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Association of arbuscular mycorrhizal fungi (AMF) with *Brachiaria precumbens* (Poaceae) in tailing and its potential to increase the growth of maize (*Zea mays*)

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[2]Graduate Study Program of Biology, Faculty of Biology, Universitas Gadjah Mada, Sleman 55281, Yogyakarta, Indonesia
[3]Faculty of Biology, Universitas Gadjah Mada, Sleman 55281, Yogyakarta, Indonesia. ♥♥email: retpeni@ugm.ac.id; annisah-endang@ugm.ac.id; rkasiamdari@yahoo.com.au

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Abstract. *Suharno, Soetarto ES, Sancayaningsih RP, Kasiamdari RS. 2017. Association of arbuscular mycorrhizal fungi (AMF) with* Brachiaria precumbens *(Poaceae) in tailing and its potential to increase the growth of maize (*Zea mays*). Biodiversitas 18: 433-441.* The role of Arbuscular Mycorrhizal Fungi (AMF) in the process of rehabilitation of degraded land is very important, including the handling of sand tailings. In the rehabilitation process, utilizing the AMF isolates from the tailings area will be easier to adapt to the habitat that will be rehabilitated. The purpose of this study was to determine AMF that associated with *Brachiaria precumbens* (Poaceae) derived from the tailings area in Timika, Papua, and its potential to the growth of maize (*Zea mays*). The methods used to determine the presence of AMF were a survey and wet sieving methods, while the calculation of percent colonization was done by slide method. The compatibility test and effectiveness of AMF inoculation on the maize growth were conducted by completely randomized design (CRD) with 4 treatments: M0: control (without mycorrhiza); M1: *Clariodeoglomus etunicatum* BGR; M2: *C. lamellosum* L1A01S; M3: *C. etunicatum* L3A12D each with eight replications. The results showed that the presence of the AMF in the rhizosphere of *B. precumbens* was found in the tailings deposition area Modified Ajkwa Deposition Area (ModADA) of a gold mine in Timika. AMF percent colonization at the root reached 73.3%, while the number of spores in the rhizosphere was 8-25 per 10 g samples of soil and increased to reach an average of 49.6 spores per 10 g soil samples by trap methods. Based on the morphological identification, AMF found in the B. precumbens rhizosphere were identified as genus Glomus, Scutellospora, Acaulospora, and Claroideoglomus, whereas based on molecular identification, two isolates (L1A01S and L3A12D) were identified as *C. lamellosum* L1A01S and *C. etunicatum* L1A12D. The compatibility test showed that the AMF was able to increase the growth of maize, and significantly affected plant height, leaf area, and relative growth rate. *C. lamellosum* L1A01S derived from the tailings had a better effect than *C. etunicatum* L3A12D and *C. etunicatum* BGR.

Keywords: Arbuscular Mycorrhizal Fungi, *Brachiaria precumbens*, tailings, Timika, *Zea mays*

INTRODUCTION

Association of arbuscular *mycorrhizal* fungi (AMF) (Phylum *Glomeromycota*) with plants is very important in the ecosystem (Brundrett and Ashwath 2013; Souza 2015). AMF is one group of fungi which were found scattered in a variety of habitats (Brundrett 1991; Bordoloi et al. 2015; Souza 2015; Pagano and Gupta 2016), including the tailings (Suharno et al. 2014, 2016). These fungi become a key success in improving the growth of plants (Smith and Read 2008; Abiala et al. 2013; Aguacil et al. 2014) and rehabilitation (Cabral et al. 2015; Gosling et al. 2016). Tailing is mine waste material and it needs a place to waste, called tailing land. Adverse effects of tailings are the change of an area used as a location for deposition.

Gold mining activities in Timika are located in the highlands (2500 m above sea level), while the tailings are deposited on the Ajkwa river (below 30 m asl), which is a Modified Ajkwa Deposition Area (ModADA). The land area of the tailing deposition as the result of the company's activities in Timika metal ore processing is 23,000 hectares or 230 km^2 (Puradyatmika and Prewitt 2012), whereas, it is of approximately 220 km^2 in the estuary area (PTFI 2007). Every day, the company is able to process approximately 220000-240000 tons of material from the activities. Only about 3% of them contains concentrates of gold, copper, and silver, while the remaining 97% is only sand tailings that should be discarded and will not be used optimally (Suharno et al. 2014, 2016).

To do the rehabilitation on tailing land, the appropriate technology is required, so it will produce the maximum results (Khan 2006; Suharno and Sancayaningsih 2013). Local plants and microorganisms of the local area can be used as materials in the revegetation process. This process will minimize the changes in the existing ecosystem (Khan 2005; Hu et al. 2013; Toju et al. 2014). *Brachiaria precumbens* (Poaceae) is one of the local plant species that grows well in the tailings area in Timika (Suharno et al. 2014). This plant can be used as an alternative in the rehabilitation process. To improve growth performance,

AMF is required, because it was able to grow and contribute to an increase in growth of plants.

The utilization of local AMF (native AMF) for land reclamation needs a test on compatibility and effectiveness on the plants. Plants used are the types that will be used directly or will be combined with other vegetation (Suharno and Sancayaningsih 2013). The aim is to see the influence of AMF in the improvement of plant growth. The purpose of this study was to determine the association of AMF with *B. precumbens* plants originating from the tailings area in Timika, Papua, and its potential to the growth of maize. This local AMF is expected to play a role in increasing crop growth so that the process of land reclamation can be achieved with the maximal outcome.

MATERIALS AND METHODS

Study area

This study was conducted in November 2014 to August 2016. Sampling was from the tailings area, namely Modified Ajkwa Deposition Area (ModADA) of PT. Freeport Indonesia (PTFI) on the West of the old and new embankment (double levee) of Timika, Mimika District, Papua (Figure 1). Sampling sites are at coordinates S: 04°29'50.9" and E: 136°54'02.3" until S: 04°34'14.0" and E: 136°54'11,7" between 24-28 miles (km 15 to 18). West levee bordering the town of Timika is made of two dams, the old levee bounds the City from Ajkwa river, while the new levee restricts the expansion of the material deposition from Otomona River which flows into the Ajkwa River. In the double embankment area, there are several locations covered by vegetation, either as a result of a succession of natural or as an artificial reclamation. A total of 300 g soil samples were cultured in a mixture media of zeolite : sand (3: 1; v: v) in pots with a capacity of 3 kg using *Shorgum bicolor* seeds as host plants.

