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Exploration and Identification of Arbuscular Mycorrhizal Fungi (AMF) in the Rhizosphere of Cocoa (Theobroma cacao. L) in West Sumatera

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Abstract

West Sumatera was declared as a center for Indonesian cocoa in the western region. Almost all areas in West Sumatra are suitable for cocoa cultivation, so the cocoa planting area covers almost all the districts in the province. However, West Sumatera cocoa production decline occurs because cocoa plantations are still mostly cultivated with smallholder plantations, and also decrease in soil quality is occurred. One of the efforts to improve soil quality biologically is to use soil microbes, one of which is AMF. The use of indigenous mycorrhizae is expected to be more effective in supporting the growth of cocoa plants to increase yields production. This study aimed to identify the AMF in the cocoa rhizosphere in three districts of West Sumatera. The results showed the number of spores and diversity of AMF. The number of spores was a range of about 67-218 spores per 20 g soil. The root colonization was in the range of 21.3 - 24.6%. three types of AMF spores that were found in this study were identified as Glomus sp., Acaulospora sp., and Gigaspora sp.

Keywords: colonization, exploration, Mycorrhizal

Introduction

Cocoa (Theobroma cacao. L) is one of the plantation commodities which is a source of foreign exchange for the country, and its export value continues to increase. According to data from the ministry of trade in 2019, the value of cocoa exports was 98.7 million US dollars, raised in 2020 by 136.6 million US dollars (The Ministry of Trade of Republic Indonesia, 2020). . The distribution of West Sumatera cocoa is almost evenly distributed in each district. In 2017, the distribution of cocoa's production is Pasaman District 17,558 tons/ha, West Pasaman Pasaman District 9,396, 29 tons/ha, Agam District 5982.76 tons/ha, Lima Puluh Kota District 4,393.02 tons/ha, Tanah Datar District 41.87 tons/ha, and Solok District 229.54 ton/ha. The total production in 2015 of about 82,104 tons/ha, while in 2017 drastically decrease to 67,843 tons/ha (BPS, 2018). The decline in West Sumatra cocoa production occurs because most of the plantations are still cultivated with smallholder plantations, the occurrence of land conversion, and the reduced carrying capacity of the land. The reduced carrying capacity of the land is including soil fertility due to the continuous use of inorganic fertilizers, which results in a decrease in the ecological quality of the land. One of the efforts to improve soil quality biologically is the use of soil biotechnology. One of the soil microbes that is often used is Arbuscular Mycorrhizal Fungi (Kumalawati et al., 2014).

Arbuscular Mycorrhiza Fungi (AMF) can live in various places and are capable of mutualistic symbiosis with plant roots and help plants absorb nutrients. The ability of one AMF species to associate with several plants is quite extensive. Still, its suitability in symbiosis with plants is strongly influenced by various soil conditions, soil types, and plant species. AMF can colonize effectively on certain plants but is not necessarily effective on other plants. Several AMF species in nature that are associated with various plant species, including cacao, have been identified (Goltapeh et al., 2013). However, indigenous mycorrhizal species in cocoa-growing areas in West Sumatera are not associated with local cocoa plants. The use of indigenous mycorrhizae) is expected to be more effective in supporting the growth of cocoa plants to increase yields production. The study aimed to identify the arbuscular mycorrhiza in the cocoa rhizosphere in three districts of West Sumatera province.

Materials and Methods

The research was carried out from November 2020 to May 2021. AMF exploration was carried out in three districts, namely: Lima Puluh Kota, Solok, And West Pasaman. Lima puluh Kota district is located in La: -0;16:13,0548, Lon: 100:34:30,5292, and an altitude 594 masl. Solok district is located in Lat: -0º49':26,1192, Lon: 100º34':13,974, and an altitude of 477 masl, While West Pasaman district is located in Lat: 0º02':11,5 and Tan:99º50':45,1, and an altitude of 81 masl.

AMF identification was carried out at the Plant Physiology Laboratory Faculty of Agriculture, Andalas University, Padang. The results of AMF observations were analyzed descriptively by comparing the effects of AMF identification to the genus level with the INVAM identification reference. AMF spores were identified based on the size and the color, reaction with Melzer solution, spore ornament (outer wall of the spores), and the shape of the hyphae attached to the AMF spore wall. The research parameters were soil chemical properties from the sampling locations, number of spores, AMF colonization percentage, AMF identity, and AMF spores characteristics.

