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Selection of Aruscular Mycorrhizal Fungi (AMF) Indigeneus in Ultisol for Promoting The Production of Glmalin and Aggregate Formation Processes

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***Abstract*— The muatualism symbiosis of arbuscular mycorrhizal fungi (AMF) with plants are able to increase the capacity of plants to absorb nutrients and water from the soil. Recently, research was indicated that AMF hyphae containing glomalin as a glycoprotein that serves to glue the between dispersed soil particles. The content of glomalin in soil was positively correlated with soil aggregate stability. The research potential of AMF species indigenous of Ultisol Darmasraya District of West Sumatra and glomalin production in experimental pots of sterile sand medium has been carried out. The purpose of this study was to determine the diversity of AMF species on Ultisol and to seeking indigenous AMF isolates that have the best glomalin production capability. AMF spores were isolated and identified from the rhizosphere soil of corn in Ultisol. AMF species that have been identified experimentally tested culture medium pot of sand and zeolite (w / w 1:1) using corn crops. The results showed that the AMF species indigenous of Ultisol Darmasraya found 9 species, namely Acaulospora scrobiculata, Glomus etunicatum, Glomus luteum, Glomus mosseae, Glomus verruculosum, Glomus versiforme, Scutellospora gregaria, Scutellospora heterogama and Gigaspora sp. AMF species that showed better colonization ability in maize is G. luteum, G. verruculosum and G. versiforme. All three species can produce glomalin was significantly higher than the other species, ie 1.29 mg.g-1; 1.17 mg.g-1; 1.15 mg.g-1 respectively.**

***Keywords*— glycoprotein, glomalin, indigenous, aggregate stability, Ultisol**

I. INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) can perform symbiosis with plants and were largely found in various ecosystems. The diversity of AMF in the soil may differ in environmental conditions from one another. AMF symbiosis with host plants can improve the absorption of nutrients and water for plants. Recent findings indicate that the role of the AMF to improved soil physical properties can be explained by increase in soil aggregation.

Activities AMF has an important function in the ecosystem in the form of contributions to the improvement of soil structure that is highly correlated with soil aggregate stability and environmental quality [35],[38],[25],[30],[5]. The role of AMF in soil aggregation is through AMF activity that produces a protein called glycoprotein as "glomalin" [40]. Glomalin first discovered in 1996 [35], in which this protein is abundant in the soil associated with the activity of AMF [37]. Rillig, [24] use a new term to describe the soil protein is called "glomalin-related soil protein (GRSP)" as the source of C (carbon) and N (nitrogen) soil are important in nutrient cycling and ecosystem [30],[12]. Rillig et al. (2001) found that approximately 4 to 5 % of the total soil C and N in Hawaii comes from glomalin. Furthermore, Lovelock et al. (2004a) reported that levels of glomalin in the soil of La Selva at a depth of 0-10 cm is 3.94 mg.g-1, which contributes to soil C and N were 3.2% and 5% respectively.

Glomalin related soil protein (GRSP) instead secretion of AMF hyphae, but more than 80 % making up the walls of hyphae and spores AMF is glomalin (Driver, Rillig and Holben, 2005). Glomalin form of adhesive (glue) is produced by the AMF for the transport of nutrients and water activity. Glomalin serve to protect the hyphae of drought and destruction attack by microbes, which after activity stalled hyphae (in weeks) apart along the hyphae and glomalin fused with minerals (sand, dust and clay) and organic matter clump stable soil aggregates [40],[24],[5],[7].

Wright and Nichols (2002) explained that the blob structures formed by binding soil glomalin is quite stable, resistant to water and wind erosion, porous enough to pass air and water. It can hold more water, preventing scale formation (crusting) in the surface layer of the soil and supports the development of plant roots.

Glomalin production by AMF hyphae during colonization of the plant root activity can differ between species with each other. Wright et al. [36] found that the amount of glomalin extracted per unit weight of hyphae differ between species Gigaspora gigantea, Glomus intraradices and Glomus etunicatum. The same was reported by Wright and Upadhyaya [38], where the total production of glomalin was significantly different between the Gi. rosea, G. caledonium and G. intraradices. Furthermore Levelock et al. [16] observed between the result of different glomalin Acaulospora morrowiae, Gi. rosea, G. etunicatum and G. intraradices, where A. morroaiae produce the highest concentration of glomalin and G. intraradices resulted in the lowest.

