($P < 0.05$).

III. RESULTS

A. AM Fungi Colonization

AM fungi colonization was observed in all *A. saman* seedlings inoculated with *Glomus* sp.1., *Glomus* sp.2., *Acaulospora* sp.1., and *G. decipiens* in nine weeks after transplanting (Table 1). AM fungi colonization was not observed in control. AM fungi colonization of *A. saman* was significantly higher when inoculated with *Glomus* sp.1, *Glomus* sp.2, and *Acaulospora* sp.1 than *G. decipiens*. There was no significant difference in the percentage of AM fungi colonization between *Glomus* sp.1, *Glomus* sp.2, and *Acaulospora* sp.1.

TABLE 1 Growth response of 70 days old *Albizia saman* seedlings to the inoculation of AM fungi grown in post coal mined spoil-bank under greenhouse conditions

Treatment	AM Colonization (%)	Height cm	Leaf number no./plant	Shoot dry weight g/plant	Root fresh weight g/plant
Control	0 c*	23.3 a	75.8 a	0.9 b	1.9 b
<i>Acaulospora</i> sp	83 a	27.6 a	99.6 a	1.7 a	3.4 a
<i>Glomus</i> sp.1	90 a	25.9 a	93.8 a	1.6 a	2.4 a
<i>Glomus</i> sp.2	93 a	27.4 a	87.6 a	1.7 a	3.0 a
<i>G. decipiens</i>	38 b	22.7 a	75.4 a	1.4 a	2.0 b

*Different letters within column indicate a statistically significant difference determined ($P < 0.05$) by the Tukey HSD test ($n=5$)

B. Shoot N, P, K, Ca, and Mg concentrations and content

There was no significant difference for shoot N concentration between inoculated and non-inoculated seedlings (Table 2). On the other hand, all seedling colonized by AM fungi resulted on higher shoot N content than control (Table 2). Among AM fungi, *Acaulospora* sp. and *Glomus* sp.2 tended to have higher shoot N content than *Glomus* sp.1 and *G. decipiens*.

Among all treatment, shoot P concentration was highest in seedling colonized by *Glomus* sp.1. (Table 2). Seedling colonized by *Glomus* sp.1, *Glomus* sp.2, and *Acaulospora* sp. had higher shoot P concentration than seedling colonized by *G. decipiens* and control seedling. Seedling colonized by *Glomus* sp.1. had higher shoot P concentration than seedling colonized by *Acaulospora* sp.1. There was no significant difference of shoot P concentration between seedlings colonized by *Glomus* sp.1 and *Glomus* sp.2. No significant difference of shoot P concentration was observed between seedlings colonized by *Glomus* sp.2. and *Acaulospora* sp.1. Among AM fungi, shoot P concentration was lowest in seedling inoculated with *G. decipiens*. There was no significant difference in shoot P concentration between seedling colonized by *G. decipiens* and control seedling. On the other hand, seedling colonized by *Glomus* sp.1 and *Glomus* sp.2 showed highest shoot P content among all treatment (Table 2). Seedling colonized by *Glomus* sp.1, *Glomus* sp.2, and *Acaulospora* sp. had higher shoot P content than control. Seedling colonized by *Glomus* sp.1 and *Glomus* sp.2 had higher shoot P content than *G. decipiens*. There was no significant difference of shoot P content between *Glomus* sp.1, *Glomus* sp.2, and *Acaulospora* sp.1. Seedling colonized by *Acaulospora* sp.1 tended to have higher shoot P content than seedling colonized by *G. decipiens*. No significant difference of shoot P content was observed between *G. decipiens* and control. Generally seedling colonized by native AM fungi had higher shoot P concentration and content in comparison to seedling colonized by nonnative *G. decipiens* and control seedling.

