

The Diversity of Mycorrhiza Arbuscular Fungi in Several Types of Peatland Utilization in Sungai Asam Village Kubu Raya District

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ABSTRACT

The purpose of this study was to determine the diversity of FMA from the rhizosphere of cassava, corn, taro, ginger, kale, pineapple which was cultivated in the Sungai Asam peatland by using a corn plant host. This research was conducted at the Soil Biology and Biotechnology Laboratory of the Faculty of Agriculture and plastic houses in the Universitas Tanjungpura Faculty of Agriculture's experimental garden. The study was conducted from August 2nd to October 21st, 2018. The procedure was done by taking soil and root samples from cassava, corn, taro, and peanut rhizosphere cultivated on peatland. A sampling of soil and roots in each rhizosphere was carried out at 4 observation points as replication with a depth of 0-20 cm and a diameter of 20 cm and then put into a plastic bag and labeled. Then, the soil samples taken were used for microscopic analysis (extraction and identification) and were analyzed to determine their chemical properties. Root samples were colorized to determine the percentage of colonization, and corns were used to cultivate. Furthermore, extraction and identification of spores were carried out using the same technique as extraction and identification of soil samples. The variables observed included the percentage of root colonization, spore density, and spore diversity. The FMA diversity of the 6 types of peatland utilization from the rhizosphere of cassava, corn, taro, ginger, pineapple, and kale in the Sungai Asam village before and after trapping showed an increase in the number of spores, diversity of FMA while the percentage value of root colonization was varied. Moreover, the number of spores increased from around 36 - 52 spores per 50 g of soil to 61 - 178 spores per 50 g of soil. The diversity of spore types increased from 10 types of Glomus, 1 type of Gigaspora, and 4 types of Acaulospora to 13 types of Glomus, 3 types of Gigaspora, and 5 types of Acaulospora. The highest percentage of infected roots before trapping ranged from 48.89% - 78.48% and after trapping ranged from 78.89% - 94.80%.

Keywords: Arbuscular mycorrhiza fungi, diversity, peatland

ABSTRAK

Tujuan penelitian ini adalah untuk mengetahui keanekaragaman FMA dari rizosfer ubi kayu, jagung, talas, jahe, kangkung, nanas yang dibudidayakan di lahan gambut Sungai Asam dengan menggunakan tanaman inang jagung. Penelitian ini dilakukan di Laboratorium Biologi Tanah dan Bioteknologi Fakultas Pertanian dan Rumah Plastik di Kebun Percobaan Fakultas Pertanian Universitas Tanjungpura. Penelitian dilakukan dari tanggal 2 Agustus 2018 hingga 21 Oktober 2018. Prosedur dilakukan dengan pengambilan sampel tanah dan akar dari ubi kayu, jagung, talas dan rizosfer kacang tanah yang diusahakan di lahan gambut. Pengambilan sampel tanah dan akar di setiap rizosfer dilakukan pada 4 titik pengamatan sebagai ulangan dengan kedalaman 0-20 cm dan diameter 20 cm kemudian dimasukkan ke dalam kantong plastik dan diberi label. Sampel tanah yang diambil kemudian digunakan untuk analisis mikroskopis (ekstraksi dan identifikasi) dan analisis tanah untuk mengetahui sifat kimianya. Sampel akar diwarnai untuk menentukan persentase kolonisasi, dan jagung digunakan untuk budidaya. Selanjutnya ekstraksi dan identifikasi spora dilakukan dengan teknik yang sama seperti ekstraksi dan identifikasi sampel tanah. Variabel yang diamati meliputi persentase kolonisasi akar, kepadatan spora, dan keanekaragaman spora. Hasil penelitian menunjukkan bahwa keanekaragaman WPP dari 6 jenis pemanfaatan lahan gambut dari rizosfer ubi kayu, jagung, talas, jahe, nanas dan kangkung di desa Sungai Asam sebelum dan sesudah penangkapan menunjukkan peningkatan jumlah spora,

keanekaragaman hayati. FMA - sedangkan nilai persentase kolonisasi akar menunjukkan variasi. Hasil pengamatan terhadap jumlah spora menunjukkan peningkatan dari sekitar 36 - 52 per 50 g tanah meningkat menjadi 61 - 178 spora per 50 g tanah. Keragaman jenis spora mulai dari 10 jenis *Glomus*, 1 jenis *Gigaspora* dan 4 jenis *Acaulospora* menjadi 13 jenis *Glomus*, 3 jenis *Gigaspora* dan 5 jenis *Acaulospora*. Persentase tertinggi akar terserang sebelum penangkapan berkisar antara 48,89% - 78,48% dan setelah penangkapan berkisar antara 78,89% - 94,80%.

