Diversity of Arbuscular Mycorrhizal Fungi from Liberica Tungkal Jambi iii

by Elis Katika

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Elis Kartika[1*], Made Deviani Duaja[1], Gusniwati[1]
Agriculture Faculty, Jambi University, Jambi, Indonesia

*elisk63@yahoo.com

Abstract, Arbuscular Mycorrhizal Fungi (AMF) diversity found in Liberica Tungkal Jambi coffee plant rhizosphere which planted on peatland in Tanjung Jabung Barat, Jambi Province has not been reported. The existence of various types of AMF is important to know because AMF can increase growth and production of coffee plant. This study aims to identify the diversity of AMF species from coffee plant rizosphere on peatlands in Tanjung Jabung Barat. The soil samples were taken from the coffee plant rhizosphere in Parit Pudin, Senyerang, and Serdang Jaya villages at a 0-20 cm depth. The isolation was carried out by Pacioni's wet screening technique and continued by centrifugation technique from Brunndret, while spore identification was performed based on morphological characterization and its response to Melzer's solution. The results showed that both in Parit Pudin and Senyerang villages found two genus, i.e. Acaulospora and Glomus, whereas in Serdang Jaya Village only Glomus was found. In Parit Pudin obtained 1 *Acaulospora sp.* and *6 Glomus sp.*, in Senyerang found 3 *Acaulospora sp.* and *6 Glomus sp.*, and in Serdang Jaya only found *6 Glomus sp.* In Parit Pudin village, the spores abundance of *Acaulospora sp.* and *Glomus sp.* were 5 and 63 spores per 50 g of soil samples. In Senyerang village, the spores number of *Acaulospora sp.* and *Glomus sp.* were 57 and 49.5 spores per 50 g of soil samples, while in Serdang Jaya Village, the number spores of *Glomus sp.* as much as 46.83 spores per 50 g of soil samples. In all locations studied, there were various types of AMF with different numbers of spores. Spores found in soil samples showed variations in spore shape, spore color, attachment of hypha stalks, and spore walls.

1. Introduction

Liberica coffee (*Coffea liberica Bull ex Hiern*) is a location-specific coffee in Jambi, it grows on peatland area in the regency of Tanjung Jabung Barat (TJB). It is marketed to Singapore and Malaysia at a higher price. Therefore it is one of the main sources of farmer income. TJB has large comfortable area that could be cultivated as Liberica farming. It is coverage of district: Pengabuan, Bram Itam, Senyerang, Kuala Betara, and Tungkal Hilir.

Liberica coffee is a kind of coffee that is suitable for peat areas. Actually, peat soil is a marginal land that has low physical, chemical and biological properties. Therefore, several efforts should be needed to optimize its growth and yield. However, this plant is poor, attacked by disease (root fungus and leaf rust) so it is not productive. However, this plant is still used as a parent tree for germplasm. One effort that could be done to overcome the problems is the application of beneficial Rhizosphere microorganisms, namely indigenous Arbuscular Mycorrhizal Fungi (AMF). AMF functions are to absorp nutrients and increase plant resistance to pathogens and drought.

It has been proven that AMF can improve nutrient absorption, especially phosphate (Bhattacharjee and Sharma, 2012; Kathlee. and Treseder, 2013; Watts-Williams Stephanie, *et al*., 2014). increase plant resistance to abiotic stress (Wu and Zou, 2010; Ndiaye, *et al*., 2011; Zhu, *et al*., 2012), increase plant

resistance to heavy metal stress (Krishnamoorthy, *et al*., 2015), increase plant resistance to biotic stress such as root pathogen attacks (Sylvia dan Chellemi, 2001), and controlling for root rot (Simanjuntak, Fahridayanti dan Susanto, 2013).

