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Endomycorrhizae and Soil Ameliorant Applications on Growth Performance of Red Jabon (*Anthocephalus macrophyllus*) in Lime Stone Post Mining Land of PT. Holcim Indonesia Tbk

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ABSTRACT

Limestone post-mining lands often exhibit low fertility levels due to limited macro and micronutrient availability, hindering the proper growth of red Jabon plants (*Anthocephalus macrophyllus*). Suppressive soil is rich in microbes can be created by inoculating endomycorrhizae (arbuscular mycorrhizal fungi, AMF) and applying soil ameliorants to promote healthier growth in these conditions. This study aimed to analyze the growth performance of red Jabon in limestone post-mining lands following the application of endomycorrhizal and ameliorants. Factorial with a completely randomized design was employed, comprising three factors: endomycorrhizal treatment (M0, M1, and M2), phosphate treatment (P0 and P1), and residual cement leaching waste (CLW) treatment (L0 and L1). With 12 treatment combinations and each treatment repeated 20 times, 240 test plants were examined. Results indicated that AMF and soil ameliorant treatments effectively improved *A. macrophyllus* growth. Single-factor treatments of AMF and phosphate significantly impacted plant height increase and chlorophyll content in leaves while exerting no significant effect on plant diameter increase and AMF colonization. The single-factor CLW treatment did not significantly affect any measured growth parameters. Interaction effects among treatments revealed a highly significant difference in chlorophyll content in leaves but no significant differences in plant height increase, diameter increase, or AMF colonization. Additionally, AMF, phosphate, and CLW treatments influenced the formation of wood anatomical tissue proportions (xylem, phloem, cambium, and pith). *A. macrophyllus* plants grown in limestone post-mining lands exhibited an average root anchor index (IJA) value (0.45-1.00) in the medium category, while the root grip index (ICA) value (0.79-0.87) was classified as low.

1. INTRODUCTION

Limestone post-mining land at PT. Holcim Indonesia has been identified as marginal land requiring improvement in macronutrient and micronutrient availability [1]. This constraint can hinder plant growth during revegetation efforts. Investigations on post-mining limestone land have detected endomycorrhizal species, including Glomus sp, Gigaspora sp, Acaulospora scrobiculata, *A.* tuberculata, *A.* foveata, and Scelerocystis sinuosa [2], which hold potential as inoculants for plant revegetation. It has been reported that arbuscular mycorrhizal fungi (AMF) significantly enhance the absorption of macronutrients (N, P, and K) and micronutrients (Mn, Zn, Cu, and Fe) under metal-contaminated conditions [3-6]. Additionally, AMF have been found to mitigate heavy metal uptake into plant tissues [5, 6]. This ability of AMF to facilitate nutrient absorption and suppress metal uptake can positively impact plant growth and biomass [7].

Inoculating plants with endomycorrhizae and supplementing with ameliorants represent promising alternative technologies for accelerating the revegetation process in limestone post-mining land. Ameliorants play a crucial role in revegetating post-mining land by providing nutrients and microbes, as well as improving soil structure, thereby stimulating root development during early growth stages [8].

Previous studies have investigated the effectiveness of AMF and soil ameliorant applications on the growth of red Jabon (*A. macrophyllus*) seedlings in greenhouse-scale limestone post-mining soil media. Generally, inoculation of *A. macrophyllus* seedlings with endomycorrhizae has been found to elicit a positive response in terms of growth and plant biomass enhancement. Consequently, further research is warranted to examine the growth performance of *A. macrophyllus* on limestone post-mining land following endomycorrhizae and ameliorant applications, with plants being directly situated on the post-mining land in the working area of PT. Holcim Indonesia.

In this study, the effectiveness of local AMF and soil ameliorant materials on plant growth performance, AMF root colonization, woody anatomical tissue formation, leaf chlorophyll content, and plant root development/geometry (root anchor index and root grip index) of *A. macrophyllus* planted on limestone post-mining land in the working area of PT. Holcim Indonesia Tbk will be investigated.

2. MATERIAL AND METHOD

2.1 Research time and location

The planting was carried out at the site of the post-mining limestone land of PT. Holcim Indonesia Tbk. The research was carried out from September 2016-March 2018. Then the data will be presented in 2023.

2.2 Research materials and equipment

The research materials used were aluminum foil, label paper, tissue rolls, H_2SO_4, 70% alcohol, 10% KOH, alkaline H_2O_2, 1% HCL, 0.05% trypan blue solution, and red Jabon plant seeds (*A. macrophyllus*) from the nursery from previous research, then planted on limestone post-mining land. Plants grown in the field are adjusted to each existing treatment. The tools used in the research were plant watering tools, Petri dishes, permanent markers, rulers, caliper, meter tape, tape measure, knife, hoe, shovel, scissors, object glass, cover glass, camera, microtome, microscope, and Chlorophyll Meter MC - 100.

2.3 Cultivation of red Jabon plants on limestone post-mining land

Mycorrhizal and non-mycorrhizal red Jabon seedlings six months old in the nursery were then transferred to the field. The average height of mycorrhizal seedlings planted was 35 cm, and the average height of non-mycorrhizal seedlings was 25 cm. Before planting, the land is cleared, the distance between the plants is measured, and the planting holes are made with 40 cm x 40 cm×40 cm. Plants are planted in holes, with a distance between planting holes of 3 m×3 m. The total combination of treatments was 12; each treatment was repeated 20 times, so the total number was 240 plants. The experimental research methods were used multi-level and multi-factor. Furthermore, maintenance activities include weeding, watering, and controlling pests and diseases. Observations and data collection were carried out once a month for 18 months.

2.4 Observed plant parameter

2.4.1 Increase in plant height

The plant height data were measured from the base of the stem to the highest growing point of the plant; measurements are made once a month for six months.

2.4.2 Increase in plant diameter

Plant diameter data were obtained by measuring the diameter of the plant at a distance of 1 cm from the root neck using calipers. Seedling diameter data were measured once a month for six months.

2.4.3 Percentage of root colonization by AMF

The percentage of mycorrhizal colonization was calculated using the Slide Method. Ten pieces of colored roots are taken randomly and then arranged on a glass object, as many as ten pieces. The colonization percentage is calculated based on the formula:

$$\% \, Colonization = \frac{Number\ of\ infected\ root\ pieces}{The\ total\ number\ of\ root\ pieces\ observed} \times 100\%$$

2.4.4 Proportion analysis of xylem, phloem, cambium, and pith

(1) Preparation of samples

The samples were taken from three seeds at each treatment and carried out the observation in the nursery. The samples were prepared by taking cross-sections or slices on the tiller stems. Slices were made at a height of 1 cm from the base of the root. Furthermore, the stem pieces were used as preparations; then, the preparations were photographed using a micro capture microscope regarding the Sass method (1958).

(2) Measurement

Microscopic observation of the test sample incisions was carried out by measuring the proportion of cells that make up the xylem, phloem, cambium, and pith tissues. The photos taken are then processed using the ImageJ software to calculate the proportion of each constituent cell in the seedling.