TABLE 2 Shoot nutrient concentrations and contents of 70 days old *Albizia saman* seedlings to the inoculation of AM fungi in post coal mined spoil-bank under greenhouse conditions

Treatment	Shoot nutrient concentration					Shoot nutrient content				
	N	P	K	Ca	Mg	N	P	K	Ca	Mg
	mg/g					mg/plant				
Control	32.60 a*	0.80 c	8.14 a	1.70 a	2.00 a	28.19 b	0.69 c	7.01 b	1.46 b	1.73 b
<i>Acaulospora</i> sp	31.28 a	1.48 b	8.00 a	1.73 a	1.84 a	52.62 a	2.47 ab	13.44 a	2.91 a	3.11 a
<i>Glomus</i> sp.1	29.41 a	2.23 a	7.95 a	1.38 a	1.93 a	45.63 a	3.59 a	12.58 a	2.28 ab	3.01 a
<i>Glomus</i> sp.2	31.42 a	1.95 ab	8.02 a	1.60 a	1.83 a	52.60 a	3.23 a	13.47 a	2.70 ab	3.09 a
<i>G. decipiens</i>	31.85 a	0.87 c	7.46 a	1.69 a	1.76 a	45.77 a	1.27 bc	10.75 ab	2.45 ab	2.54 ab

*Different letters within column indicate a statistically significant difference determined ($P < 0.05$) by the Tukey HSD test ($n=5$)

There was no significant difference for shoot K concentrations between inoculated and non-inoculated seedlings (Table 2). On the other hand, seedling colonized by *Glomus* sp1, *Glomus* sp2, and *Acaulospora* sp.1 showed higher shoot K content than control (Table 2). Plant colonized by *Glomus* sp1, *Glomus* sp2, and *Acaulospora* sp.1 tended to have higher shoot K content than seedlings colonized by *G. decipiens*. No significant difference of shoot K was also observed between seedling colonized by *G. decipiens* and control seedling.

There was no significant difference for shoot Ca concentrations between inoculated and non-inoculated seedlings (Table 2). While shoot Ca content showed that seedling colonized by *Acaulospora* sp.1 showed higher shoot Ca content than control (Table 2). Seedling colonized by *Glomus* sp1, *Glomus* sp2, *G. decipiens* tended to have higher shoot Ca content than control seedling.

No significant different in shoot Mg concentration was observed among all treatment (Table 2). On the other hand, seedling colonized by *Glomus* sp1, *Glomus* sp2, and *Acaulospora* sp.1 had higher shoot Mg content than control seedling (Table 2). Seedling inoculated with *Glomus* sp1, *Glomus* sp2, and *Acaulospora* sp.1 tended to have higher shoot Mg content than seedling inoculated with *G. decipiens*. There was no significant difference in shoot Mg content between seedlings colonized by *G. decipiens* and control seedling.

C. Plant Growth

All colonization of AM fungi in *A. saman* seedling resulted on higher shoot dry weight than control (Table 1). Among AM fungi, seedling colonized by *Glomus* sp.1, *Glomus* sp.2, and *Acaulospora* sp. tended to have higher shoot dry weight than seedling colonized by *G. decipiens*. Seedling colonized by *Glomus* sp.1, *Glomus* sp.2, and *Acaulospora* sp. had higher root fresh weight than seedling colonized by *G. decipiens* and control seedling (Table 1). There was no significant difference in root fresh weight between *Glomus* sp.1, *Glomus* sp.2, and *Acaulospora* sp. No significant difference of root fresh weight was also observed between *G. decipiens* and control. Seedling colonized by *Glomus* sp.1, *Glomus* sp.2, and *Acaulospora* sp.

tended to have higher leaf number and height than seedling colonized by *G. decipiens* and control seedling (Table 1).

IV. DISCUSSION

The application of organic soil amendment and fertilizer to restore post coal mine spoil-bank is costly and also has generated in several environmental problem. This problem can be overcome by using biofertilizer such application of AM fungi which are natural, beneficial, and ecofriendly. A greenhouse study investigation was conducted to study the effectiveness of different AM fungi in improving growth of *A. saman* grown in post coal mine spoil-bank.

All colonization of AM fungi has different effect in increasing nutrient content of *A. saman*. All colonization of AM fungi increased shoot N content of *A. saman* (Table 1). Colonization of *Glomus* sp.1, *Glomus* sp.2, and *Acaulospora* sp. increased shoot P, K, and Mg content. Highest increased of shoot Ca content was in plant colonized by *Acaulospora* sp., and followed by *Glomus* sp.1, *G. decipiens*, and *Glomus* sp.2.

