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The Utilization of Arbuscular Mycorrhizal Fungi for Planting Agarwood (*Aquilaria* spp) Seedling in Open Land

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Agarwood is a type of semi-tolerant plant, so that for planting the seedlings should be grown under the shade. For planting in open land, it requires treatment in which one of them is using seedlings inoculated with arbuscular mycorrhizal fungi. The aim of the research is to obtain information on agarwood growth that has been inoculated with fungi mycorrhizal arbuscular when planted in the open land and ability to grow between agarwood seedlings inoculated mycorrhizal that was planted in the shade and in the open area. Split plot randomized block design was applied with treatments: the first plot consisting of plant had been inoculated with mycorrhizae and without mycorrhizal inoculation, and the sub plot was the types of shading that consists of open land, paranet 60 % intensity and natural vegetation. To reduce variabilty of site topographical differences were separated as bloks. Variables measured were: plant height (cm), stem diameter (mm), number of leaves, and survival percentage of plant. The results show that the height and diameter growth of seedlings innoculated with mycorrhizae were higher than non innoculated. The seedlings innoculated with mycorrhizal fungi were planted in the paranet shading grew better and significantly different compared to the vegetation shading. Seedlings innoculated mycorrhizal that were planted in open land grew better and significantly different compared to vegetation shading. This study results indicate that planting agarwood in the open land can be done using seedlings inoculated arbuscular mycorrhizal fungi.

Key words: agarwood plant, arbuscular mycorrhizal fungi, shading and open land

Gaharu merupakan jenis tanaman yang bersifat semitoleran, sehingga hanya bisa ditanam dibawah naungan. Untuk penanaman pada lahan terbuka diperlukan perlakuan dimana salah satunya adalah menggunakan bibit yang terinokulasi fungi mikoriza. Tujuan penelitian adalah untuk memperoleh informasi mengenai pertumbuhan tanaman gaharu yang terinokulasi fungi mikoriza pada lahan terbuka, kemampuan tumbuh tanaman yang terinokulasi fungi mikoriza dibawah naungan dan di lahan yang terbuka. Metode *Split Plot Design* acak kelompok dengan perlakuan : Petak utama terdiri dari bibit tanpa mikoriza dan bermikoriza. Sebagai sub plot adalah tipe naungan yang terdiri dari : lahan terbuka, paranet 60 % dan vegetasi alam. Variabel yang diukur adalah : tinggi tanaman (cm), diameter batang (mm) jumlah daun dan persentase hidup. Hasil penelitian menunjukkan bahwa pertumbuhan tinggi dan diameter bibit yang terinokulasi mikoriza lebih baik dibandingkan dengan tanpa mikoriza. Bibit terinokulasi mikoriza yang ditanam dibawah naungan paranet tumbuh lebih baik dan berbeda sangat nyata dibandingkan dengan naungan vegetasi. Bibit terinokulasi mikoriza yang ditanam di lahan terbuka, tumbuh lebih baik dan berbeda nyata dibandingkan dengan dibawah naungan vegetasi. Hasil penelitian ini mengindikasikan bahwa penanaman tanaman gaharu di lahan terbuka dapat dilakukan dengan menggunakan bibit terinokulasi fungi mikoriza arbuskula.

Kata kunci: fungi mikoriza arbuskula, naungan dan lahan terbuka, tanaman gaharu

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Agarwood (*Aquilaria* spp) is a forest products produced from wood trunk section that specifically form aromatic resin. Because the utilization is very wide, it becomes a forest products commodity that have high economic value. More over agarwood is one of the main of forest products in international trade. Intensive logging on agarwood in natural forest, resulting in the decrease of its existence that is already endangered. One contributing factor is that genus agarwood (*Aquiliria* spp) is already included in CITES Appendix II which prohibits trade in agarwood derived from natural forests. In terms of technology to accelerate the growth of agarwood trees has been found through various studies as conducted by Muin and Muin *et al.* (Muin 2009; Muin *et al.* 2010) that must be supported by planting activities.

Due to the semi-tolerant properties of the agarwood plant people are not be able to plant this species in open lands. Difficulties in planting in the open land were obtanined from experiences of community in the District Parindu and Bonti Sanggau West Kalimantan (Muin *et al.* 2017). This information came up because peoples want to plant agarwood (*Aquilaria* spp) on open land, especially in the land rehabilitation activities (GN-RHL/GERHAN) and Community

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Plantation Forest (Hutan Tanaman Rakyat).

