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ARBUSCULAR MYCORRHIZAL FUNGI (AMF) CHARACTERIZATION IN RHIZOSPHERE of *Gigantochloa atter*

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ABSTRACT

Mycorrhizae are typical structures found in a plant's root system found symbiotic mutualism between fungi (myces) and roots (rhiza). Mycorrhizae have an essential role for plants because they can increase water and nutrient uptake, especially phosphorus absorption. The purpose of this study was to determine the mycorrhizal genera on the roots of parring bamboo plants (Gigantochloa atter) in Sabantang Hamlet, Toddopulia Village, Tanralili District, Maros Regency because there is no scientific information regarding this issue. This research was conducted with the wet filter method or sieving. The isolation of spores from the rhizosphere of Gigantochloa atter samples was carried out by referring to the pouring method and wet sieving using a stratified filter set. Staining techniques were used to observe the colonization of arbuscular mycorrhizal structures in the roots of the sample plants. The results showed that 27 spores were found, consisting of 15 spores of the Glomus genera, three spores of the Gigaspora genera, and nine Acaulospora genera. Observation of the root structure has not shown the presence of vesicular and arbuscular. However, only hyphae and spore structures were found because the mycorrhizal hyphae in Gigantochloa atter have not yet reached the infection stage to form arbuscular or vesicular structures.

INTRODUCTION

The existence of various microorganisms on earth has their respective roles and functions to help the survival of other living things and the environment around them. Mycorrhizae is a form of symbiosis between microorganisms of fungi and root systems of higher plants (vascular plants, Tracheophyte). For example, they have a crucial role in the growth of these plants (Kramadibrata et al., 2007). Arbuscular Mycorrhizal Fungi (AMF) are soil microorganisms found in almost all types of soil. This symbiont has excellent potential in increasing plant growth because it improves nutrient and water

uptake and improves soil aggregation (Piliarová et al., 2019; Nainggolan & Rahmaniah, 2001). In general, mycorrhizal fungi can associate with various plants, including crops, but it is not clear at what level these mycorrhizae can work in the field. The complexity of mycorrhizal associations requires a description of the diversity that affects the functionalization performance of these mycorrhizae. This description is essential for the use of mycorrhizae in agricultural, forestry and land restoration systems (Hartini et al., 2019; Nurbaity et al., 2017; Chalim, 2016; Irianto, 2015a; Irianto, 2015b).

Bamboo is a plant that has high biological, economic and ecological aspects. Indonesians widely use bamboo from the roots to the leaves (Walida, 2019). In Indonesia, it estimated that there are 143 types of bamboo. There are four types of bamboo developed: *Gigantochloa atter* (bamboo parring) with a potential of 8,975 stalks/ha (Liana et al., 2017; Kramadibrata et al., 2007). Bamboo parring (*Gigantochloa atter*) is the most dominant type of bamboo that grows in the Tanralili sub-district and is spread over local community lands. It is not confident what the total area of bamboo is in South Sulawesi, but it predicted that it would decline from year to year. One of the contributing factors is the increasing demand for bamboo, which has not balanced by improvements in bamboo vegetation management (Jannah et al., 2019). The decreasing number of land area for bamboo is also part of one factor (Hani, 2019). To increase the growth of bamboo, it is necessary to have the right and environmentally friendly alternatives. This alternative can take advantage of the role of symbiotic microbes. Mycorrhizae is a symbiotic microbe that can be used as an alternative to increasing water absorption (Begananada, 2019; Saleh & Atmaja, 2017; Gustian, 2011) and as biological fertilizer (Proborini et al., 2020; Widowati et al., 2020; Nafiah & Prasetya, 2019; Herliana et al., 2018; Berruti et al., 2016; Heijden, 2016) to increase plant productivity.

The diversity and distribution of AMF in the root area vary greatly, influenced by various environmental conditions (Johnson & Pfleger, 2015; Humphreys et al., 2010). Research on the diversity of the AMF genera in the rhizosphere of Parring bamboo (*Gigantochloa atter*) in Toddopulia Village, Sabantang Hamlet, Tanralili District, Maros Regency has never carried out. Therefore, this research is expected to provide initial information on the use of mycorrhizae as a biological agent suitable for the local area.

