PROPAGATION OF ARBUSCULAR MYCORRHIZAL FUNGI (AMF) SPORES FROM ARABICA COFFEE (Coffea arabica L.) PLANTATIONS IN BENER MERIAH REGENCY

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ABSTRACT

Arbuscular Mycorrhizal Fungi (AMF) is a type of fungus that is capable of mutualistic symbiosis with plant roots. The presence of AMF can increase the availability of nutrients in the soil, especially the element P, expand the nutrient uptake area with the help of mycelium, plant resistance to disease and drought, and be able to produce growth hormones. These fungi can form a symbiotic relationship and increase the growth and productivity of coffee plants. This research aims to multiply AMF spores collected from the rhizosphere of Arabica coffee plants in Bener Meriah Regency using corn as a host plant. This research uses a descriptive method. Propagation of AMF spores collected from the rhizosphere of Arabica coffee plants using a single propagation method using zeolite media and corn host plants. Counting of AMF spores resulting from multiplication and observing AMF colonization on plant roots was carried out after the corn plants were stressed. Observation of the number of spores was carried out using the wet filtration isolation method and centrifugation technique (Brundrett et al., 1996). Observation of the percentage of AMF colonization on corn plant roots using the root staining method. Data analysis was carried out descriptively. The research results showed that from 44 AMF spores originating from the rhizosphere of Arabica coffee in Bener Meriah Regency, 111 AMF spores were obtained from propagation with a colonization percentage ranging from 0 – 81%. The largest number of spores were 11 spores resulting from multiplication originating from M44 spores, while the highest percentage of root colonization (81%) with very high criteria contained host roots colonized by M1 spores. A total of 12 AMF spores colonized the host roots with high to very high criteria. The research results showed that AMF spores from the rhizosphere of Arabica coffee in Bener Meriah Regency which were propagated by pot culture on zeolite media and corn as the host plant were relatively low with the percentage of root colonization varying from none to very high.

Keywords: AMF; Propagation of AMF; Root colonization; *Arabica coffea*

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) is a form of symbiotic mutualism between fungi and plant roots. This relationship is mutually beneficial, the fungi get carbohydrates from the host plant, otherwise, the host plant can expand nutrient uptake with the help of fungi (Pulungan, 2013). The existence of massive hyphae can absorb nutrients into the smallest pores of the soil so that nutrient absorption is maximized. Arbuscular mycorrhizal fungi can infect almost all types of plants, one of which is coffee plants (Rillig and Mummey, 200)} and can increase the growth of these plants. This fungus can increase the height and root length of coffee plant seedlings (Samah and Harahap, 2019) and the dry weight of Arabica coffee plant seedlings at a rate of 40 to 50 g ha^{-1} (Sugiarti and Taryana, 2018).

Coffee cultivation in Aceh is mostly found in the Gayo highland area, one of which is Bener Meriah Regency, which is located at an altitude between 100 and 2,500 meters above sea level and covers an area of 1,941.61 km^2 (BAPPEDA Bener Meriah, 2019). One type of coffee that is widely cultivated by the Gayo Highland community is arabica coffee. Coffee plants need nutrients for their growth and production. The continuous use of inorganic fertilizers in large quantities can reduce soil fertility and even the quality and taste of coffee. Therefore, another alternative is needed, namely the use of biological fertilizers such as AMF.

The potential of AMF as a biological agent in increasing soil and plant productivity is more effective when using local AMF spores (Sasli and Ruliansyah, 2012). The lack of AMF inoculants both in terms of quality and quantity is an obstacle in the utilization of AMF as a biological fertilizer. AMF spore propagation is one of the alternatives that can be done to overcome this problem. AMF spore propagation is generally done by pot culture using media and certain types of host plants.

This study aims to determine the ability of AMF spores from the rhizosphere of arabica coffee plants in the District of Bener Meriah to propagate and colonize corn roots as host plants.

MATERIALS AND METHODS

Research Methods

The method used in this research is the descriptive method.

Work Procedure

1. Propagation of AMF Spore

Spore propagation using single culture propagation technique. FMA spores collected from the rhizosphere of arabica coffee plants in Bener Meriah Regency were propagated using the pot culture technique using zeolite as a carrier medium and corn (*Zea mays)* as a host plant.