The importance role of the AMF in coffee plants growth, it is required to isolate and identify the types of AMF in the coffee plant rhizosphere. The diversity of AMF species found in the Liberica coffee plant rhizosphere has never been reported yet. Therefore this research needs to be done because it will be applied to coffee plants. This experiment aim is to identify the AMF diversity of coffee plantations in peatland in certain village: Parit Pudin, Senyerang and Serdang Jaya.

2. Methods

This research was carried out at Seed Technology Laboratory and the Soil Fertility Laboratory, Faculty of Agriculture, University of Jambi. It is started from April to June, 2017.

Soil sample were taken from the Liberica coffee plant rhizosphere in several regions of coffee plantation on peatland located in 3 districts in the regency of TJB, namely : Parit Pudin village (Pengabuan District), Senyerang village (Senyerang District) and Serdang Jaya village (Betara District). In each site, soil was sampled from five adult coffee plants randomly. All samples were taken from a depth of 20 cm, carried out at the rhizosphere of 5 trees / site (2 kg / tree). Then, a composite sample of soil was prepared at each site.

2.1. Isolation of Spores and AMF Identification

Isolation of AMF spores was carried out by pour-filter technique and continued with centrifugation techniques, followed Brundrett *et al*. (1996). The 50 grams weighted soil samples were dissolved in 300 ml of water then stirred evenly. The suspension is filtered with multilevel sieve, starting with a 500 µm coarse sieve, 250 µm medium sieves, 125 µm fine sieve and a very fine 45 µm sieve. Medium and fine filter results were taken and poured into a petri dish. AMF spores from the filter are sorted with a dropper pipette. Then, observations were conducted under a stereo microscope. Spores were identified by using Meilzer's coloring agent, in which changes in spore color in Meilzer's solution were one indicator. Identification of spores was carried out based on morphological characteristics: shape, color, wall, hypha stalk and surface texture of AMF spores. The calculation of the number of spores per 50 g of soil samples is done manually by grouping the same type of AMF.

3. Results and Discussion

The results of spore identification showed that in the villages of Parit Pudin and Senyerang were found two genera namely the genus of Acaulospora and Glomus (Tables 1 and 2), in Serdang Jaya one genus, Glomus was obtained (Table 3). In the Parit Pudin village, it was obtained one type of *Acaulospora sp.* and 6 *Glomus sp.* (Table 1), while in Senyerang obtained 3 types of *Acaulospora sp*. and 6 types of *Glomus sp*. (Table 2), and in Serdang Jaya found 6 types of *Glomus sp* (Table 3).

Table 1. Characteristics of AMF spores from the rhizosphere of Liberica coffee plants in Parit Pudin Village, Pangabuan District, Tanjung Jabung Barat

No	Type	Shape	Colour		Spore wall layer	Stalk hyphae	Spore surface texture	Reaction with Melzer's
			Spore wall	Spore				
1	*Acaulospora sp-1a*	Globose	Yellow	Clear yellow	2	-	smooth	react
2	*Glomus sp-1a*	oval	Brown	Dark brown	3	used	smooth	not react
3	*Glomus sp-2a*	oval	Dark brown	Dark brown	3	straight	smooth	not react
4	*Glomus sp-3a*	oval	Brown	Dark brown	3	crooked	slightly coarse	not react

5	*Glomus sp-4a*	oval	Brown	Light brown	2	used	smooth	not react
6	*Glomus sp-5a*	oval	Brown	Dark brown		crooked	slightly coarse	not react
7	*Glomus sp-6a*	Globose	Dark yellow	Yellow	3	-	smooth	not react

Table 2. Characteristics of AMF spores from the rhizosphere of Liberica coffee plants in Senyerang Village, Senyerang District, Tanjung Jabung Barat

