PENGEMBANGAN TEKNOLOGI SELEKSI IN VITRO BERULANG (REPEAT CYCLING – IN VITRO SELECTION) TOLERAN STRES KEKERINGAN UNTUK PERBAIKAN MUTU GENETIK DAN PRODUKSI TANAMAN KEDELAI BERMIKORIZA

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ABSTRACT

Every plant rhizospheres in any ecosystem there are various living microorganisms including Arbuscular Mycorrhizae Fungi (AMF). An isolation and characterization is required to investigate the species or type of the AMF. This research was aimed at studying the isolation and characterization of AMF sporulation in soybean rhizospheres in Jambi Province. The results of evaluation on soil samples before trapping showed that there are spores from three genus of AMF twelve types *Glomus* , two types *Acaulospora* and one type of *Enthrophospora*. Following single spore culture in soybean rhizosphere, 5 spore types were obtained: *Glomus sp*-1, *Glomus sp*-4, *Glomus sp*-7, *Glomus sp*-8 *Glomus sp*-10.

Key words: Soil microbiology, soybean, *Glomus, Acaulospora, Enthrophospora.*

INTRODUCTION

Fungus symbiosis with the roots of plants form the external and internal structures. The external structure is a structure that is formed outside the FMA roots while the internal structure is the structure of the mold is formed in the root. In general the structure is owned by FMA can be hyphae, hyphae bight, arbuscular, vesicles and spores. At first the only existing technology capable of utilizing spore structure as the material for the identification and determination FMA (Abbott 1982), but with the development of technology characterization and identification of FMA can be done with serological tests , (Wilson *et al*. 1983; Thingstrup *et al*. 1995), fatty acid analysis (Bentevenga & Morton 1994), isozyme analysis (Sen & Hepper 1986), even for ecology and biodiversitasnya identification can be used with PCR (Giovanetti & Gianinazzi-Pearson, 1994; Clapp & Edwards 1995).

Spores isolated from the field are not optimally used as the identification and determination. This is because in addition to a limited number due to too contaminated or affected by nematodes so that spores are not intact (Smith & Read 1997). Trapping (trapping) spores is one technique to get the spores intact with environmental manipulation spore production can be increased (Brundrett *et al*. 1994).

The study of the diversity of FMA in Indonesia have been carried out. FMA Research inventory of biodiversity by identifying the spores have been conducted on stands of teak clonal seed orchards pastures are found three genera FMA namely as *Glomus, Sclerocysti*s and *Acaulospora* (Maryadi 2001). Irmawati (2001) found four genera FMA namely *Glomus, Sclerocystis, Gigaspora* and *Acaulospora* contained under teak stands and Wulandari (2001) find one genus FMA on taro. Meanwhile Retnaningsih (1998) examined the structure of paris type of infection in the root Salak some cultivars.

Research carried out aimed to isolate, characterize and purification FMA soybean plants. Research conducted has a special purpose, to isolate natural FMA isolates from soybean roots rhizofir, identify and characterize the type FMA and purify isolates were obtained through single spore culture.

MATERIALS AND METHODS

Soil sampling carried out in the zone rhizosfir soybean roots in Experimental Garden, Jambi University with a depth that is 0-20 cm, with a distance of 5-10 cm from soybean roots. Soil excavated in sections corresponding to the sampling technique used is 1, 2, 3, 4 points and roving soybean plant roots, taken 500 g soil for the first point of soil sampling. For soil sampling 2, 3, 4 points and the soil around plant roots soybean mixed well in advance just taken 500 g soil, then put in a plastic bag and labeled. The soil samples have been taken subsequently used for observation of the initial spore, spore dispersal most of each sampling point is used as the basis of soil sampling in future studies. Once known technique best soil sampling, soil sampling subsequently to be used in this study conducted at different depths are 20-40, 40-60, 60-80 and 80-100 cm. This is done considering the soybean roots deep enough.

Spores FMA Natural isolation. Spore observation activities were done at the Seed Technology Laboratory, Faculty of Agriculture, University of Jambi. Isolation of spores from the soil sample carried out following the casting method strain (Gedermann & Nicolson 1963). Furthermore, the counting of the spores obtained under the microscope. Spore extract identified/determination to genus level (Schenck & Perez 1988; Brundrett et al., 1996). Besides spores also observed morphological and are divided into several types at once numbered. Observation types and spore morphology and structure infections performed by staining the roots of example following the method Kormanik and McGraw (1982). If the results of observations show that the number of spores is very little that is likely when soil sampling sporulation not in season, then do activities trapping spores in advance.

