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Arbuscular mycorrhizal fungi and *Rhizobium* enhance the growth of *Samanea saman* (trembesi) planted on gold-mine tailings in Pongkor, West Java, Indonesia

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Abstract. *Setyaningsih L, Dikdayatama FA, Wulandari AS. 2020. Arbuscular mycorrhizal fungi and Rhizobium enhance the growth of Samanea saman (trembesi) planted on gold-mine tailings in Pongkor, West Java, Indonesia. Biodiversitas 21: 611-616.* Revegetation of severely degraded lands, such as gold-mine tailings, requires comprehensive approach including the selection of appropriate tree species and the improvement of soil fertility with the application of microorganisms. This study aimed to analyze the growth of trembesi (*Samanea saman* (Jacq.) Merr) seedlings inoculated with Arbuscular Mycorrhizal Fungi (AMF) and rhizobial bacteria (RB) on gold-mine tailings in Pongkor, Bogor, West Java, Indonesia. The AMF, RB, and mixture of AMF and RB were inoculated into *S. saman* seedlings and then the seedlings were grown on gold mine tailings land. Seedling growth, AMF infection and RB nodulation were observed after 12 weeks after planting. The results showed that the treatments facilitated AMF colonization by up to 20.7% and RB nodulation up to 22 nodules per plant. The inoculation of *S. saman* seedlings with AMF and RB significantly increased the growth on diameter, biomass, and root length, with values of root-to-shoot ratio of 3.1-4.3. The roots of *S. saman* seedlings were also able to penetrate the depth of the tailings solum. These results showed that AMF and RB application effectively increased the growth of *S. saman* seedlings in the gold tailings field.

Keywords: Arbuscular Mycorrhizal Fungi, seedling growth, *Samanea saman*, *Rhizobium*, tailings

INTRODUCTION

Tailings are waste materials produced by mining activities after the process of separating primary minerals, such as gold and silver, from ore. In mining activities, management of tailings is mostly carried out by submarine disposal (SMD) by placing it in tailings storage facility (TSF) which is a general method aimed to hold tailings to remain on land (ANTAM 2018). Generally, mine tailings are considered to have a negative value due to the lower organic matter content, mineral nutrients, cation exchange capacity (CEC), and microorganism activity (Wasis et al. 2011), while they have higher heavy metals concentration (Setyaningsih et al. 2017). For example, Hidayati et al. (2006) and Hilmi et al. (2018) found that lead (Pb) contamination in gold mine tailings was 6-20 times greater than land without tailings.

The Indonesian Government Regulation No. 78 year 2010 about reclamation and post-mining emphasizes that every company that operates mining activities is required to properly manage tailings to facilitate post-mining reclamation activities. Furthermore, if the mined land is previously a forested area, the post-mining reclamation would involve reforestation activities using groups of trees to recover the vegetation. Reforestation on post-mining land requires the right selection of type of plant to increase effectiveness and success of the reclamation (Lestari et al., 2019). The selected plants are expected to adapt with extreme biophysical soil conditions while at the same time it can help in remediation processes through the improvement of physical and chemical properties, as well as biological properties of the soil.

Trembesi (*Samanea saman* (Jacq.) Merr.) is a species from Fabaceae family which is often recommended to be planted in revegetation activities on severely degraded lands. *S. saman* is known to grow on clay or on waterlogged soils with pH ranges from 4.7 to 8.5. This plant is also often used as a shade for other plants, such as vanilla and cacao, as well as for shade of animals that are grazing. *S. saman* has also been reported to be able to absorb Pb through roots, as well as absorb Pb in the air through leaves (Dahlan 2010).

