Journal of Applied Agricultural Science and Technology
6(2): 90-97 (2022)
E-ISSN: 2621-2528
ISSN: 2621-4709

IDENTIFICATION OF THE DIVERSITY OF INDIGENOUS ARBUSCULAR MYCORRIZA FUNGI IN THE RHIZOSPHERE OF COFFEE (*Coffea sp*) ARABICA SOLOK RADJO WEST SUMATERA

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Abstract. *Arbuscular mycorrhizal fungi are a group of fungi that can be symbiotic with many types of plants. Soil factors and plant species, affect the diversity of arbuscular mycorrhizal fungi. Likewise, differences in location and rhizosphere cause differences in the diversity of species and populations of AMF. In addition, not all AMF have the same morphological and physiological characteristics, therefore it is very important to know their identity. Coffee is a plant that has economic value and the potential for symbiosis with AMF. This study aimed to identify AMF in the rhizosphere of four varieties of Solok Radjo coffee plants in the Aia Cold area of the Gumanti Valley, Solok, West Sumatra. Meanwhile, the stages of this research include: collecting and collecting data in the field, determining the location of the soil sample, analyzing soil properties in the laboratory, isolating AMF spores, and identifying AMF spores morphologically. The results showed that the diversity of AMF spores was found in 3 genera, namely Glomus sp., Acaulospora sp., and Gigaspora sp. The Kartika coffee variety has a higher AMF spore population than Sigararutang, Andongsari and Gayo.*

Keywords: *mycorrhizae; production; quality; seedlings; solok radjo*

1. Introduction

Microorganisms play a role in the balance of ecological dynamics in the soil (Tedersoo *et al*., 2020). The interaction of microorganisms with plants will improve the balance of ecosystems, plant productivity and biodiversity (Bagyaraj & Ashwin, 2017) Microorganisms that play a major role in forming a symbiosis with plant attachment systems are arbuscular mycorrhizal fungi (Van der Heijden *et al*., 2015) Mycorrhizae are known as soil fungi because their habitat is in the root area or rhizosphere. Nearly 82% of plant species can symbiotically with mycorrhizae (Davison *et al*., 2015) The symbiosis of arbuscular mycorrhizal fungi with this plant has been known for 400 million years ago (Selosse *et al*., 2015).

The symbiotic nature of the interaction between plant roots and arbuscular mycorrhizal fungi is based on the exchange of nutrients (Balestrini *et al*., 2015) mycelium extraradical mycorrhizae, which grow from roots in the soil, have access to nutrient minerals that are delivered to the host plant in exchange for carbon (Van der Heijden *et al*., 2015) Nutrients are exchanged across symbiosis between plants and fungi in the roots (Rich *et al*., 2017). Arbuscular mycorrhizal fungi help increase the absorption of nutrients such as phosphorus and nitrogen (Johnson *et al*., 2015).

Received Mei 3, 2022; Accepted August 15, 2022; Published August 30, 2022
https://doi.org/10.55043/jaast.v6i2.39

Various factors can affect the diversity of arbuscular mycorrhizal fungi. For example, plant diversity (Lee *et al*., 2013; Armansyah *et al*., 2018; Susila *et al*., 2022), soil fertility (Montiel *et al*., 2016), and environmental changes (Hazard *et al*., 2013). Differences in plant varieties also determine the diversity of arbuscular mycorrhizal fungi (Wagg *et al*., 2011; Johnson *et al*., 2015). Arbuscular mycorrhizal fungi have different species diversity and populations in the rhizosphere of plants that are not the same. This diversity is characterized by spore morphology that is not the same, therefore it is very important to know its identity (Hartoyo *et al*., 2011; Trejo *et al*., 2013).

The people's coffee plantation in the Aia Cold area, Solok Radjo, has a fairly large area of land. Research on the existence of indigenous AMF from the rhizosphere of coffee plants has not been carried out. There is no information on the study of diversity, population and morphological characteristics of AMF spores in the rhizosphere of coffee plants. This study aims to determine the presence and number of spores and to determine the morphological characteristics of AMF from the rhizosphere of several coffee plant varieties. This study will help to compare the types of AMF spores at the Aia Cold Solok Radjo community plantation on different coffee varieties.

2. Methods

The study used a field survey method. Sampling was done by Simple Random Sampling (SRS). Soil samples were taken from the root areas of 4 coffee varieties, namely Sigararutang, Andongsari, Gayo and Kartika located in Solok Radjo, Gumanti Valley Aia Cold, Solok, West Sumatra with an altitude of ± 1,458 meters above sea level.

