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Population and Diversity of Indigenous Arbuscular Mycorrhizal Fungi from the Rhizosphere of Shallots at Different Altitudes in West Sumatra

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***Abstract*--- Arbuscular Mycorrhizal Fungi (AMF) of indigenous believed to be more efficient and effective in helping the growth of plants. Observations diversity of indigenous AMF conducted at several locations of centers of shallot cultivation in West Sumatra. AMF existence is closely related to environmental factors (abiotic and biotic). Sampling was based on the different in altitude : highland (Alahan Panjang) middle land (Saniang Baka) and lowland (Kambang). Indigenous AMF identification using morphological approach. The present study was undertaken to 1) look at the population and species diversity of AMF indigenous derived from shallots rhizosphere in different altitudes in West Sumatra and determine whether there are differences on the number and type of AMF at any location, 2) identified morphological characters of AMF spores. 19 types of indigenous AMF derived from shallots cropland in West Sumatra that *Scutelospora* (4 types), *Glomus* (10 types), *Gigaspora* (2 types), *Acaulospora* (3 types). We found the genus Glomus dominant in this study. Of the three locations, the greatest of AMF types was found in the area Saniang Baka (13 species), followed by Alahan Panjang (12 types) and Kambang (10 types). The number of species did not always a correlate with the number of spores. AMF spore count decreased with increasing height above sea level. Differences in altitude correlated negatively with the number of spores produced. The lowland, Kambang having a higher spore count (817 spores) than Saniang Baka in the middle land (798 spores) and Alahan Panjang in the highland (687 spores). It appears that differences in altitude affect the production of spores.**

***Keywords*— Indigenous AMF; altitude; population; diversity.**

I. INTRODUCTION

Shallot are a leading horticultural commodity in Indonesia. However, the sustainability of supply within the country is not continuous and areas producing shallots still limited (Brebes and Cirebon) to be the cause of import commodities still have been continued [1]. The primary area production is in Brebes, Central Java, filling 41.50%(419.472 ton) of national production of 1,010,773 ton with yield areas 98,937 (2013 figures). The production of shallot from West Sumatra, is well below the national average. West Sumatra production only 42,791 ton with yield area of only 4,144 ha [2]. This figure is showed that the production of shallots West Sumatra just filling 4.23% of the national shallots production. Expansion of planting on the lowland become one of the alternatives, in an effort to increase the production of shallots in West Sumatra, given the current production center shallot West Sumatra lies on the highland. The problem is the land of drylands in lowland West Sumatra tends to has an acid reaction or a low pH which is caused by the wet climate [3].

During the growth of shallots requires sufficient nutrient elements, particularly the element Phosphorus (P). The element of P on the shallot plays a role in enhancing the development of the roots, so it can simplify and speed up the absorption of nutrient elements. The element of P is also instrumental in improving the quality and yield of shallots, in this case, by reducing shrinkage and weight loss of the bulbs [4]. P uptake by plants will run normally when soil acidity is not too high. Ions such as aluminum, iron, and manganese will be bind by H_2PO_4, making it unavailable to plants. In order for plants to grow and produce maximally, then availability the element of P in the soil needs to be increased.

For maximum growth, shallots are cultivated on dry land during the dry season. However, the short roots of shallots cause it require a lot of water in the vegetative period and

are not tolerant of drought which has a negative effect on reproductive growth. During the growth and development of bulbs needed quite a lot of water. Drought conditions that started from the vegetative phase have a negative effect on reproductive growth. A decrease in water content is available to 60% on vegetative phase raises the effect of stress drought on shallots plant [5]. The vegetative phase (11-35 day after planting) is a period of rapid growth in the shallots, it requires the availability of sufficient amounts of water. Reduced availability of water during this period may disrupt crop growth, although in subsequent growth phases the availability of water returns to normal, shallots yields continue to affected

One effort to increase the availability of P and the resistance of plants to drought stress is to utilize Arbuscular Mycorrhizal Fungi (AMF). [6] explain how AMF can act as a biofertilizer and a bioprotector, because it absorbs and translocates mineral nutrients beyond the depletion zones of plant rhizosphere (biofertilizer) and promotes tolerance to soil and environmental pressures (bioprotector). [7] reported that an effective agronomical technique for the optimization of water use efficiency in areas with limited water availability is to use a combination of artificial plant mycorrhization with irrigation during dry periods. Recent research indicates that AMF may also produce glomalin which can be significant in improving physical properties of soil [8], so the development of the bulbs in the soil can be maximized. Another advantage of the use of AMF is it is environmentally friendly. [9] reported the application of biostimulant tablets containing both of *Glomus* and *Trichoderma* on vegetable crops can increase productivity in a sustainable way.