Utilization fungi (Nouri et al. 2014) as well as bacteria (Akhtar et al. 2011; Altuhaish et al. 2014) is important to enhance plant growth under unfavorable conditions. Arbuscular mycorrhizal fungi (AMF) is a symbiotic mutualist soil-fungi with the ability to provide protection against disease to the host plants, as well as it can alleviate the limitation in plant growth caused by an inadequate nutrient supply, in exchange for photosynthates (Gosling et al. 2006; Nouri et al. 2014; Berruti et al. 2016). Rhizobia are able to form symbioses with legumes, within root nodules, where atmospheric N_2 fixation takes place to be converted into ammonium (NH_4^-), also in exchange for photosynthates (Sessitsch et al. 2002; Udvardi and Poole 2013; Wheatly et al. 2017). Therefore, rhizobia have been considered as an environmentally friendly biological fertilizer.

Preliminary experiment showed that *S. saman* inoculated with AMF was able to grow normally on the tailings media (unpublished report). The inoculation of AMF together with *Rhizobium* onto host plants is expected to have even better growth than if it is solely inoculated with AMF. However, there is no existing study on the performance of *S. saman* plants inoculated with both AMF and *Rhizobium* grown on tailings area. The objectives of this study were: (i) to examine the colonization of AMF and *Rhizobium* to *S. saman* seedling grow on tailing area; (ii) to examine the effects of AMF and *Rhizobium* inoculation to the growth of *S. saman* seedling grown on tailing area.

MATERIALS AND METHODS

Study area and period

The research activities were carried out in several places in Bogor Regency, Indonesia including the greenhouse of Silviculture Department, IPB University, Dramaga, Bogor for seedling preparation and observation of root infection and nodules, while the planting of *S. saman* seedling was carried out in reclamation land of PT ANTAM Tbk UBPE Pongkor, Nanggung District, Bogor, Indonesia. Material preparation for chemical analysis of tailing was carried out at the Chemistry Laboratory of the University of Nusa Bangsa, and then the samples were analyzed at the Indonesian Center for Biodiversity and Biotechnology (ICBB) research center, Bogor, Indonesia. The study was conducted over a period of 13 months, April 2018-May 2019).

Nursery preparation

The germination of *S. saman* seed was conducted on tube sprout filled with zeolite media in 7 days. Seedlings were selected based on uniformity, healthy-looking, normal morphology, straight stems with ± 5 cm height, minimum leaves number of two strands, and free from disease. *S. saman* sprouts were then transferred to polybags filled with latosol soil obtained from the nearby area (Cikabayan, Dramaga, Bogor, West Java, Indonesia). The nursery was carried out for 6 months in Silviculture greenhouse of IPB University, Dramaga, or until plants reached an average height of 70 cm when the plants were ready to be transferred to the tailing field.

Inoculation preparation

AMF, *Glomus manihotis*, hereafter referred to AMF, was propagated using bioassay method using *Sorghum bicolor* as a host and zeolite as a carrier. A total of 10 grams of AMF inoculum was then inoculated twice to the seedling of *S. saman* in each polybag at the nursery, and AMF inoculation was repeated when planting in the tailings field. Rhizobia inoculant, hereafter referred to RB, contained *Rhizobium* sp. was inoculated during the germination of *S. saman* seeds by applying 200 grams of RB per 4 kg of seeds.

Planting of seedling on tailings field

Location

Planting of *S. saman* seedlings was carried out at tailings field of reclamation site in the concession area of a gold mining company located at Cikabayan, Pongkor, Bogor District, West Java Province, Indonesia at geographical position of 6º38'38" S;106º34'36,1" E at an altitude of above sea level. This site had a tailing thickness of 30-40 m.

Planting preparation

The experiment was carried out using Split Plot Design with 4 treatments as the main plot i.e. Control, Mycorrhiza, *Rhizobium* and Mycorrhiza+*Rhizobium* applications. There were 50 plants as replication for every treatment. Seedlings were planted in the plot according to the treatment (control plot, AMF plot, *Rhizobium* plot, and AMF + *Rhizobium* plot).