2.1. Soil sampling

Soil samples were taken around the stems of coffee plants at a depth of 0-20 cm. The total weight of the soil samples taken for each variety was one kilogram and dried in the air. A total of 10 g of soil from each sample was used for spore identification.

2.2. AMF spore isolation

Approximately 10 g of dry homogeneous soil samples were then extracted using a wet sieve (Dandan & Zhiwei, 2007). The sample was mixed with 200 ml of water and then filtered using multi-filters of 300 m, 106 m, and 45 m. The distillate sample was put into a centrifuge tube and 60% glucose was added, then centrifuged at 2500 rpm for 3 minutes. The results of the centrifugation solution were poured into multilevel sieves (300 m, 106 m and 45 m) which had been filtered and then rinsed with water. The supernatant solution remaining in this filter is then put into a 150 ml beaker.

2.3. Identification of AMF spores

Identification of root colonization, using root staining method. Fresh root samples measuring 0.5 – 2.0 mm were taken randomly. Root samples were washed and cleaned, then soaked in 10% KOH for 24 hours, then followed by immersion with 1% HCl for 10 minutes. After that, the root samples were soaked in lactoglycerol trypan blue for 1 hour. The stained root samples were then randomly selected and cut into 10 pieces with a length of approximately 1 cm. All root pieces were arranged on a glass object, then covered with a cover slip. The stained root samples were then observed using a binocular microscope with a magnification of 100 x.

3. Results and Discussion

3.1. Soil Sampling Location

Soil samples were taken from the coffee plantation area of Solok Radjo, Gumanti Aia Cold Valley, Solok, West Sumatra (Figure 1), with an altitude of ± 1,458 meters above sea level. Soil samples were taken based on different varieties. namely Sigararutang, Andongsari, Gayo and Kartika

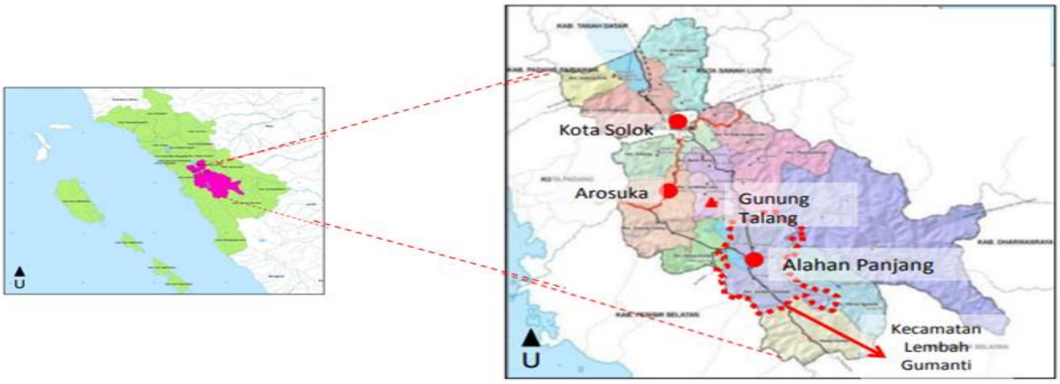

Figure 1. Map of soil sampling locations for 4 coffee varieties in Solok Radjo, Lembah Gumanti (Kementerian Pekerjaan Umum. 2013)

3.2. Number of Arbuscular Mycorrhizal Fungi Spores

Spores are part of fungi, which is an indicator of the presence of AMF in the plant rhizosphere. The development of the spore population is largely determined by the type of plant. Different varieties affect the type and population of AMF spores. The results of observations of the number of AMF spores on coffee plants in Solok Radjo showed that the number was not the same as in Table 1. The Kartika variety had the highest number of spores compared to the Gayo, Andongsari, and Sigararutang varieties. The highest number of spores in Kartika coffee also showed the highest colonization of 86.53% (Figure 2). The spores found in all coffee varieties were Glomus, Acaulospora and Gigaspora.

According to the type of AMF spores, there will be different types and numbers in plants, one of which is caused by variety and genetics (Lee *et al.* 2013) (12) Plant types also affect AMF diversity (Armansyah *et al.*, 2018; Susila *et al* 2022). Coffee varieties will determine AMF diversity in the rhizosphere. Different varieties will produce different exudates. The composition of root exudates will determine the structure and composition of microorganisms in the rhizosphere (Cesco *et al.*, 2012).

