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Population and Diversity of Arbuscular Mycorrhiza Fungi in the Rhizosphere of Kasetsart Cassava Clone Grown on Two Different Locations

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ABSTRACT

Population and diversity of arbuscular mycorrhiza fungi (AMF) are varied in the soil and influenced by biotic factors such as host plant and abiotic factors such as soil fertility, soil moisture, pH, temperature, *etc.* This study aimed to determine the population, diversity, and the dominant type of AMF in the rhizosphere of Kasetsart cassava clones obtained from LampungTimur and Tulang Bawang Barat Regencies, Lampung Province, Indonesia. Population of AMF was counted directly from the rhizosphere of Kasetsart cassava clones and the diversity of AMF was assesed using a pot culture experiment. The results showed that the population and the diversity of AMF in the rhizosphere of Kasetsart clone obtained from Tulang Bawang Barat was higher than that from LampungTimur. The predominant type of AMF found in the pot culture using soil samples from Lampung Timur was spore with S2 code that belongs to the genus Gigaspora and S4 code that belongs to the genus Glomus. On the other hand, the type of AMF found in the rhizosphere of soil samples from Tulang Bawang Barat was dominated by spore with S9 code that belongs to the genus Entrophospora.

Keywords: Arbuscular mycorrhiza fungi, cassava, diversity, population

ABSTRAK

Populasi dan keragaman fungi mikoriza arbuskular (FMA) sangat beragam di dalam tanah karena dipengaruhi oleh faktor biotik seperti jenis tanaman inang dan abiotik seperti kesuburan tanah, kelembaban, pH, suhu, dll. Penelitian ini bertujuan untuk mengetahui populasi, keragaman, dan jenis FMA yang dominan pada rizosfir ubi kayu Klon Kasetsart yang ditanam di Kabupaten Lampung Timur dan Tulang Bawang Barat. Populasi FMA dihitung langsung dari sampel tanah rizosfir ubi kayu Klon Kasetsart, sedangkan penentuan jenis-jenis FMA pada sampel tanah dilakukan melalui kultur pot. Hasil penelitian menunjukkan bahwa populasi dan keragaman FMA pada sampel tanah dari rizosfir ubi kayu Klon Kasetsart di Kabupaten Tulang Bawang Barat lebih tinggi dibandingkan dengan sampel tanah rizosfir ubi kayu Klon Kasetsart dari Kabupaten Lampung Timur. Jenis FMA yang dominan dari hasil kultur pot dengan sampel tanah Kabupaten Lampung Timur yaitu spora dengan kode S2 yang termasuk ke dalam genus Gigaspora dan kode S4 yang termasuk ke dalam genus Glomus. Sedangkan pada Kabupaten Tulang Bawang Barat didominasi oleh jenis spora dengan kode S9 yang termasuk ke dalam genus Entrophospora.

Kata kunci: Fungi mikoriza arbuskular, keragaman, populasi, ubi kayu

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is one of the most cultivated tropical food crops in Indonesia. Types of cassava grown in Lampung Province, Indonesia comprise of Clone UJ-3 (Thailand), Clone UJ-5 (Kasetsart), and local clones, such as Barokah, Manado, Klenteng, *etc*. (Direktorat Jenderal Tanaman Pangan 2012).

Agricultural management practices applied on cassava cultivation such as soil tillage, use of chemical fertilizers, and utilization of pesticides can affect soil fertility. Conventional soil tillage and harvesting has lead to the decrease in total nutrient and organic matter content in soil. This condition will affect the population and diversity of Arbuscular mycorrhizal fungi (AMF) (Opik *et al*. 2006).

Arbuscular mycorrhizal fungi (AMF) belong to endomycorrhiza that is in a mutualistic relationship with plant roots (Smith dan Read 2008). In all ecosystems, mychorrhiza plays a role in improving water and nutrient absorption of its host plant (Smith and Smith 2011; Smith *et al*. 2011). The studies of Harrison *et al.* (2010) and Nicolas *et al.* (2015) showed that AMF absorb more phosphorus to be fed to their host plants compared to other nutrients. The Gale Encyclopedia of Science (2008) showed that there are numerous evidences explaining the importance of mycorrhiza for plant nutrition, especially in nutrient-poor soil. In addition, AMF are also capable of affecting the surrounding rooting environment dynamically, called the mycorrhizosphere, an environment-conditioned under the topsoil due to the presence of the mycorrhizal fungi (Audet 2012; Churchland and Graystone 2014).