The compatibility of mycorrhizal species with the plant greatly influences the outcome of cooperation between plants with mycorrhizas in symbiosis. [10] reported that inoculation of various exogenous mycorrhizal species had the same effect with treatment without mycorrhizal inoculation. It means that inoculation mikroriza not derived from shallots rhizosfir (eksogen), not yet able to increase the growth and the result of shallots. Therefore, study the use of mycorrhizal derived from shallots rhizosphere (indigenous), expected to be more adaptive and effective development, so the ability to absorb higher nutrient elements and increase plant resistance to drought, needs to be done.

AMF diversity studies on different shallots rhizosphere location and the soil fertility is an important thing to do. In addition to obtaining information about the type and number of population in the ecosystem, reproductive ability, the ability of the infection, also in order to obtain an effective AMF isolates, which could be used to increase the productivity of the soil and plants.

The present study was undertaken to 1) look at the population and species diversity of AMF indigenous derived from shallots rhizosphere in different altitudes in West Sumatra and determine whether there are differences on the number and type of AMF at any location, 2) identified morphological characters of AMF spores.

II. MATERIALS AND METHODS

A. *Sampling sites*

Soil sampling were collected at the following three sites used for growing shallot at different altitude in West Sumatera: Alahan Panjang, a highland in the district of Solok at 1.400 m above sea level ($100^0$46'58.7" E-$1^0$04'23.5" S), Saniang Baka, a middle land in the district of Solok with 400 m above sea level ($100^0$31'32.2" E-$0^0$42'55.7"S) and Kambang, a lowland in the district of Pesisir Selatan with 10 m above sea level ($100^0$42'00.0"-$1^0$42'00.0"S). The Solok district is a major center for the cultivation of horticultural crops, especially vegetables, while in the Pesisir Selatan district. is an area palawija crops, which lately cultivated organic shallots crop.

B. *Sample collection and treatments*

Soil and root sample obtained from shallots rhizosphere by taking a composite sample as much as ± 1.0 kg. On each sampling sites were taken five samples of soil and roots were randomly for observation AMF spores extraction and colonization of roots.

Samples of soil and plant roots are taken around the shallots crop ± 10 cm from the base of the stem or rhizosphere zone at a depth of 0-20 cm. Further soil samples dried for 1 week and put in a plastic bag that is labeled to be stored at room temperature (15-20 ^{0}C) prior to analysis. While the roots of the plant for inspection AMF colonization stored in the refrigerator.

Spora from the rhizosphere soil sample were isolated through the wet-sieving method described by [11]. For each soil sample, 20 g soil was independently suspended in 150 ml water and stirred with a magnetic stirrer for 10 min. We use 60, 125, 300 dan 500 μm sieves to collect the spores. The spores on each sieve were filtered onto a filter paper and placed in a 9 cm petridish for examination under a binocular stereomicroscope (10-100x). The intact healthy AMF spores were sorted into groups and counted.

Each spore type was mounted sequentially in PVLG (*Polyvinyl-Lactic-Glycerol*) and PVLG+Melzer's Reagent (v/v 1:1) [12] for identification. The identification was based on morphological descriptions published originally and provided by the international collection of vesicular and arbuscular mycorrhizal fungi as well as various sources such as [13],[14] and [15]. AMF species were identified using digital camera Olympus SZ-16 and photo laptop. The fixed slides and images were stored as reference collections in our laboratory.

C. *Statical analysis*

Diversity is defined as a form of community both the flora and fauna that live on earth. The diversity of the AMF community can be illustrated by observing Spore Density (SD), Species Richness (SR), Isolation Frequency (IF), Relative Abundance (RA), Evenness (E) and Shannon - Wiener index of diversity (H') [11] (Table I).