(3) The amount of leaves chlorophyll

The amount of leaves chlorophyll was measured using a Chlorophyll Meter MC 100. This tool is clipped to a leaves so that the leaves chlorophyll value will automatically appear. These measurements were made on the lower, middle, and upper leaves blades. Then the values are averaged. The mean value indicates the amount of leaves chlorophyll in each 1 cm^2 area. Furthermore, each plant's area and the number of leaves are calculated to analyze the amount of chlorophyll. The area of each leaves can be measured using millimeter block paper so that the total amount of chlorophyll for each plant can be determined.

(4) Root anchor index (IJA) and root grip index (ICA)

Each sample plant was excavated and exposed to its roots to be observed, and its dimensions were measured according to the variables used. There are several variables used in observing the development of plant roots, namely: 1) the position of root penetration in the soil layer, 2) root architecture, 3) Root Anchor Index (IJA), and 4) Root Grip Index (ICA).

The position of root penetration in the soil layer for each observed plant sample can refer to the classification of the type of root penetration proposed by Gray and Sotir [9].

Root architecture can also be observed based on the branching pattern when observing the position of root penetration in the soil layer. Architectural observations can refer to the theory [10] put forward, namely VH-type, H-type, V-type, R-type, and M-type.

In this study, the rooting variables observed were Root Anchor Index (IJA) and Root Grip Index (ICA). IJA is the ratio between the diameter of the vertical roots and the diameter of the stem. At the same time, ICA is the ratio between the diameter of the horizontal roots and the diameter of the stem [11]. Each plant sample was measured for horizontal root diameter, vertical root diameter, and stem diameter. A root is classified as a horizontal root if the angle between the root and the vertical plane is more than or equal to 45°, while if it is less than 45°, it is classified as a vertical root. Root diameter, horizontal and vertical, was measured at a distance of 1 cm from the base of the root. The stem diameter of the sample plants was measured at the height of 130 cm for the tree and pole level, 25 cm from the root base for the sapling level, and 10 cm from the root base for the seedling level. The classification (Table 1) and formula for calculating IJA and ICA is as follows:

$$\mathrm{IJA} = \sum \frac{dv^2}{db^2} \quad \mathrm{ICA} = \sum \frac{dh^2}{db^2}$$

Information:
IJA = Root Anchor Index
ICA = Root Grip Index
dv = Vertical Root Diameter
dh = Horizontal Diameter of Lateral Root
db = Stem Diameter

Table 1. Classification of IJA and ICA scores [10]

Classification	IJA	ICA
Low	< 0.1	< 1.5
Medium	0.1 – 1	1.5 – 3.5
High	> 1	> 3.5

2.5 Data analysis

Several stages of analysis were carried out in this study to improve the accuracy of the research results, namely the data normality test using the Shapiro-Wilk test; after the data was known to be expected, the homogeneity test of variance was carried out. The homogeneity test of variance was calculated using the Bartlett test. After that, an Analysis of Variance (ANOVA) was carried out, and further tests were carried out with the Duncans New Multiple Range Test (DMRT) methods at 95% [12] to see the effect of each treatment being tested.

3. RESULTS AND DISCUSSION

Based on the results of the analysis of diversity at the 5% level, it was found that mycorrhizal treatment and soil ameliorant material in the test plant (*A. macrophyllus*) planted on various characteristics of post-mining land had a highly significant different effect on some of the variables measured (Table 2).

Based on the results of statistical analysis, the planting of the test plant (*A. macrophyllus*) on limestone post-mining land with the single-factor treatment of AMF inoculation and the single-factor treatment of phosphate each had a very significant effect on the variable plant height increase and the amount of leaves chlorophyll. While on the variable, plant diameter increase and AMF colonization had no significant effect. The application of residual cement leaching waste (CLW) on plant growth of *A. macrophyllus* indicated not significant results in all measured growth parameters. Meanwhile, the effect of giving a single factor of residual CLW from diverse car castings on plant growth of *A. macrophyllus* showed no significantly different effect on all growth parameters measured. Furthermore, the interaction effect of each treatment had a highly significant different effect on the parameter of the amount of leaves chlorophyll. It showed no significant effect on the increase in plant height, diameter, and AMF colonization (Table 2).

Table 2. Results of the analysis of the variation in the effect of mycorrhizae and soil ameliorant materials on several observed variables on the test plant (*A. macrophyllus*) planted on the limestone post-mining land of PT. Holcim Indonesia Tbk

Area	Observed Variable	F-count						
		M	P	L	M*P	M*L	P*L	M*P*L
Limestone	Plant Height Increase	18.34**	4.76*	1.48tn	0.26tn	1.18tn	0.00tn	1.63tn
	Plant Diameter Increase	2.41tn	1.17tn	1.22tn	0.15tn	0.18tn	0.22tn	1.54tn
	AMF Colonization	1.69tn	0.22tn	0.22tn	0.71tn	0.92tn	2.71tn	1.50tn
	Leaves Chlorophyll	177.43**	67.31**	0.43tn	21.65**	52.89**	94.6**	14.32**

Note: tn = Not significantly different (P > 0.05); M = Arbuscular Mycorrhizal Fungi (AMF); * = Significantly different (P < 0.05); P = Phosphate; ** = Very significantly different (P < 0.01); L = Leaching waste of leftover cement castings from mixer car

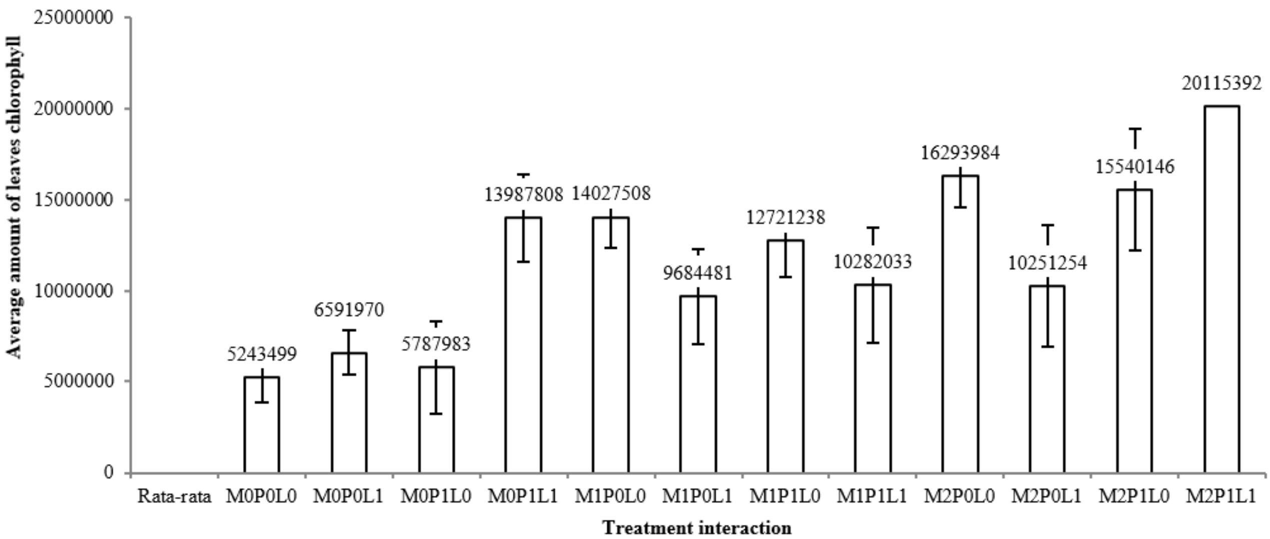

Figure 1. Effect of AMF, phosphate, and CLW treatment interaction on the average leaves chlorophyll content of 6-month-old *A. macrophyllus* plants in limestone post-mining soil

