Diversity and Abundance of Mycorrhizal Fungi Spores in Gmelina arborea Stand

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Diversity and Abundance of Mycorrhizal Fungi Spores in *Gmelina arborea* Stand

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Abstract. Mycorrhizas are the symbiotic associations between plant and fungi. Mycorrhizal fungi always associate with the roots of higher plants, indeed over 90% of plant species, including forest trees, wild grasses and many crops. This study aims to determine the type of spores associated with *Gmelina arborea* stands. This research consisted of collecting root samples, identifying fungal spores in root samples and observing arbuscular mychorriza fungal infections. The results showed that the types of spores observed in root samples were Glomus, Gigaspora, and Scutellospora. The percentage of colonization on both sites is medium.

Keywords: *Mycorrhiza, Vesicular, Arbuscular, Gmelina arborea.*

1 Introduction

Melina (*Gmelina arborea* Roxb) is one type of tree that has the potential to be developed in industrial plantations and community plantations [1]. Melina has rapid growth (fast growing species) and uses as raw material for the pulp industry, materials for making particle boards, plywood cores, matches, containers and wood craft materials [2]. The benefits of Melina cultivation are easy to plant maintenance, not complicated planting techniques and good economic value [3].

One effort to support Melina's growth is to increase the fertility of the soil which can be done by giving organic fertilizer. However, in large-scale planting, the fertilizer needed is relatively large and expensive. Therefore, an alternative method is needed to increase soil fertility through mycorrhizal utilization. True mycorrhiza comes from the Greek language, mykes, which means mushroom, and rhiza means root, so literally means root mushroom. Mycorrhizal fungi can associate with over 90% of higher plant species (including agricultural crops) and are found in soil of all continents from alpine lands to tropical forests and from grasslands to croplands [4,5]. Mycorrhiza can colonize and develop symbiotic mutualism with plant roots, so it can increase plant growth, and help suppress the development of several soil pathogens. Mycorrhizal infection can increase plant growth and its ability to utilize nutrients, especially the elements P, Ca, N, Cu, Mn, K, and Mg [6,7,8,9]. This is caused by mycorrhizal colonization in plant roots can expand the field of root uptake [10].

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Arbuscular mycorrhizas are the most common type of mycorrhizas, involving primitive aseptate fungi from Glomeromycota phylum. At the present time, arbuscular mycorrhizal fungi have been classified into four orders (Diversisporales, Glomerales, Archaeosporales and Paraglomerales) of the Glomeromycota phylum [11]. Research on arbuscular mycorrhizal identification in various types of community forest ecosystems in South Sulawesi showed that there were three types of spores found, namely the genus Glomus, Gigaspora and Acaulospora [12]. [13] conducted research on the identification of mycorrhizae in teak forests in Barru Regency. The results showed that there were four types of spores found, namely Gigaspora, Glomus, Acaulospora, and Scutellospora.

Research on the identification of mycorrhizae in Melina plants in South Sulawesi has not been reported, so research is needed on the types of mycorrhiza associated with Melina stands. This research is expected to be the basis of mycorrhizal utilization in increasing Melina plant growth.

2 Material and Methods

Root sample collection

Root sampling was carried out at five points for each location. Determination of sampling points is done by purposive sampling method. Roots are taken in four directions for each point with a depth of 0 - 20 cm. The sample root is then put into a plastic clip and labeled.

Isolation, Identification and Root colonization observation

The technique of observing mycorrhizal colonization in roots using the infected root length method [14] while identification of mycorrhizal spores was carried out using the root coloring method [15].

The first step is to choose the delicate roots of the plant and then wash the roots clean. The root sample is then soaked in FAA solution for 24 hours then discard the FAA solution and wash the root sample thoroughly. Root samples were immersed in 10% KOH solution for 24 hours then disposed of the KOH solution and washed the roots clean. Root samples were immersed in 10% H_2O_2 solution for 24 hours then disposed of H_2O_2 solution and washed the roots thoroughly. Root samples were immersed in 2% HCl solution for 24 hours then disposed of HCl solution and washed the roots thoroughly. The roots are divided into two, one part is dried for 24 hours for the method of identification of mycorrhiza, and the other part is immersed in a staining solution for 24 hours for the method of observing root colonization. Observation of colonization was carried out by cutting five root samples with a length of 1 cm in each part of the root (root base, middle root, root tip, end of root branch and base of root branch). The roots are arranged in glass preparation and observed under a microscope.