Kata Kunci: Fungi Mikoriza Arbuskula, Keanekaragaman, Lahan Gambut

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) is a form of association between fungi with a high level of plant's roots, which reflects the existence of mutually beneficial functional interactions between a plant and one or more mycobiome strains in time and space. One of the mycorrhizal-forming fungi is the endomycorrhizal group. This type of fungus is characterized by intracellular hyphae that penetrate the cortex from one cell to another (Goltapeh *et al.* 2013). The presence of arbuscular mycorrhizal fungi is important for the resilience of an ecosystem, plant stability, and maintenance. It is also important in supporting plants' diversity and for the increase in crop productivity (Moreira *et al.* 2007). It is quite essential for the availability of nutrients such as P, Mg, K, Fe, and Mn for plant growth. This has occurred through the formation of hyphae on the root surface which functions as an extension of the roots, especially in growing media that are nutrient-poor, low pH, and lack of water (Abbot and Robson 1984). The benefits of mycorrhizal fungi can be proved if the soil conditions are poor in nutrients or dry conditions, while for fertile soil conditions the role of fungi does not exist (Lakitan 2000).

Huda *et al.* (2016) AMF in its association has a relatively wide range of hosts. The AMF association reaches 80% with terrestrial plants. However, the effectiveness of each host plant varies due to certain types of AMF "some will only associate with certain types of host plants. Okon *et al.* (1994) the type of host plant and environmental conditions will greatly determine the level of root colonization, the number of spores, and the diversity of it. Burhanuddin (2012) revealed that open land conditions and high temperature cause the sporulation happens to be higher because of the tools reproduction of AMF will still be more resilient when in extreme circumstances. The spread of AMF through inoculation is reduced in soil that has already undergone mycorrhiza, but increases in non-mycorrhizal soils (Goltapeh *et al.* 2013).

One alternative to increase the production of crops is to determine ahead on which plant species to cultivate cassava, corn, taro, ginger, kale,

pineapple which are currently widely cultivated by the Sungai Asam community. According to Hakim *et al.* 1986), peat soil as a growing medium is faced with the constraints of chemical and biological properties of the soil including low fertility rates and high soil acidity due to the decomposition of organic matter which produces organic and inorganic acids which are accumulated in the soil.

The P nutrient in peat soils is mostly taken in the form of organic-P, which is available abundantly in peat soil but not for plants because they are bound to the peat organic matter (Everett, 1983). The ability of cassava, corn, taro, ginger, kale, pineapple in the absorption of nutrients is closely related to the symbiosis with Arbuscular Mycorrhizal Fungi (AMF). According to Rasyid *et al.* (2016), the number of spores is greater when closer to the root zone than far from the root zone. Place also affects the number of spore populations, this is related to soil conditions, humidity, organic matter content, water content level, soil texture, and others.

Each type of AMF has different in enhancing plant growth or root growth, therefore the selection of AMF isolates that are compatible with host plants is important (Pratifthriasari and Nurbaity 2010). Difference in location and rhizosphere will result in differences in species diversity and AMF populations (Widiastuti and Kramadibrata 2015). It becomes necessary to research the potential diversity of AMF in the rhizosphere of cassava, corn, taro, ginger, kale, pineapple planted on peatland.

This study was aimed to find and determine the diversity of AMF from the rhizosphere of cassava, corn, taro, ginger, kale, pineapple cultivated on the Sungai Asam peatland.

MATERIALS AND METHODS

Time and Place of Research

The research was conducted from August 2nd until October 21st, 2018. The samples of soil and roots were taken from peatland planted with cassava, corn, taro, ginger, kale, pineapple in the Sungai Asam Village area, Sungai Raya District. Spore extraction, identification, and calculation of the percentage of roots infected by FMA at plant roots were carried

out at the Soil Biology Laboratory and for trapping culture at Screen House, Faculty of Agriculture, University of Tanjungpura.