3.1 Growth performance of the red Jabon plant (*Anthocephalus macrophyllus*) in the limestone post-mining land of PT. Holcim Indonesia Tbk

3.1.1 Interaction effect of AMF, phosphate, and residual cement leaching waste (CLW) casting mixer car

The interaction effect of AMF, phosphate and CLW treatment on *A. macrophyllus* test plants for the parameter of leaves chlorophyll amount showed a statistically significant different effect. Based on Duncan's test results, the interaction of the three treatment factors: AMF, phosphate, and CLW, showed a very significant difference compared to the control. The highest mean value of leaves chlorophyll was found in the M2P1L1 treatment, 20115392. The lowest average value of leaves chlorophyll was found in the M0P0L0 treatment, namely 5243499 (Figure 1). The low average value of leaf chlorophyll in the M0P0L0 treatment (control) in terms of growth performance was also seen in the number of leaves produced and the size of the leaf area formed during the growth phase of *A. macrophyllus* when planted in the field.

The interaction effect of AMF and phosphate treatment on the test plants for the parameter of leaves chlorophyll amount statistically showed a significantly different effect on *A. macrophyllus* plants. Based on the results of Duncan's advanced test, the interaction of mycorrhizal and phosphate treatment that gave the highest average value of leaves chlorophyll in a row was the M2P0 treatment, namely 13272619 and the M2P1 treatment, namely 17827769. And the lowest average value of leaves chlorophyll was found in the M0P0 treatment, namely 5917735 (Figure 2).

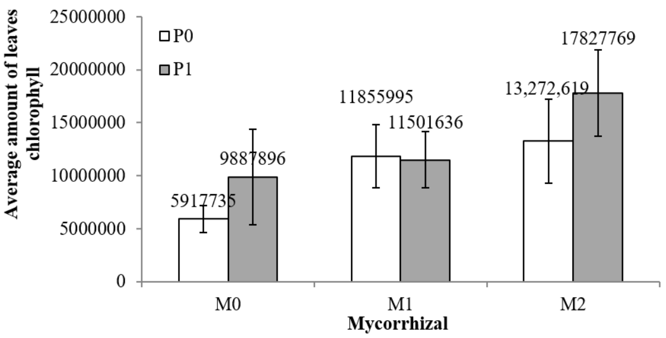

Figure 2. Effect of AMF and phosphate treatment interaction on the average leaves chlorophyll content of 6-month-old *A. macrophyllus* plants in post-mining limestone soil

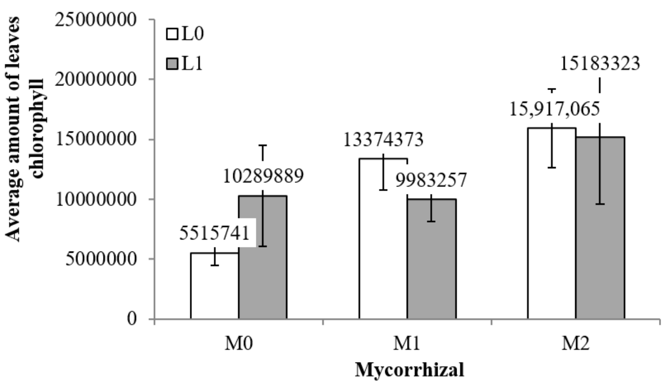

Figure 3. Effect of AMF and CLW treatment interaction on the average leaves chlorophyll content of 6-month-old *A. macrophyllus* plants in post-mining limestone soil

The interaction between AMF and CLW treatment showed significant results in increasing the amount of chlorophyll in *A. macrophyllus* leaves. The results of Duncan's further test of the interaction of FMA with CLW, which gave the highest average value of leaf chlorophyll, were the M2L0 treatment, namely 15917065, and the M2L1 treatment, namely 15183323. The lowest average value of leaf chlorophyll was found in the M0L0 treatment, namely 5515741 (Figure 3).

The interaction effect of phosphate treatment with CLW on *A. macrophyllus* test plants for the parameter of leaves chlorophyll amount showed a statistically significant different effect. Based on Duncan's test results, the interaction of phosphate treatment with CLW showed a significant difference compared to the control. The highest average value of leaves chlorophyll was in the P2L0 treatment, 15917065, and the P1L1 treatment, 14795087. The lowest average value of leaves chlorophyll was found in the P0L1 treatment, namely 8842568 (Figure 4).

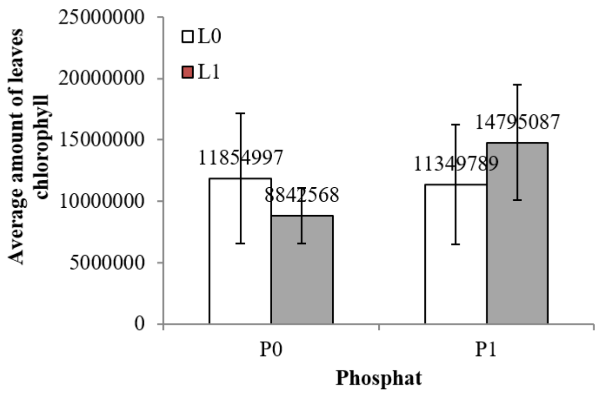

Figure 4. Effect of interaction between phosphate treatment and CLW on the average leaves chlorophyll content of 6-month-old *A. macrophyllus* plants in limestone post-mining soil

3.1.2 Effect of endomycorrhizal single-factor treatment

(1) Increase in plant height

The AMF treatment of type M2 had a very significant effect on treatment M0 (control) and no significant difference on treatment M1. The highest average increase in the height of *A. macrophyllus* plants was in the M2 treatment, which was 61.55 cm (Figure 5).

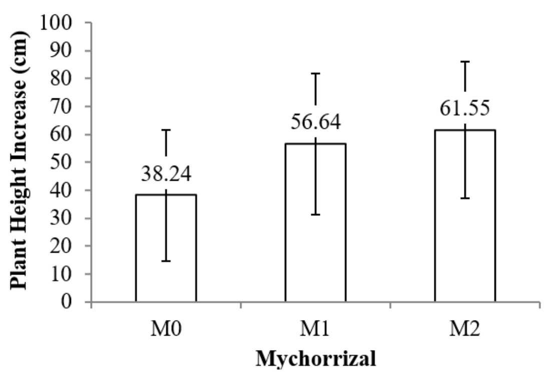

Figure 5. Effect of AMF treatment on the average height increase of 6-month-old *A. macrophyllus* plants on limestone post-mining soil

(2) Number of leaves chlorophyll

The treatment of AMF type M2 had a very significant effect on treatment M0 (control) and a significant difference on treatment M1. The highest average number of leaves chlorophyll for *A. macrophyllus* plants was in the M2 treatment, namely 15550194 (Figure 6).

