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Isolation and Identification of Vesicular Arbuscular Mycorrhizae (VAM) in the Rhizosphere of Maize (*Zea mays*) in the Village of Lekopacing, Tanralili District of the Maros Regency

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Abstract: ***Vesicular Arbuscular Mycorrhizae (VAM) is suitable to increase the potential of dry land and crop production. This study aims to determine the types of VMA in the rhizosphere of maize plants in the village of Lekopacing, Tanralili district and as an initial stage in the utilization of VAM as a biological fertilizer. The method used is descriptive-exploratory method by taking soil samples at random in the maize rhizosphere. Soil samples obtained were filtered using wet screening techniques. Identification carried out up to genus level and performed based on morphological characteristics including shape, color and ornament. From these results it can be concluded that the highest population of VAM spores in the maize rhizosphere in the Lekopacing village, Tanralili district of the Maros regency was 89 VAM / 100 g soil. Three mycorrhizal genus were found, namely Glomus, Gigaspora and Acaulospora. Glomus genus was found more than the genus Gigaspora and Acaulospora.***

Keywords: Vesicular Arbuscular Mycorrhizae (VAM), dryland, population, genus.

1. Introduction

In Indonesia, maize is planted in various agro ecological including dry land/upland, irrigated paddy, land with non technical irrigation or rainfed which are generally in suboptimal conditions. Maize cultivation in dry land often faces the problem of soil acidity, low soil fertility and drought (Miti *et al*., 2010). This low productivity is mainly due to the drought, acidity, and low soil fertility in addition to low input given (Sutoro, 2012). Khan *et al.* (2004) stated that maize is a cereal crops grown throughout the world, which is sensitive to drought.

The village of Lekopacing, Tanralili district, Regency of Maros is dominated by acid and non acid dry land largely is Red Yellow Podzolic soil classified in ultisol. The level of soil acidity (pH) is 4.5 to 7.0. Acid dry soil attributed to properties that unfavorable for plants due to high acidity level and Al content. Soil acidity and high aluminum content can damage plant roots causing inhibited ability of water and nutrient absorption, low macro and m icro nutrients content li ke N, P, K, Ca, Mg, and Mo, which causes stunted plant growth and death (Ma *et al.,* 2001; Sutjahjo, 2006).

Dry land can be defined as an expanse of land that never been flooded or have stagnant water on most of the time in a year. Acidic dry land is land with properties of low pH, cation exchange capacity (CEC), base saturation (BS) and C-organic, high content of aluminum (Al saturation), high P fixation, the content of iron and manganese approached the toxic level for the plant, susceptible to erosion, and poor biotic elements (Soepardi, 2001). In addition, soils formed generally have deep cross section, red-yellow in colors and have low natural fertility (Mulyani, 2009).

Indigenous mycorrhizal fungi have high potential to form extensive infection due to ability to recognize its host plant and more tolerant to unfavorable environmental conditions such as high stress (Delvian, 2006). Inoculum development which is based on the function of the Arbuscular Mycorrhizal Fungi (AMF) has shown adaptability in the context of a local AMF species population. In fact, local species showed better growth on their own conditions compared with introduced species (Hernadi *et al*., 2012).

The role of the Vesicular Arbuscular Mycorrhizae (VAM) indirectly increases the resistance to extreme moisture. External hyphae tissue of VAM will produce an intensive branching web of hyphae thus extending the field of water and nutrient uptake, additionally, the size of hyphae that finer than root hairs allow hyphae to penetrate into the pores of the soil are the smallest, so that the hyphae can absorb water when soil water conditions is very scarce (Auge, 2001). VAM can increases plant growth by increasing uptake of plant nutrients through the expansion of the surface area of absorption, protecting plant roots from pathogens and improve the resistance of plants to drought stress (Smith and Read, 2008), improve soil structure, enhance water and nutrients absorption (Smith *et al*., 2010), increases fertilization use efficiency (Douds *et al*., 2010). VAM has four functional roles (Nusantara *et al*., 2012) as follows: bio processor, bio protector, bio activator, and bio aggregator.