Based on the nature of the semi-tolerant tree species, the planting of agarwood could be expanded into open land. However, it require a variety of treatment, both while still in the nursery and when planted in the field. Semi-tolerant seedlings planting in open fields requiring high seedling quality index, and special treatment when planted in the field, among others by using mycorrhizal seedling and providing shade with the appropriate light intensity (Muin 2009). This information on the treatment was no available and becomes critically needful regarding the cultivation of agarwood using mycorrhizal seedlings and provision of appropriate shade to be planted in open land.

The aims of research areto obtain information an agarwood plant growth that has been inoculated with arbuscular mycorrhizal fungi when are planted in the open land, and to search the differences between the ability to grow with and without mycorrhizal seedlings are planted in the shade and on the open land. The final goal of this research is to confirm that people can plant agarwood (*Aquilaria* spp) on open land on a broader scale, thereby they can increase the production of agarwood aromatic resin and expand economic development, especially for the people who live around the forest.

MATERIALS AND METHODS

Research had been conducted at Kebun Percobaan Universitas Tanjungpura, Desa Pak Laheng Kabupaten Mempawah for mycorrhizae, seedlings inoculated with mycorrhiza and paranet with intensity 60%. In this study, the data collection was done on the plant until the age of 12 weeks. The materials used for this research are agarwood seedlings which are inoculated with mycorrhizal fungi and without mycorrhizae, paranet with 60% light intensity. The research method was using experimental split plot randomized block design which seedlings as main plot that not inoculated with mycorrhizae (F_1) and inoculated with mycorrhiza (F_2), and sub plot in the form of shade was consisting of without shade (N_1), shade paranet intensity 60 % (N_2) and the shade of natural vegetation (N_3). Blocks were used in this study due to the differences of site topography. Data that had been collected : plant height, diameter and number of leaves and survival of plants. To determine differences in the effects of each treatment, analysis of variance was parformed according to the split plot design experiment. The Least Small Different test (LSD) was used to determine the different effecst of the treatment given to the agarwood plant.

RESULTS

Plant Height (cm). Base on analysis of variance, the micorrhizal plants and shade treatment as well as the interaction has not shown any significant effect on height variable of the agarwood plant until the age of 12 weeks (3 months). Although the results of Analysis of Variance showed no significant effect, but the difference in height gain for 12 weeks of each treatment can be seen (Fig 1, 2).

The treatment without shade (N_1) showed height growth was slower compared to the treatment of paranet 60% (N_2) at the age of up to eight weeks, but became faster after more than 10 weeks old (Fig 1). The height growth of agarwood plant grown in the open land was better than the one in the shade paranet (N_2).

The plants that have been inoculated with arbuscular mycorrhizal fungi (F_2) growth better started in week ten and up to 12 weeks height growth was 10.94 cm, while plants without mycorrhiza (F_1), height growth until the age of 12 weeks only 8.85 cm. Is seems that the arbuscular mycorrhizal fungi began to affect the plant on 10^{th} weeks (Fig 2).

Stem Diameter (mm). Effect of arbuscular mycorrhizal fungi and shade treatment to plant diameter growth and its analysis of variance according to split plot design experiment results was shown (Table 2).

Based on analysis of variance that giving shade and mycorrhizal fungi treatments and the interaction of both treatment show hihgly significant effects on the growth of the agarwood plant diameter (Table 2). LSD test showed that mycorrhizal agarwood plant grown in the shade paranet 60% had the best diameter growth. The differences in the growth of plants grown in the shade of forest vegetation, and in the open land and under paranet presented (Fig 3). It shows the difference in diameter growth was taking place since the plant eight weeks old (measurement week 4). Diameter growth of plants grown in the open land turned out to be slower compared to those grown under paranet and natural vegetation (Fig 3). Significant difference between the diameter growth agarwood plant with myocrrhizal and without mycorrhizal. Growth of mycorrhizal agarwood plant was faster and started to take place in a six-week-old plants (measurement weeks to 3) (Fig 4).

