**Application of *Glomus* sp. and Mixed of *Glomus* sp. with *Gigaspora* sp. Improved Agarwood (*Aquillaria malaccensis* Lamk.) Seedling Growth in Ultisol Soil**

# Maria Viva Rini1\*, Endah Susilowati2, Melya Riniarti3 , Iing Lukman4

1Department of Agronomy and Horticulture, Faculty of Agriculture, Universitas Lampung

2,3Department of Forestry, Faculty of Agriculture, Universitas Lampung

4Department of Accounting, Faculty of Economics, Universitas Malahayati, Bandarlampung

E-mail: rinimariaviva@fp.unila.ac.id

**Abstract.** Arbuscular mycorrhizal fungi (AMF) form symbiotic mutualism with plant root, where fungi will help the host plant to absorb more nutrients and water from the soil. The objective of this paper was to evaluate the effectiveness of two species of AMF namely *Glomus* sp. and *Gigaspora* sp in increasing agarwood seedling growth. The treatments were control (m0), *Glomus* sp. (m1), *Gigaspora* sp. (m2), and mixed of *Glomus* sp. with *Gigaspora* sp. (m3) with 6 replications arranged in Completely Randomized Design. The seeds of agarwood germinated in sterilized sand for one month. The one month old seedlings were then transferred into 12 x 19 cm polybags contain sterilized Ultisol soil. During time of transplanting, the AMF of 300 spores per seedling spread onto the root surface of agarwood seedling. The seedlings remained in the greenhouse for 6 months. Results showed that AMF increased the shoot and root fresh weights and the shoot and root dry weights. Conclusion showed that agarwood seedlings treated with *Glomus* sp. (m1) and mixed of *Glomus* sp. with *Gigaspora* sp. (m3) gave the highest seedling growth which indicated by seedling height, total leave area, fresh and dry weight of shoot and root.

# Introduction

Nowadays, in Indonesia so many plantations downgraded due to continuity of the nutrient-washed. The downgraded of nutrient in the soil resulted in decreasing of the plant production. Therefore, it is required the new technology to upgrade the nutrient availability in the soil. One of the new technologies is to let the arbuscular mycorrizhal fungi (AMF) alive in the soil where the plant alive.

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

Agarwood is the resinous heartwood of the Aquilaria tree as a result of physiological metabolism when the tree become infected with a type of mould.  *Aquilaria* tree is well-known important agarwood-producing genus, which is endemic to Indonesia region. *Aquilaria malaccensis* is one of *Aquilaria* species that have potential to develop due to able to produce agarwood [5]. This tree is one of the forestrycommodities with high value in Indonesia and also in the world. The benefits of agarwood can be vary from perfumes, health, beauty, and even religion [6]. As the agarwood has a high financial value, hence the *A. malaccensis* which can produce agarwood suitable to be developed as a commodity for plantation [7].

*A. malaccensis* has been reported as slow growing tree. This tree species able to associate with the AMF proven by the availability of AMF spores in their rhizosphere [8]. In addition, AMF inoculated *A. malaccensis* was growing fast [9] and able to increase the length of stem diameter [10]. However, research on which species of AMF that suitable in colonizing and improving *A. malaccensi* growth are still very interesting. 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 *A. malaccensis* seedling growth.

# Materials and Method

This research took place in the glasshouse and Plantation Production Laboratory, Faculty of Agriculture of the Universitas Lampung from November 2018 until July 2019. The design of experiment was Completely Randomized Designs by four treatments. The four treatments were as follows: without giving AMF (m0), *Glomus sp.* (m1), *Gigaspora sp.* (m2), and the mixed of *Glomus sp. and Gigaspora sp.* (m3) and those all was repeated six-time. Every experimental unit represented by two plant such that all 48 experimental units were available. After data was collected, then the observation data was analyzed by Anova (analysis of variance) followed by testing the mean different with Least significant different at size of test of 0.01 and 0.05.