Thus the vesicular arbuscular mycorrhizal (VAM) fungi is suitable to increase the potential of dry land and crop production. This study aims to determine the types of VAM in the rhizosphere of corn plants in the village Lekopacing, district of Tanralili and as an initial stage in the utilization of VAM as a biological fertilizer. From the study it is expected to obtain indigenous VAM isolates in maize plant rhizosphere in the village of Lekopacing, thus a further

research can be conducted to explore the possibility of the VAM to be used as biological fertilizer.

2. Research Methodology

Time and Research Location

The research was conducted in the laboratory of the Forestry Research Institute of Makassar, and Soil Chemistry Laboratory of Agricultural Science Faculty, University of Hasanuddin in March-July 2015. Soil sampling location is in the rhizosphere of maize planted on dryland in Lekopacing village, district of Tanralili, Maros regency.

Materials and Tools of Research

Materials used in this study were soil samples from maize cultivation in the district of Tanralili, Maros Regency, indigenous mycorrhizal isolates, PVLG (polyvinyl alcohol-lacto-glycerol), Melzer, sugar, tissue paper, paper labels, clear plastic bags vol. 1 kg.

While the tools used were a hand shovel, analytical scales, scales, a set of soil sieves with the size of 200 µm, 50 µm, and 40 µm, stirring rod, scissors, petri dish (diameter of 5 cm, 8 cm, and 10 cm), beaker glass, volumetric flask, centrifuge tubes, spray flasks, object glass, cover slips, micro pipettes, scalpel, spore tweezers, semi-automatic calculation tools, centrifuges (Hettic Universal II, max 5000 rpm, 30 min), autoclave, a dissecting microscope (Olympus SZ -51, magnification: 0.8-4), compound microscope (Olympus CX-31, magnification of 40x - 1000x), Nikon Elipse 80i microscope with camera (magnification 40x-1000x).

Mycorrhizal Soil Sampling

The method used in this stage is a descriptive-exploratory method by taking soil samples in the mycorrhizal maize rhizosphere randomly in 3 different locations of dry land in Maros regency. Soil sampling conducted in the root zone of plants, with a distance of 20 cm radius from the plant to a depth of 10-25 cm of maize rhizosphere from the soil surface because the mycorrhizal spores are found in the top soil. The method of sampling was done randomly using stratified random sampling. 2 cm of root tip of the plants (actively growing) were taken because the mycorrhizal fungi generally infect the young roots only. A total of 1 kg of soil sample and the plant roots hence dried, then packaged in a clear plastic bag and labeled for the isolation and identification in the microbiology laboratories of Forestry Research Institute of Makassar.

Isolation and Identification of Mycorrhizal Spores

Isolation of mycorrhizal spores was carried out by wet screening methods and methods of sucrose gradient centrifugation (Walker *et al*., 1982). The wet screening technique was conducted by weighing 100 g soil samples and the dissolved in 1000 ml of water then left for 10-15 minutes to allow the sediment to settle. The suspension then filtered by pouring it into filter with diameter of 40 µm, 50 µm, and 200 µm, respectively, repeated 3 times. Spores were filtered through a sieve of 50 µm and 200 µm then inserted into the centrifuge tube. A 2500 rpm speed was used to centrifuge the suspension for 5 minutes. Supernatants were collected on the top in the exhaust up to three-quarters of the tube. The remaining solution in the tube was mixed with 60% sucrose solution then centrifuged at 1200 rpm for 2 minutes. The suspension is poured into 10 cm diameter petri dishes.

Further, the suspension between the water and the sugar was taken and placed on a 200 µm sieve, sieve sprayed with water slowly to clean up spores from the remnants of attached sugar. Clean spores were placed on a petri dish diameter of 10 cm to be observed and counted under a dissecting microscope. Subsequently, population of each of mycorrhizal fungi types was counted for 100 g soil then separated on a petri dish based on shape, color and size. Based on these observations, type, population, form and spore color of the mycorrhizae were matched with standard spores (Schenck and Perez, 1990; Brundet, 1996).

