Improvement of Early Growth of Endemic Sulawesi Trees Species Kalappia celebica by Arbuscular Mycorrhizal Fungi in Gold Mining Tailings

Husna*, F D Tuheteru, A Arif, Solomon

Departement of Forestry, Faculty of Forestry and Environmental Science, University of Halu Oleo, Kendari Southeast Sulawesi, Indonesia

*Corresponding author e-mail: husna.faad19@yahoo.com

Abstract. Kalapi (Kalappia celebica Kosterm.) is an endemic species of Sulawesi and listed in the IUCN red list as an endangered species. In addition, information about silviculture of K. celebica type in the world is still limited. One important component of silvicultural techniques to support conservation efforts of K. celebica is the application of fertilizers such as mycorrhizal biofertilizers. The aim of the study was to examine the improvement of initial growth of K. celebica by inoculation of arbuscular mycorrhizal fungi (AMF) on gold tailings media. This research was conducted in the greenhouse of the Indonesian Mycorrhizal Association (AMI) branch of Southeast Sulawesi in March - July 2019. The study was designed by using a completely randomized design with 4 AMF inoculums treatments namely control, Acaulospora delicata, Ambiospora appendicula, and Mixed AMF types. Each treatment was repeated 3 times with 5 plant units of each treatment. The results showed that the AMF colonization ranged from 42.2 to 74.6%. The colonization of AMF significantly increased the early growth of K. celebica aged 4 months. Inoculation of A. delicata significantly increased shoot height, number of leaves, shoot and total dry weight. There was no difference of AMF effect in both diameter of the stem and roots dry weight. The dependence of K. celebica on mycorrhizal fungi was high about 51-71% (Mycorrhizal inoculation effect). AMF inoculation improves the initial growth of K. celebica, therefore, it can contribute to the conservation of tropical endangered tree species.

1. Introduction

Kalappia celebica Kosterm included into family Fabaceae and growth in Malili (East Sulawesi), Kolaka Utara, Tanggetada Kolaka and Abuki Kolaka Timur (Southeast Sulawesi). This plant is an endemic species of Sulawesi and categorized as monospecies in Kalappia genus. Related to the type of wood, K. celebica is a type of legume plant that has high wood quality with class I for strength and II for durability. Listed in the IUCN red list as an endangered species, the decreasing of plant population may due to its characteristics such as required long period for flowering and limited number of seeds and seedling in natural forest. While external factors problems may come from limited plant distribution, illegal logging and over harvesting, as well as no regeneration efforts [1].

In Sulawesi, it was found 15 AMF types reported symbiotic with legume Pericopsis mooniana [2] and K. celebica [3,4], among them are Glomus, Gigaspora, and Acaulospora. The use of local AMF that was isolated from rhizosphere P. mooniana was applied to P. mooniana on mineral soil media [2], serpentine media [5,6,7], and ex-coal mining media [8]. However, studies and information on the utilization of AMF for conservation of K. celebica are still limited.
The information on the symbiosis and response of *K. celebica* with AMF is needed to find the ways for improving seedling quality, growth and species conservation. AMF was effective in improving and silviculture on endangered species [5, 8, 9, 10]. AMF also increase plant dry weight and nutrient uptake in tropical legume plants such as *Cassia siamea* Lam. [11], *L. leuephala*, *A. Marmelos*, and *P. roxburghii* [12, 13].

The compatibility between AMF species and its host as well as environmental condition determine the effectiveness of local AMF and legume plant dependency on AMF application [14]. In this study, ex mining soil or gold mining soil is in a poor soil condition. An ex-mining soils loose the fertility due to a decrease of macro nutrient content, low organic C, acidic pH, high heavy metal content and low of microbial activity [15, 16]. This media will become limited factors for plant growth. Improving plant growth on tailings media can be done with incorporate seedlings with mycorrhiza. In gold tailings condition that contaminated with Hg it was confirmed that AMF gave a benefit to increase live capability, growth and nutrient uptake [17, 18]. This study aimed to determine the effectiveness of AMF local isolated from *P. mooniana* on *K. celebica* growth at gold tailings media.

2. Method
2.1 Time and Location
This research was conducted in greenhouse of Indonesia Mycorrhizal Association Southeast Sulawesi branch, and Laboratory of Forestry, Faculty of Forestry and Environmental Science, University of Halu Oleo from March to July 2019.