Table 1. Arbuscular mycorrhizal fungal spore density in four coffee varieties with various filter sizes

Coffee Varieties	Number of Spores Per Sieve Size (μm)			Amount
	300	106	45	
Sigararutang	39	112	68	219
Andongsari	57	93	38	188
Gayo	76	126	48	250
Kartika	88	181	59	328

3.3. Arbuscular Mycorrhizal Fungi Colonization Percentage on Coffee Plant Roots

The percentage of total mycorrhizal colonization and the mycorrhizal structure associated with root colonization was determined by the presence of arbuscular, internal hyphae, and vesicles in the plant root tissue. The percentage of plant root colonies by AMF is often an indicator of the level of compatibility between plants and fungi. Colonization of the roots of coffee plants indicates that in general, the Sigararutang, Andongsari, Gayo and Kartika coffee varieties have a high to very high suitability or level of suitability. This can be seen from the percentage of colonization, all of which were above 50% as shown in Figure 2.

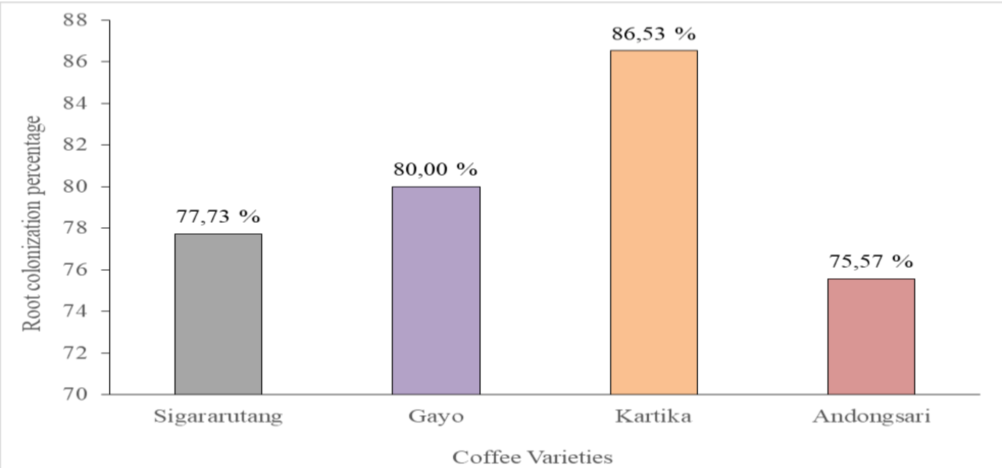

Figure 2. Percentage of Arbuscula Mycorrhizal Fungi colonies on four coffee varieties Andongsari. Kartika. Gayo and Sigararutang.

The Kartika variety showed the highest percentage of root colonization compared to the Gayo, Andongsari, and Sigararutang varieties. These results were positively correlated with the number of spores found in the rhizosphere of the Kartika variety (Table 1). Where in this variety the number of spores is the most compared to other varieties. This shows that the Kartika variety has a better level of compatibility with AMF species compared to other varieties.

Plant root colonization with AMF is different for each plant type (Horn *et al.,* 2017). Colonization is influenced by plant root exudates. The qualitative and quantitative composition of root exudates is determined by plant species, and plant age (Neumann *et al.,* 2014; Marín *et al.,* 2017). Root exudates can represent a mechanistic relationship between plant community composition and the composition and function of soil microbial communities. Increased plant diversity leads to an increase in soil microbial biomass and diversity (Lange *et al.,* 2015).

Coffee plants colonized with AMF through root staining were found to have internal hyphae, vesicular and arbuscular hyphae. However, not all coffee plant roots were colonized by AMF as shown in Figure 3.

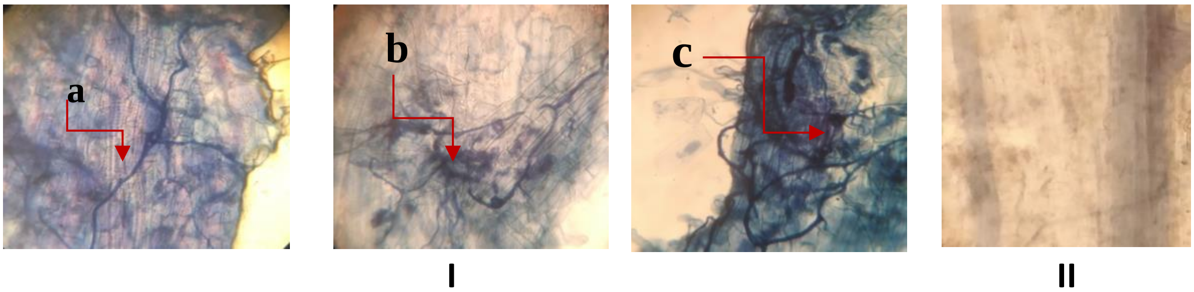

Figure 3. The structure of the roots of the coffee plant. where I. roots colonized by AMF were characterized by a) internal hyphae, b) vesicular and c) arbuscular. II. Roots not colonized by AMF.