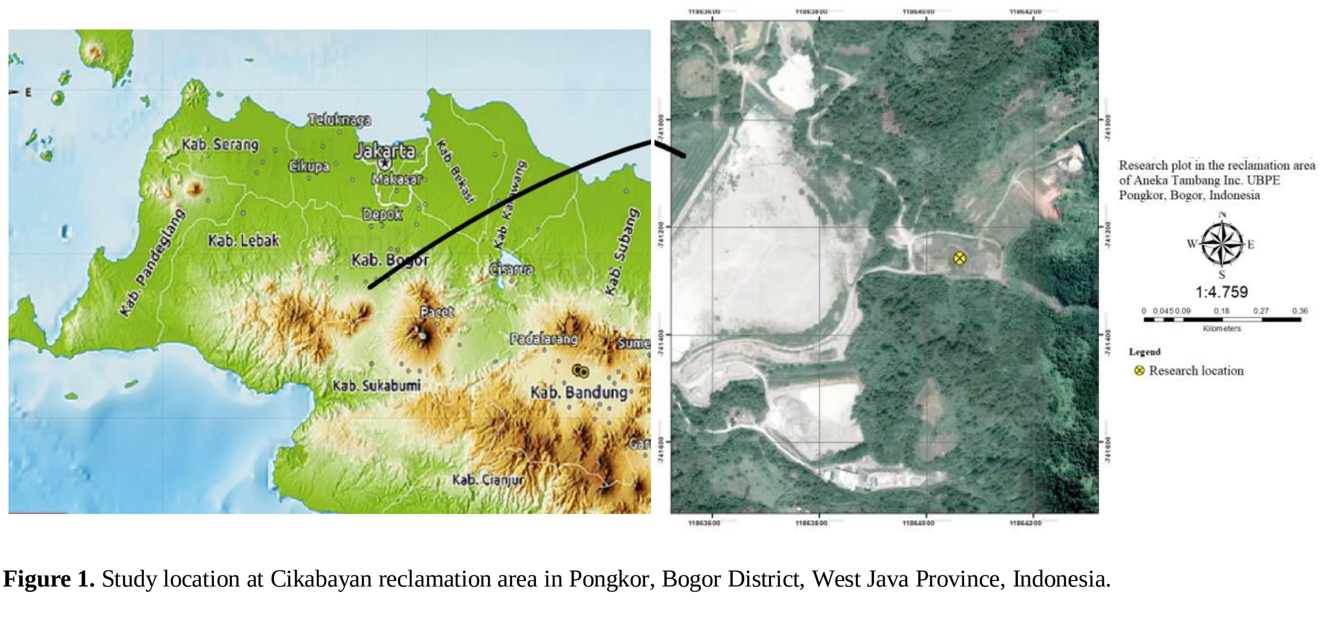

Figure 1. Study location at Cikabayan reclamation area in Pongkor, Bogor District, West Java Province, Indonesia.

Every seedling was planted in a hole of 20 x30 x 30 cm^3, with 2 x 5 m^2 space among the hole. At each planting hole, 6 kg of compost were added as basic organic fertilizer. During the planting, a five-gram of AMF and/or *Rhizobium* (depended on the treatment) was applied close to the root of the seedlings, while for control treatment nothing was added. A soil mounds as high as 3-5 cm was made on each plant to prevent waterlogging. As a marker of planting, a stick was installed to each planting hole to provide treatment information and to monitor planting. Maintenance activities were carried out including watering, pests and disease control, and fertilization during the first week of planting. Observations in the field were carried out for 12 weeks.

Soil analysis

Tailing samples were taken and analyzed to observe physical (texture: sand, dust, and clay) and chemical (pH, CCE, content of C-organic, some macronutrients: N, P, K, Mg, and a heavy metal Pb) characteristics. The material preparation was conducted in Chemistry Laboratorium of Nusa Bangsa University and then they were analyzed at ICBB research center in Bogor.