Based on the above, the management of indigenous mycorrhizal fungi as biological resources of land, is a breakthrough technology for the development of sustainable agriculture. How large is the potential diversity of AMF species indigenous to the Ultisol unknown. How can the potential of AMF species to produce glomalin in maize research needs to be done.

The purpose of this study was to determine the diversity of AMF species indigenous on Ultisol and seek isolates that have the best ability to produce glomalin.

II. Materials and Methods

A. Exploration and identification of AMF

Sampling site is on the District Darmasraya West Sumatra wich soil order is Ultisol. Soil sampling taken from two locations, sample taken from farmland planting wich corn, the Village of Pisang Berebus Sitiung and the Sungai Langkok Sitiung II. At each location taken 5 corn planting area for observation of root colonization and AMF spore extract, analysis of glomalin, aggregate stability, and soil chemical properties. Corn planting area measuring 0.5 to 1 hectare used for random sampling. Soil sampling as much as 2.0 kg in the rhizosphere of plants and their root samples were taken from each planting areas as many as 10 plants. Soil samples were taken at a depth of 5-20 cm, dried aired for one week and put in a plastic bag labeled to be stored at a temperature of 15-20 ° C prior to analysis.

Research activities begins with the observation of AMF colonization on the roots of corn plants. The roots are cleaned with tap water and cut into 2 cm. Roots immersed in potassium hydroxide (KOH) 10 % for 4 days. KOH was removed and rinsed with distilled water. Add 1 % HCl solution let stand one night. HCl then discarded, add 0.05 % trypan blue dye. Colonization is measured by the gridline intersect method [10]. Soil samples indicate the presence of infection in the roots of maize followed AMF spore isolation and identified. Every single type of spore in one group was placed on a glass preparations (glass slides) in a mixture of 1:1 (v / v) of PVLG (Polyvinyl - Lacto - Glycerol) and Melzer 's reagent for identification. Spores were identified by observing the type and morphology of spores by publication INVAM (http://invam.caf.wvu.edu/fungi/taxonomy) as well as a variety of sources such as Wilson [34], Morton [17], Morton and Benny [18] and Brundret [6].

B. Tests on indigenous AMF isolates

AMF species that have been identified do breeding on sterile sand medium with corn plants for 6 weeks. AMF inoculation treatment as much as 30 spores in suspension introduced on the roots of corn plants grown on a culture medium. AMF inoculant material from the culture medium showed colonization or spores contained in the media is used as a treatment in pot culture experiments.

Treatment of pot culture experiments are 9 species of AMF using completely randomized design (CRD) with 3 replications. Treatment of mycorrhizal inoculation, namely; F0 = non-mycorrhizal (control); F1 = *A. scrobiculata;* F2 = *G. etunicatum;* F3 = *G. luteum;* F4 = *G. mosseae;* F5 = *G. verruculosum;* F6 = *G. versiforme;* F7 = *S. gregaria;* F8 = *S. heterogama* and F9 = *Gi. sp*.

Sterilized planting medium from a mixture of fine sand and zeolite (w / w 1:1). Extraction of sand autoclaved by adding 100 mM sodium pyrophosphate pH 9.0 at 121 ° C for 1 hour. Na-pyrophosphate solution was poured and rinsed with distilled water. Mix sand and zeolite repeated sterilization by autoclave for 15 minutes. Pots filled with sterile medium and treated with 100 g of AMF inoculant at planting time.

Plants maintained for 8 weeks sprinkled with distilled water and the provision of nutrient solution. Given nutrient solution every week, starting at the age of 2 weeks using nutrient solution according to Osaki et al. [20]. Standard nutrient solution composition is : 30 mg N L^{-1} (NH_4NO_3); 0.6-1.0 mg P L^{-1} ($NaH_2PO_4.2H_2O$); 30 mg K L^{-1} (K_2SO_4 : KCl = 1:1); 50 mg Ca L^{-1} ($CaCl_2 .2 H_2O$); 20 mg Mg L^{-1} ($MgSO_4.7H_2O$); 2 mg Fe L^{-1} ($FeSO_4.7H_2O$); 0.5 mg Mn L^{-1} ($MnSO_4.4H_2O$); 0.5 mg B L^{-1} (H_3BO_3); 0.2 mg Zn L^{-1} ($ZnSO_4.7H_2O$); 0.01 mg Cu L^{-1} ($CuSO_4.5H_2O$); 0.005 mg Mo L^{-1} $(NH_4)_6.Mo_7O_{24}.4H_2O$).