In agreement to our result, increased uptake of basic cations such as K^+ , Mg^{2+} or Ca^{2+} of mycorrhizal compared with nonmycorrhizal plants had been observed on acidic soils naturally poor in these elements [32]. Higher increased of nutrient content in *A. saman* colonized by *Glomus* sp.1, *Glomus* sp.2, and *Acaulospora* sp. in general can be explained by the higher total AM colonization >83% and arbuscule >81% of these native AM fungi in comparison to colonization by nonnative AM fungi, *G. decipiens*, which only reached 37% (data not shown). A significant positive correlation was observed between AM fungi colonization and shoot N, P, K, Ca, and Mg content (Table 4). Arbuscules are important structures for translocation and transfer nutrient [33]. Despite this AM colonization formation, the higher nutrient content by native AM fungi colonization could be due to partial attribution of these fungi to an adaptation of edaphic stress. Native AM fungi were reported had higher tolerance to adverse contaminated soil [34] and more efficient in promoting plant growth than nonnative AM fungi [35]. Furthermore, the higher root fresh weight in these native AM fungi in this study (Table 1) might contribute to the higher

TABLE 3 Mycorrhizal dependency of shoot dry weight and N, P, K, Ca, Mg uptake in seedlings of 70 days old *Albizia saman* seedlings to the inoculation of AM fungi grown in post coal mined spoil-bank under greenhouse conditions

Treatment	Mycorrhizal Dependency (%)					
	Shoot Dry Weight	N	P	K	Ca	Mg
<i>Acaulospora</i> sp	48 a*	46 a	71 a	41 a	47 a	44 ab
<i>Glomus</i> sp.1	47 a	42 a	74 a	39 a	44 a	55 ab
<i>Glomus</i> sp.2	48 a	45 a	78 a	48 a	43 a	53 a
<i>G. decipiens</i>	40 a	38ab	41 b	30 ab	45 ab	33 b

*Different letters within column indicate a statistically significant difference determined ($P < 0.05$) by the Tukey HSD test ($n=5$)

nutrient uptake. A positive correlation was observed between AM fungi colonization, root fresh weight and shoot nutrient uptake (Table 4). Higher AM fungi colonization with higher root growth rate may satisfy higher nutrient requirement. In addition to root growth, different spread or length of extra radical mycelium by AM fungi that link colonized roots with the soil might also explain this different nutrient content. A relationship between soil nutrient content and hyphal spread was previously reported [36]. *Acaulospora laevis* spread hyphae faster than *Glomus* sp. and *Scutellospora calospora* and therefore transported ^{32}P over longer soil-root distances than those two others AM fungi [37]. In the other hand, *Gigaspora calospora* produced longer external hyphae than *Glomus fasciculatum* [38]. However, in this research we did not measure the extra radical mycelium of AM fungi. Nevertheless, our research showed high efficiency of native AM fungi to form high colonization to plant for nutrient uptake in adverse condition of coal mine spoil-bank. Future work should include the measurement of the extraradical mycelia which can vary greatly among AM fungi species.

Interestingly, the native inoculum treatment, *Glomus* sp.1, *Glomus* sp.2, and *Acaulospora* sp., resulted in higher shoot P concentration of *A. saman* seedling (Table 2), indicating the crucial importance of native AM fungi for nutrient acquisition in adverse unfertile coal mine spoil-bank. Furthermore, it is reported that native AM fungi accumulated high shoot P concentration in *Prosopis juliflora* grown in acidic lead/zinc mine tailing [39]. In contrast to our result, inoculation of a mixture *Glomus mosseae* (BEG95), *G. claroideum* (BEG96), and *G. intraradices* (BEG140), native to spoil bank and sedimentation pond, did not affect shoot P uptake of *Linum usitatissimum* grown in coal mine spoil-bank [40]. No effect of AM fungi colonization on shoot N, K, Ca, and Mg concentration in this study may be explain by dilution effect of higher aboveground part as a possible mechanism of

AM fungi for higher nutrient use efficiency. No different shoot N, Ca, Mg [35] and K concentration [41] was also previously observed.