The presence and symbiosis of AMF

The presence of AMF was identified by observing the samples of soil and roots of plants (Corkidi et al. 2008). The spores were observed from soil samples, whereas the AMF infections in plants were identified from the root. Samples of roots were stained with staining method (Giovannetti and Mosse 1980; Vierheilig et al. 2005). At first, the plant roots are cleaned and fixated with FAA solution (formalin-acetic acid-alcohol; v = 5: 5: 90) for 1 hour, then they were soaked by KOH 10% solution for 24 hours, followed by HCl 1% for 24 hours. After being washed with a solution of distilled water, the roots were painted with staining of tryphane blue 0.05% inside lactoglycerol for 24 hours (Vierheilig et al. 2005).

To view the percentage of infection, the slide method was carried out (Giovannetti and Mosse 1980). Roots that have been colored were cut into rectangle with 1 cm long, 30 pieces were observed microscopically (with a magnification of 100 to 400 times). The AMF Presence will be revealed by the forming structure of AMF in roots like intraradical hyphae, extraradical hyphae, vesicles, arbuscular, or spores. Mycorrhizal colonization percentage was calculated by the equation:

$$\% \text{ root colonization} = \frac{\text{The number of infected roots}}{\text{The number of all observed roots}} \times 100\%$$

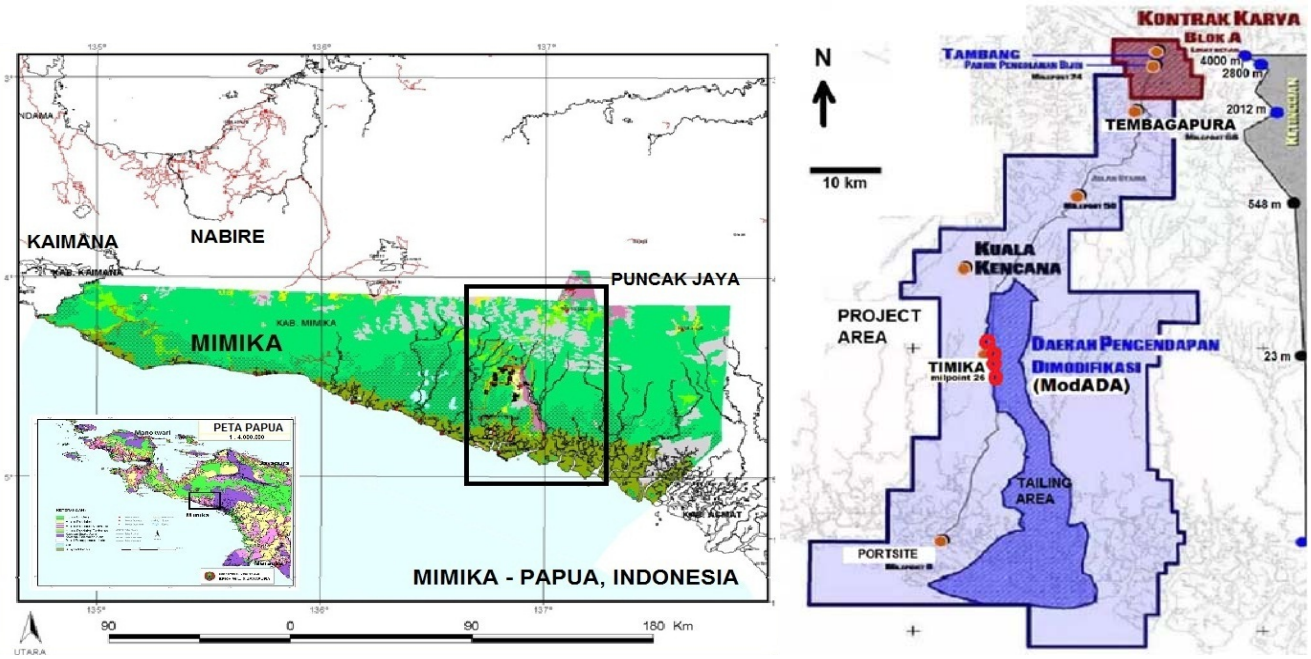

Figure 1. Area of gold mine in Mimika and tailings deposition area in Timika, Mimika District, Papua as AMF sampling locations (PTFI 2007)

The percentage of colonization was categorized by score (Dodd et al. 2001); score 0: uninfected AMF; Score 1: very little infection, the percentage of infection is less than 1%; Score 2: a bit of infection, the percentage of infection ranged from 1 to 10%; score 3: some infections, the percentage of infection ranged from 10 to 50%; score 4: a lot of infection, the percentage of infection is 50 - 90%; and score 5: abundant infections, the percentage of infections is more than 90%.

AMF spores extraction

10 g of soil samples which were from the field or from trapping process were extracted gradually using wet screening method (wet sieving). With gradient method, spores from the filtering process were separated from other materials using sucrose 60% by centrifugation (Utobo et al. 2011; Suharno et al. 2015). The spores as the results of separation process were washed with distilled water and observed under a lightened-microscope with a magnification 100-400x. The observations result was documented using a microscope camera (Olympus-CX21).

Single spore propagation and AMF identification

The determination of AMF diversity in the plant roots is done by observing the spores. Spores is a means of identification of the AMF based on morphologic characteristics (Souza 2015). Spores screening results need to be reproduced and it is done by single spore technique. Single spore is grown on zeolite media on a plastic petri dish (ϕ 9 cm, IWAKI) and uses *Desmodium heterophyllum* (Fabaceae) as host plants, while the multiplication of the inoculum is carried out with a plastic pot (Suharno et al. 2015).

Identification of the AMF is done based on morphological characteristics of spores and molecular data (Krüger et al. 2012). Identification of the morphological characteristics of spores refers to Schenck and Perez (1990) combined with several other kinds of literature (Lee et al. 2006; Karaarslan et al. 2015). Identification with molecular techniques is performed by the method of Polymerase Chain Reaction (PCR) with specific primer of AML1 (5'-ATC AAC TTT CGA TGG TAG AGA GAT-3 ') and AML2 (5'-GAA AAA CCC TTT GGT CAC TTC C-3') using spores (Lee et al., 2008). The target of this specific primers is part of small subunit (SSU) with a length of 700-800 bp on SSU rRNA gene.

About 10-20 spores are put in a 0.2 ml eppendorf tube (Lee et al. 2006) which has been filled with 10 mL of TE buffer (Kasiamdari et al. 2002; Mathimaran et al. 2008) using sterile tweezers. The spores are, then, divided with tweezers or ose needle which pin had been flattened under a stereo microscope (Kasiamdari et al. 2002; Sasvári et al. 2012). Further, it is heated at a temperature of 65 °C for 10-15 minutes and cooled on ice for 2-3 minutes (Kasiamdari et al. 2002). A total of 2 µL of the DNA extract can be used as a template in PCR (Krüger et al., 2012; Sasvári et al. 2012).