Arbuscular mycorrhizal fungi can be found almost in all types of ecosystem, including in acid and alkaline soils (Hong *et al*. 2012). However, the population level and composition of the AMF types vary considerably. The population of AMF can be affected by biotic factors such as plant species and abiotic factors such as temperature, soil pH, soil moisture, organic matter content, and concentrations of phosphorus, nitrogen and heavy metal (Brundrett and Ashwath 2013; Bedini *et al*. 2013; Omorusi and Ayanru 2011; Lekberg *et al*. 2008).

The regencies of Lampung Timur and Tulang Bawang Barat are the centres of cassava production in Lampung Province with different abiotic conditions. Accordingly, the differences of abiotic factors in both regions will affect the population and diversity of AMF in cassava plantation. In fact, cassava has been planted in Lampung Timur for ± 8 years or since 2008 in a monoculture system. The planting distance of cassava Kasetsart clone is approximately 60 cm × 70 cm. Soil tillage was conducted once before planting, and organic fertilizer (*i.e.* 20 Mg ha^{-1} cattle manure) was applied once a year.

In Tulang Bawang Barat, cassava has been planted for ± 6 years or since 2010 in a monoculture system. However, in this regency cassava and chili are planted alternately as part of crop rotation. The planting distance of cassava Kasetsart clone is 60 cm × 70 cm. Soil tillage was conducted once before planting and NPK fertilizer with a dose of 400 kg ha^{-1} was applied once during planting season. In the following year when the land was planted with chili, NPK fertilizer was applied once a week with a dose of 1 kg for 200 plants. To control the pests, an insecticide of Diafentiuron 500 g L^{-1} at a dose of 7 ml per 15 L was sprayed on the plantation. A fungicide with an active ingredient of Propineb in a concentration of 3 g L^{-1} was also applied weekly to control the pathogenic fungi.

This research aimed to determine population, diversity, and dominant species of AMF in the rhizosphere of Kasetsart clone grown in the Regencies of Lampung Timur and Tulang Bawang Barat.

MATERIALS AND METHODS

Study Sites

The study comprised of two experiments.The first experiment was conducted to determine the population of AMF in soil samples taken from the rhizosphere of Kasetsart cassava plantations in Lampung Timur and Tulang Bawang Barat Regencies, Lampung Province, Indonesia. The population of AMF obtained was tested using t-test. The second experiment was performed to explore the diversity of AMF in the soil samples using pot culture. These two experiments were carried out in April until October 2016.

Soil Sampling

Soil samples were taken from the rhizosphere of Kasetsart cassava clone grown in two different regions, *i.e.* Lampung Timur (at Braja Yekti Village, Braja Selebah Sub-District, 05°08'52.25"S, 105°44'02.64"E) and Tulang Bawang Barat (at Gunung Menanti Village, Tumijajar Sub-District, $04^0$44'57.18"S, 105°09'42.84"E) Regencies. In each regency, one plantation owned by a smallholder was chosen. For each plantation, 7 sampling points were determined. Each sampling point consisted of 12 cassava plants (taken from 3 rows and 4 plants per row). Soil samples were taken from 0-20 cm topsoil around each cassava plant sample (taken from the left and right side of the plant at ± 15 cm from the trunk). Then the soil samples taken from the 12 spots were mixed homogeneously to represent single sampling point. The obtained soil samples were used to determine the AMF population and to run the pot culture experiment.

Calculation of AMF Population

Total soil samples obtained were 14 samples in which 7 samples were derived from Lampung Timur Regency and 7 samples were collected from Tulang Bawang Barat Regency. The total population of AMF was counted using wet sieving method (Brundrett*et al.* 1996) using a 500 µm, 250 µm, 150 µm, 45 µm micro-sieve. About 50 g of soil sample

was weighed in which 3 replications were used for calculating the AMF population in each sample. The filtered spores were observed under a stereo microscope and counted manually.