Results and Discussion

Soil chemical properties of the soil from the sampling locations

The soil chemical properties from soil samples in three districts can be seen in Table 1. Soil pH can affect microorganisms in the soil, but fungi can thrive in all soil acidity levels. AMF is generally resistant to the changes in soil pH so that even on alkaline or very acidic soils, AMF can be found. Still, the amount of AMF depends on the adaptability of each AMF to develop appropriately. Then organic C is another factor that affects the number of spores. The higher the organic C, the less AMF found at that location. Samsi et al. (2017) reported that only a few mycorrhizal fungal spores were found at the maturity level of the soil with the highest organic C content. Another soil chemical property that affects the number of AMF spores is the available P content in the soil, Purwanti et al, (2019) stated that the higher the P content, the lower the number of spores and AMF colonization.

The number of AMF spore and AMF colonization percentage

The number of AMF spores observed in 20 grams of soil samples and the percentage of total AMF colonization on mycorrhizal roots observed under a microscope can be seen in Table 2.

The high AMF spore population in an area is estimated to be supported by the total P content in the soil and the environmental conditions are suitable for the development of AMF. In this study, the environmental conditions of cocoa plantations in Lima Puluh Kota are concluded as they are more suitable in supporting the growth and development of AMF spores compared to Solok and West Pasaman districts. Puspitasari et al. (2012) stated that the high population

Table 1. Soil chemical properties of the soil from the three districts in West Sumatera

District	pH		Status*	P-available(ppm)	Status*	Organic C (%)	Status*
	H20	KCl					
Lima Puluh Kota	5.97	5.93	Slightly acid	3.1	Low	4.23	Low
Solok	5.81	5.7	Slightly acid	1.8	Low	3.88	Low
West Pasaman	5.26	4.81	Acid	6.5	Medium	19.21	Very high

*source: Hardjowigeno, S, 1995.

of AMF spores was due to more suitable, optimal, and compatible the supporting environmental conditions and the possible absence of antagonistic fungi that inhibited AMF sporulation.

The presence of spores in the soil is strongly influenced by agricultural activities carried out in plantations such as tillage, fertilization, and pesticides used. Intensive tillage will damage the external hyphae network, whereas minimum tillage will increase the AMF population (Zarate and Cruz, 1995). In the cocoa plantations in three districts of West Sumatera, farmers only carry out cultivation treatments such as fertilizing, spraying pesticides, and weed clearing in addition to agricultural activities, Musfal (2008) said the intercropping farming system also influences the existence of AMF, this way of farming can improve AMF population. The average number of AMF spores in the three districts in West Sumatera is relatively low. It is because, in the case of smallholder cocoa plantations, cocoa farmers do not apply the intercropping system.

Table 2 Number of spores and percentage of AMF colonization in the cocoa rhizosphere in three districts in West Sumatera

Distric	Number of spores in 20 grams of soil (spores)	AMF colonization percentage % *	Status*
Lima Puluh Kota	218	24.6	Medium
Solok	121	22.1	Medium
West Pasaman	67	21.3	Medium

*source Category of AMF colonization rate (O'Connor et al, 2001).

From Table 2 above, it can be seen that the highest colonization percentage in the cocoa roots is the samples from Lima Puluh Kota District (24.6%). Then followed by the samples from Solok District (22.1%) and West Pasaman District (21.3 %). This data shows that mycorrhizae have different infectivity to plant roots. It is suspected that there are different cultivation treatments carried out by the cocoa farmers, such as fertilizer application and pesticide use. The percentage of root colonization in the cocoa plants according to O'Connor et al. (2001) is classified as medium The development of AMF is influenced by several factors, namely the C-organic nutrient content, Purwati et al. (2019) at a depth of soil of about 20-40 cm which has a lower percent C-organic, the root colonization is higher if compared to soil with a depth of 0-20 cm. This is because the lower the C-organic content of the soil, the less fertile the soil so that the percent of root colonization increases. This is in line with our study, wherein in the West Pasaman District, which is classified as high organic C soil, the rate of root colonization by AMF is only 21.3 % AMF colonization can be seen in the following Figure 1. From Figure 1, the AMF colonization structure formed in the roots is in the form of vesicles and internal hyphae. AMF infects cocoa roots, this can be seen by the presence of thin hyphae on the root surface and vesicles (typical oval-shaped structures). According to Wirawan et al (2014) AMF colonization begins with the formation of an appressorium on the root surface by external hyphae derived from germinated spores. The appressorium enters the root through the inter-epidermal gap, then forms intracellular hyphae along with the root epidermis and after this process vesicles and arbukule structures are formed.