Root samples for observation of spores were blended until smooth and soaked in a staining solution for 24 hours. The next step is to choose the three smoothest roots and arrange them on the glass preparation. Preparations were observed under a microscope and identified using the INVAM guide.

The observed variable

The variable observed in the study were :

1. Morphology of mycorrhizal spores
   Spores found in root samples were observed in the morphological characters in the form, color, hyphae holder and spore ornaments. Genus Spora Mikoriza
2. Relative Abundance of Spores
   The relative abundance of spores is calculated based on formulas [16]:
   Relative abundance = $\frac{\text{Number of genus}}{\text{Total of spore}} \times 100\%$
3. Root infections
   Root colonization is seen based on the percent of root infection by mycorrhizal structure calculated based on the formula [17]:
   Root infection = $\frac{\text{Number of infected root}}{\text{total observed field}} \times 100\%$

Data analysis

Data from observations are presented in the form of tabulation and image data. The classification criteria for the number of infected roots are classified into five classes and presented [18] in Table 1.

Table 1. Classification of Number of Root Infections

Percentage of Colonization	Kategori
0 – 5	Very low
6 – 25	Low
26 – 50	Moderate
50 – 75	High enough
75 – 100	High

3 Results and Discussion

The results of the observation showed that there were three types of spores found in root samples, namely Glomus, Scutellospora and Gigaspora. The type of spore has different morphological characteristics and relative abundance at each location. The types of spores found in root samples at each study location are presented in Table 2.

Table 2. Types of Spores Found in Root Samples in Each Study Site

Research site	Genus Spora
Balong village	*Glomus*
Bulukumba district	*Scutellospora*
	Gigaspora
Lamatti Riawang village	*Glomus*
Sinjai district	*Scutellospora*

Based on Table 2, there are differences in the spore genus found in the root samples of both locations. Glomus and Scutellospora can be found in root samples of both locations while Gigaspora is only found in Bulukumba root samples. According to [19], the location and rhizosphere factors greatly influence species diversity and mycorrhizal population. This is consistent with [20] study stating that the type of mycorrhizae that are associated with Eboni (*Diospyros celebica*) in Bengo-Bengo Education Forest is Gigaspora and Acaulospora, while [21] study states that the type of mycorrhiza associated with Cempaka plants (*Elmerillia ovalis*) in North Toraja community forests are Glomus and Gigaspora species. The difference in the spore genus is also caused by differences in spore tolerance to soil type, organic matter content, light intensity and altitude above sea level [17]. The forms of spores found in root samples are presented in Figure 1.

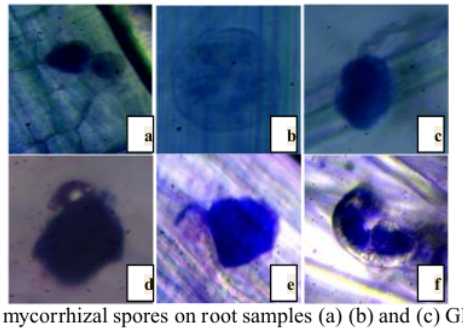

Fig 1. Identification of mycorrhizal spores on root samples (a) (b) and (c) Glomus spores, (d) and (e) Scutellospora spores and (f) Gigaspora spores

Based on Figure 1, spores were found to have different morphological characteristics. Morphological characteristics were observed in the form, color, hyphae holder and spore ornaments. There are three genera found, namely Glomus, Gigaspora and Scutellospora.