Inoculation of AM fungi increased growth of *A. saman* (Table 1) and this correlation between AM fungi colonization and leaf number, height, and shoot dry weight was observed (Table 4). Among AM fungi, inoculation by native AM fungi tended to have higher height, leaf number, and shoot dry weight. In agreement to our result, mixture of *Glomus mosseae*, *G. claroideum*, and *G. intraradices* both local and introduced AM fungi increased shoot dry weight of *Galega orientalis* grown in spoil-bank substrate which local AM fungi had highest shoot dry weight among treatment [1]. *G. intraradices*, mixture *G. intraradices* and *G. deserticola*, and native *G. mosseae* increased dry biomass of *Prosopis juliflora* grown in acidic lead/ zinc mine tailings, though *G. mosseae* tends to have the lowest biomass production among AM fungi [39]. The higher root biomass by native AM fungi colonization (Table 1) increased the nutrient uptake which resulted on the better plant growth, as this relationship between root fresh weight, shoot nutrient content, and plant growth was observed (Table 4). This result suggests that the native isolates from post coal mining area represent useful inoculums for higher biomass production, since they lead to the greater extraction of soil nutrient such as P, K, Ca, and Mg from coal mine spoil-bank soil

Variation of mycorrhizal dependency in shoot dry weight and nutrient uptake of *A. saman* was observed among AM fungi species (Table 3). All seedling colonized by native AM fungi tended to have higher dependency of shoot dry weight than seedling colonized by nonnative AM fungi. Different mycorrhizal dependency of same plant species by colonization of different AM fungi species was reported in wheat (cv. Kulin) [42]. These differences in mycorrhizal dependency of shoot dry weight might be explained by the differences in external hyphae development among AM fungi that influenced on nutrient uptake [43]. Interestingly, mycorrhizal dependency of P uptake was higher and reached more than 70% in seedling colonized by native AM fungi than nonnative AM fungi (Table 3). The mycorrhizal dependency of N, K, Ca, and Mg uptake was also observed tended to be higher in seedling colonized by native AM fungi than nonnative AM fungi. These results indicate that shoot nutrient uptake ability, particularly P is lower in seedling colonized by nonnative AM fungi. Therefore, the degree of shoot nutrient uptake particularly P, and shoot dry weight is higher in these native AM fungi. These results suggest that under low fertility and poor capacity of coal mine spoil-bank, inoculation of AM fungi, particularly native AM fungi, have high efficiency in scarce resources to biomass.

The result of our study in an overall confirmed a highly positive effect of AM fungi on plant growth, nutrient uptake and on the efficiency with which the absorbed nutrients were used in producing plant biomass of *A. saman* grown in post coal mine spoil-bank substrate. Under the low fertility of coal mine spoil-bank and the poor capacity of mining sites to retain nutrients, colonization of native AM fungi in particular, may give additional advantage in a high efficiency in converting

TABLE 4 Correlation between AM colonization (%AM), shoot dry weight (SDW), root fresh weight (RFW), shoot N, P, K, Ca, and Mg content in *Albizia saman* seedlings

	AM	Root fresh weight	Shoot dry weight	Leaf. no.	Height
	(%)	(g. plant ⁻¹)	(g. plant ⁻¹)	(no.plant ⁻¹)	(cm)
Growth parameter:					
Height (cm)	+0.458*	+0.684***	+0.616***	-	-
Shoot dry weight (g. plant ⁻¹)	+0.702***	+0.742***	-	-	-
Root fresh weight (g. plant ⁻¹)	+0.479**	-	-	-	-
Shoot nutrient content (mg. plant⁻¹):					
Nitrogen (N)	+0.607***	+0.708***	+0.914***	+0.606***	+0.441*
Phosphorus (P)	+0.821***	+0.645***	+0.807***	+0.544**	+0.621***
Potassium (K)	+0.688***	+0.807***	+0.959***	+0.464*	+0.733***
Calcium (Ca)	+0.498**	+0.631***	+0.869***	+0.362ns	+0.527**
Magnesium (Mg)	+0.697***	+0.741***	+0.933***	+0.453*	+0.561**

* 5 % level of significance; ** 1 % level of significance; *** 0.1 % level of significance; ns: non-significant.

scarce resources to biomass. This, together with the improvement of nutrient acquisition may support the general success of mycorrhiza in future restoration program.