DNA amplification is carried out through three stages, namely: denaturation, annealing, and elongation. To undergo this process, it is used PCR mix GoTag® Green Polymerase (Sasvári et al. 2012). The PCR process is carried out under conditions of predenaturation for 15 minutes, with temperature of 94 °C; denaturation for 0.30 seconds, with temperature of 94 °C; annealing for 0.40 seconds, with temperature of 58 °C; 55 seconds extension, with temperature of 72 °C; 5 minutes post extension, with temperature of 72 °C in 30x cycles. The sequencing of PCR results was performed at the First Base Laboratories Sdn Bhd (1st BASE) Malaysia. To determine the type of AMF, the BLAST is conducted via http://blast.ncbi.nlm.nih.gov/Blast.cgi website.

AMF effectiveness test on maize plant (*Z. mays*)

Media soil used in this study is the sandy soil which has a neutral pH (7.0 to 7.3), a very low content of organic C ranged from 0.10 to 0.14 %, a very low content of N (0.02 %), a very low - low ratio of C/N (4-6), a very high content of P (P_2O_5) (223.8 to 408.8 ppm), a low content of K ranged from 0.12 to 0.14 cmol / kg, and a very low CEC (cation exchange capacity) (2.08 to 2.50 $cmol.kg^{-1}$). AMF propagules are *C. etunicatum* and *C. lamellosum* which were taken from the tailings area. *C. etunicatum* BGR is used as a control and is derived from SEAMEO-BIOTROP Bogor. These three types of propagules have large numbers of spores. *C. etunicatum* BGR has an average of 89 spores, *C. lamellosum* L1A01S has an average of 96 spores, and *C. etunicatum* L3A12D has an average of 87.7 spores per 100 g sample. The results of the MPN (Most Probable Number) test the three types of AMF are respectively 1400, 2600, and 1700 propagules per gram wet weight of soil. According to Sancayaningsih et al. (2000), the density of propagules is very important in the inoculation of plant growth.

The test of compatibility and effectiveness of AMF is by inoculating AMF propagules on maize (*Z. mays*) using 4 treatments of Complete Randomized Design (CRD), namely: M0: control (without mycorrhiza); M1: *Clariodeoglomus etunicatum* BGR; M2: *C. lamellosum* L1A01S; M3: *C. etunicatum* L3A12D with 8 replications. Several measured parameters are variables of plant growth such as plant height, leaf width, fresh and dry weight of plants, the percentage of AMF colonization, as well as relative growth rate (relative growth rate, RGR). RGR is calculated by the formula:

$$RGR = [(\ln DW2 - \ln DW1) / t2 - t1],$$

Note:
DW1: dry weight plant on first-time measurement;
DW2: dry weight plant on second time measurement;
t1: first time measurement;
t2: second measurement time.

Data analysis

The data were analyzed using analysis of variance (ANOVA) with one factor using IBM SPSS (Statistical Package for Social Sciences) software version 20.0. Compared to some comparators using the Duncan's Multiple Range Test (DMRT) through post hoc test with a

confidence level of 95% (p= 0.05), data mean shows a significant difference.

RESULTS AND DISCUSSION

The existence of AMF and its association with rhizosphere of *B. precumbens*

The results showed that the AMF was found in the rhizosphere of *B. precumbens* (Table 1; Figure 5a). The number of spores found on the direct extraction of soil samples ranged from 8-25, with an average of 15.1 per 10 g soil. With the trapping method in the laboratory, the number increased 300% with an average of 49.6 per 10 g soil. Trapping treatment method showed a significant increase compared to the method of direct spores isolation in the field. With this method, spore production became the highest with the result of 33-71 spores per 10 g soil samples. According to Suharno et al. (2015) and Liu and Wang (2003), the trapping method was more effective because the laboratory conditions were more stable and more controllable for the growth of plants and fungi than the other place. In these conditions, the AMF which was unable to form spores in field conditions would have the opportunity to produce spores.

Association between the AMF and *B. precumbens* could be confirmed by the presence of AMF forming structure in the root. This structure was a special characteristic which was formed due to this associations. The observed AMF structure included intraradical hyphae, vesicles, arbuscular, while intraradical spores were not observed (Figure 5). The Percentage of root colonization ranged from 65.3 to 85.3% with an average of 73.6%. The value was categorized as high and showed a good cooperation of bio-symbion and myco-symbion. According to Wang and Qiu (2006), the symbiotic associations of arbuscular mycorrhizal fungi, from the type of *B. decumbens* and *B. humidicola*, was found on *Brachiaria* grass. This suggested that genus *Brachiaria* which was grass plant (Poaceae) could be associated with mycorrhizae. The preliminary research conducted by Suharno et al. (2014) indicated that *B. precumbens* in this area were in symbiosis with the AMF. AMF colonization percent was high. Carballar-Hernandez et al. (2013) observed colonization of AMF on *Agave potatorum* that ranged from 20-83%. The length of extraradical mycelia was 2.64 to 5.22 m / g on dry land. The number of spores ranged from 20-192 in 100 grams of soil in dry land. According to Brundrett (1991) and Yue and Liu (2016), the percent of colonization on plant roots was associated with the ecological system both AMF or host plants in their habitat. Smith and Read (2008) and Utobo et al. (2011) also revealed that the structure generated by the AMF on the root system of plants was very important in symbiosis. Three important components in the root system with mycorrhiza were its role on the root itself, intraradical hyphae which were associated with roots, and extraradical hyphae which were related to land. Suharno et al. (2014) revealed that the percent of plant root colonization by AMF varied greatly. At the same tailings lands, some plant species have a high percentage of colonization, namely *Amomum* sp., *F. adenosperma*, and *B. pilosa*. On the other hand, on some of the samples taken at the inundated habitat, there was no AMF colonization such as in plants of *Pragmites karka*.

The percent colonization of AMF on maize was also high. The results showed that AMF colonization with *C. etunicatum* BGR inoculant reached an average of 77.42%, with *C. lamellosum* L1A01S inoculants reaching an average of 77.74%, while with *C. etunicatum* L3A12D inoculants reaching 83.74%. Although *C. etunicatum* has the highest percent of colonization, but the effect on the relative growth rate of plants is not as high as the effect of *C. lamellosum* (Table 4). The colonization percent of the three treatments was not significantly different at a test level of 95% (p: 0.05). The percentage of colonization, extraradical hyphae growth and spore production depends on environmental conditions in a region (Zangaro et al. 2013; Doley and Jite 2012). It is often found that the percent of root colonization is affected by phosphorus. *Glomus fasciculatum* mycorrhizal fungi had a higher percentage of colonization due to the application of phosphorus but then mycorrhizal dependence decreased because of the increased levels of phosphorus (Doley and Jite 2012; Yuan 2015). With the addition of cadmium into the growing medium, mycorrhizal colonization percentages increased with the range of 22.7 to 72.3% in maize (Liu et al. 2014), while Valsalakumar et al. (2007) observed the colonization percentage of 28-95% on the plant of *Phaseolus aureum*.