No	Type	Shape	Colour		Spore wall layer	Stalk hyphae	Spore surface texture	Reaction with Melzer's
			Spore wall	Spore				
1	*Acaulospora sp-1b*	Globose	Yellow	clear yellow	2	-	smooth	react
2	*Acaulospora sp-2b*	Globose	Dark yellow	clear yellow	2	-	slightly coarse	react
3	*Acaulospora sp-3b*	Oval	Yellow	clear yellow	2	-	smooth	react
4	*Glomus sp-1b*	Oval	Brown	clear yellow	2	crooked	smooth	not react
5	*Glomus sp-2b*	Globose	Dark brown	Dark brown	3	straight	smooth	not react
6	*Glomus sp-3b*	Globose	Light brown	Yellow	2	Used	coarse	not react
7	*Glomus sp-4b*	Oval	Light brown	Yellow	2	crooked	slightly coarse	not react
8	*Glomus sp-5b*	Globose	Brown	Brown	2	-	smooth	not react
9	*Glomus sp-6b*	Globose	Brown	Light brown	2	crooked	smooth	not react

Table 3. Characteristics of AMF spores from the rhizosphere of Liberica coffee plants in Serdang Jaya Village, Betara District, Tanjung Jabung Barat

No	Type	Shape	Colour		Spore wall layer	Stalk hyphae	Spore surface texture	Reaction with Melzer's
			Spore wall	Spore				
1	*Glomus sp-1c*	Oval	Brown	Yellow	2	straight	smooth	not react
2	*Glomus sp-2c*	Oval	Light brown	Yellow	2	-	smooth	not react
3	*Glomus sp-3c*	Globose	brown	Reddish brown	1	Used	smooth	not react
4	*Glomus sp-4c*	Oval	Brown	dark brown	2	crooked	coarse	not react
5	*Glomus sp-5c*	Oval	Dark brown	Brown	3	-	slightly coarse	not react
6	*Glomus sp-6c*	Globose	dark brown	Dark brown	3	-	coarse	not react

This was suspected when the AMF sampling was not sporulated so that only a few genera of AMF were found. It is caused of soil taking time samples, and time identification. Presumably at the time of sampling the soil there was only propagule Glomus and Acaulospora, because the existence and diversity of AMF was influenced by environmental and plant factors. Each AMF is influenced by factors intrinsic to environmental changes such as seasons. It is suspected that there are several genera of AMF that are limited in spreading so that the genus of spores found from a type of soil in one area and at a certain time may not represent all existing spores of the AMF genus in the area.

This was supported by Cahyani, *et al*. research (2014) which only found two genera namely Gigaspora and Glomus in Pamekasan Madura district. Then, two genera namely Glomus and Acaulospora in Tlanakan Madura District, and 3 genera namely Glomus, Acaulospora and Gigaspora in Pademawu Madura District. Furthermore Nurhalimah (2014) founds three genera namely genus Glomus, Acaulospora and Gigaspora in the Districts of Larangan, Palengan and Pegantenan, Madura. The results of the research by Dewi, Sritamin and Suada (2016) showed that AMF spores found in the rhizosphere of Arabica coffee plants were Acaulospora and Glomus, while in Robusta coffee were obtained Acaulospora, Gigaspora, and Glomus. Furthermore, the results of research by Lizawati, Kartika, and Gusniwati (2017) showed that the AMF spores in Liberica coffee plants rhizosphere in the Mekar Jaya village of Betara District were only found in two genera of spores, i.e. Glomus and Acaulospora.

Color characteristics of spores *Acaulospora sp*. those found were clear yellow, on average had globose and oval shapes, had spore walls ranging from yellow to dark yellow, the surface of the spore wall varied from smooth to slightly coarse, and had 2 of spore wall. INVAM (2013) reported that Acaulospora spores are globose, rather globose, irregular to oval. The color of the spores when young is hyaline and reddish brown after mature. The spore wall consists of three layers and has a cicatrix. Spore diameter ranges from 80-380 µm._Furthermore, the color of the spores of *Glomus sp.* from light yellow to dark brown, the arrangement collects in the form of sporocarp. Spore of *Glomus sp.* those found on average have a globose to oval shape, having spore walls ranging from clear yellow to dark brown, the surface of the spore wall varies from smooth to coarse, and has 1 to 3 spore walls. Spores found are attached to hyphae and some are not. The hyphae in the spores that are found directly blend with the spore wall with a color that is almost the same as the spore wall.