Pot Culture Experiment

A pot culture experiment was performed to obtain healthy AMF spore for identification process. The host plants used in the culture were corn, sorghum, and *Pueraria javanica*. The seeds were sterilized by soaking them in a 5% chlorine solution for 30 minutes, then rinsed with distilled water. The seeds were then sprouted on the moistened filter paper and subsequently stored in room temperature for 3 days.

The medium used to grow the host plants (corn, sorghum and *Pueraria javanica*) was a mixture of sterilized sand and zeolite with a ratio of 1:1 (v/v). About 600 g of this medium was placed into a polybag. The soil samples from the field (7 soil samples from 1 regency) were composited into 1 homogenous sample. A total of ± 300 g of soil samples was placed into the polybag above a mixture of sand and zeolite medium. Two germinated seeds of host plants were planted on the soil samples and covered back with a mixture of sand and zeolite medium and kept for 3 months (corn and sorghum) and 4 months (*Pueraria javanica*) in the green house. There were 7 replications for each host plant, hence the total number of polybags used were 21 for the soil sample from Lampung Timur Regency and 21 polybags for the soil sample from Tulang Bawang Barat Regency.

For plant nurture, urea with the concentration of 2 g per litre was applied as much as 20 ml per polybag at 2 and 4 weeks after planting. NPK fertilizer with a dose of 0.3 gram per polybag was applied when the plants were 1 month old. In addition, the plants were watered daily in the morning.

Harvesting

The procedure of harvesting was remarked by stopping the watering activities for 2 weeks until the growing medium was completely dry to stimulate the formation of spores. Moreover, the stems of corn, sorghum, and *Pueraria javanica* crops were removed ± 1 cm from the surface of the growing medium. Subsequently, the polybag was cut to separate the upper part of the growing medium (soil samples from the field) and the lower part of the growing medium (a mixture of sand and zeolite). Furthermore, the bottom part of the growing medium (a mixture of sand and zeolite) was observed to calculate AMF population and diversity. This section was observed to ensure that the spores produced are new spores, not spores derived from the field. Isolation of spores from the medium was done using wet sieving method (Brundrett *et al*. 1996).

The AMF identification was done based on the characteristics of spores such as colour, shape, presence or absence of spore ornaments (bulbous, saccule, germination shield, and auxiliary cells), and spore reactions to the Melzer's solution (INVAM 2008) with the help of a stereo microscope up to 45× magnification. Accordingly, the diversity index is calculated using the Shannon-Wiener formula as follows:

$$H = -\sum(pi)\ \text{In pi} \quad \text{where} \quad pi = \frac{ni}{N}$$

, where

Note:
H: Shannon-Wiener Diversity Index
Pi: The number of individuals of a species/total number of species
Ni: Number of individual species i
N: Total number of individuals

RESULTS AND DISCUSSIONS

Number of spores in the soil samples taken from the field showed that the AMF population in the rhizosphere of Kasetsart cassava clone planted in Tulang Bawang Barat Regency was higher than that in the rhizosphere of Kasetsart cassava clone obtained from Lampung Timur Regency. The result of t-test analysis showed that the *p* value is ≤ 0.01, which means there is a difference on both AMF populations at the significance level of 1% (Table 1).

The number of AMF spores could be related to the organic matter content in the soils. The maximum number of spores can be found in soils containing organic matter 1% up to 2%, whereas in

Table 1. Results of t-test for AMF population on the rhizosphere of Kasetsart cassave clone from Lampung Timur and Tulang Bawang Barat Regencies.

Location	AMF population Per 50 g soil	*P value*
Lampung Timur	435.29	0.000
Tulang Bawang Barat	812.86	0.000

Table 2. Chemical and physical properties of soil samples taken from Lampung Timur and Tulang Bawang Barat Regencies.