2. Isolation of AMF Spores

The isolation of AMF spores is carried out on the results of propagation, carried out by wet filtration methods and centrifugation techniques (Brundrett et al., 1996).

3. Root Colonization

Root colonization observation was conducted on host plant roots after FMA spore multiplication. The observation method of root colonization was carried out with a root *staining* technique based on the method of Brundrett et al. (1996). The calculation of AMF colonization level on plant roots can be calculated using the formula:

$$\%\ \text{Root Colonization} = \frac{\sum field\ of\ view\ of\ mycorrhizal\ colonized\ roots}{\sum observed\ field\ of\ view} \times 100\%$$

4. Data Analysis

Data analysis was carried out descriptively based on the observation of the number of AMF spores, as well as the ability of AMF colonization on the roots of corn plants.

RESULTS AND DISCUSSION

1. Count of AMF Spore

The propagation results of 44 AMF spores collected from the rhizosphere of arabica coffee plants in Bener Meriah Regency can be seen in Table 1.

Table 1. Count of AMF spores from single spore propagation from the rhizosphere of arabica coffee in Bener Meriah Regency

No	Spora Code	Origin of Spores		Number of AMF Spores
		Subdistrict	**Village**	
1	M1	Pintu Rime Gayo	Rime Raya	0
2	M2	Pintu Rime Gayo	Rime Raya	0
3	M3	Pintu Rime Gayo	Alur Gading	2
4	M4	Bukit	Sidie Jadi	1
5	M5	Bukit	Sidie Jadi	1
6	M6	Bukit	Sidie Jadi	1
7	M7	Bener Kelipah	Nosar Tawar Jaya	0
8	M8	Bener Kelipah	Nosar Tawar Jaya	0
9	M9	Bener Kelipah	Nosar Tawar Jaya	0
10	M10	Gajah Putih	Pante Karya	0
11	M11	Gajah Putih	Pante Karya	2
12	M12	Gajah Putih	Pante Karya	0
13	M13	Gajah Putih	Pante Karya	1
14	M14	Gajah Putih	Pante Karya	1
15	M15	Gajah Putih	Pante Karya	0
16	M16	Gajah Putih	Umah Besi	0
17	M17	Gajah Putih	Umah Besi	4
18	M18	Gajah Putih	Pante Karya	4
19	M19	Bandar	Hakim Wih Ilang	1
20	M20	Bandar	Paya Baning	2
21	M21	Bandar	Paya Baning	2
22	M22	Bandar	Hakim Wih Ilang	1
23	M23	Bandar	Hakim Wih Ilang	3
24	M24	Bandar	Hakim Wih Ilang	4
25	M25	Bandar	Pondok Gajah	3
26	M26	Bandar	Hakim Wih Ilang	3
27	M27	Bandar	Hakim Wih Ilang	2
28	M28	Bandar	Pondok Gajah	0
29	M29	Mesidah	Simpur	4
30	M30	Mesidah	Cemparam Lama	4
31	M31	Mesidah	Cemparam Lama	5
32	M32	Mesidah	Cemparam Lama	2
33	M33	Mesidah	Simpur	5
34	M34	Mesidah	Jamur Atu Jaya	5
35	M35	Mesidah	Cemparam Lama	3
36	M36	Mesidah	Jamur Atu Jaya	6
37	M37	Mesidah	Cemparam Lama	5
38	M38	Mesidah	Gunung Sayang	8
39	M39	Mesidah	Jamur Atu Jaya	2
40	M40	Mesidah	Cemparam Lama	2
41	M41	Mesidah	Gunung Sayang	1
42	M42	Mesidah	Jamur Atu Jaya	6
43	M43	Mesidah	Simpur	4
44	M44	Mesidah	Jamur Atu Jaya	11
Total				**111**

Table 1 shows that 111 AMF spores were successfully collected from the propagation of AMF spores from the rhizosphere of Arabica coffee plants in Bener Meriah Regency. The number of spores successfully obtained from each spore code varies with the highest number of spores obtained from spore M44. The number of spores obtained is classified as very low. Spore density can be said to be high if there are 20 spores per gram of soil (200 spores/10 g of soil) (Danielsand Skipper, 1982).

