International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Diversity of Arbuscular Mycorrhiza Fungi from trapping using Different Host Plants

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Abstract

Arbuscular mycorrhizal fungi (AMF) is an obligate symbiont that can only grow and develop with the presence of host plant. A greenhouse study has been conducted to study the effect of host plants in AMF trapping process. The research design is complete randomized with 9 treatments and 3 replications. Four types of plants was used as host plant, namely kudzu (*Pueraria javanica*), sorghum (*Sorghum bicolor*), corn (*Zea mays*) and 6 varieties of soybean (*Glycine max*), ie varieties of Tanggamus, Anjasmoro, Slamet, Wilis, lines of Pangrango Godek and Sibayak Pangrango. Trapping is performed by using pot culture containing 50 g of soil taken from tidal area of Simpang Village, District of Berbak, Regency of East Tanjung Jabung province of Jambi as a source material for AMF inoculants and 150 g of zeolite as growing culture.

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The experimental results showed that there were 2 species of genus Acaulospora and 5 species of host plants of the genus Glomus. Zea mays is the best host according to variables of spore abundance, root colonization, inoculums weight, root fresh and dry weight.

Keywords: obligate symbiont; tidal swamp; Pueraria javanica; Sorghum bicolor; Zea mays; Glycine max.

1. Introduction

The main constraint on cultivation in the tidal swampland is the low P available to plants. Therefore, use AMF to take advantage of P in the form bound with other elements, to become available to plants. Utilization of AMF local is so that AMF adaptation to the environment cultivation, faster and optimal. Therefore the number of AMF spores obtained from land that will be planted with soybeans is still relatively low, it is necessary to trapping. Trapping AMF uses a host plant for the AMF are obligate symbionts.

AMF has wide host distributions, but with specific effect on plants colonized. Development of AMF colonization began with apresorium formation in the root surface by external hyphae derived from spores or roots with Mycorrhiza in the soil. Furthermore, the hyphae of the apresorium penetrate epidermal cells and spread among cells or in the cells along root cortical cells. Mycorrhiza roots will form free external hyphae network as continuation of intern hyphae that propagates in the soil. Generally AMF will form resting spore in the soil either singly or sporokarp until connect to roots of host plants. Spores can only be isolated from the soil with certain screening techniques [1].

Some of common AMF genus are *Glomus, Gigaspora, Acaulospora* and *Scutellospora* [2]. However, each type of AMF has different abilities in helping plants growth [3]. Thus, the selection of totally compatible isolates AMF with cultured plants needs to be performed. AMF living in symbiosis with responsive host plant and has many roots [4]. The types of host plants that are commonly used to propagate spores are annual crops because fast growing and produce many fibers root than perennial crops. That makes endo mycorrhiza reproduction does not need long time [5]. Annual crops such as corn and sorghum is highly compatible as AMF host [4] so that corn and sorghum used as host for AMF spores propagation [5].

According to [6], a number of mycobion strains can be associated with one plants species or varieties. Despite there is no specificity for AMF host, but association between AMF and roots can provide different levels of colonization on the root system and also in their influence on nutrient absorption and plant growth. These response differences are influenced by species and genotypes of plants, as well as the environment as soil pH and available P content in the soil. The most commonly used method to produce AMF inoculants is pot culture that is effective AMF inoculated in certain host plants on a sterile solid medium [4].

Selection of soybean as host plants is based on the reason that soybean has not much different growing environmental conditions than corn. Therefore, if soybean can be a good host, then trapping and AMF spore reproduction at the field level will be easier. Selection of several soybean varieties and strains are based on the idea that there is different AMF symbiotic for each soybean variety and strain, in addition to the host of corn, sorghum and kudzu.

The research' objectives are to obtain AMF diversity in soybean rhizosphere of tidal area, Simpang Village, District of Berbak, Regency of East Tanjung Jabung and to test the ability of some types of plants as host plant for AMF trapping.

2. Materials and Methods

In this research, trapping was carried out in Forest Ecology Greenhouse, Faculty of Forestry. The identification was carried out in Laboratory of Forest Biotechnology and Environment, Research Center for Biological Resources and Biotechnology, IPB, Kriptogam Laboratory, LIPI and Wood Anatomy Laboratory, Center for Research and Development of Forestry Engineering and Forest Products Processing, Ministry of Forestry, Bogor. The research was conducted from September - November 2013.

The materials used for culture preparation is seed of kudzu (*Pueraria javanica*), seeds of sorghum (*Sorghum bicolor*), seed of corn (*Zea mays*) and 4 varietas and 2 lines of soybean (*Glycine max*), 2 mm zeolites, PVLG and Melzer solution, aluminum foil, AMF isolates obtained from soybean rhizosphere, red Hyponex solution (0.05% by Hyponex red = 0.5 g per liter of water) and distilled water. The tool used is a multilevel filter (425 μm, 300 μm, 150 μm and 53 m), micro tweezers, sprayer bottle, and binocular microscope, plastic cup 200 g as pot culture which has been hollowed at the bottom, shelves and plastic trays.