Growth observation

The height of the *S. saman* seedlings in the field was measured once a week for 12 weeks. Measurements were made using a meter tape starting from the base of the stem to the bud, and from a predetermined point to the surface of the tailings. The growth of height was obtained by subtracting the final height to the initial height. Similarly, the diameter of the *S. saman* seedlings was measured once a week for 12 weeks using digital calipers on the stem at the point of the surface of the tailings. The diameter growth was obtained by subtracting the final diameter by the initial one. Plant biomass was determined at the time of harvest at 12 weeks by taking into account the dry weight of the roots and shoots of the plants (stems and leaves). The biomass was dried in the oven until it reached a constant weight, which was about 3 days. Total dry weight g^{-1} biomass was calculated using the formula:

$$\text{Total dry weight} = (\text{leaf dry weight} + \text{stem dry weight}) + \text{root dry weight}$$

Before measurement, roots were washed to remove soil debris. The roots were stretched maximally, then measured the length from the root base to the tip of the root. The depth of root-penetrated solum (cm) was determined using a spin technique to move or mobilize seedlings without damaging the roots (Hirfan 2016). The depth of the solum was measured from the surface of the tailings to the lowest root limit through the soil. The shoot to root ratio (SRR) was determined by comparing the shoot seedling dry weight with the root seedling dry weight with the formula:

$$\text{Shoot-Root Ratio} = \frac{\text{leaves dry weight} + \text{stem dry weight}}{\text{Root dry weight}}$$

The criteria of SRR was analyzed based on the Alrasyid (1972) criteria that the weight of plant shoot is usually 2 to 5 times the weight of root biomass.

AMF Colonization

The percentage of root colonization was determined using the Clapp method (Nusantara et al. 2012). The root sample was washed with clean water to remove soil and plant debris. Young roots (fibers) were bleached in 20% KOH solution for 24-72 hours, and rinsed with water. The roots were acidified using 0.1 M HCl for 1-5 minutes until the roots were clear yellow, thereafter rinsed with water and blue stained for 2 x 24 hours. After destaining overnight, the roots were cut into ± 1 cm long, arranged on a glass object (1 glass object for 10 pieces of root), with every 5 pieces of root covered with glass cover, in triplicate. The percentage of AMF root colonization was calculated in terms of hyphal, vesicles and arbuscular structures, using the formula:

$$\%\text{AMF Infection} = \frac{\text{Number of lateral root infection}}{\text{Total Number of lateral root}} \text{x } 100\%$$

AMF colonization rates were categorized according to de la Cruz et al. (1999) as follows: (1) high = colonization > 40%; (2) moderate = percentage of colonization 10-40%; (3) low = colonization <10

The number of AMF spores in the tailings media was determined using the sieving method (Nusantara et al. 2012). The efficiency of the rhizobial inoculation was evaluated taking into consideration the number of root nodules with a diameter ≥ 1 cm, and internal reddish color.

RESULTS AND DISCUSSION

Tailings properties

Before revegetation activities, the sample of tailings in the field was taken to soil laboratory. The physical and chemical properties of the tailings on the Cikabayan reclamation area is shown in Table 1. The content of C-organic (0.47%) and N-total (0.04%) was very low. The available phosphate (P) content (7.78 ppm) was low, although the potential P was very high (65.75 mg/100g), with potassium potential (44.95 mg/100g) was also high. Cation exchange capacity (4.77) was very low with very high levels of alkaline saturation while the pH was normal (7.42). The texture was dominated by sand (49.6%) and dust (33.7%) while the rest was clay. The content of heavy metal, Pb, (71.55 ppm) was at normal limits.

AMF colonization and rhizobial nodulation

The percentage of AMF colonization in a single AMF treatment was categorized as low, whereas the double inoculation treatment of AMF and *Rhizobium* could be considered as moderate (de la Cruz et al. 1999). The double treatment of AMF + *Rhizobium* inoculation significantly increased the colonization ($p < 0.05$) although the most number of AMF spores were found in seed media with only a single AMF inoculation (440 spores). Likewise, the highest number of nodules was significantly found in plants with a single *Rhizobium* inoculation alone (Table 2).