Trapping techniques were used to follow the method Brundrett et al. (1994) using pots of culture (200g volume). Furthermore, the culture is placed in the greenhouse and maintained for + 5 months by doing the watering, the provision of nutrient and pest control manually. Nutrient solution used is red Hyponex (25-5-20) with a concentration of 1 g / 2 liter of water. The provision of nutrient solution every week as much as ± 20 ml per pot culture.

After a 5-month-old culture, media sampling was conducted to see the level of sporulation of the culture. If from soil samples discovered new spores then was examined for spores of 50g culture media. The parameters measured were the number of spores per 50 g of growing media and spore types.

Identification and Characterization of Spore FMA. Extraction FMA done to separate the spores from soil samples enables the identification FMA. The technique used is the technique of cast-strain of the casting method filter (Gedermann & Nicolson 1963), followed by centrifugation techniques of Brundrett (1996). The results of the last filter in the filter-casting techniques in the centrifuge tube plus 60% glucose by using a pipette. Centrifuge tube was sealed and centrifuged at a speed of 2,500 rpm for 3 minutes. Furthermore, the

supernatant solution is sucked by the suction pipette and poured into a 45 μm filter, washed with running water (tap water) to remove glucose. The precipitate is left in the sieve above, is poured into a petri dish and then examined under a dissecting microscope for counting spores and spore production of preparations in order to identify existing FMA.

Identification and Characterization of Spore FMA. Extraction FMA done to separate the spores from soil samples that can be identified in order to determine the genus spores FMA FMA. The technique used is the technique of cast-strain of the casting method filter (Gedermann & Nicolson 1963), followed by centrifugation techniques of Brundrett *et al*. (1996). The results of the last filter in the filter-casting techniques in the centrifuge tube plus 60% glucose by using a pipette. Centrifuge tube was sealed and centrifuged at a speed of 2,500 rpm for 3 minutes. Furthermore, the supernatant solution is sucked by the suction pipette and poured into a 45 μm filter, washed with running water (tap water) to remove glucose. The precipitate is left in the sieve above, is poured into a petri dish and then examined under a dissecting microscope for counting spores and spore production of preparations in order to identify existing FMA.

Spores identified extraction/determination to genus level (Schenck & Perez 1988; Brundrett *et al*. 1996). Besides spores also observed morphological and differentiated into several types at once numbered. Making preparations spores using Melzer's reagent dyes and preservatives PVLG placed separately on a single glass slide. Spora- spores carefully solved by pressing the cover glass preparations using the tip of the rib. Change the color of spores in Melzer's is one to determine the type of spore.

Making the Single Spore culture. Making a single spore culture refers to methods that do Mansur (2000), namely petridish Observation Chamber (PDOC). Petri dishes that will be used as a place of investment culture first perforated (0.5 cm x 0.5 cm) on the edges that serves as the emergence of sprouts. *P. japonica* seedlings that have had 2-3 leaves (aged 7-10 days after sowing) were placed on white paper or tissue. Spores isolated from trapping that has been collected in the watch glass is taken with tweezers and placed on the roots of the seedlings. Each seed is only inoculated with the spores. Furthermore inoculated seedlings were transferred to the culture medium with the position of the seedlings placed at the edge of a plastic petri dish that has been hollowed. Culture plastic petri dish wrapped in aluminum foil, then placed in a small plastic tub that serves as a water and nutrient solution for culture. Culture will be maintained for 3 months depending sporulation happens. If the spores are formed've pretty much there will be sub-culture into pots larger culture.

RESULTS

Natural Spore isolation. Based on the experimental results of mycorrhizal spores from soil samples at various pick-up points early in the root zone soil (0-20 cm depth) seen no difference in the spread of mycorrhizal spores. Natural spore density prior to any spore trapping only 1-5 per 50 g soil (Table 1). In Table one shows that the type of spores on the location of the former land of soybean plants are one type of mycorrhizal spores which *Glomus sp*.