The season and the availability of nutrients are very influential in the development and growth phyto-symbion and myco-symbion. Fungi tend to form spores on dry season, but it will intensify the growth mycelium on the rainy season, although it is not statistically significant (Cuenca and Lovera 2010; Hu et al. 2013; Bouamri et al. 2014). This is also supported by data that shows a heavy rainfall throughout the year of 2013 reaching 280.5 to 726.3 mm/month (Suharno et al. 2014). Spores are concentrated in the top layer of soil and reduced significantly in the deepest layer of the soil profile. This condition is associated with the distribution of the root system and the availability of sources of carbon in their habitat.

Table 1. The number of spores and percentage of colonization of AMF on the roots of plants *B. precumbens* in the tailing area

Sample	Number of spores (10 g of soil)		% root colonization
	Field	Trapping method	
28-BP1	18	55	66.6
28-BP2	8	45	76.7
27-BP1	15	39	80.6
27-BP2	21	33	66.7
26-BP1	18	45	85.3
26-BP2	25	35	71.9
26-BP3	9	54	65.3
26-BP4	10	63	67.8
25-BP1	12	56	75.6
25-BP2	15	71	80.0
Average	15.1	49.6	73.65

Arbuscular Mycorrhizal Fungi (AMF) diversity

The results showed that there was 10 morphospecies of AMF from the following genus: *Glomus* (4 types), *Scutellospora* (1 species), *Acaulospora* (2 types), and *Claroideoglomus* (2 types) (Figure 2). *Claroideoglomus etunicatum* is one of the AMF identified morphologically. Two isolates are known to have large numbers of spores, reaching 36.3 and 42.7 per 10 gram of wet weight of the sample media, namely L1A01S and L3A12D isolates which are derived from the genus *Clariodeoglomus*. After the identification using molecular techniques, the results of BLAST are identified, namely *C. lamellosum* and *C. etunicatum* with similarity rate of 99 %. Identification of the AMF both morphologically and molecularly is very important to determine the certainty of one type that it results in the preciseness of identity of an organism (Schenck and Perez 1990; Lee et al. 2006; Schüßler and Walker 2010). The result of propagation on both isolates is used as an inoculant in the test of compatibility and effectiveness in maize. According to Suharno et al. (2016), there are several types of AMF found in the region deriving from several rhizospheres of plants, such as genus *Glomus*, *Claroideoglomus*, *Acaulospora*, and *Scutellospora*. Beena et al. (2001) revealed the results of a study on dry land such as desert, sand dunes and coast and it showed that AMF is able to develop in these areas. Al-Yahya'ei et al. (2011) found 25 AMF morphospecies in the desert land around the Dates plantation area, in South Arabia, and some of them have not been identified yet. Zhang et al. (2012) also observed the dynamics of AMF associated with typical plants of the desert ecosystem in Gurbantunggut, Xinjiang, China. The diversity of AMF in several parts of the world is quite high, at least there are about 200 types of AMF which have been identified (Wang and Qiu 2006), but some others still can not be identified. Schüßler and Walker (2010) mentioned 234 types of AMF as the results of the study based on the morphological and molecular characters. It can even reach more than 300 because the discovery of a new type of AMF is still continued (Blaszkowski and Czerniawska 2011; Pagano et al. 2016). Moreover, the use of molecular techniques in basic identification is common (Schüßler and Walker 2010; Young 2012). The existence of AMF morphospecies at a particular habitat can be varied (Zhang et al. 2012); Brundrett and Ashwath 2013; Afaf et al. 2015; Bordoloi et al. 2015). Brundrett (1991) suggested that AMF species varied in a habitat. It is related to the AMF capability of growing under certain conditions and the ability of fungi to form a symbiosis with various types of plants in the vicinity.

AMF effectiveness test on maize

The observation of AMF effectiveness test on maize showed that in the 3rd week after planting, the difference in growth height of the plants was not visible yet, however, at 10th week, the difference was quite significant (Figure 3; Table 2). Inoculation of *C. lamellosum* L1A01S coming from the tailing land was able to increase the growth of plant height (51.8 cm) compared to the treatment of *C. etunicatum* BGR (49.6 cm), of *C. etunicatum* L3A12D (47.8 cm), and no mycorrhizal treatment (40.7 cm). The same conditions occurred in the growth of leaf width (Figure 4; Table 2). At 7th week, the growth of leaf width might be changed and some even tended to decline because, despite the growth of young leaves, the mature leaves got old and even dried. The highest increase in plant height was 27.2% compared to controls, while the highest increase of leaf width was by the treatment of *C. lamellosum* L1A01S reaching 80.3% (327.4 cm^2) compared to controls (181.6 cm^2).

Despite an increase in the growth of shoot reaching 40.4% and of root reaching 5.1% until week 10, the response of fresh weight growth (Table 3) did not occur significantly. The difference was not significant due to the high early growth on the treatment with no AMF inoculation. This growth then decreased at week 10. On the other hand, with the treatment of AMF, the growth start slowly but it increased finally. However, this increase was not enough to affect the fresh weight significantly. The determining factor in this condition is the high water content in the fresh weight of the plants which is almost equal to treatment. On *C. lamellosum* inoculation treatment, the increase in fresh weight reached 40.4% higher than control, but it is not significant because the diversity of fresh weight coefficient is quite high. The dry weight of root did not increase significantly, but the dry weight of plant shoot showed a significant increase. The treatment of *C. lamellosum* L1A01S brought on to the highest root dry weight of 1,237g compared to 0.714 g of controls, increasing 73.2% significantly. This condition indicates that the root with mycorrhiza can increase the dry weight of shoot significantly. According to Shirmohammadi and Aliasgharzad (2013), the response to AMF treatment varied. The study showed that the role of *C. etunicatum* in increasing the fresh weight of sorghum is significant, but it is not significant on tomatoes. Doley and Jite (2012) revealed that the AMF's role is very significant to the growth of the fresh weight of the plant shoot of *Arachis hypogaea*.

