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Research Article
Potential of Native Plant Species and Indigenous Arbuscular Mycorrhizal Fungi in Post-mining Land Recovery and Revegetation

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Abstract
Background and Objective: Rehabilitation and revegetation is a post-mining activity to restore the condition of the mined land and be able to function optimally. The technology of using arbuscular mycorrhizal fungi (AMF) has been attempted to improve plant growth, especially on marginal and critical land such as mined land. This study was carried out to determine the potential of some local plants, Andalas (*Morus macroura*) and Indarung (*Trema orientalis*) that inoculated with indigenous AMF for land recovery and revegetation. **Materials and Methods:** The study followed completely randomized design (CRD). The treatments were indigenous AMF isolates from the limestone post-mining land of PT. Semen Padang, Control/without mycorrhiza, *Glomus* sp., *Gigaspora* sp. and *Acaulospora* sp. **Results:** Inoculation of indigenous AMF was able to improve the plants growth on post-mining soil and showed high percentage of roots infection. Inoculation of indigenous AMF increased the organic matter and organic carbon of post-mining soil. **Conclusion:** *Acaulospora* sp., were the most suitable type of indigenous AMF for the growth of Andalas (*M. macroura*) and Indarung (*T. orientalis*) seedlings that were able to increase plant growth better than controls and showed a high-quality index of seedlings.

Key words: Rehabilitation, revegetation, indigenous mycorrhizal, native plant, land recovery, post-mining land

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

One of the biggest types of mining activities in West Sumatra is limestone mining which is the basic raw material in cement production. Post mining is an important activity after mine closure. Mining will have a negative impact on the environment such as loss of vegetation and microflora. These conditions lead to a decline in land productivity. Mining activities can also lead to an increase of certain elements that are toxic to landfills, droughness and low level of nutrients for plant growth[1]. The process of restoring post-mining land and making the former mining land safe and useful again requires an appropriate technology. To restore the condition of the land damaged by mining and be able to function optimally again can be done through rehabilitation and revegetation. Pujiharta *et al.*[2] stated that to improve the success of the recovery of post-mining land, it is necessary to select the type of plant using appropriate microorganisms and be associated with these plants to restore and increase soil fertility.

The technology of using arbuscular mycorrhizal fungi (AMF) has been attempted to improve plant growth, especially on marginal and critical land. Mycorrhizae as soil microorganisms are key in facilitating the absorption of nutrients by plants. Chairul *et al.*[3] studied that there were several types of indigenous mycorrhizae on limestone post-mining land of PT. Semen Padang, such as *Glomus*, *Acaulospora* and *Gigaspora* with different levels of spore abundance. Glomus is the dominant taxa with the highest abundance of spore (38/50 g of soil).

Criteria for selecting plant species used in post-mining land rehabilitation efforts are having high adaptability, fast-growing plants, well-known of silvicultural techniques, availability of plant material and its symbiosis with a microorganism[4]. The choice of plant species as an effort to conduct land rehabilitation must follow the rules issued by the Ministry of Forestry, which requires the planting of local species[5]. Plants used in the revegetation process must be in line with the objectives of ecosystem restoration activity, which is protective, productive and conservative. The success of revegetation in mining land is largely determined by soil fertility, physical, chemical and biology properties[6].

Andalas (*Morus macroura*) is one of the native plants species and widely known as the West Sumatra flora mascot. This type of plant economically has high-quality of wood, resistant to termites and usually used for material as the pole in "Rumah Gadang", the traditional building of Minangkabau community and medically containing phenol compounds[7,8]. While the type of Indarung (*Trema orientalis*) is a local plant belonging to fast-growing plants. It has the potential to be used as a pioneer plant in revegetation[9]. Indarung is also known as various plants used in paper and pulp production and soil conservation because it can grow quickly on critical land[10]. Based on the description above, a study has been carried out to determine the potential growth of local plants inoculated with indigenous arbuscular mycorrhizal fungi for revegetation as an effort to restore the former of limestone mining land.

MATERIALS AND METHODS

The study was conducted from November, 2018 to May, 2019 at the Greenhouse and the Research Laboratory of Plant Physiology, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang. The study followed a completely randomized design (CRD). The treatments applied were indigenous AMF isolates from the former mining land of PT. Semen Padang with 4 treatments (Control/without mycorrhiza, *Glomus* sp., *Gigaspora* sp. and *Acaulospora* sp.) and 6 replications.