Mounting procedure was conducted as follow left hand side of a glass object was dropped into a solution of PVLG and a portion of Melzer solution was dropped onto the right hand side. Similar spores were placed on each of the solution then each surface was covered with a cover slip. Spores were crushed by pressing the cover slip surface with a toothpick (Nusantara, 2012). Mixtures of mycorrhizal spores were observed under compound microscope equipped with digital camera with enlargement of 100-400 times. The identification based on morphological characteristics of spores that is based on size, color, cell walls layer, ornaments, and hyphae form attached to the spores cell walls (bulbous suspensor, hyphae holder, or subtending hyphae) (Brundet, 1996; Nusantara, 2012; INVAM, 2015). Color change of spores in Melzer solution is one of the indicators to determine the type of spore (INVAM, 2015).

3. Result and Discussion

3.1 Result

VAM Fungi Spore Population

Calculation of the spores number of VAM in this recent study show that the highest number of spores found in the rhizosphere of maize was in the location of Tanralili L3 with 89 spores VAM/100 g soil followed by location of Tanralili L2 and Tanralili L1 (Table 1). Among the types of fungi found, in total, VAM genus *Glomus* spores were found more than genus *Gigaspora* and then followed genus *Acaulospora* (Table 2).

Table 1: Population of VAM Spores VAM (100 g soil) in the rhizosphere of Maize

Location	*Number of Population of VAM Spores per 100 gr soil*				*Total*
	1	2	3	4	
Tanralili L1	8	15	11	15	49
Tanralili L2	17	13	12	14	56
Tanralili L3	25	28	16	20	89

Table 2: Population of spores (/ 100 g soil) for each genus in the rhizosphere of Maize

VAM Type	*Population of Spores per 100 g soil*			*Total*
	Tanralili L1	*Tanralili L2*	*Tanralili L3*	
Glomus sp1.	9	13	24	46
Glomus sp2.	10	13	22	45
Glomus sp3.	11	9	8	28
Acaulospora sp.	7	7	16	30
Gigaspora sp1.	5	3	4	12
Gigaspora sp2.	4	7	9	20
Gigaspora sp3.	3	4	6	13
Total	49	56	89	

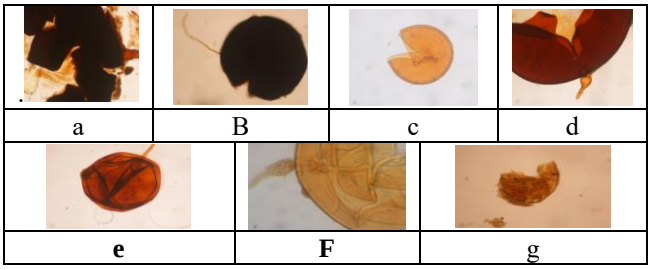
Isolation and Identification of Micorrhiza

Isolation and identification of the types of mycorrhiza in the rhizosphere of Maize was conducted at three locations in the Tanralili district of Maros Regency. In this study mycorrhizal fungi were classified based on fungi morphotype for later identification. Types of mycorrhizae that live symbiotic with maize in the district of Tanralili identified based on VAM genus. The Genus of VAM spores were determined by microscopc observation on the extraction of VAM spores from the soil. Three genus were identified from the VAM spores found at the study location i.e. *Gigaspora, Glomus* and *Acaulospora*.

The VAM spores type obtained has different characteristics of spores shapes and colors. Spores were then grouped by shape and color therefore in the District of Tanralili there are three types of genus found, namely VMA spores from genus *Glomus, Gigaspora, and Acaulospora.* Three types of spores were identified for *Glomus* type (Fig 1 a, b, c) and *Gigaspora* type (Fig 1d, e, f), respectively while only one type of *Acaulospora* spore was obtained (Figure 1g).

