Journal of Land Use Transformation System

Journal website: https://online-journal.unja.ac.id/jlasts

Identification of Arbuscular Mycorrhiza Fungi (AMF) at Rhizosphere of Jelutung Rawa (*Dyera lowii* Hook.f.) Intercroped With Oil Palm (*Elaeis guineensis* Jacq.)

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ABSTRACT

This research aims to know the diversity of the genus and the level of colonization of AMF in the rhizosphere of the Jelutung rawa (*Dyera lowii* Hook.f.) which is intercropped with the oil palm (*Elaeis guineensis* Jacq.) in Jati Mulyo Village, Dendang District, East Tanjung Jabung Regency. The study used exploratory descriptive methods, with sampling using purposive sampling techniques. The study consists of several stages, namely: soil and root sampling, AMF spore identification, and root colonization. The results of the study showed the genus AMF in the rhizosphere of the Jelutung rawa (*D. lowii* Hook.f.) which is intercropped with the oil palm (*E. guineensis* Jacq.) namely *Glomus, Acaulospora, Gigaspoora,* and *Scutellospora*. The AMF colonization rate is 100%, and this is categorized as very high.

Keywords: *Identification, Jelutung rawa, mycorrizha*

INTRODUCTION

Peatland forest areas are decreasing due to the conversion of forest into farmland, shifting cultivation, illegal logging, mining, and fires (Tawara & Turjaman, 2016). Jati Mulyo is a village located in Dendang Rural District, Tanjung Jabung Timur. The village spans an area of 1180 hectares, consisting mainly of peatland plantations. However, it has been observed that oil palm cultivation does not perform well in peatland, producing only 300 kg of palm in one harvest from an area of 1 hectare of land. As a result, the community has started to adopt intercropping techniques, growing Jelutung rawa (*Dyera lowii* Hook.f.) alongside oil palm cultivation. Jelutung Rawa is a tree species belonging to the Apocinaceae family (Tjitrosoepomo, 2013) and is an indigenous species of the peat swamp forest (Mansur, 2015). The use of mycorrhizas can improve the early growth of tree species grown in peat swamp forests (Turjaman et al., 2019).

Mycorrhizae refers to a type of symbiotic relationship between fungi and the roots of higher plants. Both parties benefit from this relationship, as fungi obtain a source of carbon from the products of photosynthesis, while plants obtain a supply of elements, particularly phosphate from the fungi. Additionally, mycorrhizae can release phosphorus, which is otherwise unavailable to the plants. This is due to the release of an enzyme called phosphatase and organic acids, such as oxalate, that help to free the phosphates (Mansur, 2013). Arbuscular mycorrhiza is a potential soil microbe that can enhance plant growth in bio-rehabilitation technology, but it has not been extensively used to support the revegetation of degraded tropical peatlands (Yuwati & Hakim, 2023). Mycorrhizal colonization was three times higher in oil palm cultivated in agroforestry systems compared to monoculture (Maia et al., 2021). This research aims to determine the level of colonization of AMF in the rhizosphere of the Jelutung Rawa (*D.lowii* Hook.f.) and to understand the diversity of the genus of Arbuscular Mycorrhiza Fungi (AMF) in the rhizosphere of Jelutung Rawa plant that is intercropped with oil palm (*Elaeis guineensis* Jacq.) in Jati Mulyo Village, Dendang District, East Tanjung Jabung Regency.

MATERIALS AND METHODS

This study utilizes a qualitative approach with a descriptive exploratory research design. Soil and roots from jelutung rawa were sampled from Jati Mulyo village, Dendang District, Tanjung Jabung Timur Regency, Jambi Province. The isolation and identification process was conducted in the laboratory of FKIP Biology Education at the University of Jambi.