On the parameters of total dry weight (Table 4), it appears that there is no significant difference until the 3rd week, but it is different at week 10. These conditions affect the relative growth rate that shows an increase significantly. The relative growth rate shows that inoculation with *C. lamellosum* L1A01S is capable of increasing to 73.3% compared to the control. Treatment with the AMF of this type is better than with *C. etunicatum* L3A12D, *C. etunicatum* BGR, and control. The AMF type and inoculum density affect the biomass increase (Valsalakumar et al., 2007; Treseder 2013), including the maize plant (Sancayaningsih et al. 2000). In peanuts (*Arachis hypogaea*), AMF role is very significant to the increase of the dry weight of plant shoot (Doley and Jite 2012). The high density of propagules determines the increase of the dry weight of the host plants in the early growth of corn plant (Sancayaningsih et al. 2000). In addition, the high population of AMF inoculum on the ground is important to achieve faster colonization of plant roots, the optimal growth, better products. It can lead to the least intake of the initial stimulus such as fertilizer or no

land management at all, and it also can avoid the longer periods of fallow and the burning of crop remnants (Smith and Smith 2011).

The effectiveness test results (Table 2, 3, 4) of AMF types derived from the tailings namely *C. lamellosum* L1A01S and *C. etunicatum* L3A15D showed that there was a significant influence of AMF on the increased growth of maize. The development of hyphae affects the level of root colonization which will further increase the absorption of nutrients and increase the rate of plant growth (Treseder 2013). AMF types that have been known for certain and able to adjust tailings land habitat are expected to play a role in the process of land rehabilitation (da Silva et al. 2012; Guo et al. 2013; Suharno et al. 2014). These types of AMF are very important in the process of revegetation of tailing lands as they are tolerant to the condition of land to be restored (Suharno & Sancayaningsih 2013).

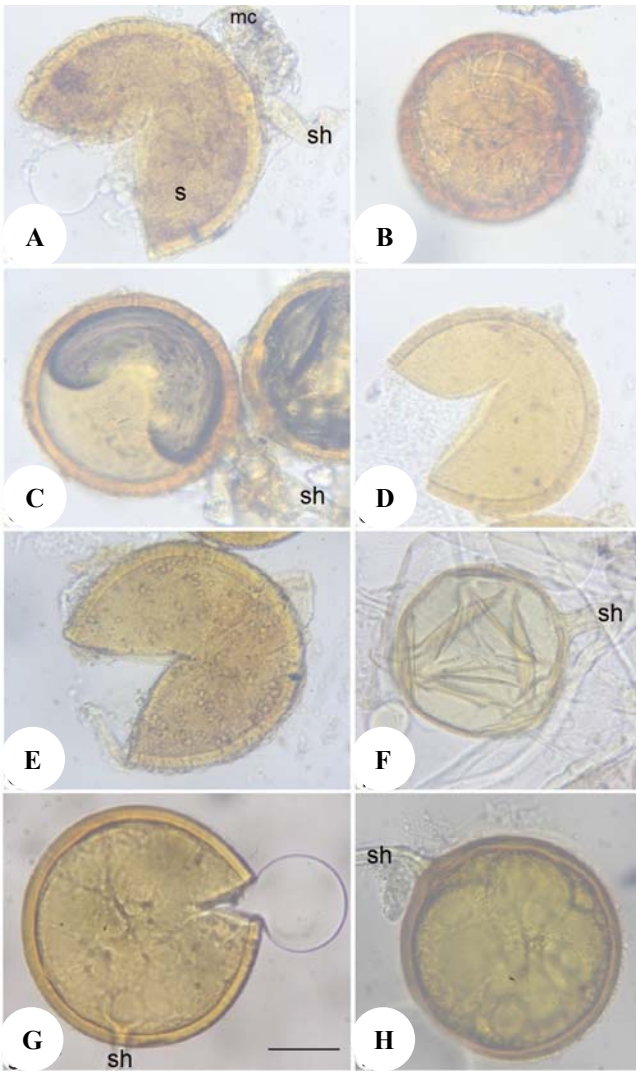

Figure 2. Some morphospecies of AMF spores which were isolated from the rhizosphere of plants *B. precumbens*. a. *Acaulospora* sp, b. *Scutellospora* sp, c-d. *Glomus* sp., E. *Acaulospora* sp2, f. *Glomus*, g. *Claroideoglomus lamellosum*, h. *C. etunicatum*. (mc: mother cell, s: spore, sh: substanding hyphae, scale bar: 50 µm).