C. Observation

Observations of soil samples is normal stability index aggregates [28], total glomalin [42] (www.ars.usda.gov/), Diversity AMF [8] includes spore density (SD), species richness (SR), Shannon-Wiener diversity index (H '), the isolation frequency (IF) and the relative abundance of spores (RA). Observations on the planting medium pot experiment includes a total glomalin and spore density (SD). Samples of plant roots were observed for the presence of AMF colonization.

Total glomalin was determined according to the USDA method (www.ars.usda.gov/). Briefly, 1 g of sample media / soil in 8 mL of 100 mM sodium pyrophosphate ($Na_4P_2O_7 . 10H_2O$), pH 9.0 and autoclaved at 121 ° C for 1 hour. Samples were centrifuged 5000 rpm for 15 min, the supernatant removed and repeat up to 3 times until the extracts is straw-colored or colorless. All supernatants were combined and measuring the volume of extract then centrifuged 10,000 rpm for 10 minutes. Move as much as 1 ml of the supernatant into a micro tube for measuring the content of glomalin.

Glomalin concentration was measured by a colorimetric Bradford protein assay (USDA method, www.ars.usda.gov/) using BSA (bovine serum albumin) as a standard. Measurements were conducted in 200 ul (micro liter) of PBS (Phosphate buffered saline) was added 10 ul of Bio - Rad dye reagent Coomassie Brilliant Blue R - 250 Staining Solution (produced by Bio - Rad Laboratories. Inc.). Color reaction is read by microplate reader at a wavelength of 595 nm after 5 min. Optical density is measured and compared against a standard curve of known concentration (1.25 - 5.0 ul) of the BSA. In the PBS standard solution reduced to the addition of 100 mM sodium pyrophosphate equivalent sample volumes (200 ul PBS - sample volume).

D. Statistical analysis

The AMF community structure indigenous on Ultisol determined by measuring the density of spores, species richness, relative abundance, frequency of isolation and the Shannon - Wiener diversity index. Spore density is a replication of AMF biomass. The relative abundance as a percentage of the number of spores of a species that demonstrates the ability of sporulation of AMF species. Frequency of isolation describe the distribution of AMF species in an ecosystem. All data were analyzed statistically using the Excel Analysis ToolPak and CoStat program version 8.0.

III. Result and Discussion

A. The diversity of arbuscular mycorrhizal fungi on Ultisol of the Darmasraya District

The AMF species found in Ultisol Darmasraya District is as much as 9 species. Characteristics of the diversity of AMF in both locations can be seen in Table 1 and Figure 1. At the location of Pisang Berebus found one species of *Acaulospora*, 5 species of *Glomus*, 2 species of *Scutellospora* and 1 species of *Gigaspora*. In the Sungai Langkok found 7 species consists of 5 species of *Glomus*, 1 species of *Scutellospora* and 1 species of *Gigaspora*. Species of the genus *Glomus* was found predominantly in the Ultisol from both locations, while the other genera found little.

At the location of Pisang Berebus found as many as 2714 spores per 100 g of soil, which is the largest spores of *G. Luteum* as many as 1009 spores and *G. versiforme* 999 spores which have relative abundance (RA) respectively 37.18 % and 36.81 % . In the Sungai Langkok found 367 spores per 100 g of soil with the highest number of spores of the species *G. versiforme* 134 spores (RA = 36.52 %) and *G. luteum* 107 spores (RA = 29.16 %). Fairly wide distribution of AMF species are at the location of Pisang Berebus (IF > 80 %), but at the Sungai Langkok, AMF species have low prevalence. The diversity of AMF species at both locations showed that the location of Pisang Berebus there are species richness (SR) 7.8 and 4.8 in the Sungai Langkok. Value of the Shannon diversity index (H ') is equal to 1.575 at the Pisang Berebus and 1,566 in the Sungai Langkok.

We found that the AMF *Glomus* is the type that has spread is quite extensive and dominant (IF> 80%) in the Ultisol Darmasraya, and more than 90% spores at both locations is derived from *Glomus*. It is almost as was also reported by Prihastuti [21], where in the acid dry land in Central Lampung found 8 species of AMF, including *G. mosseae*, *G. versiforme* and *Gi. margarita* is more dominant than others. In the hot dry valley areas in Southwestern China, Dandan and Zhiwei (2007) reported that there are six dominant species namely *G. clariodeum*, *G. Clarum*, *G. fasciculatum*, *G. verruculosum*, *G. sp 2* and *Gigaspora sp1*. They added that more than 80% of the total number of spores derived from *Glomus*. Furthermore, Wu et al. [43] stated that the agricultural areas in the Loess Plateau of China is only found from the genus *Glomus*.