The study collected data by extracting spores and coloring the roots of the jelutung plant. The sampling technique used was purposive sampling, with samples taken from 4 plots, each measuring 20x20 m. AMF spores count was conducted to determine the density and frequency of spores.

$$Spore\ Density = \frac{Number\ of\ spores}{100g}$$

Calculation of the root colonization by using the following formula Vierheilig et al., 1998 (Nusantara, 2012):

$$\%\ Colonized\ roots = \frac{\sum Mycorrizhal\ field\ of\ view}{\sum Observed\ field\ of\ view}\ x\ 100\%$$

Root colonization of AMF is categorized in the Table 1.

Table 1. Colonization rate (%) of AMF in the roots of Jelutung Rawa

Root colonization (%)	Category
0	Uncolonized
< 10	Low
10-30	Medium
>30	High

The research tools used included multilevel sieves (with 20 mesh, 60 mesh, 200 mesh, 300 mesh, and 400 mesh), stationery, hoes, shovels, cover glass, object glass, beaker glass, scissors, a camera, ruler, binocular microscope, light microscope, knife, stir bar, digital balance, Petri dishes, tweezers, dropper pipettes, centrifuges, and centrifuge tubes.

The materials used in this research included young roots of Jelutung rawa, soil from the rhizosphere of Jelutung plants, distilled water, label paper, plastic samples, 1 liter of 10% KOH solution, 1 liter of 2% HCl solution, 400 ml of glycerine, 400 ml of Lactic Acid, 0.2 g of trypan blue, 60% glucose, Melzer's solution, PVLG solution, H2O2, alcohol solution, 0.01% methylene blue, tissue, and tweezers.

Procedure:

Around 200 g of soil from the rhizosphere of Jelutung Rawa were collected and placed in a waterproof plastic bag, which was labeled for identification. Additionally, young roots of the Jelutung plant were taken, and about 10-15 cm of the roots were cut, put in a separate plastic bag, and labeled accordingly.

a. Spore Extraction

Extracting AMF spores from soil; the technique for extracting AMF spores is wet sieving and centrifugation techniques (Brundrett et al., 1996). In the first step, a 100g soil sample was mixed with 500 ml water and stirred. The mixture is filtered in a set of sieves. 5ml of the material is added from the fourth and fifth filters to separate centrifuge tubes. The material is centrifuged at 2500 rpm for 5 minutes. Then 60% glucose was added to the tubes and centrifuged again for 30 seconds. Pour the suspension into a 400 mesh filter and wash with running water. Then, transfer to a petri dish and examine under a stereo microscope.

b. Root Staining

Root staining is done with the following steps according to Vierheiling et al. (1998) method modified by Nusantara (2012). The roots are washed with distilled water (x3). The roots are soaked in hot 10% KOH for 10-30 min. Then, the roots are washed with running water (x3-5). After that, the roots were soaked in trypan blue for 12 hours and then soaked in a destaining solution. The roots are cut into 1 cm long. The roots are placed in a row on an object glass and covered it with a cover glass. then, observed each root cut under a microscope.

c. Identify AMF

To identify AMF spores, one needs to prepare mycorrhizal spore preparations. These preparations are made using Melzer's solution, which serves as an indicator in determining the genus of AMF and is also used to preserve existing spores (Yelianti et al., 2009). The identification of mycorrhiza is carried out using the Working with Mycorrhizas in Forestry and Agriculture guidebook by Brundrett (1996). The characteristics that are observed to determine the spore's identity include its shape, surface color, and walls.

RESULTS AND DISCUSSIONS

The density of AMF spores the rhizosphere of Jelutung rawa

In general, AMF are known to live freely and naturally in the rhizosphere of their host plants. Table 2 indicates that the density or average number of AMF spores in 100 g of Jelutung rawa rhizosphere is 92 AMF spores. AMF is also able to colonize almost all the roots of agricultural plants. Based on Table 3, it can be seen that the average percentage of AMF colonization on the roots of swamp Jelutung plants is 100% and is categorized as high. This shows that swamp Jelutung is a plant that is responsive to AMF.

The spore frequency in Jelutung rawa is 98.06% with a very high category (Table 3), indicating that AMF can have a symbiotic relationship with Jelutung rawa in Jati Mulyo Village. According to Handayanto and Hairiah (2007), mycorrhizal fungi can be associated with the roots of different plants, but their effectiveness is also determined by the plant type and the ecosystem origin of the inoculant.

