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Application of *Glomus* sp. and a mix of *Glomus* sp. with *Gigaspora* sp. in improving the Agarwood (*Aquilaria malaccensis* Lamk.) seedling growth in Ultisol soil

M V Rini [1*], E Susilowati[2], M Riniarti[2] and I Lukman[3]

[1] Department of Agronomy and Horticulture, Faculty of Agriculture, University of Lampung, Indonesia
[2] Department of Forestry, Faculty of Agriculture, University of Lampung, Indonesia
[3]Department of Accounting, Faculty of Economics, Universitas Malahayati, Lampung Indonesia

*Corresponding author`s e-mail address: maria.vivarini@fp.unila.ac.id

Abstract. Arbuscular Mycorrhizal Fungi (AMF) form symbiotic mutualism with the plant root, where fungi will help the host plant to absorb more nutrients and water from the soil. The objective of this study was to evaluate the effectiveness of two species of AMF namely *Glomus* sp. and *Gigaspora* sp. in increasing the agarwood seedling growth. The treatments were control (m_0), *Glomus* sp. (m_1), *Gigaspora* sp. (m_2), and a mix of *Glomus* sp. with *Gigaspora* sp. (m_3) with six replications arranged in a Completely Randomized Design (CRD). The seeds of agarwood were germinated in sterilized sand for one month. The one-month-old seedlings were then transferred into 12 cm x 19 cm polybags containing sterilized ultisol soil. During the time of transplanting, the AMF of 300 spores per seedling were spread onto the root surface of agarwood seedling. The seedlings remained in the greenhouse for six months. Results showed that AMF increased the shoot and root fresh weights and the shoot and root dry weights. The conclusion showed that agarwood seedlings treated with *Glomus* sp. (m_1) and a mix of *Glomus* sp. with *Gigaspora* sp. (m_3) gave the highest agarwood seedling growth which indicated by the seedling height, total leaf area, fresh weight and dry weight of the shoot and root.

1. Introduction

Nowadays in Indonesia, many plantations are facing the downgrade issue due to the continuity of the nutrient-leached. The downgraded of nutrients in the soil resulted in the decreasing of plant production. Therefore, it requires a new method to upgrade the nutrient availability in the soil. One of the methods is the application of Arbuscular Mycorrhizal Fungi (AMF).

AMF is one of the soil microorganisms that can create symbiotic mutualism with almost 80% of the terrestrial plant [1]. AMF hyphae could penetrate the root cortical cells and form an inner structure inside the cells known as vesicle, arbuscule, and hyphae and at the same time also growth and form outside the root structure known as an extraradical hyphae. The extraradical hyphae play the role of AMF in helping the host absorbing nutrients and water from the soil. The other benefit of AMF is able to protect the plant from biotic and abiotic distress, upgrading the carbon-deposit in the rhizosphere, and increasing the soil aggregation [2]. The beneficial effect of AMF on seedling growth has mostly been attributed to the increase of nutrients uptake especially phosphorous. The AMF hyphae extend beyond the root (extraradical hyphae) into the surrounding soil and transport nutrients directly to the plant [3]. The application of AMF can also improve chlorophyll a and b and total chlorophyll content and better cacao seedlings growth [4]. Therefore, the use of AMF is very useful in increasing the plant growth, especially if the plant has a slow growth such as agarwood tree (*Aquilaria malaccensis* Lamk.).

Agarwood is the resinous heartwood of the Aquilaria tree as a result of physiological metabolism when the tree becomes infected with a type of mold. *Aquilaria* tree is a well-known important agarwood-producing genus which is endemic to the Indonesia region. *Aquilaria malaccensis* is one of *Aquilaria* species that has the potential to develop due to its ability to produce agarwood [5]. This tree is one of the forestry commodities with high value in Indonesia and worldwide. The benefits of agarwood can be varied from perfumes, health, beauty, and even religion aspect [6]. As the agarwood has a high financial value, the *A. malaccensis* can produce agarwood that is suitable to be developed as a commodity for plantation [7].

A. malaccensis has been reported as a slow-growing tree. This species can associate with AMF proven by the availability of AMF spores in their rhizosphere [8]. AMF inoculated with *A. malaccensis* was growing faster than the uninoculated control [9] and able to increase the stem diameter of the seedling planted in ultisol soil [10]. However, the research regarding which AMF species that is suitable in colonizing and improving *A. malaccensis* growth are still very limited. Therefore, the objective of this paper was to evaluate the effectiveness of two species of the AMF namely *Glomus sp.* and *Gigaspora sp.* in increasing the agarwood (*A. malaccensis*) seedling growth.