The low number of spores obtained is thought to be due to the sporulation process not occurring optimally. This is thought to be because the nutrient needs of plants are sufficient during the growth period, so the plant's need for AMF becomes low, causing the interaction that occurs is not optimal. This follows the opinion of Hermawan et al (2015) which states that AMF will produce many spores if it experiences pressure on its environment, namely if environmental conditions are not suitable for the growth and development of host plants (Cuenca and Lovera, 2010). Suitable, optimal, and compatible environmental conditions for AMF greatly affect the spore population (Puspitasari *et al*., 2012).

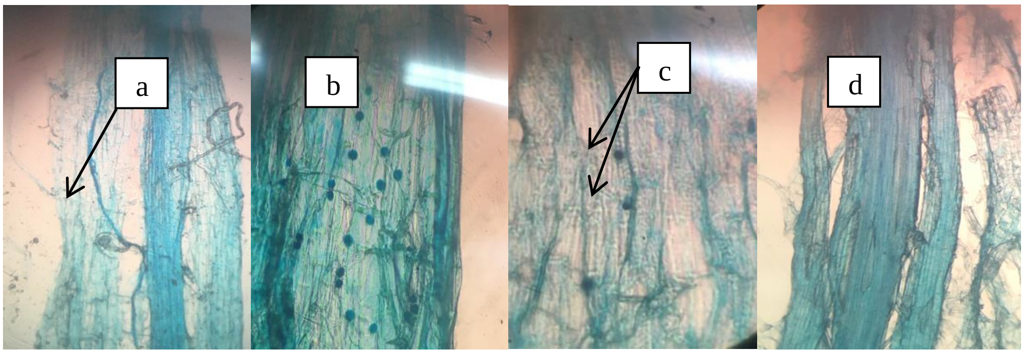
Another possibility can also be caused by spores that are formed again experiencing germination (Rini and Rozalinda, 2010). As stated by Sieverding (1991), the sporulation process is dynamic so that some spores are formed and others germinate at the same time.

Examples of successfully isolated FMA spore can be seen in Picture 1.

Picture 1. AMF spore

2. AMF colonization

Root colonization by AMF can be characterized by the presence of hyphal structures, vesicles, arbuscules and spores in plant root tissues (Setiadi and Setiawan, 2011). The symbiotic relationship has occurred even though there is only one AMF organ in the root tissue of the host plant. Roots colonized by AMF can be seen in Pictures 2.



Pictures 2. AMF-infected roots (a) hyphae, (b) vesicles, (c) spores, and (d) without infection

Picture 2 shows the root tissue of plants infected with AMF, namely hyphae and vesicle spores, and roots without infection. Hyphae are shaped like fine threads, and AMF spores are round to oval with clear to brownish color. Vesicles can be found inside or outside the parenchyma cortex layer and are formed from the bulging of AMF internal hyphae (Suamba et al., 2014).

The percentage of plant root

colonization by AMF indicates the ability of AMF to colonize the host plant. The high percentage of colonization indicates the level of need and dependence of plants on the presence of AMF (Hartoyo et al., 2011). The percentage of AMF colonization of plant roots can be seen in Table 2.