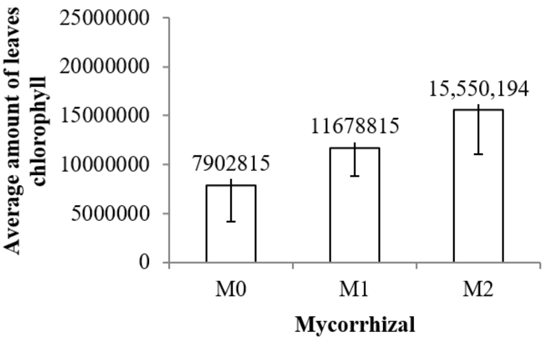

Figure 6. Effect of AMF treatment on the average leaves chlorophyll content of 6-month-old *A. macrophyllus* plants in limestone post-mining soil

3.1.3 Effect of single-factor phosphate treatment

(1) Increase in plant height

Phosphate treatment (P1) had a significantly different effect on the P0 treatment. The highest average increase in the height of *A. macrophyllus* was found in treatment P1, namely 56.38 cm (Figure 7).

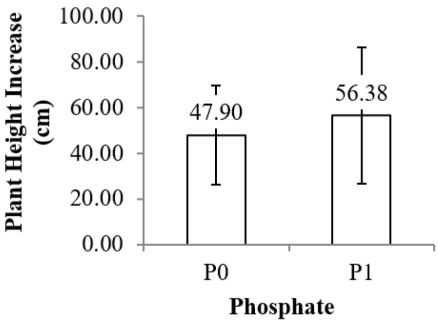

Figure 7. Effect of single factor phosphate treatment on the average height increase of 6-month-old *A. macrophyllus* plants on limestone post-mining soil

(2) Number of leaves chlorophyll

Phosphate treatment (P1) had a significantly different effect on the P0 treatment. The highest average number of leaves chlorophyll for *A. macrophyllus* plants was in treatment P1, namely 13072433 (Figure 8).

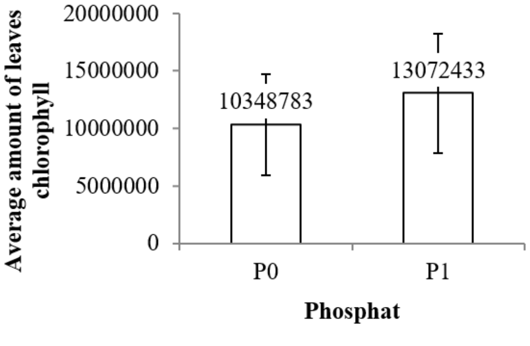

Figure 8. Effect of phosphate treatment on the average leaves chlorophyll content of 6-month-old *A. macrophyllus* plants in limestone post-mining soil

3.1.4 The proportion of xylem, phloem, cambium, and pith tissues

The results of observations of stem tissue of *A. macrophyllus* aged six months after planting on limestone post-mining land showed that AMF, phosphate, and CLW treatment affected the formation of the proportions of a wood anatomical tissue (Table 3). The effect of AMF treatment on the proportion of xylem tissue formation showed that the average proportion of xylem formed was more significant in the presence of AMF treatment compared to the control. Instead, the average proportion of phloem, cambium, and pith formed decreased.

Table 3. Proportions of xylem, phloem, cambium, and pith of 6-month-old *A. macrophyllus* plants planted on limestone post-mining soil

Variable	Proportion (%)			
	Xylem	Floem	Cambium	Pith
$M_0P_0L_0$	56.39	24.31	19.02	0.28
$M_0P_0L_1$	51.93	38.29	9.51	0.27
$M_0P_1L_0$	61.48	26.77	10.85	0.91
$M_0P_1L_1$	69.70	21.70	8.02	0.58
$M_1P_0L_0$	61.42	26.29	11.95	0.34
$M_1P_0L_1$	49.11	44.50	6.22	0.17
$M_1P_1L_0$	74.81	16.44	8.60	0.16
$M_1P_1L_1$	58.12	27.44	14.30	0.15
$M_2P_0L_0$	74.14	14.37	10.74	0.75
$M_2P_0L_1$	67.72	24.22	7.88	0.18
$M_2P_1L_0$	75.75	13.31	10.54	0.39
$M_2P_1L_1$	74.77	18.15	6.40	0.67

Note: The 1st column is a combination of treatments, columns 2, 3, 4, and 5 are the proportions of each vascular tissue.

The effect of phosphate treatment on the formation of xylem tissue showed that the average proportion of xylem and pith formed was more significant with the presence of phosphate treatment when compared to the control, and vice versa, the average proportion of phloem and cambium formed decreased. The effect of the CLW treatment on the proportion of xylem tissue formation and the proportion of pith formed showed that the mean proportion of xylem and pith formed was smaller with the CLW treatment compared to the control. Instead, the average proportion of phloem and cambium formed was more significant. The presence of AMF treatment could increase the percentage increase in the proportion of xylem by 22.08% when compared to no AMF treatment.

3.1.5 Root anchor index (IJA) and root grip index (ICA)

Table 4. Root anchorindex (IJA) and root grip index (ICA) values of *A. macrophyllus* on 6-month-old limestone post-mining soil

Variable	IJA	Category	ICA	Category
$M_0P_0L_0$	0.96	Medium	2.38	Medium
$M_0P_0L_1$	0.93	Medium	2.18	Medium
$M_0P_1L_0$	0.36	Medium	0.48	Low
$M_0P_1L_1$	0.04	Low	1.78	Medium
$M_1P_0L_0$	0.44	Medium	0.24	Low
$M_1P_0L_1$	0.66	Medium	0.13	Low
$M_1P_1L_0$	0.18	Medium	0.54	Low
$M_1P_1L_1$	0.25	Medium	0.35	Low
$M_2P_0L_0$	0.47	Medium	0.56	Low
$M_2P_0L_1$	0.46	Medium	0.29	Low
$M_2P_1L_0$	0.17	Medium	0.41	Low
$M_2P_1L_1$	0.53	Medium	0.13	Low

IJA is the ratio between the diameter of vertical roots and stem diameter. At the same time, ICA is the ratio between the diameter of horizontal roots and stem diameter [10]. IJA and ICA values can be used to describe the root distribution of a plant. The IJA and ICA values of 6-month-old *A. macrophyllus* plants planted on limestone post-mining soil are presented in Table 4.

Table 4 shows that in *A. macrophyllus*, the IJA value is included in the medium category, while the ICA value is included in the low category. The data shows that the *A. macrophyllus* plant can support the plant upright, but the plant's grip on the soil could be better. The *A. macrophyllus* plant does not yet have strong roots, so the plant is prone to toppling when disturbed (Figure 9).