METHODS

This research is qualitative research using a descriptive exploratory research approach. Soil sampling was carried out in the *Gigantochloa atter* rhizosphere area in Toddopulia Village, Sabantang Hamlet, Tanralili District, Maros Regency. Soil is taken at a depth of approximately 20 cm using a Hand-Held Soil Auger at four points for each bamboo clump. AMF extraction is carried out to separate spores from soil samples so that AMF identification can be carried out to determine the population size and genera of AMF spores. The isolation of spores from soil samples was carried out by referring to the pouring method and wet sieving using a stratified filter set. The filter sizes used were 35 μm, 45 μm, and 75 μm (Gerdemann & Nicolson, 1963) by centrifugation modification (Brundrett, 2002). The AMF spores obtained were then isolated and made preparations with Polyvinyl Lactoglycerol (PVLG) solution to identify. The isolation sensing and characterization of AMF species were carried out based on the morphological characteristics of the spores. Identification of the AMF made to genera level, which based on the shape, color, spore walls, ornaments, and the size of the spores (Schenck & Yvonne, 1990).

Observation of the colonization of arbuscular mycorrhizal structures in the roots of the sample plants was carried out through staining techniques. Root segments were cut 3-5 cm in length, then washed with running water until clean and put in a 10% KOH solution at 250℃ for 10 minutes then cooled at room temperature for 24 hours so that the roots would be white or pale. Samples were washed under running water for 5 minutes and immersed in 1% HCl solution for one night. Then the sample was immersed in a 0.05% trypan blue solution and replaced with a lactoglycerol solution for the color reduction process (destaining) for one day (Lapanjang, 2019).

RESULTS AND DISCUSSION

The *Gigantochloa atter* rhizosphere area that grows in Toddopulia Village, Sabantang Hamlet, Taranlili District, Maros Regency shows various mycorrhizae. This result expected to provide initial information on the use of mycorrhizae as a biological agent suitable for the local area. Identification of isolated spores indicated that there were 27 spores grouped into three genera, namely 15 spores of the *Glomus* genera (Figure 1), three spores of the *Gigaspora* genera (Figure 2), and nine spores of the *Acaulospora* genera (Figure 3).

The presence of spores found was predominantly of the *Glomus* species, with almost the same spore shape (round, oval) and having a smoother surface. In addition, this type has a visible spore wall, a straight hyphal attachment, but several types of *Glomus* do not have a hyphal attachment. The spores' size and color and the thickness of the spore walls were different for each species. Environmental factors influence the type of AMF in the host plant rhizosphere (Rengganis, 2013).