Glomus spores were found to be round (**Figure 1a and 1b**) and oval (**Figure 1c**) and black (**Figure 1a**), hyaline (**Figure 1b**) and blue (**Figure 1c**). The spores found were those that had hyphae seats (**Figures 1a and 1c**) and did not have hyphae (**Figure 1b**). The spore was formed singly (**Figures 1b and 1c**) and in groups (**Figure 1a**). According to [22], Glomus is a genus that has a widespread, and quite high adaptation to environmental conditions [23]. One of the adaptations carried out by the genus Glomus is faster spore germination, which only requires 4-6 days. The Glomus genus germinates faster because the smaller spore size causes the hydration phase to occur very quick so that the enzyme activity associated with the germination process will take place faster [24]. Scutellospores were found to have irregular shapes (**Figures 1d and 1e**) with black (**Figures 1d**) and blue (**Figure 1e**). The spores observed have hyphae seats. Scutellospora is usually found more in acidic soil conditions [25]. Gigaspora is found to be round and irregular and black (**Figure 1f**). Spores are produced singly and do not form the inner layer. There is no hyphae attached so that the bulbous suspensor is not visible.

Relative abundance is one determinant of qualitative assessment of spores. Relative abundance is the number of genera found at the observation location divided by the total spores

at the observation location multiplied by 100%. This data shows the amount of abundance of a type of spore in an observation location. The relative abundance of mycorrhizal spores in root samples of each location was different. The table of relative abundance of spores is presented in Table 3.

Table 3. Relative Abundance of Mycorrhizal Spores on Root Samples in Each Study Site

Study site	Relative Abundance (%)		
	Glomus	*Gigaspora*	*Scutellospora*
Balong village Bulukumba district	62,5	12,5	25
Lamatti Riawang village Sinjai district	90	-	10

Based on Table 3, Glomus has the highest relative abundance in both locations. The second genus that dominates both locations is Scutellospora. Gigaspora was only found in the root sample of Balong Village while in the root sample of Lamatti Riawang Village Gigaspora spores were not found. Glomus abundance is large because Glomus has a widespread and high adaptation to its environmental conditions [22]. This is by the results of the research by [26] which showed that Glomus had the highest percentage of abundance in plants (75.39%), followed by successively with Acaulospora (8.62%), Scutellospora (8.67%).) and Gigaspora (5.83%). The differences in the spores found were related to differences in sampling locations and rhizosphere. According to [19], differences in location will result in differences in soil types as limiting factors that influence the presence of the spore genus.

Percentage of root colonization

The results showed that the average value of the percentage of mycorrhizal colonization in both locations was different. The average percentage of mycorrhizal colonization is presented in Figure 2.

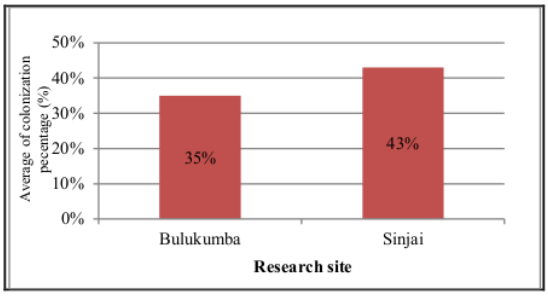

Fig 2. The Average of Percentage of Mycorrhizal Colonization in Root Samples in Bulukumba District and Sinjai District

Based on Figure 2, the average percentage of root colonization in both locations is classified as moderate. The percentage of colonization in Sinjai is slightly higher when compared to the Bulukumba region. This is because the rainfall conditions for sampling in both locations are different. The location in Bulukumba is carried out during the dry season, while in Sinjai it is carried out during the rainy season. According to [27], in conditions of high rainfall, generally, the percentage of colonization increases and spore formation decreases, whereas in the dry season new spore formation will increase. Other factors that influence the percentage of root infections are soil texture, soil pH, host plants and tillage.

4 Conclusion

Based on the results of the research conducted, it can be concluded that the spore genus associated with root Melina in Bulukumba and Sinjai Districts is Glomus, Gigaspora, and Scutellospora. Glomus has a relatively high abundance compared to other genera. The average percentage of mycorrhizal colonization in the study location is classified as moderate

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