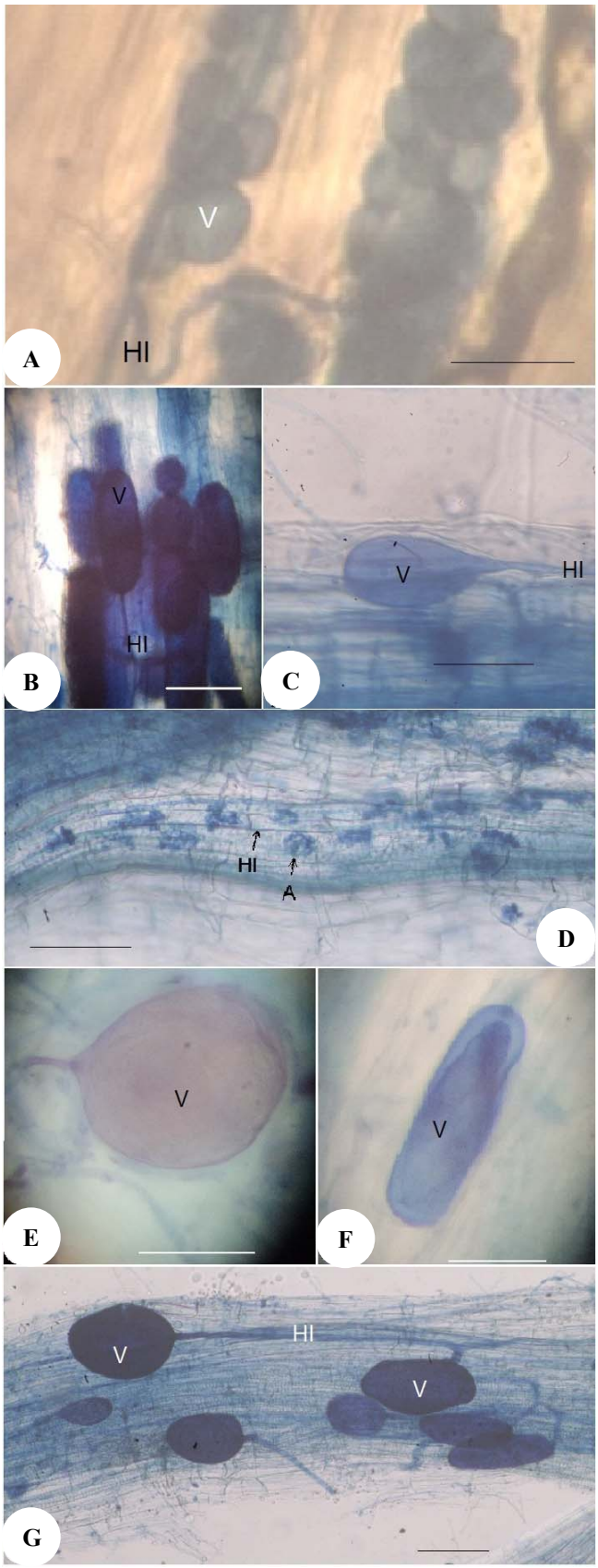

Figure 5. AMF colonization in plants. a. in *B. precumbens*, b-g. in *Z. mays*. b-c. inoculation with *C. etunicatum* BGR. d-f. inoculation with *C. lamellosum* L1A01S. g. Inoculation with AMF type of *C. etunicatum* L3A12D. *HI: hyphae intraradical., V: vesicles, A: arbuscula* (Scale bar: 100 um)

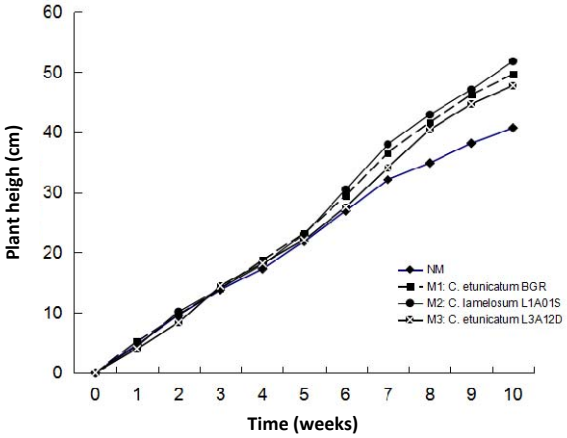

Figure 3. The height increase of corn plants which was inoculated by AMF coming from the tailings area

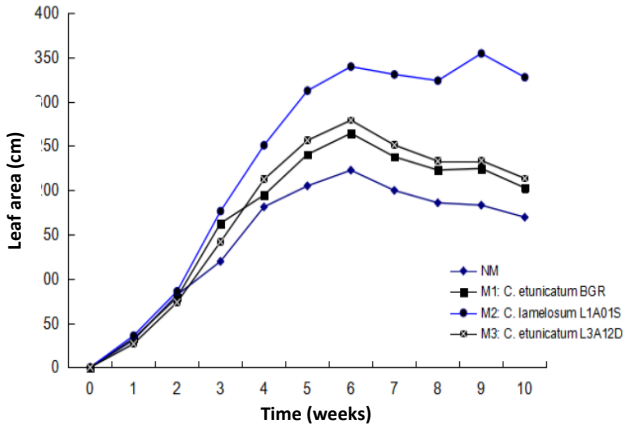

Figure 4. The increase of leaf width of maize plants inoculated by AMF coming from the tailings area

Table 2. The response of height increase and leaf width of maize plants which were inoculated by AMF

Treatment	Shoot height (cm)		Leaf width (cm^2)	
	Week-3	Week-10	Week-3	Week-10
M0 (non-mycorrhizal)	13.79 a	40.72 b	138.41 b	181.60 b
M1 (*C. etunicatum* BGR)	13.95 a	49.65 a	159.97 ab	202.59 ab
M2 (*C. lamellosum* L1A01S)	13.99 a	51.80 a	176.57 a	327.36 a
M3 (*C. etunicatum* L3A12D)	14.58 a	47.77 ab	147.18 b	213.22 ab

Table 3. The increase response of fresh weight and dry weight of maize plants inoculated by AMF 10 weeks after planting

Treatment	Fresh weight (g)		Dry weight (g)	
	Root	Shoot	Root	Shoot
M0 (non-mycorrhizal)	3.853 a	3.814 a	0.471 a	0.714 b
M1 (*C. etunicatum* BGR)	3.150 a	4.913 a	0.427 a	1.085 ab
M2 (*C. lamellosum* L1A01S)	4.051 a	5.737 a	0.603 a	1.237 a
M3 (*C. etunicatum* L3A12D)	2.677 a	4.423 a	0.368 a	0.912 ab

Table 4. The response of plant growth increases to the total dry weight and relative growth rate of maize plants which were inoculated by AMF

Treatment	% AMF colonization	Total dry weight 3 WAP (g)	Total dry weight 10 WAP (g)	RGR
M0 (non-mycorrhizal)	0.50 b	0.451 a	1.160 b	0.232 b
M1 (*C. etunicatum* BGR)	77.42 a	0.369 a	1.512 ab	0.313 ab
M2 (*C. lamellosum* L1A01S)	77.74 a	0.378 a	1.890 a	0.402 a
M3 (*C. etunicatum* L3A12D)	83.74 a	0.356 a	1.280 b	0.319 ab

Note: WAP: week after planting; RGR: relative growth rate

The exploitation and utilization of organisms, both fungi and plants, as phytoremediation are very important in the process of land reclamation. It is concerned with the effort of land rehabilitation which is environmentally friendly (Khan 2006; da Silva et al. 2012). With carrying capacity as multi-functional microorganisms to the growth of a plant on marginal condition, AMF has a big impact on the survival of plants (Jeffries et al. 2003; Zangaro et al. 2012; Dickie et al. 2013). Therefore, there are many efforts to the discovery of the AMF and its prospect of utilization of local AMF which are considered as more environmentally friendly than the other way (Khan 2006; Suharno and Sancayaningsih 2013). This process, of course, needs a long and many steps, started by the isolation of AMF types, the selection of superior type, compatibility testing on plants, propagation and utilization

in the field (Upadhyaya et al. 2010), so they are able to improve the soil structure (Aguacil et al. 2014) and to return the ecosystem like its original condition (Brundrett and Ashwath 2013; Aguacil et al. 2014).

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REFERENCES

Abiala MA, Popoola OO, Olawuyi OJ, Oyelude JO, Akanmu AO, Killani AS, Osonubi O, Odebode AC. 2013. Harnessing the potentials of vesicular arbuscular mycorrhizal (VAM) fungi to plant growth-A review. Int J Pure Appl Sci Technol 14 (2): 61-79.

Afaf N, Zohra I, Faiza BZ, Abdelkader B. 2015. Diversity of arbuscular mycorrhizal fungi in two perturbed ecosystems (dune and saline soil) in west Algeria. Intl J Agri Crop Sci 8 (3): 380-387.