2. Materials and Method

This study was conducted in the greenhouse and laboratory of Plantation Production at the Agriculture Faculty University of Lampung from November 2018 to July 2019. The Completely Randomized Design (CDR) method with four treatments was used as the experiment design. The four treatments were as follows: without AMF (m0), *Glomus* sp. isolate MV 27 (m1), *Gigaspora* sp. isolate MV 17 (m2), and a mix of *Glomus* sp. isolate MV 27 and *Gigaspora* sp. isolate MV 17 (m3). Those treatments were repeated six-time. Every experimental unit was represented by two plants such that all 48 experimental units were available. Data obtained were subjected to the Analysis of Variance (ANOVA) followed by separating treatment mean values with the Least Significant Different (LSD) test at α of 0.01 and 0.05.

A total of 300 agarwood seeds were germinated in a germination box containing sterilized sand for one month in the greenhouse. The seeds were watered daily and no fertilizer was added during this one-month period. After one month in the germination bed, the seedlings were selected based on the uniformity of its height and total leaves. Then, the selected seedlings were transferred into a polybag of 12 cm x 19 cm which contains a sterilized media-mixed of ultisol soil and sand with a ratio of 1:1 by volume. Soil sterilization was conducted by steaming it in the soil-sterilizer equipment for three hours. The AMF inoculation process was conducted during seedlings transplanting from germination bed into the polybag by pouring approximately 300 spores of the inoculum *Glomus* sp. (m_1), *Gigaspora* sp.(m_2), and a mix of *Glomus* sp. with *Gigaspora* sp. (m_3) (according to the treatment) onto the root surface of agarwood seedling in the planting hole.

After transplanting, the seedlings were kept for six months in the greenhouse and watered once a day. The weeding was done manually by pulling out weeds from the polybag. Fertilizer of urea was applied two weeks after transplanting as much as 2 g/L of water and applied for 100 agarwood seedlings or 10 ml/polybag. The urea fertilizer was applied every week until the seed reached the age of four weeks. Afterward, the NPK (N: P: K=16:16:16) was added with a dose of one g/seedling at five and 16 weeks after transplanting.

Six months after transplanting, the seedlings were pulled out from the polybag and study parameters were recorded. The percentage rate of AMF root colonization was examined after staining the roots with trypan blue [11]. The growth variables recorded were the seedling height (measured 2 cm above the soil media until the top nodes), stem diameter (measured exactly above the first root node using digital calipers), total leaf area (measured using leaf area meter equipment), the shoot and root fresh weight, and the shoot and root dry weight (measured by drying it in the oven of 80 ^{0}C until it weights constantly).

3. Results and Discussion

Table 1 shows that AMF successfully colonized the root of the *A. malaccencis* or agarwood seedlings. Agarwood seedlings responded positively to the AMF inoculation. The seedling with *Glomus* sp. and a mix of *Glomus* sp. with *Gigaspora* sp. had a higher root colonization of 91.4% and 84.8% subsequently compared to *Gigaspora* treatment and control seedling. *Gigaspora* sp. also colonized the root of agarwood as much as 47.2%, lower than *Glomus* sp. and a mix of *Glomus* sp. with *Gigaspora* sp. For the untreated control seedling, AMF colonization also detected as 22.5% of the root was colonized by the fungi. This might be due to the sterilization of the soil media process that did not eliminate the indigenous AMF present in the soil media completely.

Glomus sp. used in this study was isolated from the rhizosphere of South Sumatera oil palm, while *Gigaspora* sp. was isolated from the rhizosphere of *Jatropha* from East Java. This *Glomus* isolate might be particularly suitable to establish the symbiosis with *A. malaccencis* (seed obtained from *A. malaccencis* plantation in South Sumatera) that are also native of the similar edaphoclimatic area. A similar result also reported by [12] and [13] that AMF from the same edaphic or indigenous fungus was better than a culture collection at colonizing and increasing *Anthyllis cytisoides* growth.

Table 1. Effect of AMF inoculation on percentage of root colonization of six-month-old agarwood seedling.

AMF Treatments	Root Colonization by AMF (%)
Control	22.5 b
Glomus sp.	91.4 a
Gigaspora sp.	47.2 ab
Glomus sp. + *Gigaspora* sp.	84.8 a
LSD 1%	53.18

The numbers followed by the same letters are not significantly different according to the LSD test with confidence level (α) of 1%

In line with the AMF root colonization, the agarwood seedlings with *Glomus* sp. and a mix of *Glomus* sp. and *Gigaspora* sp. had better growth than the seedling with *Gigaspora* sp. and control which indicated by seedling height, stem diameter, and total leaf area (Table 2 and 3). The better growth might be caused by the success of AMF in colonizing agarwood root as seen in Table 1. The root colonization confirms that the association between AMF and agarwood seedling roots has happened. External hyphae of AMF might be well-developed in the soil to enlarge the absorption range of plant root so that more nutrients and water were absorbed by the seedling, and ultimately increase the seedling growth. AMF is considered as a "structure" for nutrient absorption of plants. About 75-90% P and 5-80% N in host plants are contributed by AMF [14] and [15]. Furthermore, the increase in K, Fe, Mo, Mn, and Cu absorption from the soil by AMF hyphae has also been reported [16].