V. CONCLUSION

The result of our greenhouse experiment demonstrated that native AM fungi formed better symbiosis to plant by forming higher colonization in comparison to nonnative AM fungi. Generally, native AM fungi improved better nutrient uptake and promoted better growth of *A. saman* seedling than nonnative AM fungi. These results indicate that native AM fungi from post coal mining are more effective to accelerate plant growth by enhancing nutrient uptake. These results suggest the importance of inoculation of native AM fungi in coal mine spoil-bank, and therefore can be one of promising cheap friendly environmental method of bio-fertilizer agent for restoration in post coal mining field in the future. This inoculation of native AM fungi is important especially in sites with low AM fungi inoculums potential such as in post coal mining field. Owing to the great variability condition in field, further research of AM fungi application in field condition is necessary to evaluate this AM fungi to compete with other soil microorganism in forming symbiosis and improving plant growth.

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REFERENCES

- [1] D. Puschel, J. Rydlova, R. Sudova, M. Gryndler, M. Vosatka, "The potential of mycorrhizal inoculation and organic amendment to increase yield of *Galega orientalis* and *Helianthus tuberosus* in a spoil-bank substrate," J. Plant Nutr. Soil Sci., vol. 174, pp. 664 – 672, 2011.
- [2] T.J. Bell and U.A. Ungar, "Factors affecting the establishment of natural vegetation on a coal strip mine spoil bank in Southeastern Ohio," Am. Midl. Nat., vol 105 (1), pp. 19-31, 1981.
- [3] A.N. Singh and J.S. Singh, "Experiments on ecological restoration of coal mine spoil using native trees in a dry tropical environment, India: a synthesis," New Forest, vol 31, pp. 25 – 39, 2006.
- [4] N. Pedrol, C.G. Puig, P. Souza, R. Forjan et.al., "Soil fertility and spontaneous revegetation in lignite spoil banks under different aendments," Soil Till. Res., vol. 110, pp. 134 – 142, 2010.
- [5] P.R. Salon and C.F. Miller, "A guide to conservation planting on critical areas for the northeast," United States Department of Agriculture. 1991. <http://www.nrcs.usda.gov>
- [6] J. Rydlova, D. Puschel, M. Vosatka, K. Chartavova, "Different effect of mycorrhizal inoculation in direct and indirect reclamations of spoil banks," J. Appl. Bot. Food Qual., vol. 82, pp. 15-20, 2008.
- [7] United States Environmental Protection Agency, "The use of soil amendments for remediation, revitalization, and reuse," 2007. <http://www.epa.gov>.
- [8] A. Selvam and A. Mahadevan, "Distribution of mycorrhizas in an abandoned fly ash pond and mined sites of Neyveli lignite corporation, Tamil Nadu, India," Basic Appl. Ecol., vol 3, pp. 277-284, 2002.
- [9] C.H. Wu, T.K. Wood, A. Mulchandani, W. Chen, "Engineering plant-microbe symbiosis for rhizoremediation of heavy metals," Appl. Environ. Microb., vol 27 (2), pp. 1129-1134, 2006.
- [10] L.R. Walker, B.D. Clarkson, W.B. Silvester, B.R. Clarkson, "Colonization dynamics and facilitative impacts of a nitrogen-fixing shrub in primary succession," J. Veg. Sci., vol 14, pp. 277-290, 2003.
- [11] G. Bois, Y. Piche, M.Y.P. Fung, D.P. Khasa, "Mycorrhizal inoculum potentials of pure reclamation materials and revegetated tailing sands from the Canadian oil sand industry," Mycorrhiza, vol 15, pp. 149-158, 2005.
- [12] A. Medina and R. Azcon, "Effectiveness of the application of arbuscular mycorrhizal fungi and organic amendment to improve soil quality and plant performance under stress conditions," J. Soil Sci. Plant Nutr., vol. 10 (3), pp. 354-372, 2010.