Each genus spores found at the site of research has specific characteristics (Table 3), the type of spores of genus *Glomus* show subtending hyphae and did not react with Melzer, the type of spores of genus *Gigaspora* show bulbous suspensor at the base of its hyphae and reacted with Melzer thoroughly while the type of spore genus *Acaulospora* have ornaments and reacted with Melzer.

Three genus of VAM were identified in the district of Tanralili namely *Glomus*, *Gigaspora* dan *Acaulospora*. Based on the number of spores of VAM it was found that genus *Glomus* show the most abundant followed *Gigaspora* and *Acaulospora*, respectively.



Note: (a, c, d, e, h) objective lens magnification of 20x; (b, f, g) objective lens magnification of 40x

Figure 1: Spore of Mycorrhizal Genus isolated from the rhizosphere of Maize *Glomus* (a, b, c), *Gigaspora* (d, e, f), and *Acaulosspora* (g).

3.2 Discussion

Spores Population of VAM Fungi

The presence of spores around rhizosphere of Maize in the village of Lekopacing proves that the plant is associated with vesicular arbuscular mycorrhizal fungi (VAM). The highest number of VAM spores was found in the location of Tanralili L3 (89 spores VAM/100 g soil) followed by Tanralili L2 and L1 (Table 1). According to Burhanuddin, (2014) the density of VAM spores found in each soil sample at each stage of growth under Jabon (*Anthocephalus* spp.) stands at the pole level was 3 - 77 with a mean of 42 spores and spores density of 4 - 52 with a mean of 26 spores at the tree level grown on types Ultisol/Red Yellow Podzolic (RYP). Puspitasari *et al*. (2012) stated that the abundance of VAM spores as much as 712 spores per 500 g soil was found in the soil sample collected from the rhizosphere of Maize in the Torjun village of Sampang district, Madura with sandy clay loam soil structure and at the lowest organic C content, N-total, P and CEC. This high population of VAM spores might be related to more favorable optimum environmental conditions in supporting the growth and development of VAM spores at the rhizosphere zona in the specific site as well as the possibility of the absence of antagonistic fungal that can inhibit the VAM spores formation compared to other location in the village. According to Shi *et al*. (2007), VAM is more likely to form spores when the condition prevails or host vegetation is stressed or disturbed.

Table 3: Characteristics of VAM types spores found in the rhizosphere of Maize (*Zea mays*)

Type of Vesicular Arbuscular Mycorrhiza (VAM)	*Characteristics of VAM types spores in in the Tanralili district*					
	Shape	*Color*	*Spores surface Texture*	*Special Characteristic*	*Reaction with Melzer*	
Glomus sp 1 (a)	Large round	Brownish Black	Rough	subtending hyphae	None	
Glomus sp 2 (b)	Small round	Black	Smooth	subtending hyphae	None	
Glomus sp 3 (c)	Large round	Dark yellow	Smooth	subtending hyphae	None	
Gigaspora sp1 (d)	Large round	Dark Brown	Smooth	bulbous suspensor	React thoroughly	
Gigaspora sp2(e)	Small round	Brown	Smooth	bulbous suspensor	React thoroughly	
Gigaspora sp3(f)	Large Round	Light yellow	Smooth	bulbous suspensor	React thoroughly	
Acaulospora sp1 (g)	Small round	Dark yellow	Smooth	Spores have ornaments	No reaction at outer layer, but react with Melzer's at inner layer	

Table 4: Soil analysis results, sample taken from Lekopacing village of Tanralili

Soil Analysis	Results
pH H_2O	4,48 – 5,45
N-Total (extract 1 : 2.5)	0,12
P2O5 (Olsen) (ppm)	6,88
Organic C (%)	1,39
CEC (cmol(+) kg^{-1}) (Ammonium Acetat extract pH 7.0)	10,65
Ca (cmol(+) kg^{-1}) (Ammonium Acetat extract pH 7.0)	0,22
Mg (cmol(+) kg^{-1}) (Ammonium Acetat extract pH 7.0)	1,52
Na (cmol(+) kg^{-1}) (Ammonium Acetat extract pH 7.0)	0,085
K (cmol(+) kg^{-1}) (Ammonium Acetat extract pH 7.0)	0,008
Al (cmol(+) kg^{-1}) (KCl extract 1N)	2,75
H (cmol(+) kg^{-1}) (KCl extract 1N)	1.25
Soil texture (Sand - Dust - Clay)	10 – 21 – 69
Texture clas	Clay