Table 2. Percentage of maize root colonization by FMA

No	**Spora Code**	**Origin Spora**		**Colobization (%)**	**Criteria**
		Subdistrict	**Village**		
1	M1	Pintu Rime Gayo	Rime Raya	81	Very high
2	M2	Pintu Rime Gayo	Rime Raya	73	High
3	M3	Pintu Rime Gayo	Alur Gading	73	High
4	M4	Bukit	Sidie Jadi	53	High
5	M5	Bukit	Sidie Jadi	51	High
6	M6	Bukit	Sidie Jadi	56	High
7	M7	Bener Kelipah	Nosar Tawar Jaya	48	Medium
8	M8	Bener Kelipah	Nosar Tawar Jaya	53	High
9	M9	Bener Kelipah	Nosar Tawar Jaya	48	Medium
10	M10	Gajah Putih	Pante Karya	46	Medium
11	M11	Gajah Putih	Pante Karya	60	high
12	M12	Gajah Putih	Pante Karya	24	Low
13	M13	Gajah Putih	Pante Karya	43	Medium
14	M14	Gajah Putih	Pante Karya	2	Very low
15	M15	Gajah Putih	Pante Karya	2	Very low
16	M16	Gajah Putih	Umah Besi	3	Very low
17	M17	Gajah Putih	Umah Besi	3	Very low
18	M18	Gajah Putih	Pante Karya	0	Very low
19	M19	Bandar	Hakim Wih Ilang	3	Very low
20	M20	Bandar	Paya Baning	5	Very low
21	M21	Bandar	Paya Baning	7	Very low
22	M22	Bandar	Hakim Wih Ilang	9	Very low
23	M23	Bandar	Hakim Wih Ilang	30	Medium
24	M24	Bandar	Hakim Wih Ilang	9	Very low
25	M25	Bandar	Pondok Gajah	16	Low
26	M26	Bandar	Hakim Wih Ilang	12	Very low
27	M27	Bandar	Hakim Wih Ilang	25	Low
28	M28	Bandar	Pondok Gajah	21	Low
29	M29	Mesidah	Simpur	64	High
30	M30	Mesidah	Cemparam Lama	52	High
31	M31	Mesidah	Cemparam Lama	67	High
32	M32	Mesidah	Cemparam Lama	36	Medium
33	M33	Mesidah	Simpur	28	Medium
34	M34	Mesidah	Jamur Atu Jaya	37	Medium
35	M35	Mesidah	Cemparam Lama	29	Medium
36	M36	Mesidah	Jamur Atu Jaya	51	High
37	M37	Mesidah	Cemparam Lama	26	Medium
38	M38	Mesidah	Gunung Sayang	35	Medium
39	M39	Mesidah	Jamur Atu Jaya	19	Low
40	M40	Mesidah	Cemparam Lama	23	Low
41	*M41*	Mesidah	Gunung Sayang	13	Very low
42	M42	Mesidah	Jamur Atu Jaya	12	Very low
43	M43	Mesidah	Simpur	5	Very low
44	M44	Mesidah	Jamur Atu Jaya	0	Very low

Table 2 shows the percentage of colonization by AMF spores on the roots of host plants resulting from propagation culture. The highest percentage of colonization (very high category) was found in spore code M1 which was 81%, while spores with code M18 and M44 did not successfully colonize the host plant roots.

The results showed that there was one spore that colonized the roots with very high criteria (class 5) where the percentage of root colonization ranged from 76 - 100%, and there were 11 spores with high colonization criteria (class 4) where the percentage of root infection ranged from 51 - 75%. In addition, there were also medium category (class 3) with 11 spores, low category (class 2) with six spores, and very low category (class 1) with 15 spores. The high and low percentage of root colonization expresses the level of dependence and compatibility between AMF species and host plants.

In this study, despite using the same type of host plant, the category of plant root colonization by AMF obtained varied. This is due to the ability of AMF to infect different plants because the amount of exudate released by each plant is also different so the AMF response to plants is also not the same (Muzlifa et al., 2019). In addition, differences in AMF adaptability to environmental conditions and the ability of fungi to develop after infection also affect the percentage of root infection by AMF. The results showed that there were 12 spores that had the best colonization ability, namely M1, M2, M3, M4, M5, M6, M8, M11, M29, M30, M31, and M36. The high percentage of root colonization is thought to be due to the good source of AMF inoculum used so that the AMF infection power of plants is higher.

The relationship between the percentage of AMF colonization and the number of spores is not always positively correlated. There is no close relationship between the number of spores and the level of root colonization by AMF (Elfiati and Siregar, 2010). The amount of colonization may not produce spores or increase spore production. This could be because the photosynthate used by AMF is only enough for the development of hyphae or vesicles, without the formation of spores (Rini and Rozalinda, 2010). Another possibility could be because the spores that are formed re-germinate as stated by Sieverding (1991).

CONCLUSIONS

AMF spores from the rhizosphere of coffee plants can be propagated, the results of spore propagation obtained as many as 111 FMA spores. The highest number of spores was 11 spores from M44 spores. AMf spores from the rhizosphere of coffee plants can colonize the roots of corn host plants with the percentage of colonization varying from no infection to very high. The very high colonization category came from M1 spores, which was 81%.

There is one spore that produces the most spores, namely 11 propagated spores derived from spore M44, and 12 spores that have the best colonization ability, namely spores M1, M2, M3, M4, M5, M6, M8, M11, M29, M30, M31, and M36.

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