Table 1. Number of FMA spores from soil samples at various pick-up points at a depth of 0-20 cm at each location

No	location Decision The soil samples	type FMA	Total spore FMA
1.	A1	*Glomus sp*-1a	3
	B1	*Glomus sp*-1b	2
	C1	-	-
	D1	*Glomus sp*-1d	1

Description: - not found spores
A,B,C,D = 1,2,3,4 consecutive sampling point land

Soil sampling at different depths in the soil had no effect on the spread of mycorrhizal spores. Observation of spores in soil samples found only at a depth of 0-20 cm, while the depth (20-40 cm, 40-60 cm, 60-80 cm, 80-100 cm) spores was not found. This means increasing the depth of the soil increasingly rare spore does not even exist.

Table 2. Number of FMA spores from soil samples at one point taking on the depth of 0-20, 20-40, 40-60, 60-80, 80-100 cm

location Decision The soil samples	type FMA	Total spore FMA
A1	*Glomus sp*-1a	3
A2	-	-
A3	-	-
A4	-	-
A5	-	-

Description: - not found spores
- numbers 1, 2, 3, 4 and 5 to follow the letter A represents the order of the depth of sampling

Identification and characterization of spores FMA after Trapping. Land next soybean crop marks are used for trapping mycorrhizae aimed at preserving existing mycorrhizal so well developed with zeolite media and the use of host plants Pureria Japonica. Implementation of trapping can be seen in Figure 1.

A. Age 1 month B. Age 3 month

Figure 2. Developments trapping *Pueraria japonica* plant growth as host mycorrhizal

Based on the experimental results the number of spore trapping results per 50 g of sample soil at the age of 2-3 months the number of spores that looks 1-15 spores. It is presumed that at that age spores germinate is not perfect, as shown in Table 3.

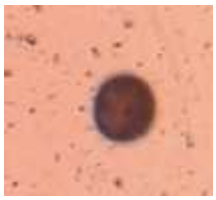
Types of spore trapping results dominated of Glomus sp. with 12 different types of spores, *Acaulospora sp* with 2 types of spores and *Enthrophospora* with one type of spore as shown in Table 4.

Tabel 3. Number of FMA spores from soil samples at various pick-up points at a depth of 0-20 cm at each location

No.	Spore Types	Total spore FMA
1.	*Glomus sp*-1	11
	Glomus sp-2	8
	Glomus sp-3	9
	Glomus sp-4	12
	Glomus sp-5	11
	Glomus sp-6	10
	Glomus sp-7	15
	Glomus sp-8	8
	Glomus sp-9	6
	Glomus sp-10	8
	Glomus sp-11	8
	Glomus sp-12	9
	Acaulospora sp-1	5
	Acaulospora sp-2	3
	Enthrophospora sp-1	1

Table 4. Characteristics of spore types were isolated from the rhizosphere of soybean plants in the garden Experiment, Jambi University, trapping results after 3 months.

No	Spore Types	Morphological Characteristics	The reaction with Melzer's
1.	*Glomus* sp-1 	Spores round, yellow, clear, smooth surface and thick-walled, funnel-shaped hyphal attachment. Spores sieve size 125 m	Do not react with the dye Melzer's
2.	*Glomus* sp-2 	Spores round, reddish, smooth surface. Spores sieve size 125 m	Do not react with the dye Melzer's

3.	*Glomus* sp-3 	Spores round (oval), yellow brownish, smooth surface. Spores sieve size 325 m	Do not react with the dye Melzer's
4.	*Glomus* sp-4 	Spores spherical, translucent yellow very light, smooth surface. Spores sieve size 325 m	Do not react with the dye Melzer's
5.	*Glomus* sp-5 	Spores round, brownish-colored, smooth surface. Spores sieve size 125 m	Do not react with the dye Melzer's
6.	*Glomus* sp-6 	spores Round, Thin walls No attachment, Brown, spores sieve size 125 m	Do not react with the dye Melzer's
7.	*Glomus sp-7* 	Round, Thin Wall No attachment, Yellow Color. Spores sieve size 125 m	Do not react with the dye Melzer's
8.	*Glomus Sp-8* 	oval shape, Thin walls No attachment, Brownish yellow color. Spores sieve size 325 m	Do not react with the dye Melzer's
9	*Glomus Sp-9* 	Round, wall thickness, No Attachment, Brown. Spores sieve size 325 m	Do not react with the dye Melzer's