Isolation and identification of VAM spores collected from maize rhizosphere in the village of Lekopacing obtain three VAM genus of *Glomus, Gigaspora and Acaulospora*. Based on the spore numbers of each genus more spores of genus *Glomus* found compare to genus *Gigaspora* and *Acaulospora* (Table 2). Previous study also show that the VAM spore types identified in the isolation and identification conducted on soil sample taken from the rhizosphere of maize (*Zea mays* L.) in the village Torjun were *Glomus* sp, *Acaulospora* sp. and *Gigaspora* sp with round and oval shapes with different colors (Puspitasari *et al.,* 2012). Likewise, based on characteristics of the spores, Yama *et al*. (2014) found two VAM genus in the rhizosphere area of *A. crassicarpa* identified as *Glomus* and *Gigaspora*. The *Glomus* genus found consists of five species (sp1, sp2, sp3, sp4 and sp5) and for genus *Gigaspora* only one species was found. Genus *Glomus* is more common than the *Gigaspora* and more dominant on peatlands (Muin, 2006). It shows that *Glomus* has a very wide adaptation to environmental conditions suboptimal.

According to Smith and Read (2010), VAM Fungi are found in almost all natural terrestrial communities and form symbiotic associations with more than 80% of the various types of plants. The highest number of spores found was on maize plants. In the rhizosphere of maize 9 types of endomicorrhizae were found, 8 types on mustard, 7 types on tomatoes, 6 types on cabbage and 4 types on pepper (Sufaati *et al*., 2011). Delvian (2006) who studied Vesicular Arbuscular Mycorrhizal fungi (VAM) in coastal forests also concluded that *Glomus* was the most dominant type of VAM and widely spread, where 25 species of 37 species found are the type of *Glomus*. *Glomus* has one of the most extensive distribution and most tolerant of soil salinity conditions. Generally high number of spores from genus *Glomus* identified in the field may be caused the great number of species in this genus than others. *Glomus* had the highest abundance in the village of Torjun. This shows that *Glomus* have a fairly high level of adaptation to a various environmental conditions (Puspitasari *et al*., 2012).

Isolation and Identification of Mycorrhiza

An indigenous mycorrhiza is a type of mycorrhiza found to perform association with plant roots naturally without human intervention in the process of initial infection between mycorrhizae with host plants (Schalau, 2002). Characteristics such as the number of spore wall, color, shape, spore size, straight or cylindrical subtending hyphae, hyphae form bulbous suspensor or not, with or without ornaments and spores react or do not react with Melzer solution. Species identification is made based on species description given by the International Culture Collection of Vesicular Arbuscular Mycorrhizal fungi (INVAM 2015).

Each genus found at the site of research has specific characteristics on the spores (Table 3). Spores of the fungi types contained in the study sites as follows: 1) spores from genus *Glomus* (Figure 1a, b, c) has a spherical shape, blackish brown, black, dark yellow with subtending hyphae and does not react with Melzer solution 2) spores of genus *Gigaspora* (Figure 1d, e, f) have a spherical shape, light brown, dark brown, light yellow, bulbous suspensor at the base of the hyphae and thoroughly reacted with Melzer solution, 3) spores of *Acaulospora* genus (Figure 1g) has a spherical shape, dark yellow, equipped with ornaments and outer layer does not react with Melzer, but the inner layer reacts with Melzer. Typical characteristics of *Glomus* spores are often visible spore walls and hyphae ends are attached to the surface of spores (substending hyphae), Genus *Gigaspora* generally have a single spore wall and suspensor attached to the outer surface of the spore wall. In some genera bulbous suspensor are found without germination sheld (Brundrett, 1996). Genus *Acaulospora* is characterized with outer layer does not react with Melzer while the inner layer reacts with Melzer (darker colors purplish-red), has various ornaments depending on the species (Nusantara, 2012). Suamba *et al*. (2014) states that microscopically each spore types were found to have distinct characteristics, such as spores of *Glomus* type is characterized by subtending hyphae, while the spore of *Gigaspora* has a distinctive characteristics such as bulbous suspensor at the base of the hyphae and do not have a layer of germination sheld, and the spore type of *Acaulospora* spores have thick walls and spores have ornaments.

