Utilization of Arbuskular Mikoriza Fungi [AMF] for growth and ready to release of three genotype gaharu [Aquilaria spp.]

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Abstract. The agarwood sapling has low viability or about 47 % cause undeveloped roots. This problem was overcome through the application of Arbuscular Mycorrhizal Fungus [AMF] at the acclimatization stage. AMF has the capability to improve root growth and coverage area, enabling a better capacity to absorb water and nutrients through its hyphae. to know the effective dose for enhancing the growth of agarwood saplings is the goal of this research. The genotypes used in this study were Aquilaria malacensis and Aquilaria microcarpa. The AMF doses were 7.5; 15; 22.5, and 30 gr/ polybag. Saplings were acclimatized in soil that had been supplemented by husk charcoal and organic fertilizer Super UPPO [product of Andalas University CSR Program 2017]. The observations including the number of the viable sapling, the height, the leaf number, the leaf length, the root number, the percentage of the AMF infection, and the ready-to-release sapling. The best performance was an application of 30 g per polybag. These doses infected 70% of root surfaces, and 90% of saplings were categorized as ready-to-release saplings.

Keywords: agarwood, AMF, hyphae, genotype, sapling.

1. Introduction

Agarwood [Aquilaria malaccensis L.] is a commercial commodity and high economic value. Recently, the availability and production are limited due to the difficulties in finding and collecting itself. Moreover, agarwood-producing take a very long time to produce itself [1][2][3].

Generative and vegetative are the ways for taking a new individual of the plant. Both of them have restrictions problems. To overcome these matters, the mass production of A malaccensis saplings can be executed through wildling considered as one of the effective ways to conserve the germplasm [4]. even though, the result of this method is poor in root development. Therefore, this kind of sapling required multi-step acclimatization, particularly through the application of arbuscular mycorrhizal fungus [AMF] to support its growth and development [5,6].

Previous studies mentioned that the root inoculation by AMF could support the plant with poor-developed roots to facilitate better nutrients uptake [7]; [8]. AMF enables to expand the root penetration coverage, support the root growth, release various unavailable elements and facilitate the nutrient and water absorption [9]. Other studies, AMF could facilitate P uptake and accelerate the growth rate of seedlings or saplings, thus reduce the maintenance period in the nursery [10]. Nevertheless, the increase of plant growth by AMF required high compatible interaction between plant, AMF, and its surrounding environment [5].
In this study, we investigated the efficiency of the AMF-Aquilaria sp. sapling association in supporting the growth. This study was aimed to identify the standard nursery method for three genotypes of Aquilaria sp. through the application of several AMF doses. The result of this study provided the prospect of AMF utilization in the growing medium of wildling-originated Aquilaria sp. saplings as well as in tomato case.

2. Materials and methods
This study was carried out in the nursery station of Agriculture Faculty of Andalas University using factorial in a completely randomized design with two factors of treatment. The first factor was the genotype of agarwood saplings consisting of three different genotypes, namely A. malacensis, A. microcarpa. Saplings were acclimatized in sterile soil medium supplemented with rice husk charcoal and organic fertilizer Super UPPO [product of Andalas University CSR Program 2017]. The second factor was the dose of AMF application started from 0 [control], 7.5, 15, 22.5, and 30 g per polybag and repeated in three replicates with 10 saplings per genotypes was plotted for each AMF dose. AMF inoculants used were multispore type composed of Glomus manihotis, Gigaspora sp. and Acteospora sp. [collection of laboratory soil science UNAND].

Saplings were placed in a semi-closed greenhouse and maintained through regular watering, intensive weeding as well as pests and diseases controlling if required. The application of 100 mg per pot NPK fertilizer into the saplings was conducted once in 2 months started from 2-weeks-old before the AMF application. Several sapling’s growth parameter were observed, such as number of the viable saplings, number of roots, enhancement on height, leaf number, leaf length, and leaf width as well as the percentage of AMF-infected root and ready-to-release sapling. All collected data were statistically analyzed using two-way analysis of variance [ANOVA]. Significance among treatments was further analyzed usingTukey’s HSD [honestly significant difference] with a p<0.05.

3. Result and discussion
3.1. Efficacy of AMF colonization on Agarwood Sapling Roots
The effect of AMF application on the growth of agarwood sapling depended on its efficacy in colonizing the roots. As shown in Table 1, the amount of AMF applied affected the efficacy of its root colonization. The genotype differences showed in different responses. Of all treatments tested, the highest root colonization up to 70% was obtained from A. malacensis treated with 30 g AMF [Table 1]. The application means that the compatibility both of plant and AMF. The AMF types could result in different responses in certain plant species as each plant produced its own root exudates required to support the AMF growth.