The shape and morphological characteristics and reaction to Melzer's solution of *Acaulospora sp.* and *Glomus sp.* in all three locations are presented in Tables 1 to 3. The diversity of AMF species in a location is strongly influenced by the type of host plant and environment and the interaction between host plants and their environment. INVAM (2013) reported that Glomus spores are globose, slightly globose, or slightly oval, having several layers of spore walls. The color of the Glomus genus spores varies transparently (hyaline), white, yellowish brown, yellowish brown, light brown, to darkish dark brown, has subtending hyphae and has a diameter of 80-320 µm spores.

Type of *Acaulospora sp*. and *Glomus sp.* in Parit Pudin Village were 14.29% and 85.71% respectively, in Senyerang were 33.33% and 66.67%, while in Serdang Jaya 100% Glomus (Figure 1). Based on this picture it appears that the type of *Glomus sp*. dominate in all three soil sampling locations. The diversity of AMF spores from each type of AMF spore in Parit Pudin Village is presented in Figure 2, in Senyerang Figure 3 and in Serdang Jaya in Figure 4.

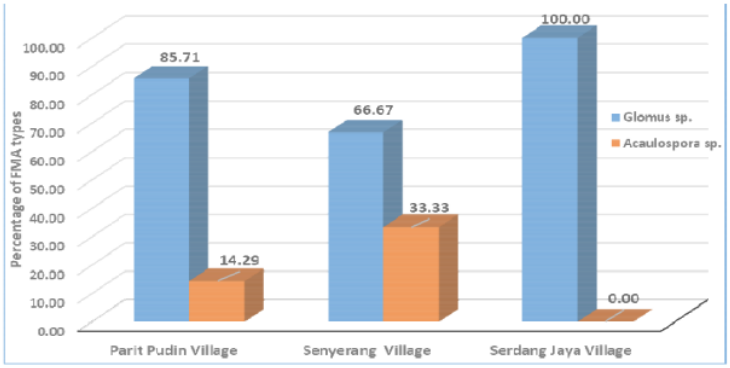

Figure 1. Percentage of AMF types in Parit Pudin, Senyerang, and Serdang Jaya Villages