TABLE I
CHARACTERISTICS OF THE DIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI

No	Arbuscular mycorrhizal fungi	Pisang Berebus			Sungai Langkok		
		Spore Number	RA (%)	IF (%)	Spore Number	RA (%)	IF (%)
1	Acaulospora Scorbiculata	35	1.29	80	0	0	0
2	Glomus Etunicatum	172	6.34	80	42	11.44	80
3	Glomus Luteum	1009	37.18	100	107	29.16	100
4	Glomus Mosseae	159	5.86	100	36	9.81	80
5	Glomus Verruculosum	183	6.74	80	32	8.72	40
6	Glomus Versiforme	999	36.81	100	134	36.52	100
7	Scutellospora Gregaria	79	2.91	100	12	3.3	40
8	Scutellospora Heterogama	42	1.55	60	0	0	0
9	Gigaspora Sp.	36	1.33	80	4	1.09	40
	Total	2714	100		367	100	

Activity of AM fungi in the Pisang Berebus is also better, which can be seen from the number of spores found 7 times more than in the Sungai Langkok. It is also supported by the content of total glomalin higher (3,604 $mg.g^{-1}$) at the location of Pisang Berebus than the Sungai Langkok (1.008 $mg.g^{-1}$). It can be understood that glomalin as building blocks in the walls of hyphae and spores [9], where an increase of activity AMF will generate high levels of glomalin. Differences of glomalin content for both sites also appear to be associated with soil aggregate stability. Soil aggregate stability was positively correlated with the content of glomalin [39],[23],[24],[41].

	***Acaulospora Scrobiculata*** Spores pale yellow color, form spores globose / 300 lm sieve rounded. Spore wall of three layers in which the outer layer is rather bright and pale yellow middle layer. Visible next to the separate components of the spore wall
	***Glomus Etunicatum*** Spores globose shaped reddish orange with a sieve size of 106 lm at 53 lm sieve. Spore wall of two layers, the outer arise turned a brownish red in Melzer's. Layer next to the colored bright yellow to arrange

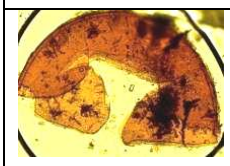
	***Glomus Luteum*** Spores form globoses yellow orange sieve 106 lm to 53 lm. Four layers of the spore wall, outside light hyaline, pale yellow on the inside and a layer of rigid, no change of color in Melzer's
	***Glomus Mosseae*** Spores brownish orange, globus and slightly rounded shape. Sieve of 300 lm to 106 lm. There are 3 layers of the spore wall, the outer bright colors and turned into a pink reddish in Melzer's. The middle layer is rather light and separate the inner brownish yellow color.
	***Glomus Verruculosum*** Color yellow orange spore globose shape, sieve size of 300 lm to 106 lm. There are two layers of the spore wall, the outside such as light-colored peel and blend with the inner layer of yellow orange
	***Glomus Versiforme*** Spores brownish red, globose shaped with a size between 53 lm and 106 lm. Spore wall of two layers, the outer rather bright and not reacting in Melzer's. The inner layer of brownish yellow
	***Scutellospora Gregaria*** Spores brownish red color, globose shaped sieve size of 500 lm at 300 lm trapped. Spore wall of two layers, the outer rigid dark brown and light brown inner visible, reacting in Melzer's reddish
	***Scutellospora Heterogama*** Spores dark red slightly rounded shape / subglobose. Spore size sieve 300 lm to 106 lm. Outer spore wall light brown and reddish brown inner
	***Gigaspora Sp.*** Pale yellow spore color, shape rounded sieve size of 500 lm at 300 lm stuck. Spore wall seen two layers, the outer brown color and a reddish orange color inside. not react in Melzer's

Fig. 1. The photograph spores of AMF indigenous on Ultisol in the District Darmasraya

The density of spores from both locations are also different (Table 1) which showed a glomalin (Figure 2). positive correlation with the content of soil

The highest total glomalin found in the Pisang Berebus locations ie from 2.14 to 4.54 mg.g^{-1} with spore density between 1824-4199 spores per 100 g soil. The Sungai Langkok location found a total glomalin <1.5 mg.g^{-1} and the number of spores between 155-637.