The response given by AMF species varied against the plant host. It can be seen from its root colonization degree [17]. The more the root was colonized, the more the host plant would benefit. In this study, the percentage of the root being colonized reached 84.8 - 91.4% (showed by *Glomus* sp. and a mix of *Glomus* sp. with *Gigaspora* sp.) which categorized as the highest colonization [18]. The high percentage of colonization will increase the absorption of nutrients and water so that the growth of agarwood seedlings such as height, stem diameter, and total leaf area were better than the control and *Gigaspora sp.* treated seedling.

Table 2. AMF effect on the plant height and stem diameter of six-month-old agarwood seedling.

AMF Treatments	Plant Height (cm)	Stem Diameter (mm)
Control	28.2 b	4.38 b
Glomus sp.	39.2 a	6.99 a
Gigaspora sp.	31.5 ab	5.13 b
Glomus sp. + *Gigaspora* sp.	37.7 a	6.67 a
LSD 1%	9.1	1.62

The numbers followed by the same letters are not significantly different according to the LSD test with confidence level (α) of 1%

Table 3. AMF effect on the total leaf area of six-month-old agarwood seedling.

AMF Treatments	Total Leaf Area (cm^2)
Control	281.4 b
Glomus sp.	430.6 a
Gigaspora sp.	265.3 b
Glomus sp. + *Gigaspora* sp.	437.1 a
LSD 5%	133.52

The numbers followed by the same letters are not significantly different according to the LSD test with confidence level (α) of 5%

Similar to the seedling height, stem diameter, and total leaf area, Table 4 shows that AMF treatment had also significantly affected the shoot and root in the fresh and dry weight. The higher shoot and root fresh weight and dry weight were obtained from the seedling with *Glomus* sp. and a mix of *Glomus* sp. with *Gigaspora* sp. Growth enhancement due to the AMF colonization is largely attributed to the extensive exploration of the external hyphae into the soil, hence increasing the root system potential to absorb nutrients as well as to increase the nutrient transfer from roots to shoots. The other mechanism that might be involved is the exudation of the phosphatase enzyme by AMF hyphae to the surrounding soil. Phosphorous is a major element often limiting plant growth as it is usually in a bound state when added or present in the soil. Phosphatase enzyme released by the AMF could break the bound and resulting in the P availability to be absorbed by the root or AMF hyphae [19].

An increase in agarwood seedling growth might be related to the root exudate. Plant and AMF association will cause the root to produce root exudate which then stimulate the microorganism development in the soil [20]. Afterward, some of these microorganisms will produce phytohormone such as Indoleacetic Acid (IAA) [21]. IAA stimulates and supports the cell multiplication so that the seedling had better root fresh weight and dry weight. Also, AMF-improved plant growth can be regulated by endogenous hormones. The increase of endogenous hormone levels (putrescine and spermidine) in mycorrhizal plant resulted in increased plant biomass and growth in Citrus [22].

Table 4. AMF effect on the shoot and root fresh weight as well as dry weight of six-month-old agarwood seedling.

	Fresh Weight (g)		Dry Weight (g)	
AMF Treatments	Shoot	Root	Shoot	Root
Control	6.74 b	2.94 b	2.02 b	0.67 ab
Glomus sp.	13.15 a	6.56 a	3.70 a	1.47 a
Gigaspora sp.	7.89 b	3.55 b	1.92 b	0.62 b
Glomus sp. + *Gigaspora* sp.	11.86 a	5.35 ab	3.23 a	1.27 ab
LSD 1%	3.49	2.56	1.06	0.72

The numbers followed by the same letters are not significantly different according to the LSD test with confidence level (α) of 1%

Root, the "hidden half" of the plant, serves a multitude of functions. They are responsible for the anchorage and supplies the nutrients and water to the plants. Mycorrhiza expanded the absorption area of the root system of the host by its external hyphae (extraradical hyphae) that developed in the soil.

Extraradical hyphae of AMF may absorb water directly from the soil and transfer it to the host and the hyphae may enter into the tiny soil pores that plant roots do not pass to take in capillary water [23]. By this mechanism, the AMF may enhance water uptake by the agarwood seedling, hence the increase in the fresh weight of root and shoot.

The seedling with *Glomus* sp. and a mix of *Glomus* sp. with *Gigaspora* sp. has a higher total leaf area (as seen in Table 3). The higher the total leaf area is, the wider the leaf surface will support the photosynthesis process. AMF colonization enables a significant increase in the rate of photosynthesis of the host plant [24]. The increase in total leaf area along with the increase in photosynthesis rate will produce more organic compounds, hence the total biomass of the host which indicated by the dry weight of the shoot and root. The dry weight described as the accumulation of organic compounds due to photosynthesis in the plant along with more absorption of nutrients from the soil.

4. Conclusion

The agarwood seedlings treated with *Glomus* sp. and a mix of *Glomus* sp. with *Gigaspora* sp. gave the highest seedling growth that is indicated by the seedling height, total leaf area, fresh and dry weight of the shoot and root.

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