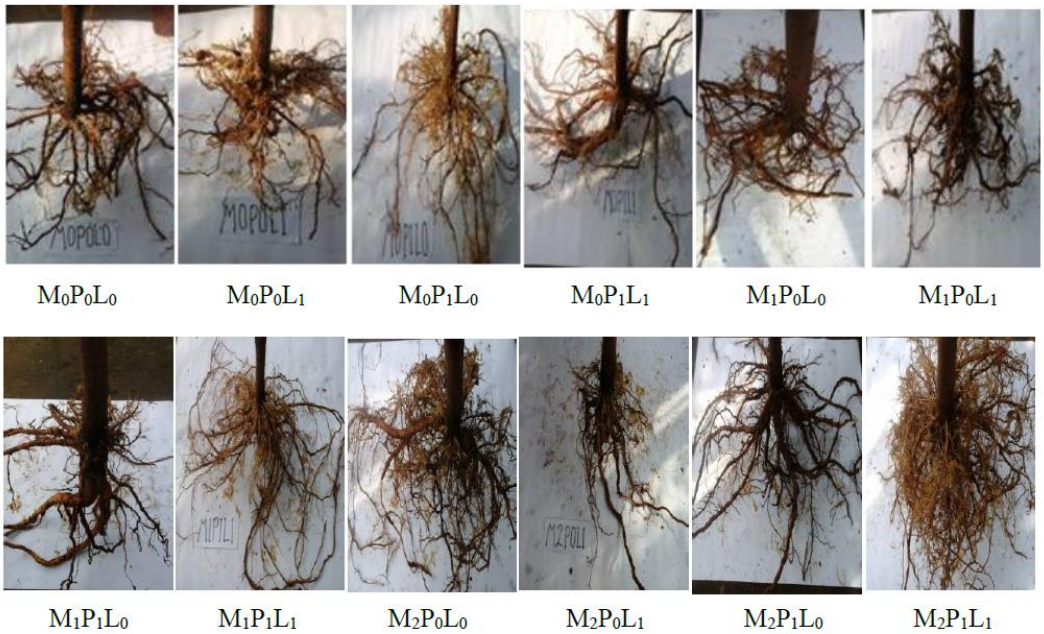

Figure 9. The form of plant roots of *A. macrophyllus* on limestone post-mining soil of PT. Holcim Indonesia Tbk

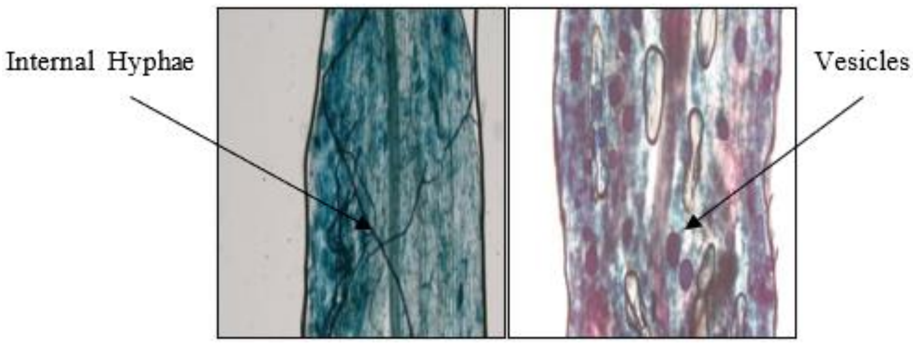

Figure 10. AMF colonization on the roots of *A. macrophyllus* aged 6 months after planting on limestone post-mining soil

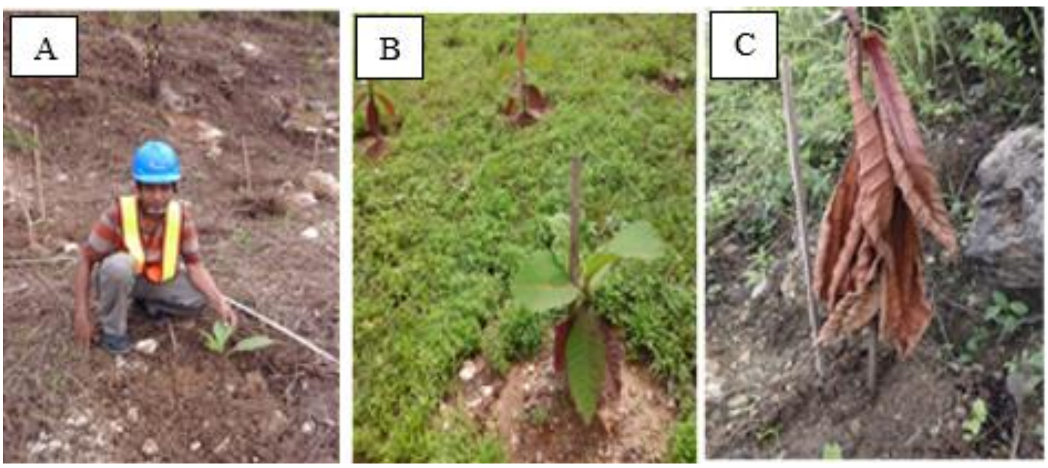

Figure 11. The condition of the new *A. macrophyllus* plants ready for planting on the limestone post-mining soil of PT. Holcim Indonesia Tbk (A), the condition of 1.5 months old plant (B), and the plant died due to ants attacking the stem (C)

Figure 12. The condition of *A. macrophyllus* plants at the age of 6 months after planting when viewed from several sides (A, B, and C) and crop yields on PT. Holcim Indonesia Tbk (treatment without AMF (D), treatment with AMF M1 (E), and treatment with AMF M2 (F))

4. DISCUSSION

4.1 Leaves chlorophyll content

Table 2 shows that the interaction of 2 factors or three treatment factors shows that the interaction only significantly affects the amount of leaves chlorophyll parameter. In contrast, the other parameters are not significantly different. In comparison, the single-factor treatment of AMF on *A. macrophyllus* plants planted on limestone post-mining land significantly affected the parameters of plant height increase. The percentage of AMF colonization and the amount of leaves chlorophyll were not significantly different from the increase in plant diameter. The effect of a single phosphate factor showed a significant effect on the height gain parameter. It was highly significant on the leaves chlorophyll amount parameter for the limestone planting area. In contrast, the influence of a single factor CLW showed no significant effect on all plant parameters.

Duncan's test results of the interaction of 3 treatment factors that gave the highest amount of leaves chlorophyll in the limestone area were the M2P1L1 treatment with a percentage increase of 283.63% compared to the control.The chlorophyll content of leaves of *A. macrophyllus* at the age of 6 months varied between AMF treatments. The highest amount of leaves chlorophyll of *A. macrophyllus* was found in the AMF treatment compared to the control. Leaves chlorophyll plays an essential role in the process of photosynthesis. The results of this study indicate that AMF symbiosis can support plant photosynthesis through the absorption of Mg as an essential component of leaves chlorophyll [13]. According to Widiastuti and Tahardi [14], AMF inoculation can increase magnesium uptake. Magnesium is the center of the chlorophyll molecule needed for photosynthesis and an activator of enzymes in photosynthesis and respiration [15, 16]. Thus, when magnesium levels increase, the essential processes it influences will also increase so plants can grow better than plants without AMF.