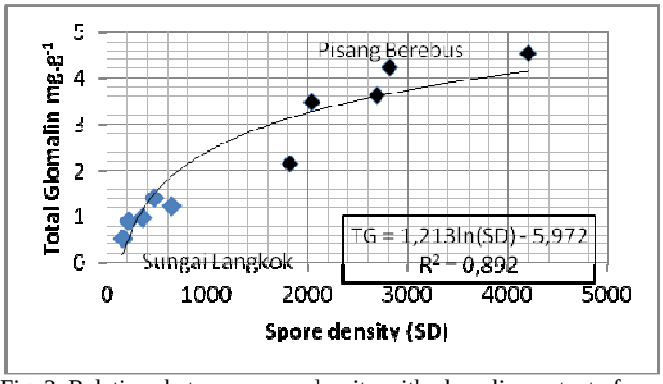


Fig. 2. Relations between spore density with glomalin content of the soil in The Pisang Berebus and Sungai Langkok

It is almost as same as was reported by Wu et. al. [43] where the density of AMF spores in the rhizosphere soil of wheat ranges from 350-1380 per 50 g of soil. He added that the content of glomalin negatively correlated with C/N ratio, but positively with the density of spores. Furthermore, Bai et al. [3] reported a significant and positive relationship between the density of spores and glomalin. Thus, the evidence indicates that glomalin contained in AMF spores and hyphae wall [9],[22], so the greater spore production by AMF may indicate higher glomalin production.

Based on the above it can be stated that the activities of the AMF can be explained from the measured total glomalin. Total glomalin higher illustrates the number of hyphae and spores produced by AMF too much. It can be understood that glomalin are the building blocks of hyphae and spores wall. This component is produced during activities symbiosis with plants, as described by Wright and Upadhyaya, [35] and Driver et. al. [9].

B. The content of Glomalin in cultur media

Each species of AMF will have the ability to grow and develop differently during activity symbiosis with the host plant. One way to determine the activity of AMF symbiosis is measured levels of glomalin produced.

The test results of 9 species showed significantly different glomalin content, especially *G.verruculosum*, *G. versiforme* and *G. luteum* (Table 2). The highest levels of glomalin is *G. verruculosum* (1.29 mg.g^{-1}), followed by *G. versiforme* is 1.17 mg.g^{-1} and *G. luteum* is 1.15 mg.g^{-1}. *Glomus mosseae* (0.65 mg.g^{-1}) showed the same effect with *S. gregaria* and *S. heterogama*. Total glomalin extracted range of 0.18 to 1.29 mg.g^{-1} soil, the lowest in the range reported by Wright and Upadhyaya [37]. Wright and Upadhyaya (1998) reported glomalin concentrations ranging from 1 to 21 mg g^{-1} soil in thirty-seven soil of five geographical locations. On the other hand, the concentration of glomalin in pot trial study by Antibus et al. [1] ranged from 1-5.5 mg.g^{-1} soil. This is also confirmed by Lovelock et al. [16] that the glomalin concentration is low for pot cultivation.

Interestingly, it turns out the concentration of glomalin or proteins are also measured on treatment without inoculation with AMF. The concentration of glomalin in the planting medium is the lowest in the treatment with no inoculation (0.0367 mg.g^{-1}), which showed no significant difference by treatment with *A.scrobiculata* inoculation. However, both of these treatments were statistically significantly different with

other treatments. According to Rosier et al. [26], a protein produced by organisms other than the AMF in the rhizosphere can be detected. He also found measuring by Bradford protein assay contained glomalin on treatment without AMF inoculation were not significantly different with the inoculation of *G. intraradices* or *Entrophospora colombiana*.