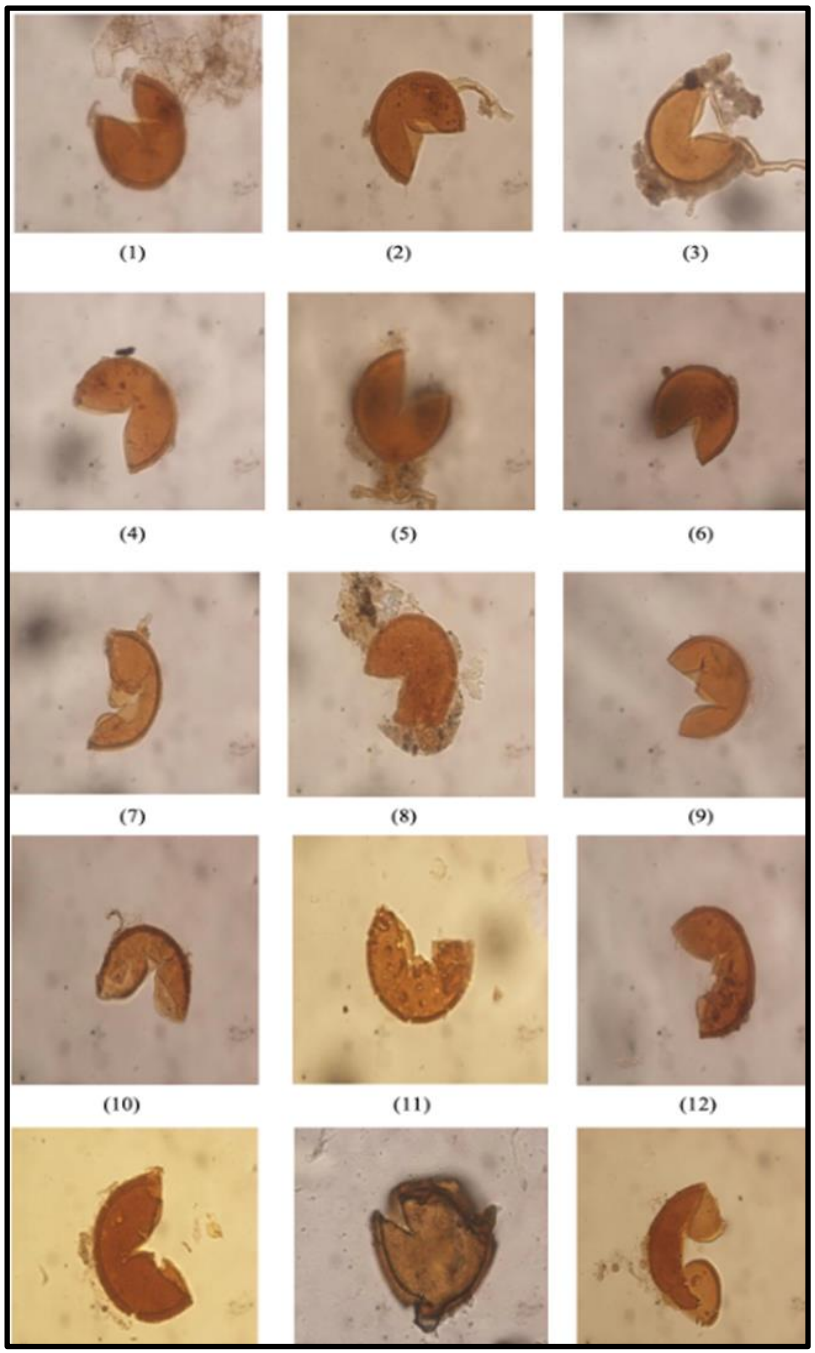

Figure 1. Arbuscular Mycorrhizal Fungi spores with Glomus genera at 400 magnification under Dissecting microscope.

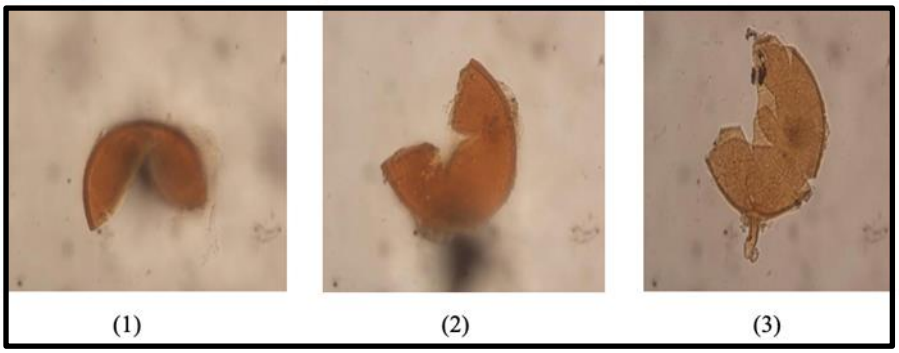

Figure 2. Arbuscular Mycorrhizal Fungi spores with *Gigaspora* genera at 400 magnification under Dissecting microscope.