Location*	Parameters									
	pH (H_2O)	Available-P (ppm)	Exchangeable-K (me $100g^{-1}$)	Total-N (%)	Fe (ppm)	Organic-C (%)	Al-dd (me $100g^{-1}$)	Fraction (%) Sand	Silt	Clay
LT	4.10	10.40	0.07	0.06	59.5	0.78	0.60	54.53	11.01	34.45
TBB	4.06	5.87	0.11	0.08	51.74	1.42	0.85	38.63	13.28	48.09

*LT: Lampung Timur Regency; TBB: Tulang Bawang Barat Regency

soils containing organic matter <0.5% the number of spores is very low (Anas 1997). The studies of Oehl *et al.* (2005) and Bedini *et al.* (2013) also reported that the highest number of AMF spores was obtained from soil with high organic matter content. The results of their studies are in line with the results of current study. The soil samples from Tulang Bawang Barat contain organic-C >1%, whereas the soil samples from Lampung Timur contain organic-C <1% (Table 2). This phenomenon is expected to be one of the factors causes the average number of AMF spores in the soil sample from Tulang Bawang Barat (812.86 spores per 50 g of soil) is higher than that in the soil sample from Lampung Timur (435.29 spores per 50 g soil).

Another factor that can affect AMF population is soil phosphorus. The results showed that the amount of available P in the soil samples from Tulang Bawang Barat (5.87 ppm) was lower than that in the soil samples from Lampung Timur (10.40 ppm) (Table 2). It can be suggested that this factor also contributes to the higher AMF spore population in the soil from Tulang Bawang Barat than that in the soil from Lampung Timur. The study of Suhardi (1989) reported that the high level of soil phosphorus may diminish AMF root colonization and hence spore production.

Based on the results of pot culture experiment, it signified that the type of AMF in the soil samples from Tulang Bawang Barat was more varied (8-11 types) than that in the soil samples from Lampung Timur (7-8 types). The number of AMF types derived from the *Pueraria javanica* host plant were higher than that derived from the other host plants for both soil samples from Tulang Bawang Barat and Lampung Timur (Table 3). The result indicates that the AMF derived from the rhizosphere of Kasetsart clone from the two regencies is more compatible to associate with the *Pueraria javanica* host plant. In addition, the *Pueraria javanica* host plant was harvested at the age of 4 months, which was much longer than the corn and sorghum host plants that were harvested at 3 months after planting. The study of Oehl *et al.* (2005) indicated that the longer period of the pot culture experiment is carried out, some AMF species will slowly sporulate. Furthermore, the high diversity of AMF found in the soil samples from Tulang Bawang Barat can also be attributed to the fact that the host plants in this location are more diverse because of the rotation

Table 3. Number and type of AMF obtained from the pot culture experiment using soil samples from Lampung Timur and Tulang Bawang Barat, and corn, sorghum and *Pueraria javanica* as host plants.

Location	Host plants	Spore 50 g^{-1} media	Number of AMF Type	Type of AMF
Lampung Timur	Corn	567.4	7	S1.S2.S3.S4.S6.S7.S8
	Sorghum	474.0	8	S1.S2.S3.S4.S5.S6.S7.S8
	P.javanica	415.1	8	S1.S2.S3.S4.S5.S6.S7.S8
Tulang Bawang Barat	Corn	400.9	8	S1.S2.S3.S4.S6.S7.S8.S9
	Sorghum	405.3	8	S1.S2.S4.S5.S6.S7.S8.S9
	P.javanica	515.0	11	S1.S2.S3.S4.S5.S6.S7.S8.S9.S10.S11

In which :
S1: *Gigaspora* sp-1 S2: *Gigaspora* sp-2 S3: *Gigaspora* sp-3
S4: *Glomus* sp-1 S5: *Glomus* sp-2 S6: *Glomus* sp-3
S7: *Glomus* sp-4 S8: *Scutellospora* S9: *Entrophospora* sp-1
S10: *Entrophospora* sp-2 S11: *Entrophospora* sp-3

of cassava plants with the chilli plants. Meanwhile, there is no rotation of cassava plants with other crops in Lampung Timur. The study of Rosendahl (2008) showed that the types of plants that exist in an ecosystem will affect the type and population of AMF. The studies of Higo *et al.* (2013) and Jansa *et al.* (2003) also indicated that the crop rotation has the potential contribution to the high AMF diversity.

Based on the calculation of Shannon-Wiener diversity indexes, generally AMF spores obtained from the pot culture experiment with different host plants have a moderate index value (1 < H <3) (Table 4), indicating the medium diversity. It can be concluded that the population of each type of spore is not that much different, and the population of each spore is distributed almost evenly.