10.	*Glomus Sp-10* 	Round, wall thickness No attachment, Brown. Spores sieve size 125 m	Do not react with the dye Melzer's
11	*Glomus Sp-11* 	Oval, Hyphal attachment Thin Walls, Color Yellow, Spore sieve size 325 m	Do not react with the dye Melzer's
12.	*Glomus Sp-12* 	Oval, wall thickness Hyphal attachment, straight shaped brownish color. Spore sieve size 325 m	Do not react with the dye Melzer's
13.	*Acaulospora sp*-1 	Spores spherical, yellow-brown, thick-walled smooth surface. Spores sieve size 325 m	Reacts with the dye Melzer's. Discoloration where the spores inside the crimson and yellow exterior
14.	*Acaulospora sp*-3 	Spores round, reddish brown, rough surface such as orange peel and thick-walled. Spores sieve size 325 m	react with the dye Melzer's
15.	*Enthrophospora sp*-1 	Spores round, brownish yellow, smooth surface, spores sieve size 125 m	Do not react with the dye Melzer's

Single spore cultures

Implementation of a single spore cultures were performed for about 5 months. Single-spore cultures can be said to be successful if visible hyphae and spores in cultured single spore culture after a 5 month old as shown in Figure 3.

Figure 3. Single-spore cultures Glomus sp-1 (A) field Glomus sp-4 (B) 5 month old, spores and hyphae were seen in cultured single spore culture

From the implementation of the culture of spores single after five months turned out spores that develop from the 12 types of spores only 5 types namely Glomus Sp-1, Glomus Sp-4, Glomus Sp-7, Glomus Sp-8, Glomus Sp-10, fully developed and can forwarded to be applied to soybean crops in future studies. Spore types can be seen in Figure 4.

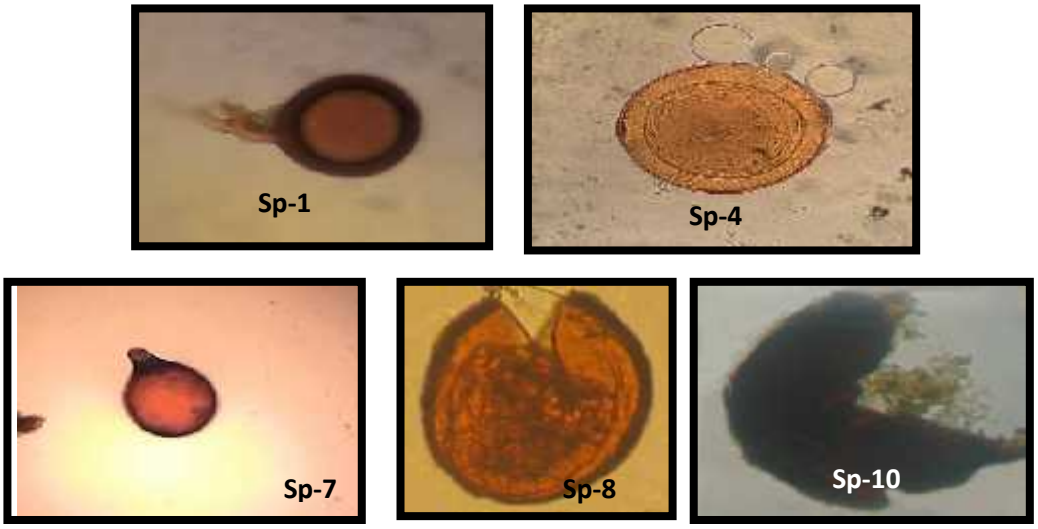


Figure 4. 5 spore type single spore culture results

Mycorrhizal infected roots can be seen in Figure 5. The infections are characterized by arbuscular, vesicles, hypha and spore or P. javanica root cortex tissue.

