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Isolation and Characterization of Indigenous AMF on Dry Land in Central Lombok

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Abstract.
This research aimed to determine the diversity of FMA species in dry land that would produce an isolate of West Nusa Tenggara (NTB). The long-term goal was to create bio-fertilizer from arbuscular fungi which is inexpensive and has the potential to increase agricultural production, particularly soybean production, in NTB dry land. The methods included a preliminary survey, isolation and morphological identification in Mataram University's microbiology laboratory, and exploration of indigenous AMF in the root rhizosphere of legume and corn plants, which took place in the Pujut subdistrict. Three mycorrhiza mushroom genera with high AMF spore density were found, specifically *Glomus* sp., *Gigaspora* sp. and *Acaulospora* sp.

Keywords: Mycorrhiza mushroom, dryland, soybean, production

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1. Introduction

West Nusa Tenggara (NTB) has unproductive agricultural land with an area of 1,807,463 ha or 84% of the area of NTB [1], used for moors, fields and plantations. Land that has the potential to be developed as productive land is 620,034.60 ha (34% of NTB dry land area) [1].

Dry land agriculture has not been prioritized in agricultural development in NTB in three decades. The government's focus is on the development of technically irrigated rice field agriculture. Dry land becomes marginal which causes various socio-economic problems, the degradation of land resources including the increase of critical dry land. Dryland farming in its development has specific challenges such as soil fertility status, undulating topography and limited water availability that will affect crop growth and production. These factors are the biggest contributors to crop failure, declining soil fertility and susceptible to the process of land damage. Sustainable dryland farming

system requires a farming strategy oriented towards efforts to improve soil fertility and overcome drought constraints.

National soybean crop production has not been able to meet domestic needs [2]. NTB soybean and corn production fluctuates. NTB Province soybean production in 2019 reached 29,698-ton dried beans or about 62% of the target of 60,241-ton dry weight [3]. Low soybean production is caused by less fertile and degraded land, less capital, non-fixed production prices [4]. Soybean production can be increased with several solutions, among others, by utilizing biological fertilizers on dry land. Bio-fertilizers that have a high contribution in improving fertilization efficiency are Mikoriza Vesicular Arbuskular (MVA) [5]. Arbuscular vesicular mycorrhizal is importantly applied to less fertile soils with low nutrient content. MVA spores inoculated at the roots of the host plant are expected to interact positively by increasing plant growth and yield through the provision of nutrients and plant growth hormone [6], facilitating the absorption of various types of nutrients, phytohormone synthesis and antagonistic to pathogenic bacteria and fungi [7].

2. Methodology

This research is an exploratory descriptive study by characterizing MVA Indegenus on dry land in Central Lombok in the land of corn and legumes of Sukadana Village, Kuta Village, Mertak Village and Lengser Village of Pujut District of Central Lombok Regency of West Nusa Tenggara which is a hilly area with a height of 100 to 355 m from sea level. This study aims to find superior isolates on some corn and legume planting land on dry land in Pujut Subdistrict (Figure ??.3). Soil sampling takes into account environmental factors (pH, soil temperature and humidity) and various commodities. Soil samples are taken in a layer of processing 0-20 cm from the ground level according to Widiastuti reference [8].

Soil samples were taken from 45 points (9 points on the rhizosphere of corn plants, 9 points on the rhizosphere of soybean plants, 9 points on rhizospheres of peanut plants, 9 points on rhizosphere green bean plants, and 9 points on rhizosphere long bean plants, which were taken randomly. Measurements of soil temperature, pH and humidity are performed at each sampling point. Soil samples are taken at each point as much as 2 kg using a soil drill, then labeled a number to facilitate control at the time of sampling of the sub-sample