Seedlings preparation: *Morus macroura* and *T. orientalis* seedlings were propagated using shoot cuttings method. The shoot were soaked in a 100 ppm IBA for 15 min and transferred into the planting media with mixed compost and sand, ratio of 1:1. 2-months- old seedlings are transferred to a polybag that has been filled with treatment media (limestone post-mining soil).

Trapping of indigenous AMF: AMF trapping was done using the "open pot culture method" according to Brundrett *et al.*[11]. Maize was used as the host plant for AMF trapping and indigenous AMF were collected from post-mining soil of PT. Semen Padang[3]. The culture was placed in a greenhouse and maintained for 2 months. AMF culture was watered and fertilized with $\pm$10 mL of red hyponex solution with a concentration of 1 g L^{-1} of water every 3 days. After 2 months, the host plants for trapping were cutted and dried up. Furthermore, the spores were harvested.

Inoculation: The indigenous AMF isolate was inoculated by sprinkling the AMF inoculant to the area around the plant's root according to the treatment given and covered again with planting media, the planted seedlings were placed in a greenhouse and maintained about 4-5 months.

Observation parameters

Soil analysis: Soil chemical analysis was measured by taking soil samples before and after treatment. This observation is carried out by measuring pH, organic C, N-total and available P, Ca exchanged, Mg exchanged and K exchanged.

Plant growth parameters: Andalas and Indarung plant growth parameters include increasing plant height, increasing the number and area of leaves, increasing of diameter, number and length of roots, fresh weight and dry weight of plants were measured.

AMF infection (%): The calculation of AMF infections with plants root based on Sieverding[12] technique (Table 1).

The percentage of infections was calculated using the equation:

$$\text{Infection (\%)} = \frac{\text{Number of infected roots}}{\text{Number of observed roots}} \times 100$$

Chlorophyll analysis (mg L^{-1}): Chlorophyll a, b and total were determined using spectrophotometric methods. The fresh samples of leaf about 0.25 g were cut into small pieces and added with 25 mL of 80% acetone, then crushed until the chlorophyll was dissolved which was indicated by the presence of white leaf pulp. The sample was filtered using filter paper and the extract solution was added with 80% acetone until the volume reaches 25 mL. The extract solution was put into a centrifuge tube and centrifuged at 2500 rpm for 15 min. Supernatant was inserted into the cuvette and OD (Optical Density) was determined using the following equation according to Arnon[13]:

$$\text{Chlorophyll a (mg L}^{-1}) = \{(12.7\times OD_{663})\text{-}(2.69\times OD_{645})\}\times\frac{V}{1000\times W}$$

$$\text{Chlorophyll b (mg L}^{-1}) = \{(22.9\times OD_{645})\text{-}(4.68\times OD_{663})\}\times\frac{V}{1000\times W}$$

$$\text{Total chlorophyll (mg L}^{-1}) = \{(20.2\times OD_{645})+(8.02\times OD_{663})\}\times\frac{V}{1000\times W}$$

Table 1: Criteria for the effectiveness of the percentage of AMF infection

Infections (%)	Criteria
76-100	Very high
51-75	High
27-50	Medium
6-26	Low
0-5	Very low

where, OD is the optical density on a spectrophotometer, V is the 80% acetone volume used to extract chlorophyll (mL) and W is the fresh weight of extracted plant tissue (g).

Dickson quality index (DQI): The seedlings quality index was determined using the equation according to Dickson *et al.*[14]:

$$DQI = \frac{TDW}{\left(\frac{SDW}{RDW}\right)+\left(\frac{SH}{SBD}\right)}$$

where, TDW is the total dry weight (g), SH is the shoot height (cm), SDW is the shoot dry weight (g), SBD is the stem base diameter (mm) and RDW is the root dry weight (g).

Statistical analysis: Data analysis was carried out on the average increase in plant height, plant diameter, number of leaves, total leaf area, number and length of roots, fresh and dry weight and chlorophyll content using analysis of variance. The significantly different effect was analysis with Duncan's new multiple range test (DNMRT) at $p<0.05$ using SPSS statistics program. Analysis of soil chemistry, the percentage of AMF colonization and the seedlings quality index were descriptively presented in the form of tables.