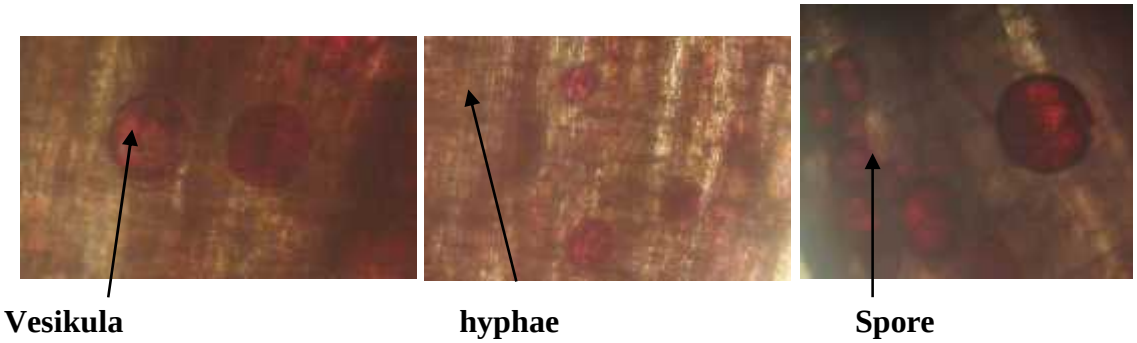


Figure 5. The root of P. javanica infected mycorrhizal

DISCUSSION

The density of spore trapping is only found naturally after 6-15 spores / 50 g soil. This result is lower than the results of the study Nadarajah and Nawawi (1997) which found 33-63 spores / 50 g soil, Delvian (2003) 31-134 spores / 50 g soil in the coastal forests and Widiastuti (2004), 3-103 / 100 g soil and Kartika (2006) 1-10 spores, on oil palm rhizosphere. The low density of natural spores in soybean rhizosphere sporulation is suspected FMA yet, but contains more other propagules like hyphae. As shown in Figure 3 above.

FMA species diversity of soil sampling can not be observed at the initial soil sample for the amount of spores that there is very limited. But spore types can already be identified at the beginning of the isolation of spores land where it was found five types of spore that is the type Glomus, but after trapping spore types are found there are 12 types of Glomus. The diversity

of species in the get was the same as research Hanafi ((2000) who found five types of spores (2 Gigaspora sp and 3 Glomus sp) on the ground latosol that disawahkan is rainfed. The existence of the diversity of types and spore types that exist in the observation location, resulting because of the type of vegetation that is under soybean crop stands and their management as well as soil sampling time.

At the time of the initial land acquisition for characterization are generally found only one genus Glomus spores that. But after trapping genus found spores remain the same, but the type that grow are of 5 types before trapping and 12 types after trapping. This is presumably due to the spread of spores genus are limited, so the possibility genus spores were found at a particular location at a particular soil type and time may not be representative of the entire spore genus FMA in the area.

The results of this study may not have been able to provide a clear picture of the diversity in the rhizosphere of plants, given at the time of this study lasted soy (1 time observations) at any time after the harvest, the spores were recovered from soil samples is very limited so it could not be detected genus. These variations show a pattern that is not equal to the change in seasons (precipitation). While it is generally not fluctuate spore density is high with the change of seasons (precipitation). While the observations were made during the dry season.

Spore formation or sporulation FMA is also influenced by environmental factors, host plants and other fungi. In many cases the factors that stimulate or inhibit the process of colonization of roots also stimulate or inhibit the formation of spores FMA (Bardgett et al. 1999). But despite colonization and sporulation, closely related, according to Abbott and Gazey (1994), these two phenomena can not be said to always have a positive relationship. In this research shows the trend of increase in the number of spores in a reduction in the amount of rainfall.

CONCLUSION

1. In general, soil sampling at four different depths point that is 0-20 cm, 20-40 cm, 40-60 cm, 60-80 cm, 80-100 cm and soil sampling at the root around not affect the amount, types and spore dispersal patterns.

2. Type FMA successfully isolated and identified on soybean rhizosfir before trapping found only one genus FMA is *Glomus*, with five types of spore

3. Diversity FMA spore types and dynamics sporulasinya in rhizosfir soybean influenced by season and soil fertility. The most dominant spore types are *Glomus.*

4. Type FMA has been identified and characterized in rhizosfir soybean after trapping (trapping) is one type of FMA is *Glomus sp* with 12 different types, *Acaulospora* with two types of spores and *Enthrophospora* with one type of spore.

5. Type FMA successfully isolated and propagated from a single spore culture in soy rhizosfir there are 5 types of spores that *Glomus sp*-1, *Glomus sp*-4, *Glomus sp*-7, *Glomus-sp8, Glomus sp*-10.

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