TABLE II.
LEVELS OF GLOMALIN (MG.G-1) IN THE GROWING MEDIA

No	Inoculation Treatment of FMA	Average of glomalin levels ($mg.g^{-1}$)
1	Glomus verruculosum	1.2900 a
2	Glomus versiforme	1.1667 a
3	Glomus luteum	1.1533 a
4	Glomus mosseae	0.6467 b
5	Scutellospora gregaria	0.4833 bc
6	Scutellospora heterogama	0.4633 bcd
7	Gigaspora sp.	0.4400 cd
8	Gigaspora etunicatum	0.2733 de
9	Acaulospora. Scrobiculata	0.1767 ef
10	Tanpa FMA	0.0367 f

The numbers followed by the same lowercase letter are not significantly different, which is followed by different lowercase letters are significantly different

Glomus tend to produce higher concentrations of glomalin, where the results are more varied with different AMF species. Therefore, differences in glomalin production by nine of indigenous AMF species were statistically different. The same thing Nichols and Wright [19] also reported that glomalin concentrations varied between five species of AMF, namely : *G. etunicatum*, *G. viscosum*, *G. caledonium*, *Gi. rosea*, and *Gi. gigantea*. In fact, they reported differences between isolates of the same species. Lovelock et al. [16] reported that glomalin production varies significantly across species of AMF in pot culture with maize as a host plant. They found that *A. morrowiae* produced glomalin higher than *Gi. rosea*, *G. etunicatum* and *G. intraradices*, and glomalin production by *G. intraradices* significantly lower than *Gi. rosea* and *G. etunicatum*. The differences in glomalin production may be due to differences in the activity of fungi [4], and environmental stress conditions can affect the production of glomalin [11].

C. Soil Aggregate Stability

Observations on the aggregate stability of both sites shows that the location of Pisang Berebus has an average value of NSI higher than the Sungai Langkok. The relationship between the content of glomalin to soil aggregate stability of the two locations can be seen in Fig. 2.

Aggregate stability for the location of Pisang Berebus is better than the location of Pisang Berebus with NSI values> 0.8 (0,82 – 1,0). Althought, the NSI values at the location of Sungai Langkok ranged from 0.6 - 0.74. Total glomalin at both the location also show the relationship with the NSI values are positively correlated. Aumtung, [2] have reported that the total glomalin positively correlated with soil aggregate stability. He added that the cultivation of upland rice on dry land containing a total glomalin higher than in lowland rice.

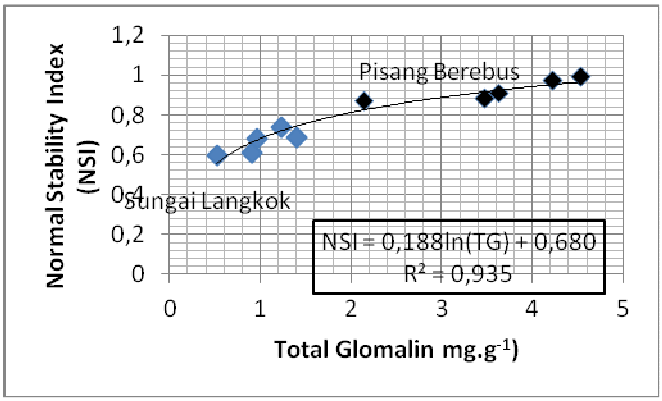


Fig. 3. Relations glomalin content with normal satibilitas index of soil aggregates on the Pisang Berebus and Sungai Langkok

The existence of AMF in the soil turned out play an important role in soil aggregation through hyphae. Subowo [29] has explained that the fungal hyphae capable of uniting the soil aggregates bind to each other, so it is not easily damaged and resistant to physical stress or erosion. Hyphae and spores of the AMF is composed of 80% glomalin [9] that functions as an organic adhesive uniting stable soil aggregates [40]. Hoorman et al. [13] have explained that the three processes simultaneously soil aggregation by AMF hyphae. The first physically unite the hyphae of soil particles. Second, fungi physically protect the clay and organic particles that make up the micro-aggregate. Third the plant root and fungal hyphae form glomalin that glues micro-aggregates and smaller macro-aggregates together to form larger macro-aggregates. Although bacterial effects on soil aggregate formation are found primarily on microaggregates but mycorrhizal effects are more evident on macroaggregates [25].

IV. CONCLUSIONS

This study is the first to determine the mycorrhizal diversity in Ultisol and glomalin production of corn from farmland in the Darmasraya District ,West Sumatra province. Our results indicate that the density of spores and AMF community is varied in the soil. We found there were nine of indigenous AMF species, where Glomus is relatively dominant in the rhizosphere soil of corn. In pot culture test, the results showed that the species of G. Verruculosum, G. versiforme and G. luteum produces glomalin was significantly higher than the other species. Total glomalin of the three species are 1.29; 1.17 and 1.15 mg.g-1 of soil respectively.

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