Table 2. The density of AMF spores in 100 g of the rhizosphere of Jelutung rawa.

No	Plot	Number of spores on the sieve size (mesh)/100g		Density
		300	400	
1.	Plot 1	197	238	435
2.	Plot 2	140	172	312
3.	Plot 3	117	212	329
4.	Plot 4	141	258	399
Total spores		595	880	1475
Average of density		37	55	92

Note: 4 plant/plot

Table 3. Percentage and categories of AMF colonization in the root of Jelutung rawa

Plot	Repeatation (%)				Average (%)	Category
	1	2	3	4		
1	100	98	100	94	98	very high
2	100	100	98	100	99.5	very high
3	90	100	100	92	95,5	very high
4	100	100	97	100	99.25	very high
Total average					98.06	very high

Structure of FMA and types of spores in the rhizosphere of Jelutung Rawa

Arbuscular Mycorrhizal Fungi (AMF) is a type of fungus that belongs to the endomycorrhizal group. AMF is classified as a mycorrhiza-forming fungus and is widely distributed as it can be found in almost all ecosystems (Kartika, 2006). Table 4 demonstrates that all the rhizosphere samples of Jelutung rawa showed the presence of AMF spores.

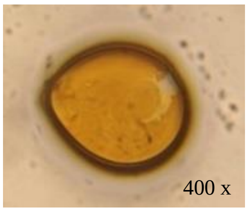
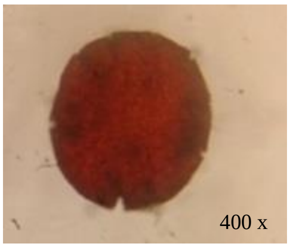
Spores are structures that form Arbuscular Mycorrhizal Fungi (AMF) and function as propagules to survive in the soil (Brundrett et al., 1996). According to Yusriadi et al. (2017), the water content in soil is a physical property that may influence the number of AMF spores. The lower the water content in a land, the larger the number of spores found. Conversely, the higher the water content, the fewer spores were found. Abdelmoneim et al. (2014), found that the total spores and mycorrhizal colonization of plants correlated with water availability. The availability of large amounts of water can reduce the number of spores and mycorrhizal colonization on plants. Mycorrhiza can be used as an alternative technology for growth, productivity, and plant quality. This was explained by Hasruddin and Husna (2014), who stated that mycorrhizae have an essential role in the agricultural sector. They are biological controllers, soil improvers, abiotic stress reducers, and organisms that play a role in rehabilitating degraded land. Tuheteru (2012) also added that mycorrhizal fungi have a role in the sustainability of plant regeneration and make a positive contribution to the existence of plant species in a community AMF spore density in the rhizosphere of Jelutung rawa.

Identification of arbuscular mycorrhizae by examining the physical traits of the spores such as their shape, size, ornamentation, and color. It was discovered that there are 5 different types of spores in the rhizosphere of Jelutung Rawa that is intercropped with oil palms. These spores belong to the genera *Glomus, Acaulospora, Gigaspora*, and *Scutellospora. Glomus* has the highest number of spore types, with 22 types, followed by *Acaulospora* with 4 types, *Gigaspora* with 2 types, and with 4 types. According to Kartika (2006), the population and diversity of AMF in acid-mineral soils in Indonesia are high, with *Glomus, Acaulospora, Gigaspora*, and *Scutellospora* being the dominant genera.

From the research carried out on AMF identification on oil palms intercropped with Jelutung rawa in Jati Mulyo village, the results obtained about 15 types of spores consisting of two genera, namely *Glomus* with 14 types and *Acaulospora* with 1 type (Table 4).