RESULTS

Soil analysis: Limestone post-mining has poor quality of soil. The limestone post-mining soil has nutrient content which is classified as very low-low, only K and C/N are classified as high with acidic soil pH and low cation exchange capacity (Table 2). Inoculation of indigenous AMF was able to improve soil organic material and carbon.

***Morus macroura* growth response to indigenous AMF inoculation:** The treatment of AMF showed significantly different effect on the number of leaves, leaf area and number of roots of *M. macroura* (Table 3) with the best results obtained on the type of *Acaulospora* sp. although the other growth parameters did not show significantly different results, *Acaulospora* sp. showed higher average results compared to other treatments. According to Table 3, clearly showed that AMF has very high criteria of roots colonization in *M. macroura*. AMF infection in plant roots of *M. macroura* was identified by the formation of hyphae structures and vesicles (Fig. 1). The treatment of indigenous AMF influenced the quality index of *M. macroura* seedlings with the

Table 2: Soil chemical analysis before and after treatment with AMF inoculants

		After treatment		
Elements of analysis	Before treatment	*Glomus* sp.	*Gigaspora* sp.	*Acaulospora* sp.
pH	6.05 (somewhat sour)	5.96 (somewhat sour)	5.69 (somewhat sour)	6.02 (somewhat sour)
KCl	5.45	5.37	5.37	5.26
N-total (%)	0.109 (low)	0.043 (very low)	0.046 (very low)	0.058 (very low)
P-available (ppm)	1.025 (very low)	0.548 (very low)	0.679 (very low)	0.421 (very low)
K, dd (me/100 g)	0.606 (high)	0.516 (medium)	0.476 (medium)	0.499 (medium)
Ca, dd (me/100 g)	1.182 (very low)	0.801 (very low)	0.733 (very low)	0.723 (very low)
Mg, dd (me/100 g)	0.726 (low)	0.758 (low)	0.813 (low)	0.983 (low)
Organic-C (%)	1.851 (low)	2.039 (medium)	2.288 (medium)	2.443 (medium)
B organic (%)	3.189	3.515	3.934	4.211
C/N	16.981 (high)	47.418 (high)	49.739 (high)	42.121 (high)
Water content (%)	11.645	2.274	2.439	3.096
Water content correction (%)	1.116	1.023	1.024	1.031

Table 3: *Morus macroura* growth response to indigenous AMF inoculation

	Treatments			
Parameters	Control	*Glomus* sp.	*Gigaspora* sp.	*Acaulospora* sp.
Plants height (cm)	27.03^{a}	28.27^{a}	28.53^{a}	31.65^{a}
Leaf number	4.67^{a}	7.17^{b}	9.50^{c}	12.33^{d}
Diameter (mm)	1.43^{a}	2.21^{a}	2.22^{a}	2.40^{a}
Leaf area (cm^2)	22.91^{a}	30.59^{b}	30.52^{b}	32.92^{b}
Roots length (cm)	36.90^{a}	37.56^{a}	37.80^{a}	44.60^{a}
Roots number (strands)	23.50^{a}	32.33^{b}	28.33^{ab}	32.00^{b}
Fresh weight (g)	4.13^{a}	5.49^{a}	5.86^{a}	5.90^{a}
Dry weight (g)	1.45^{a}	1.78^{a}	2.23^{a}	2.17^{a}
Chlorophyll a (mg L^{-1})	0.87^{a}	0.95^{a}	0.93^{a}	0.99^{a}
Chlorophyll b (mg L^{-1})	1.11^{a}	1.28^{a}	1.29^{a}	1.37^{a}
Total chlorophyll (mg L^{-1})	1.98^{a}	2.22^{a}	2.23^{a}	2.37^{a}
AMF infections (%) and criteria of infection	0.00 (very low)	82.22 (very high)	86.67 (very high)	87.78 (very high)
Seedling quality index and increase (%) on control	0.08 (0.00%)	0.11 (37.50%)	0.13 (62.50%)	0.14 (75.00%)

Treatment followed by the same letter in the same column is not significantly different t-test, $p<0.05$