The amount of leaves chlorophyll of *A. macrophyllus* was significantly affected by AMF treatment (Table 2). The amount of leaves chlorophyll of *A. macrophyllus* inoculated with AMF was more than without AMF. The presence of chlorophyll in the leaves can take advantage of low light intensity. The mechanism of photosynthesis of individual plants and leaves is genetically controlled and can adapt to specific environments [17]. Furthermore, the exceptionally high light intensity can also cause excessive transpiration, causing plant stems to shorten, leaves to become thick with small sizes, increase the number of water-carrying tissues and decrease growth. The high light intensity can also increase leaves temperature so that it can cause stomata to close and decrease photosynthesis. Photosynthesis can produce enough carbohydrates to be sent to the root area and soil microbes. Plants with sufficient chlorophyll content can carry out the process of photosynthesis, which produces assimilation for further plant growth [18].

4.2 Plant height increase

Based on Duncan's test results, the single AMF treatment that increased the height of *A. macrophyllus* plants in the limestone area was the M2 treatment, with a percentage increase of 60.95% compared to the control (Figure 5). A single phosphate treatment with the highest increase in the height of *A. macrophyllus* plants in the limestone area was the P1 treatment, with a percentage increase of 17.70% compared to the control (Figure 7).

The presence of AMF in plants will increase P uptake [19, 20] due to external hyphae spreading widely into the soil [21]. In addition to P, the presence of AMF can also help increase calcium absorption [14], which plays a role in cell division and elongation [16]. The increase in plant height is also influenced by the activity of several hormones, such as gibberellin and cytokinins [22]. According to Anas and Santosa [23], AMF on plant roots can produce several growth regulatory hormones, such as gibberellin and cytokinins, so the presence of AMF on plant roots can play a role in the process of stem elongation. Thus the presence of AMF can increase the average increase in plant height better compared to the control; this is in line with the results of this study. Besides that, the role of AMF can increase the ability of plant life both in suitable habitats and habitats on marginal lands. Various types of AMF are also capable of symbiotic and play a role in stabilizing and absorbing heavy metals in polluted land [24]. Other studies have also shown that Kayu Kuku plants with local AMF applications are suitable for post-mining nickel land, planted in the shade and the open [25]. The growth performance of the red Jabon plant on the location of post-mining limestone land is quite good (Figure 13).

Figure 13. The performance of *A. macrophyllus* plants aged 18 months after planting on limestone post-mining land of PT. Holcim Indonesia Tbk

4.3 Plant diameter increase

The increase in plant diameter is a secondary growth strongly influenced by nitrogen [22]; with the presence of AMF, the N content also increases and ultimately plays a role in increasing the diameter. N uptake was more significant in mycorrhizal plants than in plants without mycorrhizal. It is evident from the tissue analysis results conducted in previous research on the *A. macrophyllus* test plant [2]. The increase in plant diameter is strongly influenced by photosynthesis and the availability of H_2O supply [26]. The presence of AMF can help increase the supply of H_2O with the help of external hyphae, which can enter into soil cavities smaller than the root diameter [27].

The presence of AMF is significant for the availability of nutrients such as P, Mg, K, Fe, and Mn for plant growth. It occurs through the formation of hyphae on the root surface, which function as root extensions, especially in areas with poor nutrient and water conditions. Mycorrhizal plants' roots increase nutrient absorption from the soil faster than non-mycorrhizal plants [28, 29]. The benefits of these mycorrhizal fungi are seen if the soil conditions are poor in nutrients or dry conditions, whereas in fertile soil conditions, the role of these fungi is insignificant [30, 31].

However, in field studies, the results were statistically different from the greenhouse scale, where the treatment effect showed no significant difference in the increase in plant diameter (Table 2). This study suggests that physiologically young plants are more likely to optimize vertical growth first rather than diameter growth.

4.4 AMF colonization percentage

The root system of a 6-month-old *A. macrophyllus* plant grown on limestone post-mining land has been colonized by AMF (Figure 10). In post-mining limestone land, AMF treatment was able to colonize plant roots ranging from 69.66-100%, while plant roots treated without AMF were also colonized by AMF, which was relatively high, ranging from 67.79-96.66%.

The presence of AMF structures on the roots of plants that were not treated with AMF is thought to be colonized by AMF, which naturally occurs in the post-mining land. AMF structures, such as internal and external hyphae and vesicles, can contribute to the growth of *A. macrophyllus* plants post-mining.

AMF treatment can increase plant growth compared to control (Table 2). The inoculated AMF formed a symbiosis with the root system of *A. macrophyllus*aged six months. The statement is evidenced by the discovery of AMF structures in plant roots, such as internal and external hyphae and vesicles. These AMF structures have different roles. External hyphae absorb nutrients and water needed by plants [32]. The existence of the AMF structure can help improve water and nutrient status and increase plant growth.

The increase in plant growth of mycorrhizal *A. macrophyllus* was strongly associated with the role of AMF in improving the nutrient status and water absorption (Figures 11 and 12). The host of AMF infection on the plant root system will produce hyphae intensively so that mycorrhizal plants can increase their capacity to absorb nutrients and water [32, 33]. Referring to the results of several studies conducted by other researchers, it was reported that AMF was significantly able to help the absorption of macronutrients (N, P, and K) and micro (Mn, Zn, Cu, and Fe) under conditions of metal contamination [3-6]. In addition, AMF also reduces the entry of heavy metals into plant tissues [5, 6]. The ability of AMF to absorb nutrients and suppress metal uptake into plant tissues can have a positive impact on plant growth and biomass [34].

However, what is no less critical in transferring nutrients from AMF to the roots is the efficiency of AMF [32]. With a high percentage of colonization but low efficiency, the presence of AMF will only help the plants a little. The statement follows [35] which shows that the significant value of colonization in the roots is only sometimes followed by better plant growth. Plants associated with AMF are determined by the size of the percentage of AMF colonization in the roots and the efficiency and effectiveness of AMF in transferring nutrients to the root cells.

The size of the percentage of AMF colonization in the roots only partially reflects its ability to assist the absorption of nutrients for plants [36]. Barber [19] stated that the internal hyphae of AMF in the roots transfer nutrients from the external hyphae into the root cells of the host plant. However, the structure of AMF, which is no less critical in this process, is the external hyphae responsible for absorbing nutrients from the soil. Calculating the percentage of AMF colonization in the roots only determines whether or not AMF colonizes the roots and passes external hyphae scattered in the soil [36].

There is a considerable difference between AMF in the colonization ratio of internal hyphae (in the roots) to external hyphae in the soil. A large percentage of AMF colonization in roots does not necessarily have a lot of external hyphae and vice versa [36]. Thus, a close relationship between the percentage of AMF colonization and plant growth parameters can be obtained if an overall calculation is carried out on AMF colonization in the roots and those widely distributed in the soil. In addition, AMF efficiency and effectiveness tests involving compatibility at the molecular level with host plants need to be carried out. With the association of AMF with *A. macrophyllus* test plants, this study shows that AMF can be used as an alternative to increasing the growth of forestry plants on post-mining land and marginal land, which has so far been less popular.

4.5 *Anthocepalus macrophyllus* Wood Anatomy Tissue Proportions

Observation of stem tissue development of *A. macrophyllus* was carried out at the age of 6 months after planting in the field. Observations were made by cutting across the stem of the plant. Generally, the stem consists of two parts, the outside and the middle. The outer part of the stem consists of a protective layer, namely the epidermis and cortex. The middle part (stele) consists of the inner xylem and outer phloem; there is cambium tissue between the two tissues, and the stele's innermost part is called the pith [37].