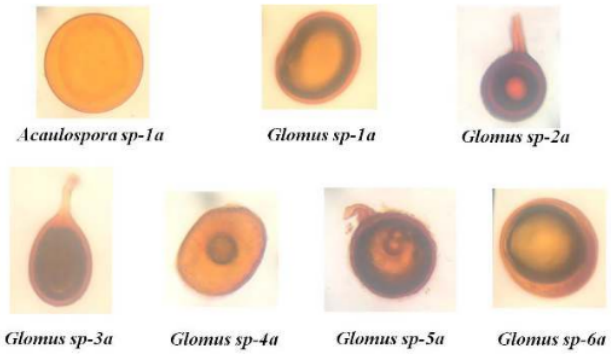

Figure 2. Diversity of AMF types in Parit Pudin Village

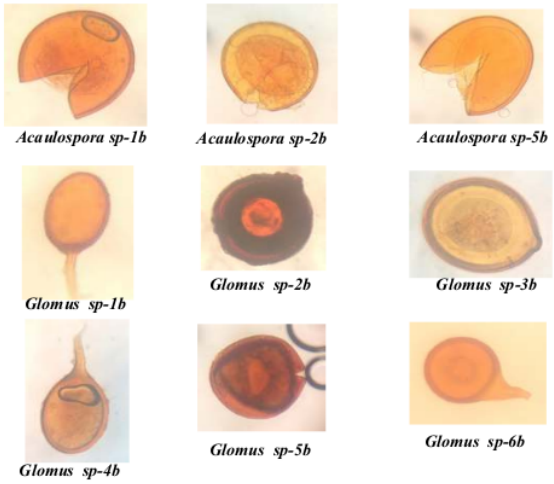

Figure 3. Diversity of AMF types in Senyerang Village

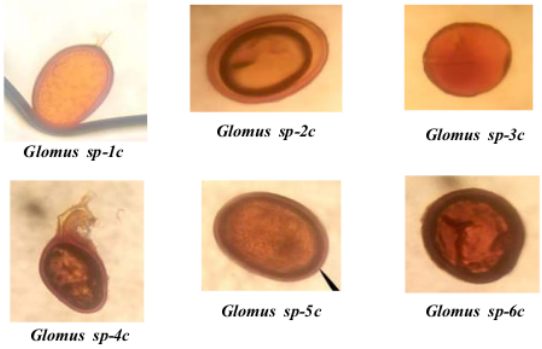

Figure 4. Diversity of AMF types in Serdang Jaya Village

The results showed that the type of *Glomus sp.* more than *Acaulospora sp.* That's means the type of *Glomus sp*. dominate in three locations (Figures 1). This is consistent with the results of Sasli and Ruliansyah (2012), that AMF *Glomus sp*. have a high level of adaptation to acidic soil environments.

This result is supported by soil analysis in three locations, indicating that in Parit Pudin Village has a pH 3.33, Senyerang Village has a pH 3.69 and Serdang Jaya Village has a pH 3.77. Then Puspitasari, *et al.* (2012) in Torjun Village, Sampang District, Madura; Husna, *et a*l., (2014) in Southeast Sulawesi; Cahyani, *et al*. (2014) in Pademawu District, Madura Regency; Nurhatika *et al.* (2014) in the Pamekasan Madura area; Diastama, *et al.* (2015) in Sanur Kaja Village, and Rifa, *et al*. (2017) in Mapanget and Tateli Menado also showed that the genus Glomus is a dominating genus compared to other genera. This shows, genus Glomus has a high level of adaptation to various environmental conditions. Differences in location and rhizosphere cause differences in species diversity and AMF population. Overall Glomus has the highest number compared to other genera. The abundance of Glomus due to mycorrhizae is suitable for their habitat. The diversity of AMF spores is due to differences in soil fertility, organic matter content, light intensity and altitude above sea level (Setiadi, 1989).

The results of spore counting based on microscopy showed that the average number of spores of *Acaulospora sp*. from Pudin Village (5 spores / 50 g soil samples) was lower than the average number of spores of *Acaulospora sp.* (57 spores) from Senyerang (Table 4). While the average number of spores of *Glomus sp.* in the village of Parit Pudin (63 spores) higher than the average number of spores of *Glomus sp*. in Senyerang (49.5 spores) and Serdang Jaya (46.83 spores). The range of AMF spores obtained in the Liberica coffee plant rhizosphere is 5-124 spores in every 50 g of soil samples (Table 4). This result is higher than the results of the study by Al-Areqi (2013) who obtained 52-100 spores in every 100 grams of soil in the *Coffea Arabica* rhizosphere, then Puspitasari's research (2012) showed an abundance of AMF spores in Torjun village as many as 712 spores per 500 g of soil samples with sandy clay structure and the lowest C-organic content, N-Total, P and CEC.

Table 4. AMF spore density from the rhizosphere of Liberica coffee plants in Parit Pudin, Senyerang, and Serdang Jaya Villages, Tanjung Jabung Barat