Soil and Root Sampling

Soil and roots samplings in each rhizosphere of cassava, corn, taro, ginger, kale, pineapple in the Sungai Asam Village area were carried out in 3 plots. Each plot consists of five soil sampling points, hence a total of 90 samples were collected. The method used to collect the sample was the non-proportional method and the soil plus roots were taken at a depth of 0 - 20 cm and a diameter of 20 cm and then put into plastic and labeled (Nusantara *et al.* 2012). In this case, the total of soil samples was only 18 samples, not 90 samples. The soil samples taken were then used for microscopic analysis (extraction and identification) and soil analysis to determine the chemical properties of the soil. The root samples were stained to determine the percentage of AMF colonization.

Isolation and Characteristics of AMF Spore Types

Isolation of FMA spores from soil samples was carried out by wet sieving method (Pacioni 1994) and centrifugation (Brundrett *et al.* 1996). Steps in isolating spores are as follows. Firstly, soil samples were taken as much as 20 g then 500 ml of distilled water was added and stirred until smooth. Secondly, the soil suspension was poured into a multilevel filter from top to bottom with a filter size of 500, 125, and 63 µm. Thirdly, the filtered soil suspension in 125 and 63 µm sieves was inserted into a test tube and then added with a 60% sucrose solution as much as 1/3 of the part, then centrifuged at 2.500 rpm for 3 minutes. Then, a rather clear liquid in the middle of the tube which is a transition between sucrose solution and water aspirated using a pipette and washed then filtered with a 63 µm size filter. Finally, the filter was put into a petri dish and observed under a microscope for calculating spore density.

Spore specimens are preserved using Polyvynil Alcohol Lactoglycerol (PVLG) and Melzer solution which is placed on the glass object. Observations were made by looking at the morphological features of the spores based on their shape, size, color, and spore walls (Nusantara *et al.* 2012). The observations of AMF spores were characterized to genus level and AMF spore density in soil samples. The color change in the Melzer solution is an indicator to determine the genus of spores that exist.

Observation of Root Colonization

Root staining was done in six stages: 1). The roots were washed clean using distilled water; 2). The roots were soaked in 20% KOH for 48 hours; 3). The roots were washed with water until clean by using a filter, then soaked in 0.1 M HCl solution; 4). Then without washing, the roots were soaked in trypan blue solution for 48 hours; 5). Next, the roots were soaked in a distaining solution for 24 hours; 6). The roots were cut to 1 cm in size and arranged parallel to the object-glass and covered with a glass cover. The number of root samples for each preparation were 10 pieces. Root samples were observed under a microscope to determine mycorrhizal infection. Next, a photo was taken on a microscope.

Trapping culture

The trapping culture refers to Brundrett *et al.* (1996) which using polybags. The planting medium used a mixture of 50 g soil and 120 g zeolites measuring 1-2 mm in diameter. The source of the soil samples came from the rhizosphere of cassava, corn, taro, ginger, kale, pineapple, which were cultivated in the peatlands of Sungai Asam village. Corn plants were used as the trapping culture. The cultures consisted of 6 levels of treatment and repeated 4 times. In total, there were 24 experimental units.

Maintenance of culture included watering, fertilizing, and controlling pests and diseases. The fertilizer given was red Hyponex (25-5-20) with a concentration of 1 g L⁻¹ of water. Fertilization was carried out every week with a dose of 20 mL plant⁻¹. After 6 weeks, old plant watering activities were stopped to make the conditions of culture stress drought. The drying period was carried out for 2 weeks. Harvesting was done after the formation of new spores was assumed to be good enough. Furthermore, spore extraction and identification were carried out using the same technique as extraction and identification of soil samples.

Observation variable

$$\% \text{ colonizati on} = \frac{\sum \text{colonized field of view}}{\sum \text{overall field of view}} \times 100\%$$

$$\text{Spore density} = \frac{\text{Number of spores (spores)}}{\text{Weight of soil analyzed (g)}}$$

Diversity of spores (genus) = Number of genera at 10 g of soil

Data Analysis

Data in the form of percentage of root colonization, spore density, and diversity status of AMF were analyzed descriptively.

RESULTS AND DISCUSSION

Percentage of Root Colonization

The percentage of root colonization in several types of peatland utilization in Sungai Asam village shows an association between AMF and roots that form hyphae in root cells. According to Okon and Kalpunik (1986) during the first three days, colonization takes place mainly on the root elongation zone, on the base of root hairs and, to a lesser extent, on the surface of young root hairs. The results of observations on the percentage of root colonization at various locations for soil sampling can be seen in Figure 1.