The results of observations of stem tissue of *A. macrophyllus* aged six months after planting on limestone post-mining land showed that AMF, phosphate, and CLW treatment affected the formation of the proportions of a wood anatomical tissue (Table 3). The effect of AMF treatment on the proportion of xylem tissue formation showed that the average proportion of xylem and pith formed was more significant in the presence of AMF treatment compared to the control. Conversely, the average proportion of phloem and cambium formed was smaller. The effect of phosphate treatment on the formation of xylem and pith tissue showed that the average proportion of xylem and pith formed was more significant with phosphate treatment compared to the control, and vice versa, the average proportion of phloem and cambium formed decreased. The effect of the CLW treatment on the proportion of xylem tissue formation and the proportion of pith formed showed that the mean proportion of xylem and pith formed was smaller with the CLW treatment compared to the control. Conversely, the average proportion of phloem and cambium formed was more significant.

The anatomical structure of wood includes shape, size, nature, function, proportion, and arrangement of the cells that make up the wood. In contrast, wood properties are nothing but a measure of the quality or description of the wood itself. The properties of wood are determined and depend on its anatomical structure. In other words, the nature of wood is inherent in the structure of the cells that make up the wood. The relationship between the anatomical structure of wood and the nature, use, and processing in the context of optimal and proper utilization of wood is very close. The properties of wood depend heavily on its anatomical structure and are inherent in the structure of the cells that make up the wood. The overall properties of wood together will determine its use, and the processing that will be applied must be adapted to the anatomical structure of the wood [38].

4.6 Root anchor index (IJA) and root grip index (ICA) values

IJA (Index of Root Anchoring) is the ratio between the diameter of vertical roots and stem diameter. At the same time, ICA (Index of Root Binding) is the ratio between the diameter of horizontal roots and stem diameter [11]. IJA and ICA values can be used to describe the root distribution of a plant.

Table 4 shows that the *A. macrophyllus* plants planted on limestone post-mining land have IJA values in the medium category, while ICA values are in a low category. It indicates that the *A. macrophyllus* plant can support the plant upright, but its grip on the soil is not good. The *A. macrophyllus* plant does not yet have strong roots, so the plant is prone to toppling when disturbed. One of the essential root characteristics of the growth and control of landslides, such as the condition of the limestone post-mining land, is that it can be seen from the ability of roots to penetrate the soil layer. Vertical and horizontal roots that can penetrate deeper soil layers will provide stability to plant growth. The role of the roots will be more effective if the roots cut through the landslide area on unstable soils [39]. Reubens et al. [40] also stated that roots penetrating deep soil layers would provide good shear strength and bending effects. The result will support the soil layer above it and is quite effective in controlling landslides so that the roots' grip on the soil is firmer than before. Horizontal roots that spread in the surface layer of the soil will grip the soil, and vertical roots as anchors will support the tree upright so that the movement of the soil mass does not easily topple it. Plant vigor is one factor that affects a plant species's survival. The level of plant sturdiness can be determined by dividing the plant height by the plant diameter [41]. A high value of toughness will indicate a low survivability due to an unequal ratio between height and diameter. At the location of the limestone post-mining land, it can be seen that the ICA value is relatively low, so the grip of the roots of the *A. macrophyllus* plant on the soil looks not good; this is suspected because the soil layer/soil solum on the limestone post-mining land tends to be very thin so that the roots do not grip the ground less.

5. CONCLUSIONS

AMF treatment and soil ameliorant effectively increased the growth of *A. macrophyllus* plants grown on limestone post-mining land of PT. Holcim Indonesia Tbk. The treatment of single-factor AMF and single-factor phosphate significantly affected the variable plant height gain and the amount of leaves chlorophyll. They did not significantly affect the variable plant diameter increase and AMF colonization. The single AMF treatment that gave the highest increase in the height of A. *macrophyllus* plants was the M2 treatment, with a percentage increase of 60.95% when compared to the control. The single phosphate treatment that gave the highest increase in the height of A. *macrophyllus* plants was the P1 treatment, with a percentage increase of 17.70% compared to the control. AMF treatment was able to colonize the roots of A. *macrophyllus* around 69.66-100%.The effect of a single CLW factor on plant growth of *A. macrophyllus* showed no significantly different effect on all measured growth parameters. The interaction effect of each treatment had a highly significant effect on the parameter of leaves chlorophyll amount. It was insignificant in plant height increase, diameter increase, and AMF colonization.

The treatment of AMF, phosphate, and CLW affected the formation of the proportions of a wood anatomical tissue (xylem, phloem, cambium, and pith). *A. macrophyllus* plants planted on limestone post-mining land had an average root anchor index (IJA) value (0.45-1.00) which was in the medium category, while the root grip index (ICA) value (0.79-0.87) was in a low category.

REFERENCES

[1] Asmarahman, C., Budi, S.W., Wahyudi, I., Santoso, E. (2017). Species diversity of undergrowth vegetation in former silica and limestone mining land at pt. holcim indonesia tbk, west java. Indonesia. International Journal of Sciences Basic and Applied Research, 31(1): 206-223.

[2] Asmarahman, C., Budi, S.W., Wahyudi, I., Santoso, E. (2018). Identifikasi mikroba potensial fungi mikoriza arbuskula (FMA) pada lahan pascatambang PT. holcim indonesia tbk. Jurnal Pengelolaan Sumberdaya Alam dan Lingkungan, 8(3): 279-285. https://doi.org/10.29244/jpsl.8.3.279-285

[3] Leyval, C., Turnau, K., Haselwandter, K. (1997). Effect of heavy metal pollution on mycorrhizal colonization and function: Physiological, ecological and applied aspects. Mycorrhiza, 7(3): 139-153. https://doi.org/10.1007/s005720050174

[4] Wang, F., Lin, X., Yin, R., Wu, L. (2006). Effects of arbuscular mycorrhizal inoculation on the growth of Elsholtzia splendens and Zea mays and the activities of phosphatase and urease in multi-metal-contaminated soil under unsterilized condition. Applied Soil Ecology, 31(1-2): 110-119. https://doi.org/10.1016/j.apsoil.2005.03.002

[5] Vivas, A., Biro, B., Nemeth, T., Barea, J.M., Azcon, R. (2006). Nickel-tolerant brevibacillus brevis and arbuscular mycorrhizal fungus can reduce metal acquisition and nickel toxicity effect in plant growing in nickel supplemented soil. Soil Biology and Biochemistry, 38(9): 2694-2704. https://doi.org/10.1016/j.soilbio.2006.04.020

[6] Chen, B.D., Zhu, Y.G., Duan, J., Xiao, X.Y., Smith, S.E. (2007). Effects of the arbuscular mycorrhizal fungus Glomus mosseae on growth and metal uptake by four plant species in copper mine tailings. Environmental Pollution, 147(2): 374-380. https://doi.org/10.1016/j.envpol.2006.04.027

[7] Tuheteru, D.F., Husna, H. (2011). Pertumbuhan dan biomassa Albizia saponaria yang diinokulasi Fungi Arbuskula Mikoriza Lokal Sulawesi Tenggara. Jurnal Silvikultur Tropika, 2(3): 143-148. https://doi.org/10.29244/j-siltrop.2.3.%25p

[8] Delvian. (2004). Aplikasi Cendawan Mikoriza Arbuskula dalam Rekalmasi Lahan Kritis Pasca Tambang. Universitas Sumatera Utara, Medan.