Identification and characterization of AMF spores

The diversity of host plant species, spore types, and environmental conditions such as soil conditions directly showed different responses to the percentage of colonization, the number of spores, and the

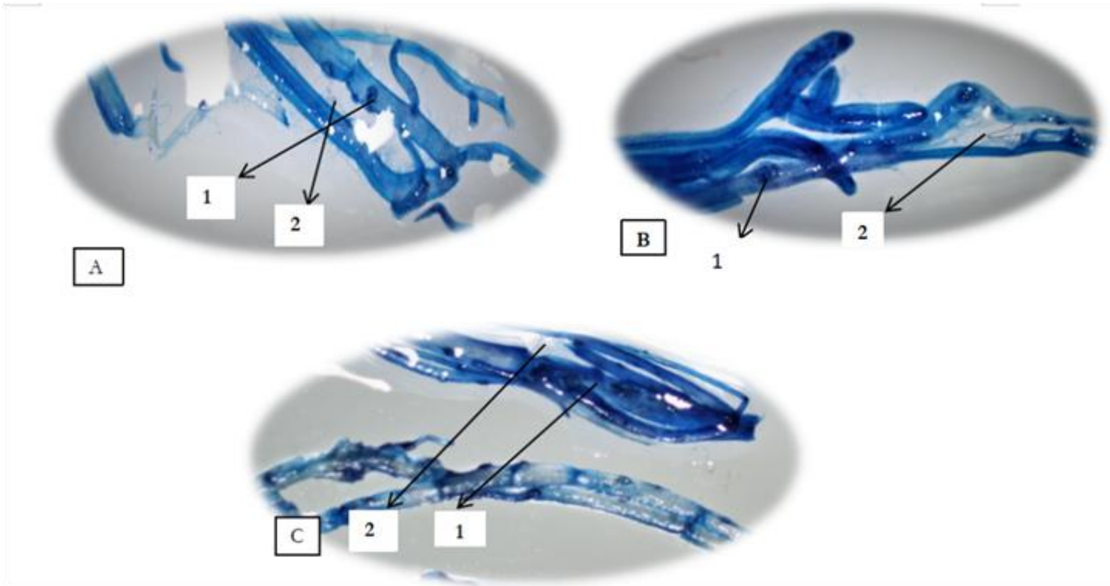


Figure: 1. Microscopic visualization of the AMF colonization on the roots of cacao. A: Cacao sample from West Pasaman District, B: Cacao sample from Solok District, C: Cacao sample from Lima Puluh Kota District. 1: Vesicle Structure. 2: Hyphae Structure. This figure was visualized in 10 x microscope magnification

diversity of spore types (Quilambo, 2003). In this study, we identify the AMF under a microscope with a magnification of 400x. The purpose of this stage is to find out what types of mycorrhizae were found in cocoa plantations in three districts of West Sumatera. There are three genera of AMF spores found in three districts in West Sumatera, namely Glomus sp., Acaulospora sp., and Gigaspora sp. The following three AMF spore genera found in three districts of West Sumatera can be seen in Table 3.

Gigaspora sp.

Gigaspora is a genus of mycorrhizae belonging to the family *Gigasporaceae.* Genus *Gigaspora* is characterized by its characteristic bulbous suspensor. *Gigaspora* spores are relatively large and have a spherical shape with a size ranging from 117.5 -147.5 µm. The color of the spores varies from yellow, greenish-yellow, brownish-yellow to yellowish-brown In addition, it only has one layer of the spore wall, namely the outer wall layer, while the inner layer is absent (INVAM, 2021). Spores *Gigasspora* found were round, brownish-yellow, had only one layer, while the inner layer is absent. *Gigasspora* sp. was found in soil samples from Lima Puluh Kota. and Solok district, where the soil pH ranged from 4.35-6.00. Large spores that were found indicated *Gigasspora* sp. In the soil sample of West Pasaman district, only a few spores were seen, this is presumably because the soil analysis results from West Pasaman district contained a high C-Organic 19.21%.