The result of spore identification showed that the predominant AMF in the soil samples from Lampung Timur are the S2 code that belongs to genus Gigaspora (*Pueraria javanica* as the host plant) and the S4 code that belongs to genus Glomus (corn and sorghum as the host plants) (Table 5). The higher proportion of sand in the soil from Lampung Timur (Table 3) is suitable for the development of Gigaspora species. The study of Baon (1998) reported that sandy soils form larger soil pores so that this condition is allegedly appropriate for the development of the genus Gigaspora in high numbers. The high number of Gigaspora spore in the pot culture experiment using soil samples from

Table 4. Shannon-Wiener AMF diversity indexes of spores from pot culture experiment using corn, sorghum and *Pueraria javanica* as host plants grown on soil samples from Lampung Timur and Tulang Bawang Barat.

Host plants	Shannon-Wiener AMF Diversity Index	
	Lampung Timur	Tulang Bawang Barat
Corn	1.343	1.548
Sorghum	0.928	1.079
P. javanica	1.456	1.360

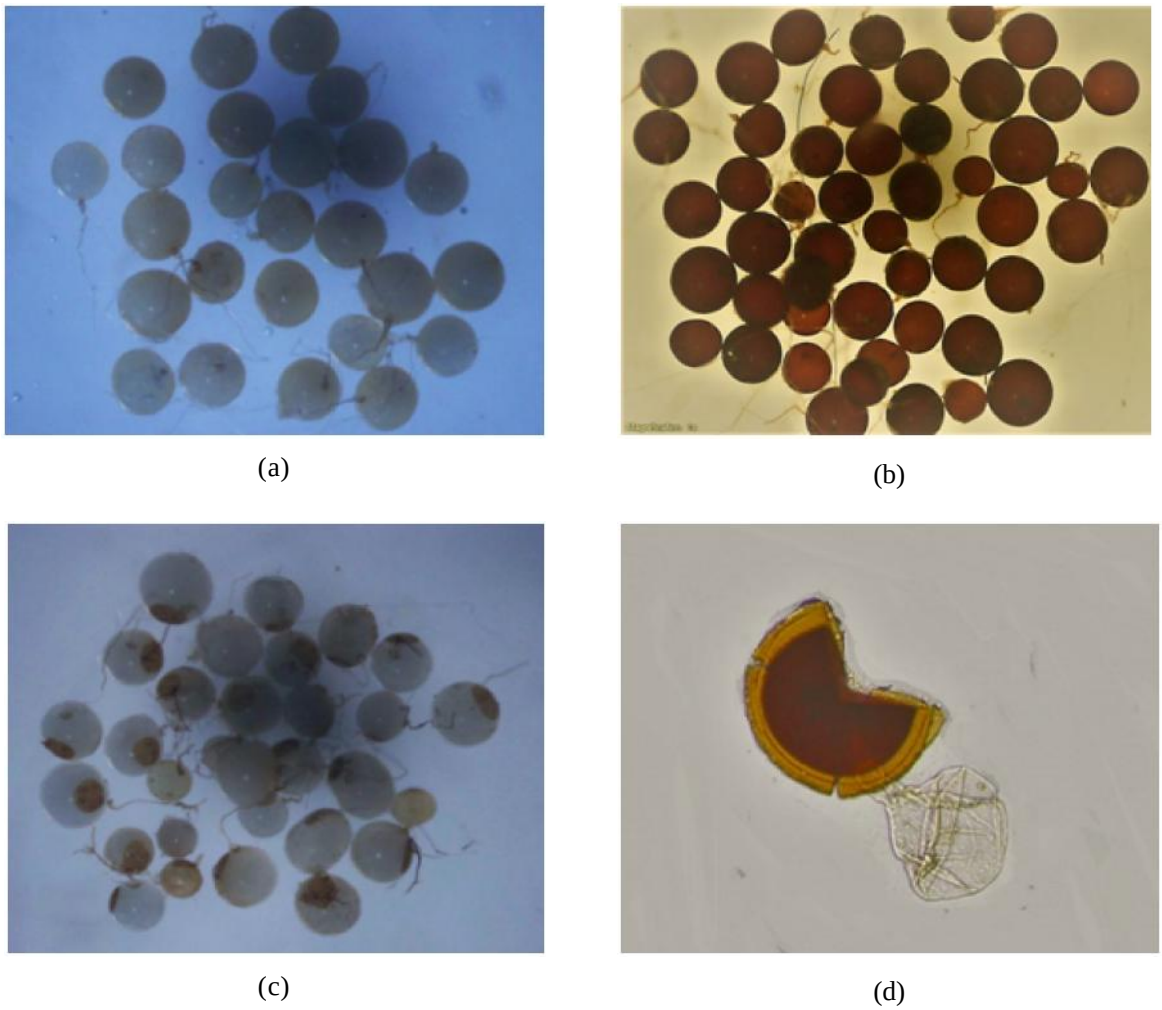
In which: Low diversity: H<1, high diversity: H>3, and medium diversity: 1<H<3