Amount of Leaves Growth. Analysis of Variance

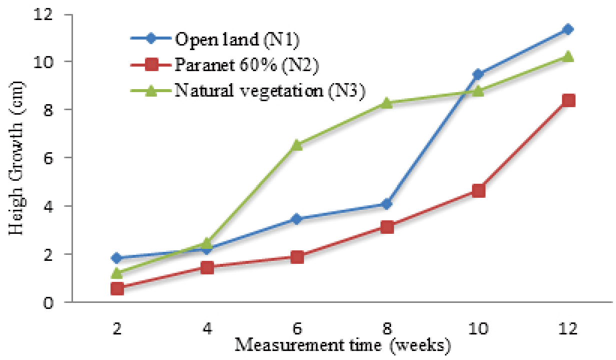

Fig 1 Graph of mean height growth (cm) of agarwood plant with treatment in open land (N_1), under shading of paranet 60% (N_2) and under shading of natural vegetation (N_3) until age 12 weeks.

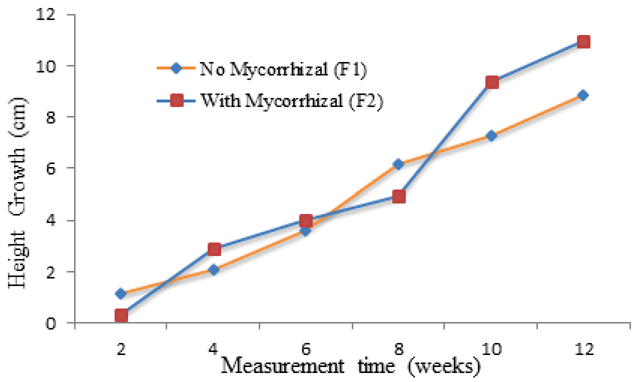

Fig 2 Graph of mean height growth (cm) of agarwood plant without and with mycorrhizal treatments, until age 12 weeks.

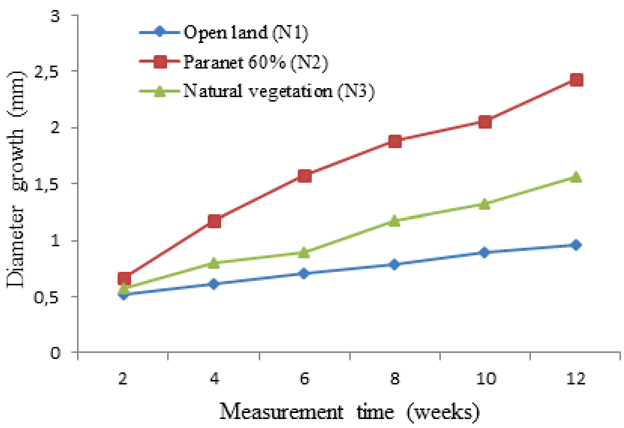

Fig 3 Graph of average agarwood diameter growth (mm) in open land (N_1), under paranet shading 60% (N_2) and under natural vegetation (N_3) until 12 weeks old.

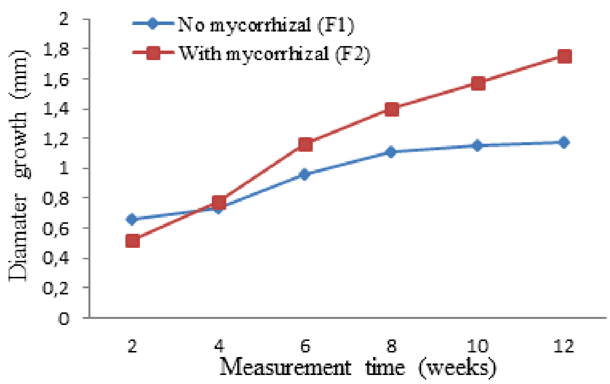

Fig 4 Mean of diameter growth (cm) of agarwood plant without mycorrhizal and with mycorrhizal treatment until age 12 weeks.

results showed that the treatment given to agarwood plants very significant effect on the increase of the amount of leaves. However, interaction of the two treatments did not affect on the increase of the amount of leaves. The difference in the leaves of 12 weeks old the agarwood plant (sixth weeks measurement) presented in Table 3 which shows more leaves on mycorrhizal plants grown in the open land. Mycorrhizal agarwood plant increased as much as 13 leaves, while mycorrhizal agarwood plant grown in the shade paranet only grown 5 leaves and whereas leaves grown under natural vegetation shading were 9.