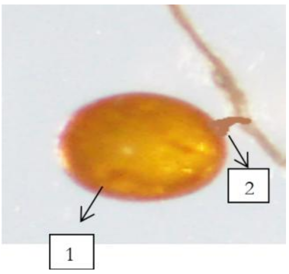
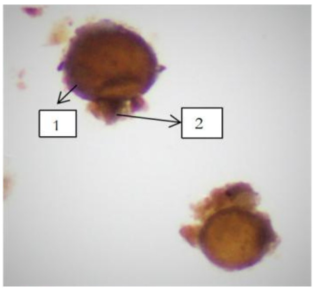
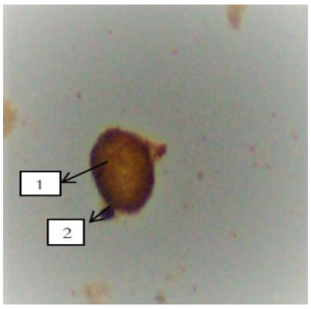
Acaulospora sp.

The genus *Acaulospora* is characterized by having a globus, sub-globose, irregular to the elliptical shape. The spore wall consists of 2 layers where the innermost spore wall is equipped with a germination orb. The color of the spores varies from yellow, orange, brownish, dark red to brownish red (INVAM, 2021). *Acaulospora* spores that were found have a round and elliptical shape, orange and faded yellow, and the spore walls consisting of 2 layers. *Acaulospora* were found in three districts in West Sumatera in pretty large numbers. The difference in the distribution of spore types in the three districts is thought to be influenced by the characteristics of the three sampling locations in the different cocoa rhizosphere, both from soil pH, vegetation, and land-use types. Widiastuti and Kramadibrata (1992) stated that differences in plant location and rhizosphere caused differences in species and population diversity of AMF. Environmental factors (soil moisture and soil fertility) also influence spore formation (Rainiyati, 2007). The optimum pH for the development of *Acaulospora* sp is about 4-5 (Tuheteru, 2003).

Glomus sp.

In the *Glomus* spores that were found in the study, some of them had ornaments in the form of papillae-like protrusions on the surface of the spore walls. Subtending hyphae which are wavy in shape and have 2-4 layers of the spore wall. The outermost spore wall is dark brown with an inner layer of yellow color. The spore shape is round, globose, and subglobose.The size of the spores found ranges from 90-200 µm Not all *Glomus* spores found during this study had hyphae and ornaments on the spore wall surface, only a few spores had hyphae, and ornaments on their spore walls. AMF from Lima Puluh Kota, Solok, and west Pasaman district showed that the genus *Glomus* sp. generally has a relatively high spore density compared to other genera. This is in accordance with Nadarajah and Nawawi (1999) research, which stated that the highest spore density in plantations in Malaysia was *Glomus fasciculatum*, with 32 spores in 100 g of soil. The same thing was also found in the soil of the mangosteen tree (*Garcinia mangostana* L.), which was dominated by *Glomus tunicate* with a total of 857 spores in 100 g of soil (Silviana et al. 1999).

Table 3. Identification and AMF spores characteristics

Types of spores	Spore photos	AMF spores characteristics*
Gigaspora sp.		Spores *Gigasspora* found were round, brownish-yellow, had only one layer, while the inner layer is absent. Single spore does not form sporocarp, bulbous suspensor (1) is attached to subtending hyphae (2), spore wall consists of only 1 layer, does not have a germinal wall and germinal shield, spore Size ranges from 100-140 µm (400x magnification)
Acaulospora sp.		Spores round, red-orange spots, Single spore, spores form chlamydospores (1) attached to sporiferous saccule (2), spore wall consists of 2 layers, straight hyphae shape, spore size ranges from 120-130 µm (400x magnification)
Glomus sp.		Spores round, blackish brown, thick spore surface, Single spore, spores form chlamydospores (1) attached to subtending hyphae (2), hyphae are wavy, spore wall consists of 2 layers, rough spore wall surface shows ornaments (jewelry), spore size ranges from 100-110 µm (400x magnification)

**Source: INVAM (2021)*

Conclusion

In this study, the genus of AMF spores found in Lima Puluh Kota, Solok, and west Pasaman districts was *Glomus sp, Gigaspora sp*, and *Acaulospora sp. Glomus sp.* spores have the highest relative abundance. The percentage of mycorrhizal colonization in the three districts is classified as a medium because the type and number of spores in the whole sample is relatively small in further study it is necessary to increase the spore population by trapping spore propagation so that the identified material is more accurate. Then it is essential to use a molecular approach to get an overview and data about the complexity of mycorrhizae.

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