The number of spores was identified (in 100 g of soil) is a reflection of spore density, the number of AMF species were identified in the soil samples was defined as species

TABLE I

DIVERSITY MEASURES USED TO DESCRIBE AMF COMMUNITIES

Spora Density (SD)	The number of spores in 100 g soil
Species Richness (SR)	The number of identified AMF species per soil sample
Isolation Frequency (IF)	$IF = \frac{\text{the number of soil sample where a species (genus) occured}}{\text{the total number of soil samples}} \times 100\,\%$
Relative Abundance (RA)	$RA = \frac{\text{spore numbers of a species (genus)}}{\text{the total number of identified spore samples}} \times 100\,\%$
Evenness (E)	$E = \frac{H'}{H_{max}}$
Shannon-Wiener index of diversity(H')	$H' = -\sum Pi \ln Pi$

Pi is the relative abundance of each species identified per sampling site and calculated by the following formula Pi = ni / N, where ni is the spore numbes of a species and N is the total number of spores identified spore sample. H'_{max}= is the maximal H' and calculated by following formula H'= ln S, where S is the total number of identified species per sampling site.

richness. Percentage of spore number of a species, which indicated the different sporulation ability of different species of AMF as relative abundance. The frequency of existence of each spore of FMA found was calculated based on the presence of particular FMA indigenous spore on each soil sample, which revealed the extent of distribution of a given AMF of the species according to relative abundance (RA> 3%) and isolation frequency (IF> 40%) [11]. In the AMF community, diversity and evenness were reflected by Shannon-Wiener index of diversity.

Qualitative (AMF morphology) and quantitative (AMF diversity and colonization AMF) data were collected. All data were analyzed descriptively by arranging and displaying in a simple form of tables, graphs, and pictures comparing data between the observation location. To see relationship between spore density and species richness, relative abundance and isolation frequency, Pearson correlation analysis was used.

III. Result and Discussion

A. The diversity of arbuscular mycorrhizal fungi

1) The number, type, and frequency of presence AMF

A total of 19 species AMF were identified from the shallots rhizosphere at the 3 study sites with different altitude in west Sumatera: 4 of these species were from the genus *Scutelospora*, 10 from *Glomus*, 2 from *Gigaspora* and 3 from *Acaulospora*. Twelve species were found at Alahan Panjang (AP), 13 at Saniang Baka (SB) and 10 at Kambang (KB) (Table II, and Fig 1). In the identified AMF species, 7 AMF species were encountered at all three sites, 1 species at both AP and SB (*Acaulospora* sp2), 1 at both AP and KB (*Scutelospora* sp3). Three species were found only at AP (*Glomus* sp5, *Acaulospora* sp3, *Glomus* sp9), 5 only at SB (*Acaulospora* sp1, *Scutelospora* sp2, *Gigaspora* sp2, *Glomus* sp7 and *Glomus* sp8), and 1 only at KB (*Glomus* sp

The results showed that the number of types of AMF and the number of spores on each of the locations were not positive correlation (r = -0.32). More spores is not necessarily an indication of a greater types of AMF. However there was a clear relationship between altitude and spore count. A total of 2,302 spores were wet sieved, from which 687 spores were from AP (highland), 798 spores from SB (middle) and 817 spores from KB (lowland) each from a 100 gram soil sample. These spore counts show a strong negative correlation with altitude (r = -0.963). Higher altitudes have lower spore counts. This is consistent with the results of previous research [16], where from the three sample sites concluded that tested colonization and spore production of AMF decreased with increasing altitude. Differences in altitude cause the difference in temperature and humidity affecting the production of spores. Lowland have high temperatures and high humidity facilitating the growth classified of fungi like AMF better than the conditions in the middles and highland.

Mycorrhizal growth is strongly influenced by environmental factors and a relatively high temperature will result in increased activity of fungi. AMF association process with the host plant in three stages: germination of spores, hyphae penetration into the root cells and the development of hyphae in the root cortex. The optimum temperature for germination of the spores varies depending on the species. Some *Gigaspora* isolated from soil in Florida (subtropical) have an optimum germination temperature of 34 ^{0}C, while for *Glomus* species that come from cold climates,the optimum temperature for germination is 20 ^{0} C.

Temperature not only effects the development of spores, hyphae penetration of the root cells and the development of the root cortex but also the resistance and symbiosis. The higher the temperature the greater the colonization and production of spores. [17] stated that the best temperature for the development of the arbuscular phase at 30 ° C, but for the best mycelial colony is at a temperature 28-34^o C, while the development of the vesicles at 35 ° C.