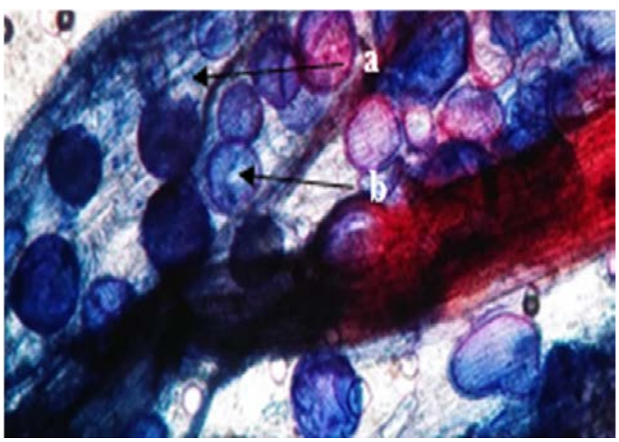

Fig. 1: AMF infection in *Morus macroura* roots (10X magnification)
a: Hyphae, b: Vesicle

percentage increase in plant quality index ranges from 37.50-75.00% out of control where the highest percentage was obtained in the treatment of *Acaulospora* sp.

***Trema orientalis* growth response to indigenous AMF inoculation:** *Trema orientalis* seedlings with indigenous AMF showed significantly different results on plant height, diameter, number and leaf area, length and number of roots, fresh and dry weight in *T. orientalis*. Clearly showed that *Acaulospora* sp. is the most effective inoculant in increasing *T. orientalis* growth and has high compatibility criteria. Indigenous AMF also shows different results on the levels of chlorophyll a, chlorophyll b and chlorophyll *T. orientalis* with the best result was observed with inoculation of *Acaulospora* sp. and *Gigaspora* sp. The percentage of AMF infection in *T. orientalis* roots showed the criteria of high-very high (Table 4), with the highest percentage of infection obtained in the type of *Acaulospora* sp. and was observed by the form of vesicle structure in the roots of *T. oreintalis* (Fig. 2). Inoculation of indigenous AMF influenced the quality index of *T. orientalis* seedlings. The percentage increase in the quality index of seedlings ranged from 60.00-160.00% where the highest percentage was obtained in the treatment of *Acaulospora* sp. which was able to improve the quality of seedlings more than 100% out of the control.

Table 4: *Trema orientalis* growth response to indigenous AMF inoculation

	Treatments			
Parameters	Control	*Glomus* sp.	*Gigaspora* sp.	*Acaulospora* sp.
Plants height (cm)	11.67[a]	18.27[b]	24.32[c]	25.40[c]
Leaf number	4.67[a]	6.17[ab]	7.50[b]	8.00[b]
Diameter (mm)	0.60[a]	1.05[bc]	0.84[ab]	1.28[c]
Leaf area (cm^2)	18.14[a]	25.33[b]	26.99[b]	29.68[b]
Roots length (cm)	22.32[a]	30.93[ab]	45.10[c]	40.93[bc]
Roots number (strands)	3.67[a]	5.17[a]	7.33[b]	8.17[b]
Fresh weight (g)	2.35[a]	3.85[ab]	6.03[bc]	7.10[c]
Dry weight (g)	1.62[a]	2.09[ab]	3.39[bc]	3.71[c]
Chlorophyll a ($mg\ L^{-1}$)	0.85[b]	0.69[a]	0.93[bc]	0.96[c]
Chlorophyll b ($mg\ L^{-1}$)	1.15[b]	0.85[a]	1.37[c]	1.32[c]
Total chlorophyll ($mg\ L^{-1}$)	2.01[b]	1.53[a]	2.29[c]	2.27[c]
AMF infections (%) and criteria of infection	0.00 (very low)	66.67 (high)	84.17 (very high)	86.67 (very high)
Seedling quality index and increase (%) on control	0.05 (0.00%)	0.08 (60.00%)	0.09 (80.00%)	0.13 (160.00%)

Treatments followed by the same letter in the same column is not significantly different t-test, $p<0.05$

Fig. 2: AMF infection in *T. orientalis* roots (40X magnification)

DISCUSSION

Based on the parameters of soil chemical properties (Table 2) especially the content of C-organic, N, P, K and CEC and soil fertility status, it can be said that the soil has the poor quality to support plant growth. Low soil fertility will be an obstacle in revegetation on the restoration of post-mining land. One of the ways to increase soil fertility is by using microorganisms such as mycorrhizae. Mycorrhizae are known to function in maintaining soil stability. External hyphae found in mycorrhizae play a role in forming macrostructure and soil micro aggregates[15,16]. Mycorrhiza can improve the quality limestone post-mining land, accelerate and increase the content of organic matter and organic carbon[17]. As found in this study where after the treatment with a different type of mycorrhizae can increase the content of organic matter and organic carbon, although some elements such as N, P, K and others are included in the category of very low, low to medium. The different types of mycorrhizae also showed an increase in the content of different soil elements.