2.2 Research design
This research used complete randomized design (CRD) with four AMF treatments: (1) Control (without AMF), (2) *Acaulospora delicata*, (3) *Ambiospora apendicula*, and (4) combination of *A. delicata* and *A. apendicula* (combined AMF). Each treatment was repeated three times using five units of plants, bring a total of 105 units of plants.

2.3 Research procedures
Seeds of *K. celebica* were collected from mother tree in Abuki Natural Forest, Konawe Regency at 2016. After applied hot water treatment (temperatures ± 50°C) for 24 hours, seeds were germinated. Sprouts were transferred to sterile tailings of gold mine in polybag of 15 cm x 20 cm and inoculated by 10 grams AMF inoculums closed to the sprouts roots. Macro and micro nutrients of soil media is analyzed.

Height (cm) and diameter (mm) of seedling measured one cm from the surface of media. Number of leaves and plant dry weight measured after dried in oven on 70°C for 2x24 hours. AMF colonization and mycorrhizae inoculation effect (MIE) observed using the following formulas:

Percentage of AMF Colonization = \( \frac{\sum \text{number of fields of view colonized}}{\sum \text{total observed field of view}} \times 100\% \) \hspace{1cm} (1)

Mycorrhiza Inoculation Effect = \( \frac{\text{dry weight of mycorrhizal plants} - \text{dry weight of non mycorrhizal plants}}{\text{dry weight of mycorrhizal plants}} \times 100\% \) \hspace{1cm} (2)

Data analyzed by using ANOVA. Differences between treatments means evaluated by using Duncan Multiple Range Test (P<0.05).

3. Result and discussion
3.1 AMF colonization and MIE
AMF inoculation revealed a significant effect for both AMF inoculation and MIE. Inoculated seedling experienced higher percentage of colonization than un-inoculated seedlings (control). In greenhouse condition, colonization of AMF in *K. celebica* roots after 4 months showed that AMF treatments resulted to high percentage of colonization (42.2-74.6 %) (Table 1). The highest value found for combined AMF (74.6 %), however, there were no differences found between *A. delicata* and *A.
appendicula in roots colonization of K celebica. Internal and external mycelium that colonized in K. celebica roots indicates the presence of AMF colonization.

Table 1. Effect of treatments on AMF colonization and MIE values of K. celebica seedlings after 4 months

| Treatments                  | AMF colonization (%) | MIE (%) |
|-----------------------------|----------------------|---------|
| Control (without AMF)       | 5.7c                 | -       |
| Acaulospora delicata        | 42.2b                | 71.3    |
| Ambiospora appendicula      | 54.8b                | 51.3    |
| Combined AMF                | 74.6a                | 64.1    |

Value that followed by different letters in the same column is significantly different at 0.05 DMRT test level.

According to Suharno et al. [19], the presence of AMF in root tissues is important for symbiosis process, in which mycelium can assist plant growth in taking nutrient and water and increase the heavy metal tolerance [20, 21]. The research showed that combined AMF treatment gave higher percentage of colonization than single inoculum treatments. We detected that un-inoculated seedlings K. celebica also colonized by AMF, may due to contamination from wind or water.

High values of MIE (51.1-71.3 %) indicated that K. celebica has high dependency on AMF inoculation. In addition, environment stress such as limited soil nutrient may bring the important of the AMF for plant to help its growth.

3.2 Seedling growth and plant dry weight

Growth of the plant was significantly affected by the appearance of AMF. AMF inoculation increase height, diameter and leaves number of K celebica seedlings aged 4 months (Table 2). Inoculated seedlings with A. delicata treatment resulted to the highest growth on height and leaves number. However, there were no significant differences in diameter between A. delicata and others.

Table 2. Effect of treatments on height, diameter and number of leaves of K. celebica seedlings after 4 months

| Treatments    | Height (cm) | Diameter (mm) | Leaf number (blade) |
|---------------|-------------|---------------|---------------------|
| Control       | 4.8c        | 0.77b         | 3.0c                |
| A. delicata   | 12.3a       | 1.68a         | 9.5a                |
| A. appendicula| 10.5b       | 1.55a         | 5.3b                |
| Combined AMF  | 10.5b       | 1.52a         | 6.2b                |

Value that followed by different letters in the same column is significantly different at 0.05 DMRT test level.