Besides the temperature factor, intensive farming systems such as the excessive use of pesticides and chemical fertilizers also affect the growth of these fungi, inhibiting

TABLE II

RELATIVE ABUDANCE (RA) AND ISOLATION FREQUENCY (IF) OF AMF

Sp. No.	Arbuscular Myccorhizal Fungi (AMF)	Alahan Panjang (AP)			Saniang Baka (SB)			Kambang (KB)		
		Spore number	RA (%)	IF (%)	Spora number	RA (%)	IF (%)	Spora number	RA (%)	IF (%)
	Scutelospora	**242**	**34.59**	**50**	**255**	**31.95**	**50**	**202**	**24.07**	**75**
1.	*Scutelospora sp1*	235	34.22	100	250	31.32	100	170	20.82	100
2.	*Scutelospora sp2*	-	-	-	5	0.63	33	-	-	-
3.	*Sutelospora sp3*	7	0.37	33	-	-	-	17	2,04	33
4.	*Sutelospora sp4*	-	-	-	-	-	-	15	1.84	67
	Glomus	**411**	**58.51**	**70**	**493**	**61.81**	**70**	**595**	**72.86**	**60**
5.	*Glomus sp1*	147	21.36	100	207	25.89	100	330	40.41	100
6.	*Glomus sp 2*	177	25.13	100	178	22.34	100	150	18.37	100
7.	*Glomus sp3*	37	5.34	100	62	7.72	67	83	10.20	100
8.	*Glomus sp4*	10	1.46	67	33	4.18	100	27	3.27	67
9.	*Glomus sp5*	2	0.24	33	-	-	-	-	-	-
10.	*Glomus sp6*	-	-	-	-	-	-	2	0,20	33
11.	*Glomus sp7*	-	-	-	5	0.63	33	-	-	-
12.	*Glomus sp8*	-	-	-	5	0.63	67	-	-	-
13.	*Glomus sp 9*	13	1.34	33	-	-	-	-	-	-
14.	*Glomus sp10*	25	3.64	33	3	0.42	33	3	0.41	33
	Gigaspora	**32**	**4.61**	**50**	**43**	**5.43**	**100**	**20**	**2.45**	**50**
15.	*Gigaspora sp 1*	32	4.61	100	40	5.01	100	20	2.45	67
16.	*Gigaspora sp2*	-	-	-	3	0.42	67	-	-	-
	Acaulospora	**4**	**0.48**	**67**	**7**	**0.83**	**67**	**0**	**0**	**0**
17.	*Acaulospora sp1*	-	-	-	2	0.21	33	-	-	-
18.	*Acaulospora sp2*	2	0.24	33	5	0.63	33	-	-	-
19.	*Acaulospora sp3*	2	0.24	33	-	-	-	-	-	-
	Total AMF = 19 species									
	Number of spores	**687**	**100,00**		**798**	**100,00**		**817**	**100,00**	
	Species Richness (SR)	**12**			**13**			**10**		
	Value SR	**1.68**			**1.80**			**1.34**		

the growth of spores. In Kambang, shallot farming is now becoming more organic with a reduction of chemical fertilizers and pesticides. This may be another reason for the better growth of AMF spores compared to the other two locations (Saniang Baka and Alahan Panjang). [18] stated that agricultural practices such as tillage, agriculture systems, enrichment with organic materials, fertilizer and pesticide use affect the presence of mycorrhizae.

The diversity of AMF observed in AP (13 AMF species and SB (12 AMF species) was greater than in KB (10 AMF species), and the greater range of different host plants [19] could be the reason for the relative higher diversity of AMF. AP and SB, located in the same district is Solok. AP and SB are both areas of cultivation of a large variety of horticultural crops, especially vegetables, while KB located in the Pesisir Selatan District is a regional producer of food crops such as rice and corn. Soil samples at KB were taken from former land of rice and maize fields now being used to grow organic shallots crops. AP and SB have shorter vegetable crop rotations of 1-4 months compared to an average rotation of 4 months at KB resulting in the smaller types of AMF as a smaller variety of plants are grown in the same soil in a fixed period of time there. [20] says that the diversity of AMF is closely linked to environmental changes. A rich plant biodiversity will support a higher biodiversity of AMF species while AMF diversity is also dependent on the physical environment [21].