[9] Gray, D.H., Sotir, R.B. (1996). Biotechnical and soil bioengineering slope stabilization: A practical guide for erosion control. John Wiley & Sons.

[10] Yen, C.P. (1987). Tree root patterns and erosion control. In Proceedings of the international workshop on soil erosion and its countermeasures. Soil and Water Conservation Society of Thailand, Bangkok, pp. 92-111.

[11] Hairiah, K., Widianto, D.S. (2008). Adaptasi dan Mitigasi Pemanasan Global: Bisakah agroforestri mengurangi resiko longsor dan emisi gas rumah kaca. Kumpulan makalah INAFE. UNS. Surakarta.

[12] Gomez, K.A., Gomez, A.A. (1995). Prosedur statistik untuk penelitian pertanian Edisi Kedua. Sjamsuddin E, Baharsjah JS, penerjemah. UI pr. Terjemahan dari: Statistical Procedures for Agricultural Research, Jakarta .

[13] Marschner H, Dell B. (1994). Nutrient uptake in mycorrhizal symbiosis. Plant Soil, 159(1): 89-102. https://doi.org/10.1007/BF00000098

[14] Widiastuti, H., Tahardi, J.S. (1993). Effect of VAM Inoculation on the growth and nutrient uptake of micropropogation oil palm. Menara Perkebunan, 61(3): 56-60.

[15] Bonner, J., Galston, A.W. (1952). Principles of Plant Physiology. W.H. Freeman and Company, San Francisco, US.

[16] Gardner, F.P., Pearce, R.B., Mitchell, R.L. (1991). Fisiologi Tanaman Budidaya. UI Press, Jakarta.

[17] Daniels, B.A.H., Trappe, J.M. (1980). Factors affecting spora germination of the VAM fungus, Glomus epigaeus. Mycologia, 72(3): 457-463. https://doi.org/10.1080/00275514.1980.12021207

[18] Wijaya, K.A. (2008). Nutrisi Tanaman Sebagai Penentu Kualitas dan Resistensi Alami Tanaman. Penerbit Prestasi Pustaka, Jakarta.

[19] Barber, S.A. (1984). Soil Nutrient Bioavailability a Mechanistic Approach. John Wiley and Sons, Inc, Canada (CA).

[20] Agrios, G.N. (1988). Plant Pathology. Third edition. San diego, Academic Press, Inc, California, US.

[21] Redhead, J.F. (1979). Soil Mycorrhiza in relation to soil fertility and productivity. Soils research in agroforestry: Proceedings of an expert consultation held at ICRAF in Nairobi.

[22] Goldsworthy, P.R., Fisher, N.M. (1992). Fisiologi Tanaman Budidaya Tropik. Gadjah Mada University Press, Yogyakarta, ID.

[23] Anas, I., Santosa, D.A. (1993). Mikoriza Vesikular-Arbuskular. Di dalam: Anas I, editor. Pupuk Hayati (Biofertilizer). Jurusan Tanah, Fakultas Pertanian. IPB, Bogor, ID.

[24] Suharno, Ningsih, S.P. (2013). Fungi Mikoriza Arbuskula: Potensi teknologi mikoriza remediasi logam berat dalam rehabilitasi lahan tambang. Bioteknologi, 10(1): 31-42. https://doi.org/10.13057/biotek/c100104

[25] Husna. (2015). Potensi fungi mikoriza arbuskula (FMA) lokal dalam konservasi ex-situ jenis terancam punah kayu kuku [Pericopsis mooniana (Thw.) Thw.] [Dissertation]. Institut Pertanian Bogor, Bogor.

[26] Kramer, P.J., Kozlowski, T.T. (1960). Physiology of Trees. McGraww Hill. Book Company, USA.

[27] Fakuara, Y. (1994). Peranan mikoriza dalam peredaran hara dan peningkatan kualitas bibit. Laporan Program Penelitian Biologi dan Bioteknologi mikoriza. Semeo Biotrop, Bogor.

[28] Abbott, L.K., Robson, A.D. (1982). The role of VA mycorrhizae fungsi in agriculture and the selection of fungi for inoculation. Australian Journal of Agricultural Research, 33(2): 389-408. https://doi.org/10.1071/AR9820389

[29] Abbott, L.K., Robson, A.D. (1984). The Effect of Mycorrhizae on Plant Growth. Boca Raton. CRC Press

[30] Setiadi, Y. (2001). Peranan mikoriza arbuskula dalam reboisasi lahan kritis di Indonesia. makalah seminar penggunaan CMA dalam sistem pertanian organik dan rehabilitas lahan. Bandung.

[31] Lakitan, B. (2000). Dasar-dasar fisiologi tumbuhan. PT. Raja Grafindo Persada, Jakarta.

[32] Smith, S.E., Read, D.J. (2008). Mycorrhizal symbiosis. Third ed. Academic Press, New York.

[33] Brundrett, M., Bougher, N., Dell, B., Grove, T., Majalaczuk. (1996). Working With Mycorrhizas in Forestry and Agriculture. Australian Centre for International Agriculture Research, Canberra.
[34] Tuheteru, D.F., Husna, Alimuddin, L.D. (2011). Respon pertumbuhan dan tingkat ketergantungan Albizia saponaria (Lour.) Miq terhadap fungi mikoriza arbuskula lokal Sulawesi tengara. Biota, 16(2): 252-261. https://doi.org/10.24002/biota.v16i2.107
[35] Setyaningsih, L. (2007). Pemanfaatan cendawan mikoriza arbuskula dan kompos aktif untuk meningkatkan pertumbuhan semai mindi (Melia azedarach Linn) pada media tailing tambang emas Pongkor [Thesis]. Institut Pertanian Bogor, Bogor.
[36] Hart, M.M., Reader, R.J. (2002). Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. New Phytologist, 153(2): 335-344. http://dx.doi.org/10.1046/j.0028-646X.2001.00312.x
[37] Pandit, I.K.N., Hidayat, R. (2002). Anatomi Kayu. Pengantar Sifat Kayu Sebagai Bahan Baku. Institut Pertanian Bogor, Bogor.
[38] Wahyudi, I. (2013). Hubungan Struktur Anatomi Kayu Dengan Sifat Kayu, Kegunaan dan Pengolahannya. Makalah. Litbang Anatomi Kayu Indonesia, Bogor.
[39] Hardiyatmo, H.C. (2006). Penanganan tanah longsor dan erosi. Gadjah Mada University Press, Yogyakarta.
[40] Reubens, BJ., Poesen, F., Danjon, G., Geudens, Muys, B. (2007). The role of fine and coarse roots in shallow slope stability and soil erosion control with a focus on root system architecture: A review. Trees, 21(4): 385-402. https://doi.org/10.1007/s00468-007-0132-4
[41] Jayusman. (2005). Evaluasi keragaman genetik bibit surian di persemaian. Pusat Penelitian dan Pengembangan Hutan Tanaman, Yogyakarta.