Figure 1 shows that the highest percentage of root colonization before trapping was indicated by the type of peatland use for pineapple cultivation (78.48%), while the lowest was by the type of peatland use for corn cultivation (48.89%). Furthermore, the highest percentage of root colonization after trapping was shown in AMF originating from the rhizosphere of corn plants (94.80%), while the lowest percentage of root colonization was shown in AMF originating from the rhizosphere of taro plants (78.89%). The criteria for the percentage of root colonization in various types of utilization of the Sungai Asam peatland before and after the trapping were classified as moderate to very high. According to O'Connor *et al.* (2011), that the percentage of root colonization ranging from 0-25% was classified as low, 26 - 50% moderate, 51 - 71% high, and 76-100% very high.

Moreover, Wani and Lee (1995) indicated that the maximum root colonization would be achieved on less fertile soils.

The AMF association with roots in each type of peatland utilization that causes infection in the host plant roots can be known by the presence or absence of structures produced by AMF, namely hipa, vesicles, and arbuscular. Baptista *et al.* (2011) stated that the root colonization process is divided into 4 stages, namely before infection, penetration of hyphae in the roots of the host plant, hyphae growing and developing in root cells and the final stage of AMF will carry out its function to help absorption of nutrients and water for host plants

AMF Spore density

The lowest spore density before the lowest trapping was carried out in the land of kale water rhizosphere which was 36 spores per 50 g soil and the highest was in the rhizosphere of corn which was 53 spores per 50 g soil. The lowest spore density after trapping was done on rhizosphere soil of ginger plants as many as 61 spores per 50 g soil and the highest spores in the rhizosphere soil of maize plants were 178 spores per 50 g soil. The increasing number of spores in Trapping culture is likely due to the treatment of stressing or stopping watering so that it has a positive influence on the development of AMF. The average density of spores before and after trapping can be seen in Table 1.

According to Suharno *et al.* (2015), the increase in the number of trapping spores was supported by controlled and stable greenhouse environment conditions, thus providing the opportunity for spores isolated from the germinated field to undergo germination and forming new spores. According to Oehl *et al.* (2011), the increase in the number of spores is due to the trapping treatment

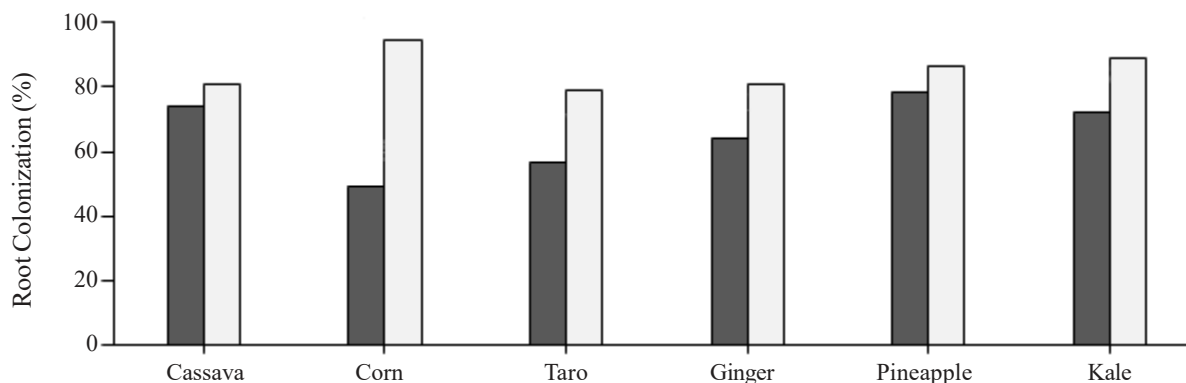


Figure 1. Root Colonization in several types of peat land utilization before and after trapping.

which aims to stimulate sporules in the soil originating from cultivated land. Each type of AMF will be active at different periods, some types of AMF will be abundant in the rainy season, while others are in the dry season and there are types of AMF that will be active throughout the year. The inoculum source from the rhizosphere of corn plants has high effectiveness spores. This is in line with the percentage value of root colonization which is 94.80%. In addition to the level of effectiveness of spores that support the number of spores in the combination treatment, it is also possible because the number of origin spores from the rhizosphere inoculum of the corn plant showed the highest number compared to other sources of inoculum, 53 spores per 50 g soil samples.

The infectivity of AMF from the same rhizosphere with the type of host plant is compatibility or compatibility. According to Santoso (1994), compatibility or compatibility of AMF with host plants varies greatly depending on species AMF, host plant species, and environmental conditions.