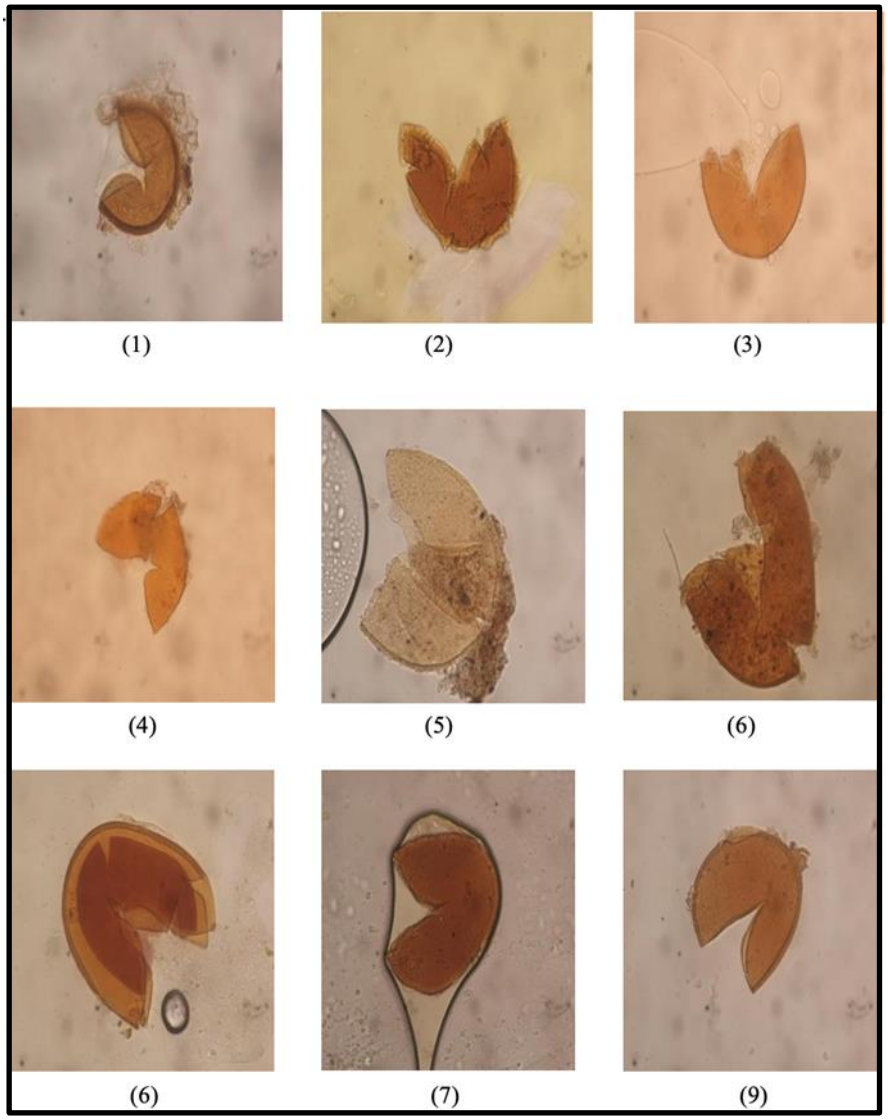

Figure 3. Arbuscular Mycorrhizal Fungi spores with *Acaulospora* genera at 400 magnification under Dissecting microscope.

The three genera of AMF that inhabit the area of the Gigantochloa atter rhizosphere are: 1) The *Glomus* genera, which is mycorrhizal of the *Glomeraceae* family,

has the highest diversity of species. The spore development process starts from the enlarged tip of the hypha until it reaches its maximum size and spores formed (Kurnia et al., 2019; Lapanjang, 2019; Helber et al., 2011); 2) *Gigaspora* genera, which belongs to the family *Gigasporaceae*. The spores develop blastically from the swollen tip of the hypha and become "sporogenous cells". Once the sporogenous cells reach their full size (usually around 25–50 μm in most species), spores begin to develop at the ends of the sporogenous cells. The outer layer and the laminate layer develop simultaneously and are often indistinguishable in young spores without the aid of Melzer staining (Hartini et al., 2019; Prihastuti, 2007); 3) *Acaulospora* genera, which included in the *Acaulosporaceae* family. *Acaulospora* spore development process as if from the tip of the hypha, although it is not the case. First of all, there are hyphae with enlarged ends whose spore-like structure is called the saccule. Then the saccule develops accompanied by a small dot appearing between the terminus hyphae and the subtending hyphae. The small spheres will develop from the subtending side of the hypha into spores (Kurnia et al., 2019; Prihastuti, 2007).