According to Luginbuehl *et al.,* (2017), colonized plants begin with the formation of an appressorium on the root surface by external hyphae derived from germinating spores. Brundrett & Tedersoo, (2018) stated that the appressorium enters the root through the inter-epidermal gap, then forms intracellular hyphae along the root epidermis and after this process vesicles and arbuscular structures are formed. AMF in root tissue will form intracellular coils and branching hyphae.

3.4. Arbuscular Mycorrhizal Fungi Spore Morphology

Identification of spore morphology steps to determine the type of AMF that develops in the rhizosphere of coffee plants. In the rhizosphere of 4 varieties of coffee plants, various colors, shapes and sizes of AMF spores were found. These results indicate that the spores that develop in the rhizosphere of coffee plants are quite diverse.

The original AMF spore species found in 4 coffee varieties were 5 species. This species is grouped into 3 genera, namely Glomus, Acaulospora, and Gigaspora. Glomus consists of 2 species, namely Glomus sp 1 and Glomus sp 2. Acaulospora consists of 2 species, namely Acaulospora sp

1 and Acaulospora sp 2. While Gigaspora is only 1 species. These findings indicate that each rhizosphere of Indigenous AMF coffee plants is not the same.

Glomus sp spores were found to be round to oval in shape, the color of the spores ranged from brown to reddish brown and glossy black and the spore walls were smooth. According to Invam (2020), AMF spores of the genus glomus are characterized by a round to oval shape, the spore wall consists of more than one layer, smooth. The color of the Glomus genus spores varies from brownish yellow, reddish brown, and light brown, to dark brown and black.

Acaulospora sp spores found in the rhizosphere of coffee plants have a globus, sub-globose, spherical shape. The spore wall consists of 2 layers where the innermost spore wall is equipped with a germination ball (Figure 4). According to Invam, 2020). Acaulospora spores are generally round and elliptical. The color of the spores varies from yellow, orange, brownish, and dark red to brownish red-orange and pale yellow. The spore wall consists of 2 layers.

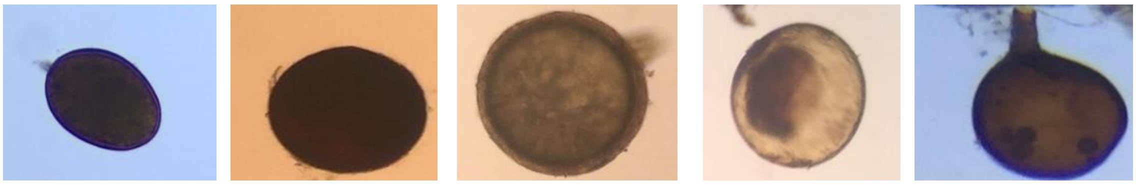

Figure 4. Different Types of Glomus sp Derived from Several Rhizosphere of Coffee Plants.

Gigaspora sp spores found in the rhizosphere have round and bulbous. Spores are round and slightly rounded. The color of the spores is yellow, reddish brown to black. It has a smooth single layered spore wall, without ornamentation. The spores are relatively large. The genus Gigaspora has the characteristics of; a single spore in the soil, round or sub-round; yellow, brownish yellow, to blackish, slimy white, and golden yellow. Invam, (2020) explained that Gigaspora spores contain a fluid like oil, do not have ornaments, hyphae form spherical suspensors or round hyphae holders, and spores grow at the end of the suspensory "bulb" (hyphae bulge like small spores) germ tube emerges directly from the spore wall.

4. Conclusions

The conclusion of the research that has been done is as follows. The original AMF spore population in the rhizosphere of 4 coffee plant varieties was quite high. The highest spore population was found in Kartika variety, followed by Gayo, Sigararutang and Andongsari varieties. The exploration of the morphology of Indigenous AMF in the rhizosphere of 4 varieties of AMF coffee plants found three genera, namely Glomus sp, Acaulospora sp and Gigaspora sp.

Acknowledgement

We thank the PNBP Fund Manager of the Faculty of agriculture Andalas University who has financed this research, by the Research Contract Number: 01/PL/SPK/PNP/FAPERTA-Unand/2021 the Fiscal Year 2021.

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