Growth of *Samanea saman* seedlings

The growth investigation was conducted at 12 weeks after planting on tailing field. On average, transplanting to the field conditions had a survival rate of 95% (Table 3). The height growth was not significantly different among all treatments. The application of AMF increased significantly ($p < 0.05$) the diameter growth (8.13 mm) and solum depth (26.8 cm) (Table 3 and Figure 2). The inoculation of *Rhizobium* did not promote the growth of the plants. Whereas, the treatments of both inoculation of AMF and *Rhizobium* significantly increased the growth of *S. saman* seedlings, including diameter (8.13 mm), dry weight (55.75 g) (Table 3), root length (41.88 cm) and depth of the solum (28.22 cm) (Figure 2).

The shoot-to-root ratio (SSR) value of *S. saman* seedlings inoculated with AMF and *Rhizobium* can be seen in Figure 3. The *S. saman* seedlings planted on the Pongkor gold tailings field had SRR values of 2.05-4.31 at the age of 12 weeks after planting.

Discussion

Gold mine tailings contain very low C-organic, low cation exchange capacity, and low macronutrients. One function of organic matter is to form stable aggregates, improve cation exchange capacity, regulate immobilization and release of nutrients (Craswell and Lefroy 2001, Gruba and Mulder 2015). The soil analysis showed that the texture of three fractions (sand, dust, and clay) was dominated by sand (49.62%), followed by dust (33.73%), and clay (16.65%). This fact indicates that the tailings in the study area might cause the plants underwent lacking in water and nutrient absorption. This finding clarifies Barker and Pilbeam (2015) that fine-size fractions (i.e. dust and clay) play an important role in holding water and nutrients to make them available to plants. The low value of cation exchange capacity (CEC) causes nutrients in the soil to be easily bound by heavy metal elements (Gruba and Mulder 2015; Widyatmoko 2017). In our study, this was indicated by the presence of lead metal (Pb) which reached a concentration of 71.55 ppm. Although the Pb content was still within the normal range, Pb^{2+} concentration at 10 ppm can cause plant stress and at the concentration of more than 100 ppm (critical threshold) has strong potential to cause stunted growth (Barker and Pilbeam 2015). Therefore, adding compost to the tailings is very important to ensure that the plants get adequate growth media.

The application of AMF and *Rhizobium* in tailings significantly increased colonization by AMF in the roots, the number of nodules in the roots of the seedlings and the presence of AMF spores in the tailings rhizosphere. This finding strengthens the existing knowledge that AMF association with legume plants has a great influence on the development of roots and shoots and the absorption of P which links to the occurrence of nodulation and nitrogen fixation (Pierre et al. 2014). It is of great importance to note that microbial colonization was not found in seedlings that were not inoculated, pointing out the absence of microbiome in the tailings. On the other hand, the establishment of AMF and *Rhizobium* strains can be considered as indicators of the ability of introduced microbes to survive under the prevailing physico-chemical tailings conditions. Indigenous AMF strains tend to be more adaptive than foreign strains (Pierre et al., 2014). The compatibility of AMF types of *Glomus manihotis* and *Glomus etunicatum* inoculum have also been reported to be developed with jabon (*Anthocephalus cadamba*) seedlings on tailings media in polybags (Setyaningsih et al., 2017), as well as with *Typha angustifolia* (Setyaningsih et al., 2018a).

Table 1. Chemical and physical properties of tailing at Cikabayan reclamation area in Pongkor before planting

Properties	Value	Category*
C-organic (%)	0.47	Very low
N-total (%)	0.04	Very low
C/N ratio	11	Average
P_2O_5 available (ppm)	7.78	Low
P_2O_5 potential (mg/100g)	65.75	Very high
K_2O potential (mg/100g)	44.95	High
Cation exchange (cmol (+)/kg)		
K^+	0.32	Low
Na^+	0.55	Average
Ca^{++}	15.7	High
Mg^{++}	0.86	Low
Capacity of Cation Exchange (cmol (+)/kg)	4.77	Very low
alkaline saturation (%)	100	Very high
pH H_2O	7.42	Neutral
Texture 3 fraction (%)		
Sand	49.62	
Dust	33.73	
Clay	16.65	
Lead (Pb) (ppm)	71.55	Normal limit

*= Status of physical and chemical properties based on Eviati and Sulaeman (2009).