Based on relative abundance and isolation frequency, the 5 dominant species in AP were (*Scutelospora sp1, Glomus sp1,Glomus sp2, Glomus sp3, Gigaspora sp1)* , the 6 dominant species in SB were (*Scutelospora* sp1, *Glomus sp1*, *Glomus sp2*, *Glomus sp3*, *Glomus sp4*, *Gigaspora sp1*) and the 5 dominant species in KB were (*Scutelospora* sp1, *Glomus sp1*, *Glomus sp2*, *Glomus sp3*, *Glomus sp4*) (Tabel II). [11] states that there is a significant positive correlation between RA and IF of AMF species. Species producing more spores usually have a wide distribution, while species with small geographic ranges usually produced fewer spores.

However, a few AMF species such as *Glomus sp4* (1.46 % RA, 67 % IF) in AP, *Glomus sp9* (0.63 % RA, 67 % IF), *Gigaspora sp4* (0.47% RA, 67% IF) in SB and *Sutelospora sp4* (1.84 % RA, 67 % IF*), Gigaspora sp1* (2.45% RA, 67% IF) in KB had low RA but were widely distributed with a relatively high IF (Table II). In contrast,

Glomus sp11 (3.64% RA, 33% IF) in AP, was not present with high IF, but it was dominant in sporulation compared with other species at that site (Table II). Thus it is important to consider the spread and sporulation ability of AMF in determining its dominance in a community [11]. Some AMF spores are found in 3 locations with different altitude as show in Fig.1.