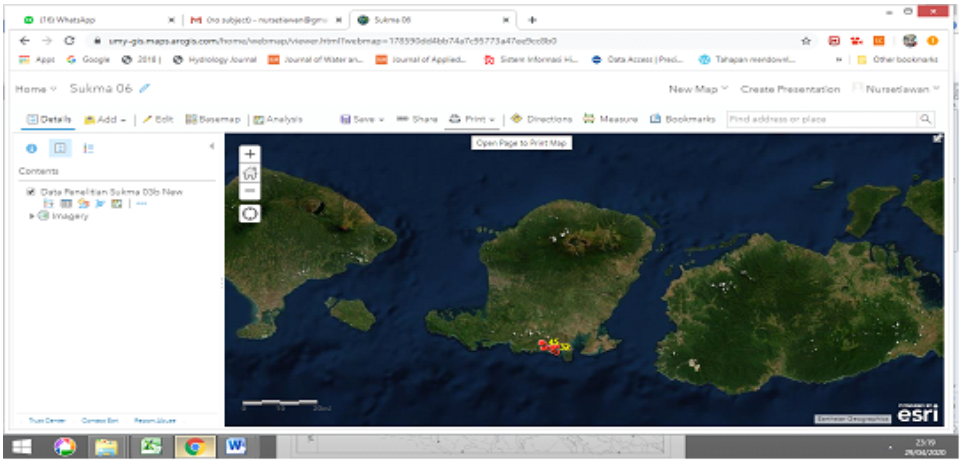


Figure 1: Map of Mikoriza Vesicular Arbuskular Indegenus Exploration Location in Central Lombok NTB.

3. Result and Discussion

The results of the identification of MVA Indegenus spores from the characterization of Spora MVA Indegenus Sukadana Village, Mertak Village, Lengser Village and Kuta Village in rhizosphere of legume and corn plants on dry land in Central Lombok West Nusa Tenggara based on morphological identification results are as many as 16 species of MVA Indegenus spores of various genera presented in the table below

The classification of 16 species of MVA Indegenus spores is based on the morphological character of the spores i.e. the color of the spores, the number of cell walls, the size of the spores, the shape of the spores and the surface of the Indegenus MVA spores. The diversity of MVA Indegenus on the dry land of Central Lombok NTB is quite high. [9] in his research managed to identify as many as 13 species in the dry land of mente plantations in Bali. Armini *et al*. [10] has identified 3 types of MVA Indegenus from batur village, Kintamani subdistrict, Bangli regency.

This research shows that the genera *Glomus, Gigaspora* and *Acalauspora* have a fairly wide spread in some legume and corn crop commodities. *Glomus, Gigaspora* and *Acalauspora* are a genus found in every rhizospheree of corn, long beans, green beans, peanuts and soybeans cultivated in exploration areas. [11], states that the species *Glomus* sp. *Gigaspora* sp. and *Acalauspora* sp. These are types of Indegenus MVA that have good adaptability to their environment so that spores can survive in dry and jagged environments faster than *Archaespora* sp species. *Ambisphora* sp. and *Entrophospora* sp.

The genera *Acalauspora, Gigaspora, Glomus, Sclerocystis* and *Scutellospora* are genera that belong to the family Glomeraceae, the order Glomerales phylum Zygomycota. The researchers agreed to place MVA not in the Zygomycota class but rather

Table 1: Results of Identification of Spore Diversity Mikoriza Vesicular Arbuskular Indegenus Sukadana Village, Mertak, Lengser and Kuta based on Morphological Characterization of Spores.

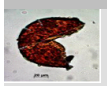
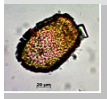
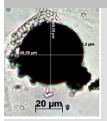
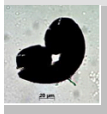
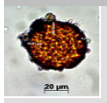
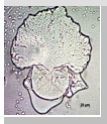
NO	Spore Photo Form	Description of Spore Morphology
1		*Glomus multicaule* A single spore, in the form of ellips. size 67.97 x 66.17µm. The spore wall has a single layer 8.6-34 µm thick with a cell wall thickness of 8.77.
2		*Glomus pansihalos* Spores are ellipsed in yellow brown. Size 120.01 x 114.09 µm. The spore wall consists of 3 layers (L1, L2, L3) but only L1 and L2 can be observed. The outermost layer (L1) of the hyaline. In water this layer is stiff with a thickness of 3-5 µm. L2 layer is stiff with orange yellow color, yellow-orange L3 layer with a thickness of 1-2 µm
3		*Glomus ambisphora* Dark brown sporocarp, subglobos shape with a size of 109.85 x 112.01 µm. It consists of a single layer of spores derived from the central nucleus of the thick interwoven hyphae, lacking basidium. Sporocarp collects up to a size of 18x12x2 mm.
4		*Glomus clavisporum* The species was previously classified into the Sclerocystis group and later transferred to Glomus by Almeida and Schenck (1989). In glomus spores formed single, ellips-shaped and sustained will increase into sporocarps complex
5		*Gigaspora decipiens* The cell wall consists of 3 layers (L1, L2 and L3). L2 layer consists of sublayers whose number and thickness are increasingly, stiff, some plasticity when damaged. As we age and the color of the spores will become dark brownish yellow, 34.8 µm thick and 48.59x 49.35 µm in size.
6		*6.Gigaspora rosea* Spores consist of 3 layers. Permanent rigid outer layer, hyaline, 1.5-2 µm thick with a smooth surface. Layer 2 consists of hyaline sublayers whose numbers increase in number and are 18-27 µm thick. Very dark colored sublayers Size 93.41 x 96.1 µm
7		*7.Gigaspora margarita* The size of the spores is large, the shape of globos. The size of the body is 77.08 x 63.15 µm. Smooth-thick spore walls 14-21 µm. Sprout tubes are produced directly from the walls of spores, thorny and thin-walled complementary cells. Yellow buffer hyphae is 6-16 µm in diameter with a funnel-like structure that comes out of the spores to the hyphae at the spore buffer.
8		*8. Gigaspora gigantea* It consists of 3 layers of L1, L2, L3, with thickening of L2 as a spore wall. L3 differentiates as the beginning of tube formation. Permanent rigid outer layer, pale yellow thickness 2.8-3.6 µm, smooth surface. Spore color from yellow to yellowish brown