Alguacil MDM, Torrecillas E, Lozano Z, Torres MP, Roldan A. 2014. *Prunus persica* crop management differentially promotes arbuscular mycorrhizal fungi diversity in a tropical agro-ecosystem. PlosOne 9 (2): e88454. DOI: 10.1371/journal.pone.0088454.

Al-Yahya'ei MN, Oehl F, Vallino M, Lumini E, Redecker D, Wiemken A, Bonfante P. 2011. Unique arbuscular mycorrhizal fungal communities uncovered in date palm plantations and surrounding desert habitats of Southern Arabia. Mycorrhiza 21: 195-209.

Beena KR, Arun AB, Raviraja NS, Sridhar KR. 2001. Association of arbuscular mycorrhizal fungi with plants of coastal sand dunes of west coast of India. Tropical Ecology 42 (2): 213-222.

Błaszkowski, J. and Czerniawska, B. 2011. Arbuscular mycorrhizal fungi (Glomeromycota) associated with roots of *Ammophila arenaria* growing in maritime dunes of Bornholm (Denmark). Acta Societatis Botanicorum Poloniae 80 (1): 63-76.

Bordoloi A, Nath PC, Shukla AK. 2015. Distribution of arbuscular mycorrhizal fungi associated with different land use systems of Arunachal Pradesh of Eastern Himalayan region. World J Microbiol Biotechnol 31 (10): 1587-1593.

Bouamri R, Dalpé Y, Serrhini MN. 2014. Effect of seasonal variation on arbuscular mycorrhizal fungi associated with date palm. Emir J Food Agric 26 (11): 977-986.

Brundrett M. 1991. Mycorrhizas in natural ecosystem. Advances in Ecological Research 21: 171-313.

Brundrett MC, Ashwath N. 2013. Glomeromycotan mycorrhizal fungi from tropical Australia III. Measuring diversity in natural and disturbed habitats. Plant Soil 370: 419-433.

Cabral L, Soares CR, Giachini AJ, Siqueira JO. 2015. Arbuscular mycorrhizal fungi in phytoremediation of contaminated areas by trace elements: mechanisms and major benefits of their applications. World J Microbiol Biotechnol 31 (11): 1655-1664.

Carballar-Hernandez S, Palma-Cruz FJ, Hernandez-Cuevas L, Robles C. 2013. Arbuscular mycorrhizal potential and mycorrhizal fungi diversity associated with *Agave potatorum* Zucc. in Oaxaca, Mexico. Ecol Res 28: 217-226.

Corkidi L, Evans M, Bohn J. 2008. An introduction to propagation of arbuscular mycorrhizal fungi in pot cultures for inoculation of native plant nursery stock. Native Pl J 9 (1): 29-38.

Cuenca G, Lovera M. 2010. Seasonal variation and distribution at different soil depths of arbuscular mycorrhizal fungi spores in a tropical sclerophyllous shrubland. Botany 88: 54-64.

da Silva DK, Pereira CMR, de Souza RG, da Silva GA, Oehl F, Maia LC. 2012. Diversity of arbuscular mycorrhizal fungi in restinga and dunes areas in Brazilian Northeast. Biodivers Conserv 21: 2361-2373.

Dickie IA, Martínez-García LB, Koele N, Grelet G.-A, Tylianakis JM, Peltzer DA, Richardson SJ. 2013. Mycorrhizas and mycorrhizal fungal communities throughout ecosystem development. Plant Soil 367: 11-39.

Dodd JC, Clapp JP, Zhao B. 2001. Arbuscular mycorrhizal fungi in plant production systems : detection, taxonomy, conservation and ecophysiology. Laboratory of Agricultural Microbiology, Huazhong Agricultural University, China.

Doley K, Jite PK. 2012. Response of groundnut ('JL-24') cultivar to mycorrhiza inoculation and phosphorous application. Notulae Scientia Biologicae 4 (3): 118-125.

Giovannetti M, Mosse B. 1980. An evaluation of techniques for measuring vesicular-arbuscular infection in roots. New Phytol 84: 489-500.

Gosling P, Jones J, Bending GD. 2016. Evidence for functional redundancy in arbuscular mycorrhizal fungi and implications for agroecosystem management. Mycorrhiza 26 (1): 77-83.

Guo W, Zhao R, Yang H, Zhao J, Zhang J. 2013. Using native plants to evaluate the effect of arbuscular mycorrhizal fungi on revegetation of iron tailings in grasslands. Biol Fertil Soil 49: 617-626.

Hu Y, Rillig MC, Xiang D, Hao Z, Chen B. 2013. Changes of AM Fungal abundance along environmental gradients in the arid and semi-arid grasslands of Northern China. PLoS ONE 8 (2): e57593. DOI:10.1371/journal.pone.0057593.

Jeffries P, Gianinazzi S, Perotto S, Turnau K, Barea J.-M. 2003. The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biol Fertil Soils 37: 1-16.

Karaarslan E, Uyanöz R, Doğu S. 2015. Morphological identification of vesicular-arbuscular mycorrhiza on bulbous plants (Taurus Mountain in Turkey). Arch Biol Sci Belgrade 67 (2): 411-426.

Kasiamdari RS, Smith SE, Scott ES, Smith FA. 2002. Identification of binucleate *Rhizoctonia* as a contaminant in pot cultures of arbuscular mycorrhizal fungi and development of a PCR-based method of detection. Mycol Res 106 (12): 1417-1426.

Khan AG. 2005. Role of soil microbes in rizhospheres of plants growing on trace metal contaminated soils in phytoremediation. Journal of Trace Elements in Medicine and Biology 18: 355-364.

Khan AG. 2006. Mycorhizoremediation-an enhanced form of phytoremediation. J Zhejiang Univ Sci B 7 (7): 503-514.

Krüger M, Krüger C, Walker C, Stockinger H, Schüßler A. 2012. Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. New Phytol 193: 970-984.

Krüger M, Stockinger H, Krüger C, Schüßler A. 2009. DNA-based species level detection of *Glomeromycota*: one PCR primer set for all arbuscular mycorrhizal fungi. New Phytol 183: 212-223.

Lee, J., Lee, S., and Young, J.P.W., 2008. Improve PCR primers for the detection and identification of arbuscular mycorrhizal fungi. FEMS Microbiol Ecol 65: 339-349.

Lee, J., Park, S.-H., and Eom, A.-H. 2006. Molecular identification of arbuscular mycorrhizal fungal spores collected in Korea. Mycobiology 34 (1): 7-13.

Liu L, Gong Z, Zhang Y, Li P. 2014. Growth, cadmium uptake and accumulation of maize (*Zea mays* L.) under the effects of arbuscular mycorrhizal fungi. Ecotoxicology 23: 1979-1986.