- [13] S. Yooyongwech, N. Phaukinsang, S. Cha-um, K. Supaibulwatana, "Arbuscular mycorrhiza improved growth performance in *Macadamia tetraphylla* L. grown under water deficit stress involves soluble sugar and proline accumulation," *Plant Growth Regul.*, vol. 69, pp. 285-293, 2013.
- [14] A. Karthikeyan and N. Krishnakumar, "Reforestation of bauxite mine spoils with *Eucalyptus tereticornis* Am. seedlings inoculated with arbuscular mycorrhizal fungi," *Ann Forest Research*, vol. 55 (2), pp. 207-216, 2012.
- [15] R.T. Koide and I.A. Dickie, "Effect of mycorrhizal fungi on plant populations," *Plant Soil*, vol. 244, pp. 307-317, 2002.
- [16] D.L. Mummey, P.D. Stahl, J.S. Buyer, "Microbial biomarkers as indicator of ecosystem recovery following surface mine reclamation," *Appl. Soil Ecol.*, vol. 21, pp. 251-259, 2002.
- [17] M.A. Loree and S.E. William, "Colonization of western wheatgrass (*Agropyron smithii* Rybd.) by vesicular arbuscular mycorrhizal fungi during the revegetation of a surface mine," *New Phytol.*, vol. 106, pp. 735-744, 1987.
- [18] P.D. Stahl, S.E. William, M. Christensen, "Efficacy of native vesicular-arbuscular mycorrhizal fungi after severe soil disturbance," *New Phytol.*, vol. 110, pp. 347-354, 1988.
- [19] F. Caravaca, J.M. Barea, J. Palenzuela, G. Figuerola, M.M. Alguacil, A. Roldan, "Establishment of shrub species in degraded semiarid site after inoculation with native or allochthonous arbuscular mycorrhizal fungi," *Appl. Soil Ecol.*, vol. 22, pp. 103-111, 2003.
- [20] R.S. Oliveira, M. Vosatka, J.C. Dod, P.M.L. Castro, "Studies on the diversity of arbuscular mycorrhizal fungi and the efficacy of two native isolates in a highly alkaline anthropogenic sediment," *Mycorrhiza*, vol. 16, pp. 23-31, 2005.
- [21] J.C. Dodd and B.D. Thomson, "The screening and selection of inoculant arbuscular-mycorrhizal and ectomycorrhizal fungi," *Plant Soil*, vol. 159, pp. 149-158, 1993.
- [22] B. Enkhtuya, J. Rydlova, M. Vosatka, "Effectiveness of indigenous and non-indigenous isolates of arbuscular mycorrhizal fungi in soils from degraded ecosystem and man-made habitats," *Appl. Soil Ecol.*, vol. 14, pp. 201-211, 2000.
- [23] M. Turjaman, Y. Tamai, E. Santoso, M. Osaki, K. Tawaraya, "Arbuscular mycorrhizal fungi increased early growth of two nontimber forest product species *Dyera polyphylla* and *Aquilaria filaria* under greenhouse conditions," *Mycorrhiza*, vol. 16, pp. 459-464, 2006.
- [24] G.W. Staples and C.R. Elevitch, *Samanea saman* (rain tree). "Species Profile for Pacific Island Agroforestry" 2006. www.traditionaltree.org.
- [25] M.H. Wishnie, D.H. Dent, E. Mariscal, J. Deago, N. Cedeno, D. Ibarra, R. Condit, P.M.S. Ashton, "Initial performance and reforestation potential of 24 tropical tree species planted across a precipitation gradient in the Republic of Panama," *Forest Ecol Manag.*, vol. 243, pp. 39-49, 2007.
- [26] J.S. Hall, B.E. Love, E.J. Garen, J.L. Slusser, K. Saltonstall, S. Mathias, Michiel van Breugel, D. Ibarra, E.W. Bork, D. Spaner, M.H. Wishnie, M.S. Ashton, "Tree plantations on farm: Evaluating growth and potential for success", *Forest Ecol Manag.*, vol. 261, pp. 1675-1683, 2011.
- [27] E. Truog, "The determination of the readily available phosphorus of soils," *American Society of Agronomy*, vol. 22, pp. 874-882, 1930.