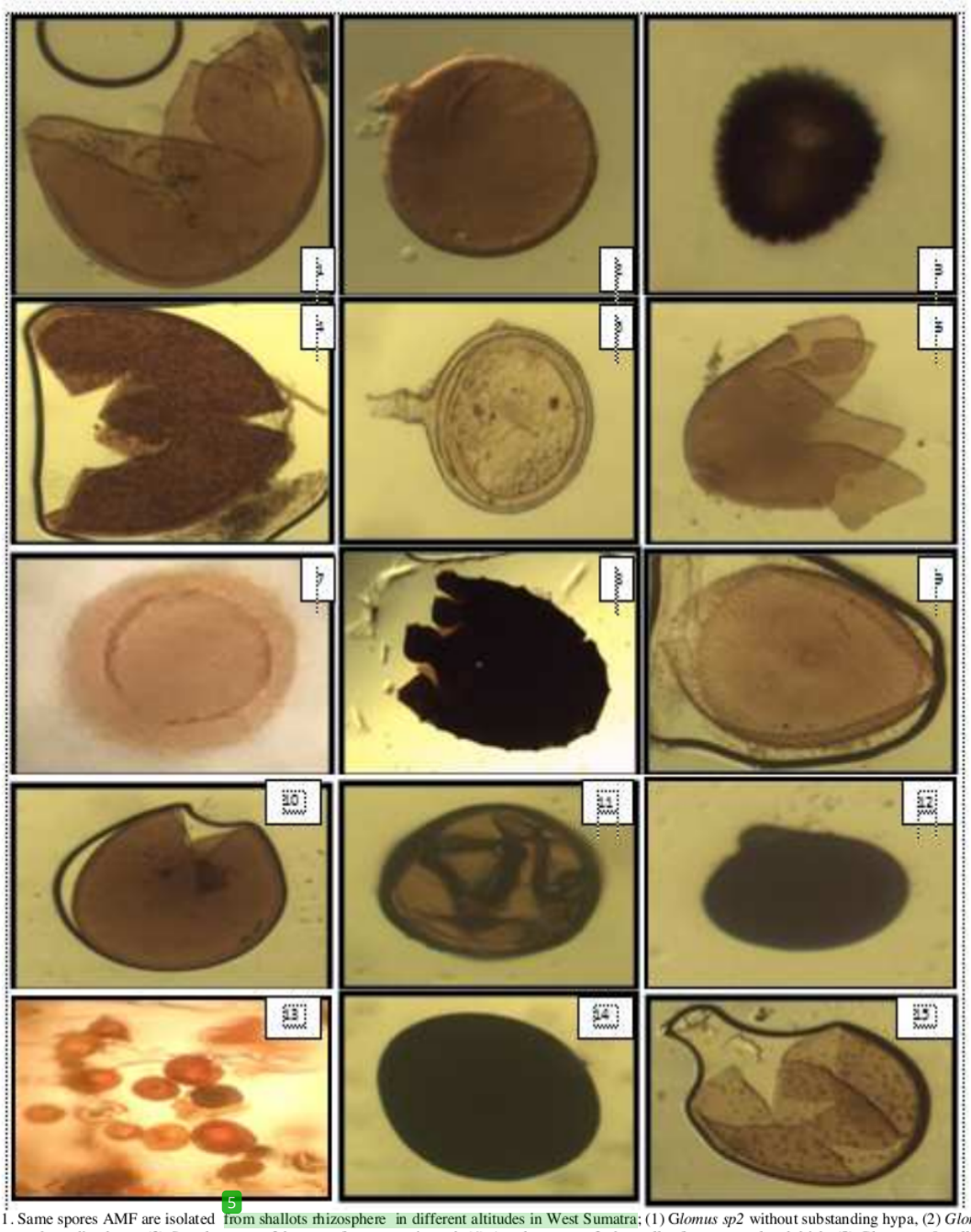

Fig 1. Same spores AMF are isolated from shallots rhizosphere in different altitudes in West Sumatra; (1) *Glomus sp2* without substanding hypa, (2) *Glomus* sp7 are substanding hypa, (3) *Scutelospora* sp2 has a surface spores jagged, (4) *Acaulospora* sp 2, the walls of orange peel and thick, (5) *Glomus* sp 8 walls of spores plated 4, (6) *Glomus* sp has a wall of slick, (7) *Scutelospora* sp4 has a center circle, (8) *Acaulospora* sp1 with walls spores smooth and has frills (concertina), (9) *Glomus* sp 6-covered walls and concertina, (10) *Glomus* sp3 , (11) *Scutelospora* sp3, spore-containing (12) *Gigaspora* sp2, have bulbous suspensor, (13) *Glomus* spores in clusters, (14) *Gigaspora* sp1 has a black color, reacts with Melzers be redness, (15) *Acaulospora* sp3 wall of orange peel and layered thin.

2) Evenness (E), and Shannon-Wiener index of diversity (H')

Evenness of AMF from KB (E=0.704) was higher than from AP and SB (E= 0.676 and 0.659). Because of there is the dominance symptom between the variety in a community or an ecosystem. This phenomenon is caused the use of land without a good crop rotation, as a result, it will make dominance of types of AMF on an ecosystem.

The land from SB had the highest H' among AP and KB. Many types of AMF are found in SB but not found in AP and KB. Seven types of AMF that found at many various sample locations; *Scutelospora sp1*, *Glomus sp1*, *Glomus sp2*, *Glomus sp3*, *Gigaspora sp1*, *Glomus* sp4 and *Glomus sp10*. It means that all species found in all locations, has a wide adaptation, so has a higher geographic effect and These appear to be more adaptable to different habitats than other varieties that are not found in various locations. See Fig. 2.

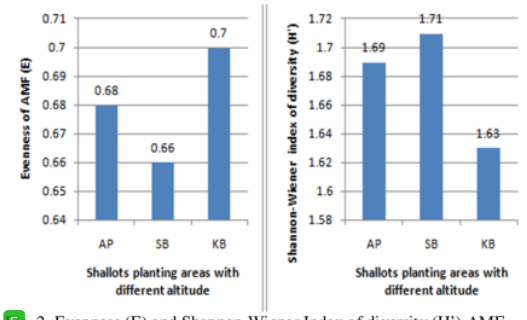

Fig. 2. Evenness (E) and Shannon-Wiener Index of diversity (H') AMF from shallots rhizosphere in different altitudes in West Sumatra

Value diversity Indigenous of AMF from shallots rhizosphere in different altitudes is presented in Table III.

TABEL III
VALUE DIVERSITY INDIGENOUS OF AMF FROM SHALLOTS RHIZOSPHERE OF VARIOUS DIFFERENT ALTITUDE

No	AMF	Number of spora AMF ($100g^{-1}$) air-dried soil samples		
		AP	SB	KB
1.	Spora Density (SD)	687	798	817
2.	Spesies Richness (SR)	12	13	10
3.	Value SR	1.68	1.80	1.34
4.	Evennes (E)	0.680	0.659	0.704
5.	Shannon-Wiener index of diversity	1.69	1.71	1.63

In general, the result of observations is showed that there are different of diversity index at each site with different altitude. These differences could be result of many factors such as the different temperature because of different altitude, differents lands use patterns, such as AP and SB lands that often plant with horticulture crop (specially vegetable), whereas the recent KB land cultivated shallots, which previously cultivated food crops (rice, cassava and corn) on a regular basis. The chemicals in the soil and soil pH also could be an influence on the diversity of AMF at these sites..