AMF treatments can increase shoots, roots and total dry weight of K. celebica aged 4 months (Table 3). The best treatment was A. delicata that gave shoots and total dry weight highest than other AMF treatments. However, on roots dry weight, there was no difference between A. delicate, A. appendicula and combined AMF treatment.

Table 3. Effect of treatments on plants dry weight of K. celebica seedling after 4 months

| Treatments    | Plant dry weight (g) | Roots | Shoots | Total |
|---------------|----------------------|-------|--------|-------|
| Control       |                      | 0.08b | 0.17c  | 0.25c |
| A. delicata   |                      | 0.18a | 0.69a  | 0.87a |
| A. appendicula|                      | 0.15a | 0.53b  | 0.68b |
| Combined AMF  |                      | 0.18a | 0.51b  | 0.69b |

Value that followed by different letters in the same column is significantly different at 0.05 DMRT test level.
Table 4. Effect of treatments on seedlings quality index and shoot-root ratio K. celebica seedling after 4 months

| Treatments       | seedlings quality index (SQI) | Shoot-root ratio (SRR) |
|------------------|-------------------------------|------------------------|
| Control          | 0.033b                        | 2.14                   |
| A. delicata      | 0.076a                        | 4.21                   |
| A. appendicula   | 0.066a                        | 3.57                   |
| Combined AMF     | 0.071a                        | 2.83                   |

Value that followed by different letters in the same column is significantly different at 0.05 DMRT test level.

AMF treatments gave the significant effect for seedlings quality index (SQI) variable (Table 4). However, the value did not reach the proper one (about 0.09). This may indicate that seedlings are not feasible yet to be transferred to the field. There was a positive trend that AMF treatments gave better SRR values. However, treatments did not give the significant effect for SRR values.

Choosa-Nga et al. [22] revealed that in natural ecosystem the Fabaceous trees produce poor regeneration and slow growth species includes K. celebica, and there was no nodulation in its roots. This greatly indicate that this plant highly required AMF to improve the growth. This study clearly showed that the AMF treatments increase significantly the height, diameter and leaves number of K. celebica seedlings. AMF treatments also increased plant dry weight in roots, shoots and total. A. delicata treatment can increase the total of dry weight of 248% than control. The study provided an evidence that AMF can enhance growth of K. celebica as a result of improving of mineral and water uptake. Plant growth was related with nutrient supply, where AMF can facilitate water and nutrient uptake through external mycelium roles in mine soil when nitrogen and phosphorus decreased. This result was in line with Turjaman et al. [23] that found AMF colonization can increase plant growth, concentration of shoot nutrient and rates of survival of D. polyphylla and A. filarial seedlings.

This study clearly demonstrates that inoculated seedlings with monospecies A. delicata gave the highest results on plant growth that different with other treatments. This result was in line with Suharno et al. [19] that the ability of AMF in support plant growth determined by AMF type. They also found that when Acaulospora type was mixed in the same genus, it resulted to the reduction of biomass compare than inoculated seedlings with only single species of Acaulospora. It assumed that the lower efficiency may due to competition [24]. Furthermore, this study proved that K. celebica seedlings can grow better in gold mining soil with AMF inoculation. This study was in line with some of researches that also showed that AMF increased plant adaptation in heavy metals stress condition. There are some mechanism occurred when plant incorporated with AMF such as binding mechanisms, chelating of heavy metals, sequestration and reduction of heavy metal accumulation in plant by AMF [24].

The AMF type of Acaulospora sp. is potential to be developed as biofertilizer to improve the quality of seedling and the early growth of K. celebica and other endangered plant species on greenhouse and nurseries scales. Enhancing seedling quality of endangered species can support the conservation effort of K. celebica in tropical area.

4. Conclusion
Inoculation of AMF improves the early growth of K. celebica and species of A. delicata is the best treatment to improve the plant growth in gold mining tailings.