Plant Survival (%). The treatment of mycorrhiza and shade effected on the percentage of plant survival until the age of 12 weeks (Table 4). The percentage of plants survival grown in open fields is lower and significantly different compared to the one planted in the shade of natural forest vegetation and paranet. Dead plants on open land occured until the age of two weeks, including the mycorrhizal plant. Drought and high air temperatures (40 °C) for 4 weeks during the study cause agarwood plants in the open land experiencing wilting and death.

DISCUSSION

The results showed the effects of treatments on the growth of mycorrhiza and shade height, diameter, number of leaves and the plant survival. Significant effect only occurs in diameter and increase the number of leaves parameters, while the height growth and survival had not yet significant differences. However, high growth mycorrhizal agarwood plant grew faster since the age of the plant was 10 weeks (the fifth measurment), especially plants grown in the open land (without shade) (Fig 2). Meanwhile the growth of mycorrhizal agarwood plant started faster on a 6 weeks old plants (measurement to three). This result shows that arbuscular mycorrhizal fungi (AMF) inoculated on seedlings in nursery can be used as an instrument in accelerating the growth of the agarwood plant after planting in the field. Seedlings grown in nutrient-poor of ultisol soil with a low pH requires forming associations with mycorrhizal fungi. By nature, the type of plants forming mycorrhizal symbiosis with fungi grow on marginal land that is poor in nutrients and low pH, such as red-yellow podzolic soil (Muin *et al*. 2016). Research conducted by Nurul Huda, Muin and Fachrizal (2016) showed that the 3 year sold agarwood plants planted in the Satong village has been in symbiosis with arbuscular mycorrhizal fungi. Results of Nurul Huda *et al.* (2016) showed that the seedlings inoculated with arbuscular mycorrhizal fungi (AMF) grew faster with higher quality index. This result indicates that the growth of agarwood from nursery to be planted in the red yellow podzolic soil requires association with mycorrhizal fungi forming. This symbiosis confers benefits directly to the host plant's growth and development through the acquisition of Phosphorus (P) and other mineral nutrients from the soil by the AMF (Aggarwal *et al*. 2011).

Role of arbuscular mycorrhizal fungi (AMF) in global sustainable development. AM fungi facilitate plant uptake of mineral nutrients such as phosphorus and nitrogen by increasing the absorbing surface area and by mobilizing sparsely available nutrients. In turn, plant hosts supply AM fungi with a carbon source that is essential for fungal growth (Wang *et al*. 2017). Fungal hyphae normally transport posphorous over longer distances from depleted zones compared to roots. The AM especially benefit plants grown in soils where P is likely to limit plant growth. A reduced effectiveness of AM colonization of roots often occurs when soluble soil P levels increase this review gives an overview on the role of mycorrhizae in absorbing phosphorous (Lizadeh 2011). Arbuscular mycorrhizal fungi (AMF) improve the plant mineral nutrition in particular the acquisition of phosphorus and some nitrogen and minor nutrients (Karti *et al.* 2018).

Arbuscular Mycorrhizal Fungi (AMF) constitute a group of root obligate biotrophs that exchange mutual benefits with about 80% of plants and provide the host with water, nutrients, and pathogen protection, in exchange for photosynthetic products (Berruti *et al*. 2016). Arbuscular mycorrhizal fungi (AMF) are ubiquitous organism that forms association with the root of most terrestrial plants and influence soil fertility through the enhancement of chemical, biological and physical content (Syibli *et al*. 2013; Armadal *et al*. 2016).

The percentage of plants survival in the open, including the mycorrhizal plants was very low, because when planted in February 2016 (up to age 3 weeks) drought with extremely high temperatures (40 °C) was leading to high soil temperatures and increasing evaporation on the leaves. High soil temperatures (36 °C) and dry soil conditions causes the amount of water absorbed less than evaporation, so that the plant become wilted and died. The high light intensity could be disadvantage to plants, because the soil became hot, while too low light can reduce the amount of

Table 1 Height of agarwood plant (cm) with mycorrhizal and shading treatment until 12 weeks olds

Shading Treatment	Mycorrhizal Treatment		Average height under shading
	Without Mycorrhizal	With Mycorrhizal	
Open Land (N_1)	10.06	11.80	10.93
Paranet 70 % (N_2)	8.68	9.27	8.98
Natural vegetation (N_3)	8.26	11.75	11.75
Average height with mycorrhizal	9.37	10.94	10.16

Table 2 Diameter (mm) of 12 weeks old agarwood plant with mycorrhizal and shade treatments