Table 2. Infection of AMF and *Rhizobium* to *Samanea saman* seedlings at 12 weeks after planting at Cikabayan reclamation area in Pongkor

Infection	Control	AMF	RB	AMF x RB
Colonization (%)	0.0^a	9.26^b	-	20.74^c
Spore number (n/gram of soil)	1.0^a	440.0^d	24.0^b	111.0^c
Nodule number/plant	24.0^a	20.0^a	52.0^b	22.0^a

Note: Numbers followed by the different letters in the same line show significant differences based on the DMRT test with an error rate of 5%

Table 3. The growth of *Samanea saman* seedling with AMF and *Rhizobium* applications 12 weeks after planting at tailing field Cikabayan reclamation area in Pongkor

Growth	Control	AMF	RB	AMFxRB
Survival rate (%)	91.3^a	97.2^b	94.5^a	97.6^b
Height (cm)	22.90^a	18.74^a	20.62^a	19.59^a
Diameter (mm)	6.03^a	8.08^b	5.62^a	8.13^b
Dry weight (g)	52.33^a	50.31^a	49.61^a	55.75^b

Note: Numbers followed by the different letters in the same line show significant differences based on the DMRT test with an error rate 5%

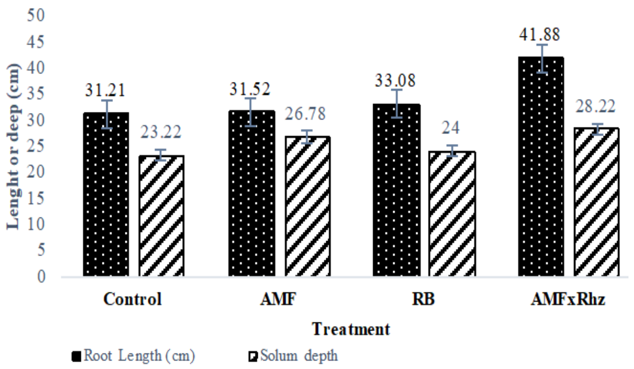

Figure 2. Root length and solum depth of *Samanea saman* seedling age at 12 weeks after planting at gold-mine tailing at Cikabayan reclamation area in Pongkor with various treatments of AMF and *Rhizobium* inoculations.

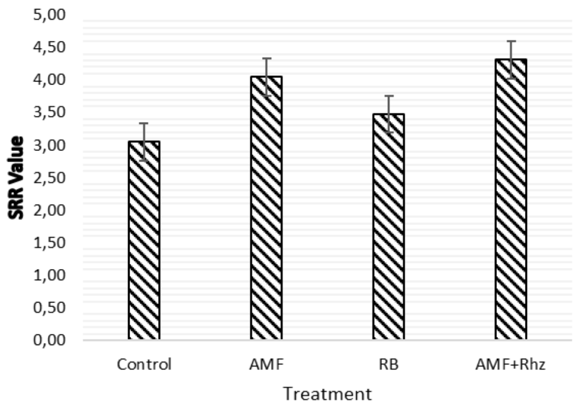

Figure 3. Shoot-to-root ratio of *Samanea saman* seedlings at the age 12 weeks after planting at gold mining tailing field at Cikabayan reclamation area in Pongkor with various treatments AMF and *Rhizobium* inoculations.