Acknowledgments
The authors would like thanks to the Indonesian Ministry for Research and Technology for providing the funding for this research (RISTEK DIKTI), Research and Community Services Institution, Halu Oleo University (LPPM UHO) and PT. Panca Logam.
References
[1] Liam AT, Arif A, Clark RP, Girmansyah D, Kintamani E, Prychid CJ, Pujirahayu N, Rosmarlinansiah, Brearily FQ, Utteridge TMA, Lewis GP 2019 *Ecology* **100**.
[2] Husna, SW Budi R, Mansur I, C Kusmana 2015 *Penulianan Tanaman Hutan* **9**, 131-148
[3] Arif, A., Tuheteru, FD., Husna. 2015. The conservation of endemic and endangered species *Kalapppia celebica* Kosterm through cutting propagation and AMF potential assessment. In: Sukamerta, IM., Wiswasta A., Soewandhi, SN., Darussalam, AB., Widnyana, IK., Tamba, IM., Sumantra, IK. ICSD proceeding 2015. p. 53-65.
[4] Arif, A., Tuheteru, FD., Husna, Kandari, AM., Mekuo, IS., Masnun. 2016 *European Journal of Sustainable Development* **2**(2): 395-402.
[5] Husna, SW Budi R, Mansur I, C Kusmana 2016 *Pakistan Journal of Biological Science* **19**, 158-170
[6] Husna, Tuheteru FD, E Wigati 2017a *Nusantara Bioscience* **9**, 57-61
[7] Husna, Tuheteru FD, Arif A 2017b Arbuscular mycorrhizal fungi and plant growth on serpentine soils. In : Wu QS (Eds.), Arbuscular mycorrhizas and stress tolerance of plants. (Singapore: Springer).
[8] Husna, Mansur I, SW Budi R, Tuheteru FD, Arif A, Tuheteru EJ, Al Basri 2019 *Asian Journal of Plant Sciences* **18**, 101-109.
[9] Sharma D, Rupan K, Bhatnagar AK 2008 *World J Microbiol Biotechnol* **24**, 395-400
[10] Zubek S, Turnau K. Tsimilli-Michael M, Strasser RJ 2009 *Mycorrhiza* **19**, 113-123
[11] Giri, B., Kapoor, R., Mukerji, KG 2005 *New Forest* **29**(1): 63-73
[12] Wulandari D, Saridi, Cheng W, Tawaraya K 2014 *International Journal of Forestry Research*
[13] Kumar, N., Kumar, A., Shukla, A., Kumar, S., Uthappa, AR., Chaturvedi, OP. 2017 *International Journal of Current Microbiology and Applied Sciences* **6**(7):3885-3892. ISSN: 2319-7706
[14] Estaun V, Cinta C, Camprubi A 2010 Effect of differences among crop species and cultivars on the arbuscular mycorrhizal symbiosis [Chapter 13]. In: Koltai, H. and Kapulnik, Y. (Eds.). *Arbuscular Mycorrhizas: Physiology and Function* (New York: Springer).
[15] Husna. 2010. Pertumbuhan bibit kau kuku (*Pericopsis mooniana* THW) melalui aplikasi fungi mikoriza arbuskula (FMA) dan ampas sagu pada media tanah bekas tambang nikel [thesis]. Universitas Halu Oleo Kendari
[16] Agus C, Primananda E, Faridah E, Wulandari D, Lestari 2018 Role of arbuscular mycorrhizal fungi and *Pongania pinnata* for revegetation of tropical open-pit coal mining soils. *International Journal of Environment Science and Technology*
[17] Orlowska E,D Orlowski, J Mesjasz-Przybyłowicz, K Turnau 2011 *International Journal of Phytoremediation* **13**, 185–205
[18] Madejon E, Doronila AI, Madejon P et al 2012 *Agrofor Syst* **84**, 389–399
[19] Suharno, Kasiamdari RS, Soetarto ES, Sancayaningsih RP 2016 *International journal of environmental bioremediation and biodegradation* **4**(1): 1-7
[20] Yang Y, Han X, Liang Y, Ghost A, Chen J, Tang M 2015 *Plos One* **10**(12).
[21] Smith, SE., Read, DJ. 2008. Mycorrhizal Symbiosis. 3rd Edn., Academic Press, London, UK., ISBN-13: 978-0123705266, Pages: 800
[22] Choosa-Nga P, Sangwanit U, Kaewgrajang T 2019 *Biodiversitas* **20**(2): 405-412
[23] Turjaman M, Tamaí Y, Sitepu IR, Santoso E, Osaki M, Tawaraya K 2008 Improvement of early growth of two tropical peat-swamp forest tree species *Ploiarium alternifolium* and *Calophyllum hosei* by two arbuscular mycorrhizal fungi under greenhouse conditions.
[24] Crossay T, Majorel C, Redecker D, Gensous S, Medevielle V, Durrieus G, Cavaloc Y, Amir H 2019 *Mycorrhiza* **29**(4): 325-339