This study found that the spores of the *Glomus* genus were much more common than other genera, making up 22 out of a total of 33 spore types. This accounts for approximately 66.67% of all AMF found. This high frequency of *Glomus* spores indicates that they are well-suited and able to form a symbiotic relationship with Jelutung Rawa. The diversity of AMF species in various types of trees in the Deudap Pulo mountains of Aceh, Aceh Besar Regency was also analyzed by Hasyiati et al. (2018). The study found that *Glomus* was the most dominant genus found, with 34 different mycorrhizal species belonging to it. Based on the research conducted by Subari (2019) regarding the identification of AMF in the rhizosphere of oil palm intercropped with Jelutung rawa, it showed more genus variations than the genera found in oil palm. This is because Jelutung rawa is an indigenous plant species in peat swamp land, so it has good adaptability to live in a peat swamp environment.

Table 4. Types of AMF spores in the rhizosphere of Jelutung rawa (*D. lowii* Hook f.)

No.	Spores type	Morphological characteristics	Reaction With Melzer's
1.	 *Glomus sp. (1)*	The spores are ovoid with two layers of spore walls, bright yellow in color, and pass through a 200 mesh sieve.	No
2.	 *Glomus* sp.*(2)*	The spores are ovoid, yellow in color, have two layers of spore walls, have Hypha subtending, and pass through a 300 mesh sieve.	No
3.	 *Gigaspora* sp.	The spores are round (globose) red in color, have ornaments in the form of small protrusions (punctates) on the surface of the spores, and pass through a 300 mesh sieve.	No
4.	 *Acaulospora* sp.	The spores are round (globose), bright yellow in color, consisting of 4 layers of spore walls, and pass through a 200 mesh sieve.	Positive

The dominant presence of *Glomus* spores was also explained by Delvian (2006) who stated that *Glomus* spores are generally smaller than other genera. This will influence the hydration phase to be faster so that the activity of the enzymes associated with germination will take place earlier. So, *Glomus* germination will occur faster than other mycorrhizal genera. Besides that, differences in the frequency of AMF in an ecosystem are influenced by several factors as explained by Husin et al. (2012), namely that mycorrhiza is influenced by temperature, soil water content, soil pH, organic matter, light, and nutrients, heavy metals and trace elements, others in the soil as well as fungicides.

Microscopically, each genus of spores found in the rhizosphere of Jelutung plants has distinctive characteristics, such as the *Glomus* spore type which has multi-layered cell walls, the shape tends to be round, elliptical, and ovoid, the spores are hyaline, reddish-yellow, brown and black. Apart from that, *Glomus* has hyphae stand or what is usually called subtending hyphae and does not react with Melzer's solution. This is in line with Nusantara's statement (2012) that *Glomus* spores are round, clear, hyaline (transparent), white, yellow, and brown, do not react with Melzer's solution, and do not have ornaments, and have subtending hyphae. Apart from that, it was stated by Brundrett et al. (1996) that some glomus spores have several layers of spore walls.

In contrast to *Glomus*, in general, the type of spores found in the Genus *Acaulospora* are round (globose) with yellow, red, and brown colored spores. *Acaulospora* has spores with several layers of quite thick walls. Apart from that, the outer layer of *Acaulospora* did not react with Melzer's solution, while the inner layer showed a reaction after being given Melzer's solution, which was characterized by a color change to dark red and brown. Nusantara et al. (2012) found that the outer layer of the spores of the Genus *Acaulospora* does not react with Melzer. However, the inner layer reacts with Melzer with a darker color.

The spores of the *Gigaspora* genus that were found have characteristics including having a bulbous suspensor, bright yellow, red, and brownish. *Gigaspora* has a thin spore wall layer and reacts thoroughly with Melzer's solution. Nusantara et al. (2012) stated that *Gigaspora* spores are usually bright yellow, the spore wall layer is thin and reacts with Melzer's solution thoroughly, has no ornaments, and the hyphae form bulbous suspensors or rounded hyphae stands.

The characteristics of the spores of the *Scutellospora* genus are that the spore wall layer is thin, reacts with Melzer completely, has an ornament in the form of a germination shield, the hyphae form bulbous suspensors or rounded hyphae seats (Nusantara et al., 2012). In this study, spores of the Genus *Scutellospora* were found with characteristics including being round (globose), ovoid (ovoid), and irregular (angular). Apart from that, several types of *Scutellospora* have germination shields, bulbous suspensors, and ornaments on the surface of the spores.