As much as 300 seed of Agarwood were germinated in a germination box containing sterilized sand for one month in the glasshouse. The seed were watered daily and no fertilizer was added during this one month period. After one month in germination bed, the seedlings were selected based on the uniformity of it height and total leave. Then, the selected seedlings were transferred into polybag of the 12 x 19 cm size which contain of sterilized media-mixed of ultisol soil and sand of ratio 1:1 by volume. Soil sterilization was conducted by steaming it in the soil-sterilized equipment as long as 3 hours. The AMF inoculation process was conducting during seedlings transplanting from germination bed into polybag by pouring approximately 300 spores of the inoculum *Glomus sp*. (m1), *Gigaspora sp.* (m2), and the mixed of *Glomus sp*. with *Gigaspora sp*. (m3) (according to treatment) onto the root surface of Agarwood seedling in the planting hole.

After transplanting, the seedlings were kept as long as six-month in the glasshouse and watering once a day. The weeding were done manually by pulling out weeds from polybag. Fertilizer of urea was applied two weeks after transplanting as much as 2g/L of water and applied for 100 seedlings of Agarwood or 10 ml/polybag. The urea fertilizer was applied every week until the seed reached age of four weeks. Afterwards, the NPK (N: P: K=16:16:16) was added with dose of one g/seedling at 5 and 16 weeks after transplanting.

Six month after transplanting, the percentage rate of AMF root colonization was examined after staining the roots with trypan blue [11]. The growth variables recorded such as the seedling height (it was measured 2 cm above the top soil until the top nodes), stem diameter (was measure exactly above the first root node using digital calipers), total leaf area (was measured using leaf area meter equipment), the shoot and root wet weight, the shoot and root dry weight (was measured by drying it in the oven of 800C degrees until it weight constant).

# Result and Discussion

Data in Table 1 showed that AMF successfully colonized the root of the *A. malaccencis* or Agarwood seedlings. Agarwood seedlings responded positively to AMF inoculation. The seedling treated with *Glomus* sp and mix of *Glomus* sp with *Gigaspora* sp. had higher root colonization of 91,4% and 84,8% subsequently. *Gigaspora* sp. also colonized the root of *A. malaccencis*  as much as 47,2%, lower than *Glomus* sp and mix of *Glomus* sp. with *Gigaspora* sp. In the untreated control seedling, AMF colonization also detected as 22,5% of the root was colonized by the fungi. This might be due to sterilization of soil media process not completely eliminated indigenous AMF present in the soil media.

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

Table 1. Effect of AMF inoculation on percentage of root colonizationn of six-month old *A. malaccencis* seedling

|  |  |
| --- | --- |
| AMF Treatments | Root Infected 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 |

Notes:

Numbers followed by the same letters were not significantly different in accordance with the LSD at the α= 1%

In line with AMF root colonization, Agarwood seedlings treated which *Glomus* and mix of *Glomus* sp and *Gigaspora* sp had better growth than that seedling treated with *Gigaspora* sp. and control which indicated by seedling height, stem diameter and total leaf area (Table 2 and Table 3). This better growth may be due to the successful of AMF in colonizing Agarwood root as seen on Table 1. The root colonization confirm that the association between AMF and Agarwood seedling roots has happened. External hyphae of AMF might 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 an “structure” for nutrient absorption of plants. About 75-90% P and 5-80% N in host plant are contributed by AMF [14, 15]. Furthermore, 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 were vary against the plant host. It can be seen from its root colonization degree. The more the root were colonized, the more the host plant will benefit. In this study, the percentage of root being colonized reached the 84,8 - 91.4% (showed by *Glomus* sp. and mix of *Glomus* sp. with *Gigaspora* sp.) which categorized as highest colonization [17]. The highly percentage of colonization will increase the absorption of nutrients and water so that the growth of Agarwood seedling such as height, stem diameter, and total leaf area were better than control and *Gigaspora sp.* treated seedling.