Location	AMF type (before trapping)	Spores Number per 50 g of soil sample
Pangabuan District (Parit Pudin Village)	*Acaulopspora sp-1a*	5
	Average	**5**
	Glomus sp-1a	33
	Glomus sp-2a	79
	Glomus sp-3a	92
	Glomus sp-4a	42
	Glomus sp-5a	105
	Glomus sp-6a	27
	Average	**63**
Senyerang District (Senyerang Village)	*Acaulopspora sp-1b*	23
	Acaulopspora sp-2b	90
	Acaulopspora sp-3b	58
	Average	**57**
	Glomus sp-1b	70
	Glomus sp-2b	12
	Glomus sp-3b	31
	Glomus sp-4b	75
	Glomus sp-5b	29
	Glomus sp-6b	80
	Average	**49.5**
Betara District (Serdang Jaya Village))	*Glomus sp-1c*	124
	Glomus sp-2c	7
	Glomus sp-3c	14
	Glomus sp-4c	56

	Glomus sp-5c	39
	Glomus sp-6c	41
	Average	**46.83**

Furthermore, Cahyani, *et al.* (2014) found that the number of spores in Pademawu was 11 spores / 100 g of soil samples, in Pamekasan 7 spores, and in Tlanakan 9 spores This results was supported by Nurhalimah, *et al.* (2014) in the District of Larangan, the number of AMF spores was 7 spores / 100 g of soil samples, in Palengaan Subdistrict 9 spores, and in Pegantenan subdistrict 6 spores. Pangaribuan (2014) reported that before trapping, the density of natural spores in the soil of the Rantau Rasau and Jawai peat in West Kalimantan was only 18-30 spores in every 50 grams of soil samples. Furthermore, Suharno, *et al.* (2015) showed that the number of spores in the *Setaria italica* (L.) rhizosphere before trapping 33 spores per 10 g of sample and increasing to 54 spores after trapping and much higher than the results of this study. The results of Dharmaputri, Wijaya and Adiartayasa (2016) research showed that in the rhizosphere of lamtoro plants, there were 94 spores in 100 g of soil samples, and 99 spores in the Kaliandra plant rhizosphere. Furthermore, Dewi, Sritamin and Suada (2016) found 59 spores at Arabica coffee and 67 spore at Robusta coffee rhizosphere.

This is consistent with the theory that low nutrient availability will optimize the role of mycorrhizae by expanding the absorption area and stimulating the roots to penetrate the nutrient depletion zone. The high population of AMF spores is thought to be due to environmental conditions that are more suitable, optimal, and compatible in supporting the growth and development of AMF spores. possible absence of antagonistic fungi that inhibit AMF sporulation. It is suspected that in areas that have higher nutrients have a variety of species and the numbers are relatively lower. This is because when nutrients are sufficient, plant roots can act as nutrient absorbing organs so can plants accumulate nutrients in high amounts. This condition will cause a negative response to mycorrhizal colonization. The number of AMF spores on agricultural land varies depending on the season each year and also depends on several factors such as plant growth, edafic factors, weather patterns each season and management of fertilization.

4. Conclusion

Based on the results obtained in this study we conclude that:

1. Types of AMF spores result from isolated and identified from the rhizosphere of Liberica coffee plants in the village of Parit Pudin, Senyerang and Serdang Jaya are *Acaulospora sp*. and *Glomus sp.*
2. In Parit Pudin Village, there is one type of *Acaulospora sp*. and 6 types of *Glomus sp*., in Senyerang found 3 types of *Acaulospora sp.* and 6 types of *Glomus sp*., while in Serdang Jaya obtained 6 types of *Glomus sp.*
3. The result from three locations showed the AMF spore were dominated by *Glomus sp*. namely in Parit Pudin Village at 85.71%, in Bunga Tanjung at 66.67% and in Serdang Jaya 100%.
4. In Parit Pudin village, the spores abundance of *Acaulospora sp*. and *Glomus sp.* were 5 and 63 spores per 50 g of soil samples. In Senyerang village, the spores number of *Acaulospora sp.* and *Glomus sp*. were 57 and 49.5 spores per 50 g of soil samples, while in Serdang Jaya Village, the number spores of *Glomus sp*. as much as 46.83 spores per 50 g of soil samples.
5. AMF Spores found showed variations in spore walls, shape, color and attachment of the hypha stalks.

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