- [28] S.R. Olsen and L.E. Sommers, "Phosphorus. In: Page AL (ed) *Methods of soil analysis*". Part 2. Chemical and Microbiological Properties. Soil Science Society of America, Madison, 1982, pp 403-430
- [29] C. Plenchette, J.A. Fortin, V. Furlan, "Growth response of several plant species to mycorrhizae in a soil of moderate P fertility. I. Mycorrhizal dependency under field conditions," *Plant Soil*, vol. 70, pp. 199 - 209, 1983.
- [30] K. Tawaraya, K. Hashimoto, T. Wagatsuma, "Effect of root exudate fractions from P-deficient and P-sufficient onion plants on root colonization by the arbuscular mycorrhizal fungus *Gigaspora margarita*," *Mycorrhiza*, vol. 8, pp. 67-70, 1998.
- [31] M. Giovannetti and B. Mosse, "An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots," *New Phytol.*, vol. 84, pp. 489-500, 1980.
- [32] R.B. Clark, R.W. Zobel, S.K. Zeto, "Effects of mycorrhizal fungus isolate on mineral acquisition by *Panicum virgatum* in acidic soil," *Mycorrhiza*, vol. 9, pp. 167 - 176, 1999.
- [33] G. Cox and P.B. Tinker, "Translocation and transfer of nutrients in vesicular arbuscular mycorrhizas. I. The arbuscule and phosphorus transfer: A quantitative ultrastructural study," *New Phytol.*, vol. 77 (2), pp. 371 - 378, 1976.
- [34] C. Leyval, B.R. Singh, E.J. Joner, "Occurrence and infectivity of arbuscular mycorrhizal fungi in some Norwegian soils influenced by heavy metals and soil properties," *Water Air Soil Poll.*, vol. 84, pp. 203 - 216, 1995.
- [35] A. Tchabi, D. Coyne, F. Hountondji, L. Lawouin, A. Wiemken, F. Oehl, "Efficacy of indigenous arbuscular mycorrhizal fungi for promoting white yam (*Dioscorea rotundata*) growth in West Africa," *Appl. Soil Ecol.*, vol. 45, pp. 95 - 100, 2010.
- [36] I. Jakobsen, L.K. Abbot, A.D. Robson, "External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 1. Spread of hyphae and phosphorus inflow into roots," *New Phytol.*, vol. 120, pp. 371 - 380, 1992.
- [37] I. Jakobsen, L.K. Abbot, A.D. Robson, "External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 2. Hyphal transport of ³²P over defined distances," *New Phytol.*, vol. 120 (4), pp. 509-516, 1992.
- [38] L.K. Abbot and A.D. Robson, "Formation of external hyphae in soil by four species of vesicular-arbuscular mycorrhizal fungi," *New Phytol.*, vol. 99, pp. 245 - 255, 1985.
- [39] F.A. Soli-Dominguez, A. Valentin-Vargas, J. Chorover, R.M. Maier, "Effect of arbuscular mycorrhizal fungi on plant biomass and the rhizosphere microbial community structure of mesquite grown in acidic lead/zinc mine tailings," *Sci. Total Environ.*, vol. 409, pp. 1009-1016, 2011.
- [40] D. Puschel, J. Rydlova, R. Sudova, M. Gryndler, "Cultivation of flax in spoil-bank clay: Mycorrhizal inoculation vs. high organic amendments," *J. Plant Nutr. Soil Sci.*, vol. 171, pp. 872 - 877, 2008.
- [41] H. Perner, D. Schwarz, C. Bruns, P. Mader, E. George, "Effect of arbuscula mycorrhizal colonization and two level of compost supply on nutrient uptake and flowering of pelargonium plants," *Mycorrhiza*, vol. 17, pp. 469-474, 2007.
- [42] J.H. Graham and L.K. Abbot, "Wheat responses to aggressive and non-aggressive arbuscular mycorrhizal fungi," *Plant Soil*, vol. 220, pp. 207 - 218, 2000.
- [43] F.A. Smith, I. Jakobsen, S.E. Smith, "Spatial differences in acquisition of soil phosphate between two arbuscular mycorrhizal fungi in symbiosis with *Medicago truncatula*," *New Phytol.*, vol. 147, pp. 357-366, 2000.