Inoculation of AMF significantly improved the growth of *M. macroura*, such as total leaves, leaf area and roots number with inoculation of *Acaulospora* sp. and although the other growth parameters did not show significantly different results, *Acaulospora* sp. showed higher average results compared to other treatments. This is due to *Acaulospora* sp. has good compatibility with *M. macroura* (Table 3). AMF was able to increase the growth of host plants through increased absorption of element P which is a macronutrient that is very necessary for the vegetative growth of plants by increasing the area of nutrient uptake through hyphae[18,19]. The high criteria of roots colonization on *M. macroura* indicate that there is compatibility between AMF and *M. macroura* roots. According to Widiastuti *et al.*[20], plants that has good compatibility with AMF showed by the capability of spores on infecting roots and germinate, then spreads the infection into plants root tissues. AMF infection in plant roots can be identified by the formation of hyphae structures, misellia, arbuscular, vesicles or spores[21]. The quality index value ranges from 0.11-0.14 with percentage of increase 37.50-75.00% out of control where the highest percentage was obtained in the treatment of *Acaulospora* sp. And according to Dickson *et al.*[14], variations in the quality index range depend on plant species and environmental conditions and in general, seedlings are qualified to have good quality if they have a quality index>0.09. *M. macroura* which is inoculated by indigenous AMF has good quality of seedling.

The inoculation of indigenous AMF on *T. orientalis* seedlings also showed significantly different results on growth parameters with the best result was found on seedling with *Acaulospora* sp. inoculant It showed that *Acaulospora* sp. is the most effective inoculant in increasing *T. orientalis* growth on limestone post-mining soil. AMF increased the availability and absorption of the essential element for plant growth, N, P and K. AMF also able to extract Ca, Mg and other essential elements to support plant growth especially on marginal land. Hyphae of AMF able to increase the absorption of nutrients and water, not only macronutrients but also micronutrients, as well as being able to improve soil aggregates and it affect crop productivity. AMF hypha can absorb water from the pores of the soil and hypha had a very broad spreading area and its allowing plants to take more water[22]. The percentage of AMF infection in *T. orientalis* roots showed the criteria of high-very high with the highest percentage of infection obtained in the type of *Acaulospora* sp. According to Faiqoh and Setiadi[23], each type of crop on the root system can form an association with one or more of the types of mycorrhizae. Mycorrhizae do not have a specific host plant specificity, the ability to infect and colonize roots differs from one species to another.

Indigenous AMF influenced the quality index of *T. orientalis* seedlings. Seedlings with *Acaulospora* sp. Inoculant has a quality index value 0.13 and showed a quality index value>0.09. According to quality index criteria by Dickson *et al.*[14], where the seedlings are qualified to have good quality if they have quality Index value>0.09. According to Komala *et al.*[24], the quality Index value indicated the worth of a plant to be planted. The higher the value of seedlings in the quality index, the better quality of seedlings able to support the survival ability in the field. Lumbantobing[25] studied the provision of AMF can increase the height of Trembesi seedlings, total dry weight and QI value of Trembesi by 45-50%. AMF-infected roots are known to increase the growth of trees found in tropical forests. The survival rate of AMF-inoculated plant seedlings is higher compared to seedlings without AMF[26].

CONCLUSION

Acaulospora sp. is the type of AMF that is most suitable for the growth of Andalas (*M. macroura*) and Indarung (*T. orientalis*) seedlings that can increase plant growth better than controls and showed a high-quality Index of seedlings.

SIGNIFICANCE STATEMENT

This study discover the effectiveness of indigenous AMF inoculation to support plant growth on post-mining land and improved the quality of seedlings for revegetation that can be beneficial for land recovery. This study will help the researcher to uncover the use of indigenous AMF in bioremediation for land recovery especially on rehabilitation of post-mining land through revegetation using native plant species. Finding the compatible type of indigenous AMF and native plants species is a necessary step in phytoremedial strategy in rehabilitation.

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