AMF Diversity Spores

The results of isolation and identification of spores carried out on the 6 types of utilization of the Sungai Asam peat soil were found in three genera of spores, namely *Glomus*, *Acaulospora*, and *Gigaspora*. The most common genus found was the genus *Glomus* with details of *Glomus* consisting of

8 types of spores, *Acaulospora* 1 type of spore and *Gigaspora* 1 type of spore. The diversity of host species, spore type, and environmental conditions such as soil conditions directly showed different responses to the percentage of colonization, number of spores, and diversity of spore types (Quilambo 2013).

The results of observations on trapping cultures showed an increase in spore type. The diversity of the genus *Glomus* spore types in soil samples before the trapping consisted of 8 types of spores then developed into 13 types of spores, the genus *Acaulospora* which initially only found 1 type of spore increased to 5 types of spores and genera of *Gigaspora* from 1 type of spore to 3 types of spores. Several genera found in the sampling and trapping locations can be seen in Table 2.

The results of the identification showed that there were 3 genera of AMF found in the sample soil and trapping results, namely *Glomus*, *Gigaspora*, *Acaulospora*. Identification was carried out with different characteristics, morphological characteristics (shape, cell wall thickness, surface ornaments), and spore reactions to Melzer. *Glomus* was found in all observation sites, while *Acaulospora* and *Gigaspora* were found in only a few locations. *Glomus* sp. is dominant on peatlands (Ervayenri 1998).

The types of AMF spores in each genus found in the 4 types of peat soil utilization in Sungai Asam Village can be seen in Table 3. It can be seen that

Table 1. AMF spore density before and after Trapping.

Type of peat land utilization	AMF Spore density per 50 g of soil	
	Before Trapping	After Trapping
Cassava	52	118
Corn	53	178
Taro	41	64
Ginger	38	61
Pineapple	47	122
Kale	36	68

Table 2. Spore diversity in 6 locations of peat land utilization in Sungai Asam Village.

Soil sample of peat land utilization	FMA Diversity Value
Cassava	3 (<i>Glomus</i> , <i>Gigaspora</i> , <i>Acaulospora</i>)
Corn	3 (<i>Glomus</i> , <i>Gigaspora</i> , <i>Acaulospora</i>)
Taro	2 (<i>Glomus</i> , <i>Acaulospora</i>)
Ginger	2 (<i>Glomus</i> , <i>Gigaspora</i>)
Pineapple	3 (<i>Glomus</i> , <i>Gigaspora</i> , <i>Acaulospora</i>)
Kale	1 (<i>Glomus</i>)

Table 3. Number of AMF Spore Types in each of the 4 genus Types of Peat land Utilization in Sungai Asam Village.

Plant Type	Spore type					
	Glomus		Acaulospora		Gigaspora	
	Field	Trapping	Field	Trapping	Field	Trapping
Cassava	5	9	2	4	1	3
Corn	7	13	3	5	1	2
Taro	4	5	2	2	-	-
Ginger	5	7	-	-	1	1
Pineapple	6	10	2	5	1	2
Kale	4	11	-	-	-	-

the genus *Glomus* spores always dominate the diversity of spores before and after trapping cultures. According to Widiastuti and Kramadibrata (2015), the genus *Glomus* spores are the most dominant type of spore found in several ecosystem conditions because this type of AMF has a broad host range. The most dominant type of spore is from the genus *Glomus* as many as 13 types of spores, *Acaulospora* respectively in 5 types of spores, and *Gigaspora* as many as 3 types of spores. Spore density, diversity, and AMF infection are negatively correlated with soil pH (Lee et al. 2012).

CONCLUSIONS

The AMF diversity of the 6 types of peatland utilization from the rhizosphere of cassava, corn, taro, ginger, pineapple, and kale in the Sungai Asam village before and after trapping showed an increase in the number of spores, diversity of AMF, while the percentage values of root colonization are varied.

The results of observations on the number of spores showed an increase from around 36 - 52 per 50 g of soil to 61 - 178 spores per 50 g of soil. The diversity of spore types is from 10 types of *Glomus*, 1 type of *Gigaspora*, and 4 types of *Acaulospora* to 13 types of *Glomus*, 3 types of *Gigaspora*, and 5 types of *Acaulospora*. The highest percentage of infected roots before trapping ranged from 48.89% - 78.48% and after trapping from 78.89% - 94.80%.

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