Environmental or edaphic conditions largely determine the distribution of arbuscular mycorrhizal genera. The *Glomus*, *Acaulospora* and *Gigaspora* genera are three different genera and indirectly have different environmental adaptations (Kurnia et al., 2019; Sukmawaty et al., 2016).

In general, the microscopic appearance of the root tissue of agricultural plants through the staining process shows that there are spherical structures called vesicles and arbuscular. The appearance of these structures indicates that there has been an infection or symbiotic colonization between plant roots and AMF. The staining results on the structure of the arbuscular mycorrhizal fungi on the roots of the *Gigantochloa atter* plant observed under the microscope. Only spore and hyphae structures were found. The hyphal structure appears blue because the hypha structure absorbs the staining solution, namely Trypan blue solution.

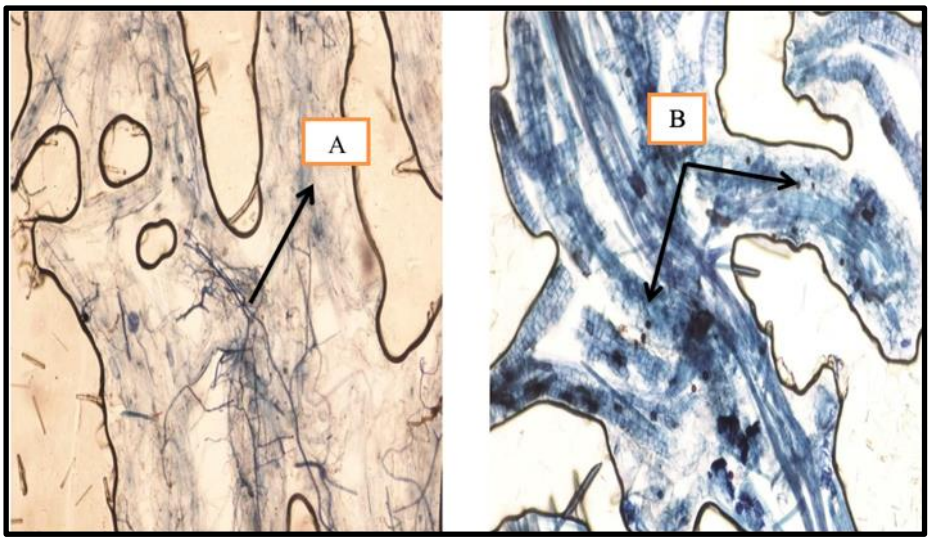

Figure 4. Structure of (A) hyphae and (B) spores on *Gigantochloa atter* roots at 10x magnification on Compound microscopy observation

Imaging the root structure did not reveal the presence of vesicular and arbuscular but only hyphae and spore structures were found. This is because the hyphae found in the roots of *Gigantochloa atter* have not yet reached the infection stage to form arbuscular or vesicular structures. This process refers to AMF spore infection, which begins with the presence of infective AMF propagules in the form of hyphae or hyphal fragments on the roots and spores (Linderman, 2015). Spores of AMF that germinate will produce hyphae, and hyphae will infect the host plant's roots by forming an appretory hyphae structure. Then the hyphae will develop in the root cortex cells (external hyphae), and some hyphae develop to form arbuscular and vesicular structures (Davison, 2015; Heijden, 2015; Johnson, 2010; Johnson et al., 2010). As with the fact that arbuscular mycorrhizal fungi infection is influenced by the sensitivity of the host to infection, climatic factors and soil factors in plants (Hartini et al., 2019).

CONCLUSION

This research has revealed the diversity of the AMF genera found in the rhizosphere of Parring bamboo (*Gigantochloa atter*) in Toddopulia Village, Sabantang Hamlet, Tanralili District, Maros Regency. Based on the morphological characteristics of the spores, the shape and color of the spores, mycorrhizal spores have been found with a yellow round shape, precise round shape and yellow oval. In the rhizosphere of the Parring bamboo plant, 27 AMF spores were found consisting of 15 spores of the *Glomus* genera, three spores of the *Gigaspora genera* and nine spores of the *Acaulospora genera*.

Meanwhile, the root structure observations were not found vesicular and arbuscular, but only hyphae and spore structures were found.

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