Table 1: (continued).

NO	Spore Photo Form	Description of Spore Morphology
9		*9.Acalauspora scrobiculata* Single round spores, size 92-156 µm, no spore stem cells, are yellowish brown in color. The walls of the liding spores consist of two layers, a jagged outer layer (scrobicula), a second layer of smooth, hyaline-yellowish color, 2-2.5 µm thick.
10		*10.Acalauspora denticulata* There are 3 layers of L1, L2 and L3, the outer part is connected with the neck wall of the induk soporiferus sakula and the latter two are synthesized as soon as the spore wall is re-formed. L1, hyaline thick 0.6 - 1.6 µm. L2 as a single layer with a thickness of 0.6-0.8 µm with a yellow-brown color
11		*11.Acalauspora foveata* Single spores, round oblong shaped, dark yellow-reddish-brown color, diameter 124-246 x 148-256 µm, spore walls consist of two layers. The first layer is 12-13 µm thick red brown, the second layer is thin 3-7 µm like a clear colored membrane.
12		*12.Acaulospora delicata* It consists of 2 layers (L1 and L2) where the outermost layer is connected with the neck wall of the sporiferus and the inner layer of dysentesis locally with the onset of spore formation. L1 hyaline with a thickness of 0.4 - 0.8 µm. Pale and thick color 1.8 - 3.2. Size 120.96 x 117.94 µm
13		*13.Acalauspora colombiana* It consists of two layers: L1 and L2. L1 is a hyaline layer that connects with the saccule neck wall with a thickness of 0.5-0.8 µm. The L2 layer is a layer consisting of a yellowish-brown color derived from L1 with a thickness ranging from 2.4-4.4 µm. The size of the spores is 81.06 x 80.3 µm
14		*14.Ambisphora leptoticha* This species is completely different from Glomus or Acaulospora (Redecker et al., 2000). These species are from a new family, Arkeosporaceae (Morton et al., 2001). Walker et al. (2007a) identified other dimorphic species and Glomus callosum as the most closely related all in the new genus Ambispor.
15		*15 Enthosphora infrequent* Hall Single, round, brown spores, measuring 220-300 x 220-300 µm. The surface has jewelry in the form of short and rough protrusions, relatively tight jewelry, 1-2 µm thick. Size 114.66 x 75.14 µm

appropriately included in the new phylum Glomeromycota based on morphological and molecular identification [12]. Arbusicular vesicular mycorrhizal in the new phylum is grouped into 10 genera namely *Acalauspora, Archaeospora, Entrophospora,*

Table 1: (continued).

NO	Spore Photo	Form	Description of Spore Morphology
16			*16. Archaeospora schenckii* It consists of 3 layers of hyaline (L1, L2, L3). The spore wall contains many folds of L1 thin hyaline layer, a thickness of <1µm that is connected to the neck wall of the sporiferus. L2 layers of thin hyaline thickness < 1 µm, usually attached to the deepest layers. L3 hyaline is thinner than L1 or L2, ranging in thickness from 1.3 to 4 µm, along with L2 in the endospore. The size of the spores is 84.87 x 84,733 µm

Geosiphon, Gigaspora, Glomus, Diversispora, Pacispora, Paraglomus and *Scutellospora* [12]. [13] stated the genus *Acalauspora* is grouped in the family Acaulosporaceae order Diversisporales and genus *Gigaspora*, *Scutellospora* belongs to the family Gigasporaceae order Diversisporales.