Liu R, Wang F. 2003. Selection of appropriate host plants used in trap culture of arbuscular mycorrhizal fungi. Mycorrhiza 13: 123-127.

Mathimaran N, Falquet L, Ineichen K, Picard C, Redecker D, Boller T, Wiemken A. 2008. Microsatellites for disentangling underground networks: Strain-specific identification of *Glomus intraradices*, an arbuscular mycorrhizal fungus. Fungal Genet Biol 45: 812-817.

Pagano MC, Gupta VK. 2016. Overview of the recent advances in mycorrhizal fungi. *In:* recent advances on mycorrhizal fungi (Pagano MC, Ed). Springer, Switzerland.

Pagano MC, Oehl F, Silva GA, Maia LC, Silva DK, Cabello MN. 2016. Advances in arbuscular mycorrhizal taxonomy. In: Pagano MC (ed). Recent Advances on Mycorrhizal Fungi. Springer, Switzerland.

PTFI. 2007. Report on the Implementation and Management of Environmental Monitoring. First Quarter, 2007. PT. Freeport Indonesia. Jakarta. [Indonesian]

Puradyatmika P, Prewitt JM. 2012. Tailings reclamation trials at PT. Freeport Indonesia in Mimika, Papua, Indonesia. Proceeding of the

Sevent International Conference on Mine Closure. Brisbane, 25-27 September 2012.
Sancayaningsih RP, Setiadi Y, Moeljopawiro S, Soedarsono J. 2000. Effect of density of propagules and application of the inoculum on the level of colonization and the dry weight of maize. Biologi 2 (10): 567-581. [Indonesian]
Sasvári Z, Magurno F, Galanics D, Hang TTN, Ha TTH, Luyen ND, Huong LM, Posta K. 2012. Isolation and identification of arbuscular mycorrhizal fungi from agricultural fields of Vietnam. Amer J Plant Sci 3: 1796-1801.
Schenck NC, Perez Y. 1990. A Manual for identification of vesicular arbuscular mycorrhizal fungi in VAM. 3rd Edition. University of Florida, Gainesville, Florida.
Schüßler, A., and Walker, C. 2010. The *Glomeromycota.* A species list with new families and new genera. With correction on July 2011. [online]. The Royal Botanic Garden Kew, Botanische Staatssammlung Munich, and Oregon State University.
Shirmohammadi E, Aliasgharzad N. 2013. Influence of *Glomus etunicatum* and *Glomus intraradices* fungi inoculums and micronutrients deficiency on root colonization and dry weights of tomato and sorghum in perlite bed culture. Afr J Biotechnol 12 (25): 3957-3962.
Smith FA, Smith SE. 2011. What is the significance of the arbuscular mycorrhizal colonisation of many economically important crop plants? Plant Soil 348: 63-79.
Smith SE, Read D. 2008. Mycorrhizal Symbiosis. 3rd ed. Elsevier, New York.
Souza T. 2015. Handbook of arbuscular mycorrhizal fungi. Springer, Switzerland.
Suharno, Kasiamdari RS, Soetarto ES, Sancayaningsih RP. 2016. Presence of arbuscular mycorrhizal fungi on fern from tailing deposition area of gold mine in Timika, Indonesia. Intl J Environ Bioremed Biodegrad 4 (1): 1-7.
Suharno, Sancayaningsih RP. 2013. Arbuscular Mycorrhizal Fungi: potential of heavy metals mikorizoremediasi technology in the rehabilitation of mining areas. Bioteknologi 10 (1): 31-42. [Indonesian]
Suharno, Sancayaningsih RP, Soetarto ES, Kasiamdari RS. 2014. The presence of arbuscular mycorrhizal fungi in the tailings of mining gold Timika as an attempt of environmentally friendly. J Manusia dan Lingkungan 21 (3): 295-303. [Indonesian]
Suharno, Tanjung RHR, Agustini V, Sufaati S. 2015. Diversity of arbuscular mycorrhizal fungi in pokem [*Setaria italica* (L.) Beauv.] plant rhizosphere with trapping methods. Jurnal Biologi Papua 7 (2): 68-77. [Indonesian]
Toju H, Sato H, Tanabe AS. 2014. Diversity and spatial structure of belowground plant-fungal symbiosis in a mixed subtropical forest of ectomycorrhizal and arbuscular mycorrhizal plant. PlosOne 9 (1): e86566. DOI:10.1371/journal.pone.0086566.
Treseder KK. 2013. The extent of mycorrhizal colonization of roots and its influence on plant growth and phosphorus content. Plant Soil 371: 1-13.
Upadhyaya H, Panda SK, Bhattacharjee MK, Dutta S. 2010. Role arbuscular mycorrhiza in heavy metal tolerance in plants: Prospect for phytoremediation. J Phytol 2 (7): 16-27.
Utobo EB, Ogbodo EN, Nwogboga AC. 2011. Techniques for extraction and quantification of arbuscular mycorrhizal fungi. Libyan Agric Res Cen J Intl 2 (2): 68-78.
Valsalakumar N, Ray JG, Potty VP. 2007. Arbuscular mycorrhizal fungi associated with green gram in South India. Agron J 99(5): 1260 - 1264.
Vierheilig H, Schweiger P, Brundrett M. 2005. An overview of methods for the detection and observation of arbuscular mycorrhizal fungi in roots. Physiologia Plantarum 125: 393-404.
Wang B, Qiu Y-L. 2006. Phylogenetic distribution and evolution of mycorrhizas in land plant. Mycorrhiza 16: 299-363.
Young JPW. 2012. A molecular guide to the taxonomy of arbuscular mycorrhizal fungi. New Phytol 193 (4): 823-826.
Yuan LH. 2015. Effects of arbuscular mycorrhizal fungi on *Elaeagnus mollis* Diels seedlings' growth and root. Intl J Agric Innov Res 3 (4): 2319-1473.
Yue H, Liu Y. 2016. Research progress on the process and mechanism of arbuscular mycorrhizal fungi colonizing roots. Agric Sci Technol 17 (2): 433 - 437.
Zangaro W, Ansanelo AP, Lescano LEAM, de Almeida Alves R, Rondina ABL, Nogueira MA. 2012. Infection intensity, spore density and inoculum potential of arbuscular mycorrhizal fungi decrease during secondary succession in tropical Brazilian ecosystem. J Trop Ecol 28: 453-462.
Zhang T, Tian CY, Sun Y, Bai DS, Feng G. 2012. Dynamics of arbuscular mycorrhizal fungi associated with desert ephemeral plants in Gurbantunggut Desert. J Arid Land 4 (1): 43-51.