Shading Treatment	Mycorrhizal Treatment		Mean diameter with shading
	Without Mycorrhizal	With Mycorrhizal	
Open Land (N_1)	0.64	1.12	0.88
Paranet 60 % (N_2)	1.52	2.35**	2.44**
Natural vegetation (N_3)	1.34	1.78	1.56
Mean diameter with mycorrhizal	1.17	1.75**	1.63

Note: **) Significant difference at 1% level

Table 3 Number increasing of leaves (number of) 12 weeks old agarwood plant with mycorrhizal and shade treatments

Shading Treatment	Mycorrhizal Treatment		Mean leaves number with shading
	Without Mycorrhizal	With Mycorrhizal	
Open Land (N_1)	9.33	13.33	11.33**
Paranet 60 % (N_2)	6.00	5.00	5.50
Natural vegetation (N_3)	5.33	9.33	7.33
Mean leaves number with mycorrhizal	6.88	9.22	8.05

Note: **) Significant difference at 1% level

Table 4 Survival of agarwood plants with mycorrhiza and shade treatments untill 12 weeks old

Shading Treatment	Mycorrhizal Treatment		Mean survival with shading
	Without Mycorrhizal	With Mycorrhizal	
Open Land (N_1)	78.25	60	69.13
Paranet 60 % (N_2)	90	90	90
Natural vegetation (N_3)	90	90	90
Average leaves number with mycorrhizal	86.08	80	83.04

mycoorhizal rooting due to lack of supply of carbohydrates resulted on photosynthesis. The establishment of AM symbiosis involves recognition of the two partners and bidirectional transport of different mineral and carbon nutrients through the symbiotic interfaces within the host root cells (Wang *et al*. 2017). Intriguingly, recent discoveries have highlighted that lipids are transferred from the plant host to AM fungus as a major carbon source. However, by week four weather returned normal with rainfall and then a normal air temperature (28 °C - 32 °C), the plants began to grow normally. In the dry season, almost all crops grew well in the shade and yet mostly in the open land, they did not but in process stagnant growth (slow). This is because the seedlings was planted in this study had a high quality index. According Muin (2009) that planting semi-tolerant seedlings in open land requiring high seedling quality index, and special treatments when planted in open land as it is done on ramin (*Gonystylus bancanus* Miq. Kurz) seedlings. One of the weaknesses in this study, the seedlings planted were not adapted well to field conditions. The provision of shade to new seedling planted in the field in needed so that the plant does not receive direct sunlight and soil temperatures are not too high. The soil temperature is too high (over 32 °C) will affect negatively to the development of mikroiza arbuscular fungi (Muin *et al*. 2016). Plants that are semitoleran need shade when they are in seedlings growth level and requires full light after approaching adult levels (sapling). However with mycorrhizal fungi inoculation agarwood plant (*Aquilaria* spp) are able to grow in the open, as is the case in plants ramin peat swamp forest.

Arbuscular mycorrhizal fungi inhabiting soil play an important role for vascular plants. Interaction between arbuscular mycorrhizal fungi, plants and soil microorganisms leads to many mutual advantages. However, the effectiveness of mycorrhizal fungi depends not only on biotic, but also abiotic factors such as physico-chemical properties of the soil, availability of water and biogenic elements, agricultural practices, and climatic conditions (Jamiołkowska *et al*. 2018).

Research results of Muin (2009) showed that the mycorrhizal seedlings of ramin (semitoleran) planted in the logged open land grew faster whitin the three months compared without mycorrhizae. The results of research showed that mycorrhizal fungi can enhance the growth of agarwood in the open land. Most vascular plants form symbiotic relationships with arbuscular mycorrhizal fungi (AMF), root symbionts that provide soil nutrients to plants in exchange for carbohydrates, which may reduce the effects of environmental stresses on plants (Pischl and Barber 2017). AM fungi facilitate plant uptake of mineral nutrients such as phosphorus and nitrogen by increasing the absorbing surface area and by mobilizing sparsely available nutrients. In turn, plant hosts supply AM fungi with a carbon source that is essential for fungal growth (Wang *et al*. 2017). Arbuscular mycorrhizal fungi (AMF) are ubiquitous organism that forms association with the root of most terrestrial plants and influence soil fertility through the enhancement of chemical, biological and physical content (Syibli *et al*. 2013).

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