Based on the assessment criteria soil chemical properties (Hardjowigeno, 1995), sample taken from Lekopacing village of Tanralili district (Table 4) contains soil pH H_2O of 4.48 to 5,45 (acidic), P_2O_5 Olsen of 6.88 ppm (very low), 1.39% organic C (very low), N-Total (extract 1 : 2.5) 0.12% (low), CEC (Extract Ammonium Acetat pH 7.0) 10.65 (cmol (+) kg^{-1}), (low)and Ca, Mg, Na, K (Extract Ammonium Acetat pH 7.0) 0,22(cmol (+) kg^{-1}) (low), 1,52 (cmol (+) kg^{-1}) (low), 0,085 (cmol (+) kg^{-1}) (very low), 0.008 (cmol (+) kg^{-1}) (very low), Al (Extract KCl 1 N) 2.75 (cmol (+) kg^{-1}) (very low). Sjoberg, (2005) states that mycorrhizal diversity in a region is caused by the response of the types of different mycorrhiza on soil properties, such as pH. The number of spores and mycorrhizal species closely related to the condition of the soil chemistry. The amount and type of mycorrhizal spores is associated with chemical soil conditions. When the soil pH, P, and organic-C increases, the number and type of VAM will increase (Muzakir, 2011). Study om the diversity of vesicular arbuscular mycorrhizal fungi species under Jabon trees (*Anthocephalus* spp) only found spores of the genus *Glomus, Gigaspora* and *Acaulospora*, and found no other genus. This suggests that the presence of the type of VAM is strongly influenced by environmental factors such as pH, Ultisol soils with acidity ranged from 4.1 to 4.9 are directly related to the availability of nutrients. The higher the acidity of the soil, indicated by soil pH, the higher Al content in the soil that will impact on the decrease in the number and types of mycorrhizal (Burhanuddin, 2014).

In addition to soil pH, soil texture also influences the development and growth of spores. Sari *et al*. (2014) reported that soil with texture tends to be clayey mud are suitable for development and growth of *Glomus* spores, therefore this genus is more common in rural agricultural land of Cabbiya village of Poteran island, Sumenep, Madura. Types of *Glomus, Acaulospora* and *Gigaspora* were found in the village of Torjun with soil structure of sandy clay loam. Land dominated by loam fraction (clay) is a condition considered to be suitable for the development of *Glomus* spores, whereas in the sandy soil *Gigaspora* genus were also found in high abundant (Puspitasari, 2012). Octavianti and Ermavitalini (2014) reported a field study conducted in the village of Poteran, Poteran Island, Sumenep Madura that indentified three mycorrhizal genus, namely *Glomus, Gigaspora* and *Acaulospora*. Similarly, type of mycorrhiza classified in the genus of *Glomus* found was more compared to the genus *Gigaspora* and *Acaulopsora* which could be related to the soil structure in the village of Poteran which is clay sandy soil. Zarei *et al*. (2010) states that the population of VAM spores decrease with increasing soil moisture.

4. Conclusion.

From the results of the recent study it can be concluded that: The number of spores in the Maize rhizosphere was highest in Lekopacing village, districts of Tanralili, Maros regency with VAM spores of 89 spores/100 g soil.

Three mycorrhizal genus were found in the Maize rhizosphere, namely *Glomus, Gigaspora* and *Acaulospora*. More *Glomus* genus was found compares to *Gigaspora* and *Acaulospora*.

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