Mycorrhiza does the mutualistic symbiosis both of itself and the epidermis host [5]. The exudate from itself contains auxin to stimulate growth and help to solubilize phosphate thus make it available to be absorbed by the roots [5]. To support, the AMF must be compatible with the host. These compatibility labs the AMF in capability and efficacy to infecting plant roots, thus enabling better efficacy in absorbing nutrients from the soil [11].

3.2. Increase of Root Number
The application of AMF on agarwood saplings contributed a higher number of formed roots compared to the one with no AMF applied. The higher the dose of AMF applied, the more the number of roots increased. The increase in this parameter varied among agarwood genotypes. The three genotypes tested, the sapling of A. malacensis exhibited the most number of roots reaching 8.5 pieces [Table 2].

The efficacy of AMF colonization varies among plant species and depends on the compatibility between the AMF hosts. Association of plant roots and AMF could fix the root system with poor-developed roots [12];[13];[14]. AMF was able to expand the root coverage area, support the root growth, release the unavailable nutrients for plants, and vast absorption area [15]. The presence of
AMF also triggered faster growth of seedling or sapling and enhance the absorption of P, thus reduced the maintenance period in the nursery [16];[17];[18];[19]. Several previous studies have been outlined the benefits of AMF application towards plant growth, including improves the nutrients uptake and protects plants from pathogen infection, drought as well as heavy metal [20] and [8].

3.3. Number of Viable Agarwood Saplings

The application on agarwood saplings did not affect the number of viable saplings. Even the saplings with no AMF applied showed the same percentage of viable saplings [Table 3]. These results indicated that agarwood saplings were naturally viable. Therefore, this application did not give a significant impact on its viability.

3.4. Increase of Sapling Height

Similar to the number of viable saplings, the AMF application also did not contribute to a significant increase of the sapling height. All genotypes revealed insignificantly different heights, where the highest sapling was obtained from *A. malacensis* about 14 cm after the application of 30 g of AMF [Table 4]. This result suggested that the sapling had obtained sufficient nutrients from the soil leading to optimum growth, so the impact of AMF application was less noticeable.

3.5. Increase of Agarwood Leaf Number and Length

These indicated that the presence of AMF and its efficacy in supporting plant growth occurred by stimulating the leaf growth. However, in this study, no significant increase in sapling height resulted from the AMF application suggesting no direct correlation between the increase in plant height and leaf number.

The effect of AMF application showed a significant impact on the increase of both leaf number and leaf length. In the case of leaf number, the highest dose of AMF increased the number of growing leaves from 5 to 8 pieces in all genotypes compared to the saplings with no applied [Table 5]. The best response exhibiting the highest number of leaves has resulted from *A. malacensis* sapling treated with 30 g AMF with 15 leaves [Table 5]. Similar to leaf number, the increase of leaf length was significantly triggered by the presence of AMF. As the AMF dose rose, the elevation of leaf length was getting higher. As shown in Table 6, the addition of leaf length could reach 1.50 – 2.70 cm after the application of 30 g AMF. The longest leaf was obtained from *A. malacensis* treated with 30 g AMF with 4 cm.

The increase of leaf number and length due to the effect of AMF colonization indicated that better nutrients uptake resulted from the AMF-plant interaction directed the better development of vegetative parts. The leaf development is commonly dominated by the presence of nitrogen [the biosynthesis of chlorophyll] [21];[22];[23]. Other studies also reported that sufficient N availability during leaf development would result in greener leaf as the amount of synthesized chlorophylls increase [24];[25];[26].

In this study, the effect of AMF application did not result in the increase of sapling height [Table 4]. In line with this study, the application of *multispore* AMF in various doses did not stimulate the increase in the height of tea seedling. [25] and [26] mentioned that the association of AMF and plant roots did not always affect all plant growth aspects.

3.6. Number of Ready-to-release Agarwood Saplings

The application of AMF revealed a significant impact on the number increase of ready-to-release saplings. As shown in Table 7, the number of ready-to-release sapling increased along with the increase of AMF doses applied. This increasing number reached 30% higher than the one resulting from the AMF-untreated sapling. The untreated sapling only showed 45-60% ready-to-release saplings, while the sapling treated with 30 g AMF exhibited 75-90% ready-to-release saplings [Table
These results suggested that AMF stimulated better growth of the sapling leading to well-developed sapling characteristics that matched to the market demand.

**Table 1.** The effect of various AMF doses and agarwood genotypes on the efficacy of AMF colonization on roots.

| Genotypes       | Efficacy of root colonization [%] on each AMF dose [g/polybag] |
|-----------------|---------------------------------------------------------------|
|                 | 7.5 | 15 | 22.5 | 30 |
| *A. malacensis* | 50 c | 55 bc | 60 b | 70 a |
| B               | C   | B   | AB   |
| *A. microcarpa* | 45 c | 50 bc | 55 b | 65 a |

CV* [%] = 21.80 %

Numbers followed by the same lowercase in the same row and/or by the same uppercase in the same column were insignificantly different according to Tukey’s HSD with a p<0.05.