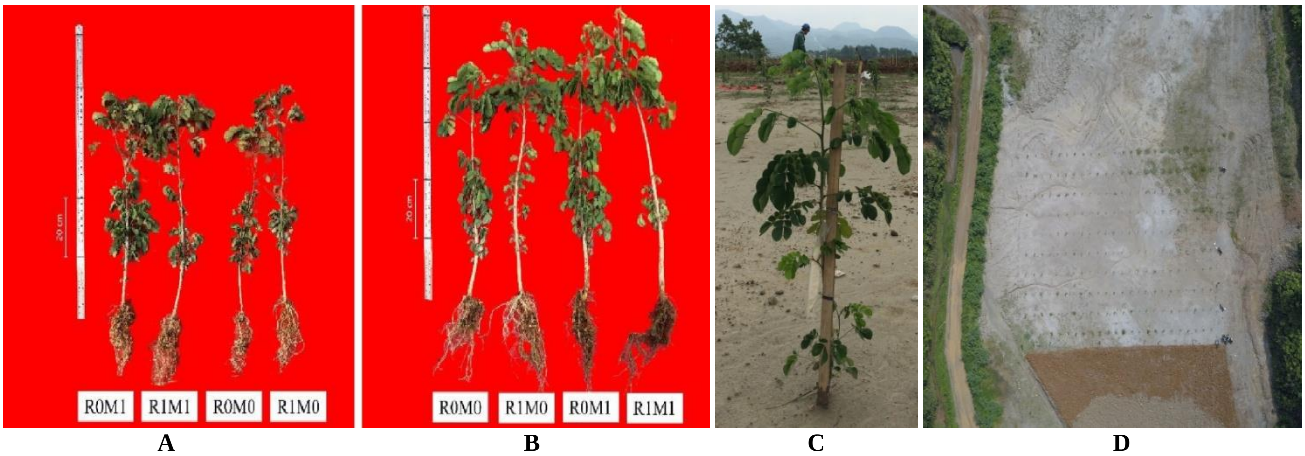

Figure 4. Performance of *Samanea saman* seedlings harvested at age 8 (A), age 12 (B) weeks after planting, and appearance of single seedling on the ground (C) and row of plants from the air (D) on gold mining tailing, Pongkor with various treatments AMF and *Rhizobium* applications. R0 = no *Rhizobium*, R1 = with *Rhizobium*, M0 = no AMF, M1 = with AMF

The 95% of survival rate of *S. saman* was greater than that was reported for other local plant species in West Kalimantan, such as *Hevea brasiliensis*, *Archidendron pauciflorum*, *Shorea leprosula* and *Vitex pinnata* (Ekyastuti et al., 2016; Jusran 2016). The dominance of plant species and the growth rate of certain plants in a mining area, especially tailings, is strongly influenced by the level of contamination, soil physical and chemical properties, the level of plant adaptation, and other environmental factors in mine tailings (Anawar et al., 2013; Ekyastuti et al., 2016; Setyaningsih 2018b, Lestari et al., 2019), including the adaptability of plants to limit the absorption of heavy metals at the roots (Reichman 2002; Setyaningsih et al., 2012)

Dual inoculations with AMF and *Rhizobium* triggered a better growth of the *S. saman* seedlings than single inoculation treatment, as previously reported in a number of different plant species (Yaseen et al., 2016; Havugimana et al., 2016). AMF and *Rhizobium* inoculations can also play a pivotal role in increasing plant growth in areas with high heavy metal content through improved plant nutrition (Pierre et al., 2014). AMF increases nutrient and water uptake, and increases nutrient mineral transporters in plant roots, while rooted nodules fix N_2 and also produce antibiotics and phytoalexin. The combination of the two microbes will provide better benefits for plants (Akhtar et al., 2011; Wang et al., 2017). However, more investigations are needed as the compatibility between the two microbes may vary, for instance between *Glomus intraaridices* and *Rhizobium* sp. NR4 strain, or between *Glomus coronatum* and *Rhizobium* sp. NR9 strain. This compatibility is important for management consideration if they are used as biofertilizers (Pierre et al. 2014). Results obtained in this investigation suggest the potential application of dual inoculation of *S. saman* seedlings with AMF and *Rhizobium* for the revegetation of gold mine tailing fields.

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