Table 5. Number of spores of each type of AMF obtained from pot culture experiment using corn, sorghum and *Pueraria javanica* as host plants grown on soil samples from Lampung Timur and Tulang Bawang Barat.

Spore type	Amount of spore per 350 g media					
	Lampung Timur			Tulang Bawang Barat		
	Corn	Sorghum	*P.javanica*	Corn	Sorghum	*P.javanica*
S1	780	25	637	347	30	118
S2	706	186	1,152	44	26	144
S3	95	25	7	59	0	12
S4	2,030	2,479	516	679	438	343
S5	0	213	4	0	40	6
S6	270	346	497	692	377	279
S7	29	7	79	49	67	48
S8	62	37	14	9	2	10
S9	0	0	0	927	1,857	2,226
S10	0	0	0	0	0	361
S11	0	0	0	0	0	58
Total	3,972	3,318	2,906	2,806	2,837	3,605

In which :

S1: *Gigaspora* sp-1 S2: *Gigaspora* sp-2 S3: *Gigaspora* sp-3
S4: *Glomus* sp-1 S5: *Glomus* sp-2 S6: *Glomus* sp-3
S7: *Glomus* sp-4 S8: *Scutellospora* S9: *Entrophospora* sp-1
S10: *Entrophospora* sp-2 S11: *Entrophospora* sp-3



(a) (b)

(c) (d)

Figure 1. Some of AMF spores isolated from pot culture experiment: a) AMF type S2 (*Gigaspora* sp.2); (b) AMF type S3 (*Gigaspora* sp.3); (c) AMF type S8 (*Scutellospora* sp.) and (d) AMF type S9 (*Entrophospora* sp.1) in the Melzer's solution.

Lampung Timur also indicates that the number of spore of this genus is naturally high in the soil samples from Lampung Timur, and the Gigaspora genus is compatible with the host plant of *Pueraria javanica* used in the pot culture experiment.

Spore of S4 code that belongs to the genus Glomus was found predominantly in the soil samples from Lampung Timur with corn and sorghum as host plants. The finding is likely happened due to the fact that Glomus has a large number of fungal species that belong to this genus and this genus also has a wide range of ecosystem adaptation (Smith and Read 2008).

The predominant AMF type observed in the soil samples from Tulang Bawang Barat was the S9 code that belongs to Entrophospora genus. This type of AMF was only found in this Regency. It can be suggested that the more host plant present in Tulang Bawang Barat other than cassava (chili as an alternative host plant) has lead to the more type of AMF present in the soil. The biotic factor (*i.e.* the host plant) is confirmed affecting the population and the diversity of AMF observed in the current study. Some of AMF spores isolated in this study are presented in Figure 1.

CONCLUSIONS

The population of AMF in the rhizosphere of Kasetsart cassava clone obtained from Tulang Bawang Barat Regency is higher than that from Lampung Timur Regency. Based on the Shannon-Wiener Diversity Index, the variety of AMF in the cassava rhizosphere of Kasetsart clone from Tulang Bawang and Lampung Timur are classified as moderate with the value of diversity index of $1<H<3$. Spores with the code of S2 belonging to genus Gigaspora and S4 code belonging to genus Glomus

are the predominant types of AMF found in the soil samples obtained from Lampung Timur Regency. On the other hand, the spore with S9 code belonging to genus Entrophospora is the predominant type of AMF found in the soil samples obtained from Tulang Bawang Barat Regency.

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