*Coefficient of variance

**Table 2.** Effect of various AMF doses and agarwood genotypes on the increase of root number.

| Genotypes       | Number of root on each AMF dose [g/polybag] |
|-----------------|--------------------------------------------|
|                 | 0 | 7.5 | 15 | 22.5 | 30 | Mean |
| *A. malacensis* | 6.70 | 7.00 | 7.60 | 8.00 | 8.50 | 7.56 |
| *A. microcarpa* | 5.70 | 6.50 | 6.50 | 7.20 | 7.80 | 6.74 |
| Mean            | 6.20 | 6.75 | 7.05 | 7.60 | 8.15 |
| CV [%]          | 9.32% |

Numbers followed by the same letters were significantly different at p<0.

**Table 3.** Effect of various AMF doses and agarwood genotypes on the number of viable agarwood saplings.

| Genotypes       | Number of viable sapling [%] on each AMF dose [g/polybag] |
|-----------------|------------------------------------------------------------|
|                 | 0 | 7.5 | 15 | 22.5 | 30 | Mean |
| *A. malacensis* | 100 | 100 | 100 | 100 | 100 | 100 |
| *A. microcarpa* | 100 | 100 | 100 | 100 | 100 | 100 |
| Mean            | 100 | 100 | 100 | 100 | 100 |
| CV [%]          | 9.32% |

**Table 4.** Effect of various AMF doses and agarwood genotypes on the height of agarwood saplings.

| Genotypes       | Sapling height [cm] on each AMF dose [g/polybag] |
|-----------------|-------------------------------------------------|
|                 | 0 | 7.5 | 15 | 22.5 | 30 | Mean |
| *A. malacensis* | 11.00 | 12.50 | 13.00 | 13.50 | 14.00 | 12.80 |
| *A. microcarpa* | 10.00 | 11.50 | 13.00 | 13.10 | 13.50 | 12.22 |
| Mean            | 10.50 | 12.00 | 12.83 | 13.10 | 13.50 |
Table 5. Effect of various AMF doses and agarwood genotypes on the leaf number of agarwood saplings.

| Genotypes    | Leaf number on each AMF dose [g/polybag] |
|--------------|-----------------------------------------|
|              | 0           | 7.5         | 15          | 22.5        | 30          |
| A.malacensis | 9.20 b      | 9.50 b      | 9.50 b      | 10.50 b     | 15.00 a     |
|              | B           | B           | B           | A           | B           |
| A.microcarpa | 5.30 b      | 5.60 b      | 6.50 b      | 9.00 a      | 13.50 a     |
|              | A           | A           | A           | A           | B           |
| CV [%]       | = 12.50%    |             |             |             |             |

Numbers followed by the same letters were significantly different at p<0.

Table 6. Effect of various AMF doses and agarwood genotypes on the leaf length of agarwood saplings.

| Genotypes    | Leaf number on each AMF dose [g/polybag] |
|--------------|-----------------------------------------|
|              | 0           | 7.5         | 15          | 22.5        | 30          |
| A.malacensis | 1.30 b      | 1.50 b      | 1.80 b      | 2.90 a      | 4.00 a      |
|              | A           | A           | A           | B           | B           |
| A.microcarpa | 1.50 b      | 1.55 b      | 1.60 b      | 1.65 b      | 3.00 a      |
|              | A           | A           | A           | A           | AB          |
| CV [%]       | = 16.50%    |             |             |             |             |

Numbers followed by the same letters were significantly different at p<0.

Table 7. Effect of various AMF doses and agarwood genotypes on the number of ready-to-release agarwood saplings.

| Genotypes    | Number of ready-to-release saplings [%] on each AMF dose [g/polybag] |
|--------------|-----------------------------------------------------------------------|
|              | 0           | 7.5         | 15          | 22.5        | 30          |
| A.malacensis | 60.00 c     | 70.00 b     | 75.00 b     | 85.00 a     | 90.00 a     |
|              | B           | C           | C           | C           | B           |
| A.microcarpa | 55.00 c     | 60.00 b     | 65.00 bc    | 70.00 ab    | 75.00 a     |
|              | AB          | B           | B           | B           | A           |
| CV [%]       | = 25.60%    |             |             |             |             |

Numbers followed by the same letters were significantly different at p<0.05

4. Conclusion
This study proved that the association between AMF and Aquilaria sp. sapling had stimulated more leaves, longer leaf size, and a higher number of ready-to-release saplings. From the three genotypes studied, the sapling of A.malaccensis exhibited the best growth performance. Regarding AMF application dose, 30 g conferred the highest support to the sapling growth, indicating that it is recommended to use this amount of AMF for similar purposes on the same plant species. According to these results, it is suggested to apply the AMF during the planting of this sapling in the field to support the sapling growth under uncontrolled growing conditions until the root system is well-adjusted with
the field condition.

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