The characteristics of the *Sclerocystis* genus were found to have a wall layer that was clustered and did not react with Melzer's solution. This is in line with the statement of Nusantara et al. (2012) that the characteristics of the spores of the genus *Sclerocystis* include a dominant red color with a size of 100-200 µm. Has bunches of spores (sporocarp). The wall layers are clustered, do not react with Melzer's solution and have layered and uncoated ornamentation.

Based on Table 4, it is known that the spore wall layers in the *Glomus, Gigaspora* genera do not react to Melzer's solution, while the *Acaulospora* and *Scutellospora* genera reacted with Melzer's solution.

Melzer's solution is one of the solutions used to identify AMF. Melzer's contains iodine as an active dye in this solution which will react with polysaccharides such as starch in cell walls. Brundrett et al. (1996) explained that the AMF spore cell wall consists of one or more layers. The layers have variations in structure thickness, surface, and coloring reaction. *Acaulospora, Entrophospora,* and *Scutellospora* have a complex wall structure consisting of a thicker outer wall and a thinner inner layer. One or more of these wall layers can be stained with Melzer's solution, while *Glomus* and *Gigaspora* have simpler structures than other genera. Although some species of the *Glomus* genus also have layered cell walls. Young Glomus will have a weak response to the new reagent Melzer's solution, and will not react to old spores. This is because young Glomus spores have an outer wall layer that is fragile and easily destroyed as the spores age.

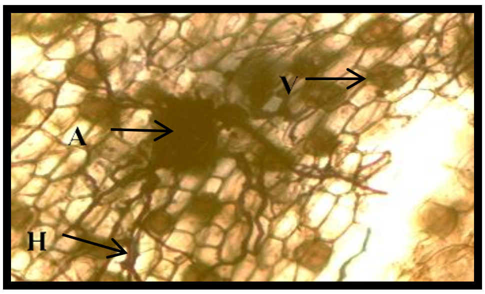


Figure 1. Structure of FMA on the roots of Jelutung rawa (Magnification 40 x 100)
V = Vesicule, A = Arbuscular, H = Hyphae

According to Brundrett et al. (1996), AMF consists of typical structures, namely hyphae that branch in the root cortex, arbuscules, and some form of storage structures called vesicles. The results of root staining and observation under a microscope with a magnification of 400 times show that there is AMF symbiosis in the roots of swamp Jelutung plants. This is characterized by the discovery of several AMF structures in root samples, namely hyphae, vesicular and arbuscular.

Hyphae are structures that are often found in the root cortex cells of many plants (Brundrett et al., 1996). Internal hyphae function mainly to partition nutrients from both outside and inside the AMF tissue (Husin et al., 2012). So, it has an important role in the development of mycorrhiza and the symbiotic relationship between mycorrhiza and host plants.

Based on observations, vesicular were found in all samples of Jelutung rawa roots that were observed in round and oval shapes. According to Husin et al. (2012) vesicular functions as a food storage organ or develops into chlamydospores. It also contains a lot of fat and functions as a food storage organ for AMF, the vesicular also functions as a reproductive organ.

The arbuscules found in the roots of the Jelutung rawa have a tree-like structure that has many branches. According to Brundrett et al. (1996), arbusculars are finely branched hyphae, formed by repeated dichotomous branching, so that they resemble trees in the host cell. Husin et al. (2012) added that arbuscular is an AMF structure that functions as a place for nutrient exchange between host plants and fungi.

Conclusion

The AMF genera in the rhizosphere of Jelutung rawa (*Dyera lowii* Hook.f.) which are intercropped with oil palm (*Elaeis guineensis* Jacq.) are *Glomus, Acaulospora, Gigaspoora*, and *Scutellospora* with high levels of AMF colonization.

Further research needs to be carried out regarding the influence of soil chemical content on mycorrhizal growth and the effectiveness of AMF on the growth of swamp Jelutung plants intercropped with oil palm.

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