Ambisphora leptothica, Archaespora schenchii and *Entrophospora* are three genera that are rarely found in exploration areas so it can be said that the genus is a ”rare” species in this study. [14] states that the species *Entrophospora infrequent, Ambisphora leptoticha, Archaespora* sp. It is a species that is rarely found in nature and has a low adaptability to be able to jagged in nature, especially on very clay and dry textured soils known as ’rare’ species. [15], also stated that *Entrophospora infrequent* is a ”rare” species found in dry areas in the Mente Munti Plantation of Mount Karang Asem Bali and the existence of such species only in the rhizosphere of mente plants. This study states that the species *Ambisphora leptothica, Archaespora schenchii* and *Entrophospora* are spores that are very rarely found and only found in the Kuta area of Central Lombok. *Ambisphora leptoticha* is found in the rhizosphere of green bean plants with a spore density of 48 spores /25 g of soil in The Village of Gerupuk Sukadana Village. *Archaespora schenchii* is found in the rhizosphere of green bean plants in Sukadana Village Institution Hamlet with the density of spores is 9 spores / 25 g of soil. *Entrophospora ifrequent* exists. on the soybean rhizosphere in Lengser Village with a spore density of 60 spores / 25 g of soil

The genera *Glomus, Acaulospora*, and *Gigaspores* that are widely found in rizospher corn crops are thought to be due to environmental factors suitable for the development of spores, so the genus MVA can thrive. [16] state the genus MVA found and the effectiveness of MVA against host plants is determined by soil type and host plant type. [17], also stated that the pattern of spread of MVA each genus varies depending on the ability of MVA spores in germination and is influenced by the types of host plants that are compatible with MVA types. Certain genera have a very wide spread and there

are also genera with limited distribution. The genus that had the most widespread dispersion in this study was the genus *Glomus, Gigaspora* and *Acalauspora* whose high numbers are thought to be caused by environmental conditions that are more suitable, optimal, and compatible in supporting the growth and development of spores. Antagonistic fungi that inhibit MVA sporulation were not found.

Indegenus MVA spores are widely discovered after extraction allegedly because MVA Indegenus has been speculated and supported by appropriate environmental factors (soil conditions, soil pH soil porosity) [18] and climate (rainfall, temperature, soil moisture). Factors of difference in place, season and low rainfall also greatly affect the number of spores [19]. The temperature at the time of soil sampling in the root rhizosphere is lowest at 29^{o}C and the highest is 35^{o}C. Soil temperature of 29^{o}C was found in green bean legume plants at 5 sampling points. The soil temperature in the peanut rhizosphere at 4 sampling points is 29^{o}C. The soil temperature in the long bean rhizosphere at 4 sampling points is in the range of 28^{o}C and 29^{o}C and in the soybean rhizosphere the average soil temperature is 28^{o}C. The sample temperature in the rhizosphere of corn crops ranges from 32-36^{o}C. Indegenus arbusicular veciscular mycorrhizal can develop at exploratory sampling sites because temperatures in the rhizosphere legume and corn range from 5^{o}C and 36^{o}C. [20] states that the presence of vesicular arbusicular mycorrhizal Arbusicular Indegenus can be observed in the form of spores under unfavorable conditions. Vesicular Mycorrhizal Arbusular Indegenus can sustain its life in the form of spores and germinate after the condition allows, which begins with the process of root infection. High soil temperatures strongly support the amount of MVA in the soil. In spring or early dry season MVA can be observed in the form of spores at the time of root dormancy.

In addition to environmental conditions, the presence of MVA spores is also influenced by soil nutrient levels. The pH range in the exploration area is the soil pH of 7.18 - 8.85 with the total N nutrient content of the soil which is 0.00 - 0.19% (very low), the total organic C nutrient content of the soil which is 0.47 - 2.83% (low), the total P nutrient content of the soil which is 1.52 - 114.88 ppm. Low nutrient levels will increase the effectiveness of MVA's work in infecting plant roots. [21], stated that the infection of corn plant roots will increase even without the provision of organic matter. Mycorrhizal plants grow 6.4 times larger than plants without mycorrhizal [22]

4. Conclusion

This A total of 16 species of MVA Indegenus have been identified from the dry land of Sukadana Village, Mertak Village, Lengser Village and Kuta Village of Kuta District of Central Lombok Regency of West Nusa Tenggara which can be used as a biological fertilizer. Morphologically identified species are: *Glomus multicaule, Glomus pansihalos, Glomus ambisphorum, Glomus clavisporum, Gigaspora decipiens, Gigaspora rosea, Gigaspora gigantea, Gigaspora margarita, Acalauspora delicata, Acalauspora denticulata, Acalauspora scrobiculata, Acalauspora colombiana, Acalauspora foveata, Ambisphora leptchaoti, Archaespora schenchii, Enthospora*. Among the 16 species 3 species found as rare species are *Enthroposphora infrequent* species, *Ambisphora leptoticha* and *Archaespora schenchii*

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