3) The colonisasi AMF with plant root

The colonization AMF with plant roots is the most easily observed parameter to know the association between AMF and plants. Base on the result of observation (Table IV), the highest percentage of infection is showed in Kambang location. This location also has the highest spo counts (Table I). In this case, the root infection has a positive correlation with the number of spores. However, the SB samples have a higher number of spores than the AP location, but a lower percentage of infection. Hence the number and diversity of AMF types do not always correlate with the percentage and degree of AMF infections in plants as other factors may be more significant. Soil chemical properties can also directly influence the growth and exudate produced by plants. [21] reported that chemical fertilizer significantly reduces the frequency and intensity of mycorrhization compared to a control and a sample fertilized with fowl droppings.

The intensity of infection was observed to determine the effectiveness of the symbiosis of the AMF with shallots. Assessment the intensity of root infection is done through mikroskospis looking for the presence of hyphae, vesicles and arbuscular both inside and outside the root cortex cells. From these observations, it was found that the intensity of infection in AP area is higher than KB and SB area. It means that the effectiveness of symbiosis AMF in AP area better than in KB or SB area (Fig.3).

Based on the phenomenon that occurs naturally above should be seen the relationship between the number and type of FMA with some chemical properties of soil. P levels and P uptake are growth parameters that are often associated with the percentage of AMF colonization with plant roots. In addition, soil pH is also often associated with symbiosis between AMF and plants. From the research [22], demonstrated that differences in the level of colonization of AMF with plant roots resulted from different N and P dosing.

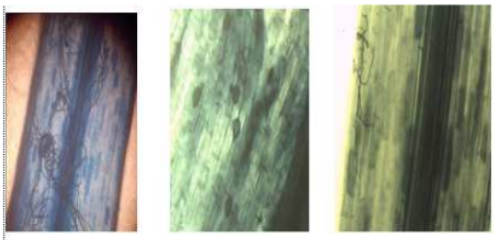

Fig 3. AMF colonization in the roots of shallots crop in AP, SB and KB

[23] reported the average colonization level of *M. saccharifierous* was 23-28% in natural soil, whereas no colonization was found in sterilized soil. In addition to nutrient status in the soil (including fertilization and pesticides), there are various other factors that affect the effectiveness of AMF with the plants such as the sensitivity of host plants to the AMF colony, propagule density, competition between AMF with other microorganisms and climatic factors.

TABEL IV

THE PERCENTAGE OF INFECTION AND THE INTENSITAS INFECTION OF INDIGENOUS AMF FROM SHALLOTS RHIZOSFIR IN DIFFERENT ALTITUDES

The sample locations	Percentage		Average	
	Root infection	Intensity	Infection	Intensity
Alahan Panjang (AP)				
A1	80	51	74	35.6
A2	80	41		
A3	90	28		
A4	80	32		
A5	40	26		
Saniang Baka (SB)				
B1	50	8.5	50	10.9
B2	80	21,5		
B3	40	8		
B4	40	7.5		
B5	40	9		
Kambang (KB)				
C1	70	30	75	28.6
C2	60	30		
C3	75	30.5		
C4	90	27		
C5	80	30.5		

IV. CONCLUSION

A total of 19 types of indigenous AMF were derived from shallots rhizosphere at different altitudes in West Sumatra: *Scutelospora* (4 types), *Glomus* (10 types), *Gigaspora* (2 types) and *Acaulospora* (3 types). We found the genus *Glomus* dominant in this study. Of the three locations, the greatest of AMF types was found in the area Saniang Baka (13 species), followed by Alahan Panjang (12 types) and Kambang (10 types). The number of species did not always a correlate with the number of spores. AMF spore count decreased with increasing height above sea level. Differences in altitude correlated negatively with the number of spores produced. The lowland, Kambang having a higher spore count (817 spores) than Saniang Baka in the middle land (798 spores) and Alahan Panjang in the highland (687 spores). It appears that differences in altitude affect the production of spores.

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