Table 2. Effect of AMF on 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 |

Notes:

Numbers followed by the same letters were not significantly different in accordance with the LSD at the α= 1%

Table 3. Influence of AMF on leaf wide of the Agarwood seed of six-month age

|  |  |
| --- | --- |
| AMF Treatments | Total Leaf Area (cm2) |
| 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 |

Notes:

Numbers followed by the same letters were not significantly different in accordance with the LSD at the α= 5%

Similar to seedling height, stem diameter, and total leaf area, from Table 4 can be seen that AMF treatment had also significantly affected shoot and root fresh weight and shoot and root dry weight. The higher shoot and root fresh weight and dry weight were obtained from the seedling treated with *Glomus* sp. and mix of *Glomus* sp. with *Gigaspora* sp. Growth enhancement due to AMF colonization is largely attributed to the extensive exploration of the external hyphae into the soil, hence increasing the potential of the root system to absorb nutrient as well as to increase nutrient transfer from roots to shoots. The other mechanism that might involved is from exudation of phosphatase enzyme by AMF hyphae to the surrounding soil. Phosphorous is a major element often limiting plant growth because it usually in a bound state when added or present in the soil. Enzyme phosphatase could break the bound and make P become available and can be absorbed by the root or by AMF hyphae [18].

Increase in Agarwood seedling growth might also be related to root exudate. Plant associated with AMF will cause the root to produce root exudate which will stimulate the development of the microorganism in the soil [19]. Afterwards, some of these microorganisms will produce phyto hormone such as Indoleacetic Acid (IAA) [20]. IAA stimulates and supports the multiplication of cell so that the seedling had better root fresh and dry weight. In addition, AMF-improved plant growth can be regulated by endogenous hormones. Increase of endogenous hormones levels (putrescine and spermidine) in mycorrhizal plant resulted in increase plant biomass and growth in Citrus [21].

Table 4. Influence of AMF on shoot wet weight, shoot dry weight, root wet weight and root dry of Agarwood seed of six-month age

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| AMF Treatments | Shoot Wet Weight (g) | Shoot dry Weight (g) | Root wet Weight (g) | Root dry  weight (g) |
| Control | 6,74 b | 2,02 b | 2,94 b | 0,67 ab |
| *Glomus sp.* | 13,15 a | 3,70 a | 6,56 a | 1,47 a |
| *Gigaspora sp.* | 7,89 b | 1,92 b | 3,55 b | 0,62 b |
| *Glomus* sp + *Gigaspora* sp | 11,86 a | 3,23 a | 5,35 ab | 1,27 ab |
| LSD 1% | 3,49 | 1,06 | 2,56 | 0,72 |

Notes:

Numbers followed by the same letters were not significantly different in accordance with the LSD at the α= 1%

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | 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 |

Root the “hidden half” of the plant, serve a multitude of function. They are responsible for anchorage, supplier of nutrient 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 [22]. By this mechanism, the AMF may enhance water uptake by the Agarwood seedling hence increase in fresh weight of root and shoot.

The seedling treated with *Glomus* sp. and mix of *Glomus* sp. with *Gigaspora* sp has higher total leaf area (as seen on Table 3). The higher total leaf area, the wider the leaf surface that will support photosynthesis process. In addition, AMF colonization enable significant increase in the rate of photosynthesis of the host plant [23]. Increase in total leaf area together with the increase in photosynthesis rate will produce more organic compound and hence total biomass of the host which indicated by dry weight of shoot and root. The dry weight described the accumulation of organic compound due to photosynthesis in the plant along with more absorption of nutrients from the soil.

# Conclusion

The Agarwood seedlings treated with *Glomus* sp. (m1) and mixed of *Glomus* sp. with *Gigaspora* sp. (m3) gave the highest seedling growth which indicated by seedling height, total leave area, fresh and dry weight of shoot and root.

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