The research design is complete randomized with 9 treatments and 3 replications. Four types of plants was used as host plant, namely kudzu (*Pueraria javanica*), sorghum (*Sorghum bicolor*), corn (*Zea mays*) and 6 varieties and 2 lines of soybean (*Glycine max*), ie varieties of Tanggamus, Anjasmoro, Slamet, Wilis, lines of Pangrango Godek and Sibayak Pangrango.The parameters measured were AMF type, spores abundance, roots colonization, wet biomass, dry biomass, roots wet biomass, dry root biomass, inoculum weight as roots colonization multiplied with wet root biomass weight, roots nutrient, shoots nutrient, plant nutrient content and sporulation dynamics. The data were analyzed using analysis of variance and followed by Tukey test.

Trapping techniques followed [2] using cultures pots with a size of 200 g. Planting medium is soil from research sites as much as ± 50 g and 1-2 mm zeolite rocks as much as ± 150 g. Culture media are culture pot filled with zeolite 100 g, then put soil samples 50 g and covered with zeolite 50 g. The planting medium is composed of zeolite- soil samples- zeolite. Seed of *Pueraria javanica*, *Sorghum bicolor*, *Zea mays* and *Glycine max* for host plant soaked previously in a solution of sodium hypochlorite (NaOCl) 5:25% for 10 minutes for surface sterilization. The seeds are soaked in warm water and allowed to stand for ± 24 hours to break dormancy that may occur. The seeds are sown in culture tub for ± 10 days. Once it sprouts, transferred directly into culture pots. Culture maintenance includes watering, nutrients application and manually pest control. Nutrient solution used is red compound fertilizer containing low P (25-5-20) with concentration of 0.5 g per liter of water (0.05%). Nutrient solution application conducted two times every week as much as ± 20 mL per pot culture. After three months old, the culture harvesting to get spores that will be used in the next experiment namely compatibility test source of arbuscular mycorrhizal fungi inoculant on soybean plant with saturated soil culture and conventional cultivation.

3. Results and Discussion

The use of trapping culture methods are intended to obtain new spore that had not experiencing sporulation previously. After trapping, the new types of spores will appear and can be various types and have high viability when cultured again. Maximum root colonization will be achieved in less fertile soil condition. Both N and P would reduce the roots colonization when present in high levels of availability. Colonization will increase if the content of N and P were moderate, but on high condition of P, the addition of N will be the obstacle to the formation of root infection and the number of spores. This research indicates that trapping with various types of host plants increase the number of AMF species. Early research in the characterization and identification of AMF on the land to be planted with soybean found two species of genus *Glomus* namely *Glomus fasciculatum* and *Glomus fecundisporum*. After trapping, it can be found 7 AMF species from 2 genus, namely *Glomus* and *Acaulospora*. For genus Glomus, there were five species namely *Glomus fasciculatum, Glomus Clarum, Glomus macrocarpum, Glomus fecundisporum* and *Septoglomus constrictum*. For genus *Acaulospora*, there were two species namely *Acaulospora scrobiculata* and *Acaulospora tuberculata*. Observations on the number of spores show that spore ans species obtained before trapping on soybean rhizosphere from tidal swampland is lower than after trapping. This is presumably because before trapping, mycelium and roots have not grown well. After trapping, mycelia in the soil develop properly supported by environmental conditions and sufficient nutrient metabolite.

Table 1: Type and abundances of AMF spores at 90 days after planting (HST) from different host plants

Host type	Type of spore	Spore abundance	Number (spore)
Zea mays	*Glomus clarum*	190.00	203.33 a
	Acaulospora tuberculata	13.33	
Pueraria javanica	*Glomus macrocarpum*	1.33	2.00 b
	Acaulospora scrobiculata	0.67	
Sorghum bicolor	*Glomus clarum*	168.67	168.67 a
Glycine max var Tanggamus	*Glomus fecundisporum*	0.33	0.33 b
Glycine max var Anjasmoro	*Septoglomus constrictum*	0.33	0.33 b
Glycine max var Selamet	*Septoglomus constrictum*	1.33	1.33 b
Glycine max strain Pangrango Godek	*Glomus fasciculatum*	0.33	0.33 b
Glycine max strain Sibayak Pangrango	*Glomus clarum*	0.33	1.00 b
	Septoglomus constrictum	0.67	
Glycine max varietas Wilis	*Septoglomus constrictum*	0.33	0.33 b

Note: Numbers followed by the same lowercase showed no significant difference in the level of 5% of Tukey test

AMF spores produced by two type of host vary in type and number. Zea mays produces two species namely *Glomus Clarum* and *Acaulospora tuberculata* with the number of spores higher than any other host plants. In terms of the number of spores, only *Sorghum bicolor* was able to balancing *Zea mays* but there was only one species can be trapped by *Sorghum bicolor*, namely *Glomus Clarum*. *Glycine max* produces low type and number of spores. Each tested variety or strains of *Glycine max* as host plant only produces one species and amount ranges between 0.33 to 1.33 spores. Interesting phenomenon is type of host plants interact with different types of AMF, even different varieties or strains of *Glycine max* interact with different types of AMF (Table 1).

Characteristic of AMF found in this study are presented in Table 2 and visually presented in Figure 1. These characteristics compared with the criteria in the guidelines proposed by [7] to determine the type of AMF found.

The type of host plants affects root colonization. The highest root colonization is corn and sorghum, 56.40% higher and significantly different compared to the colonization of Willis and significantly different from all other host plants. Weight of fresh root is influenced by the type of host plant. *Sorghum bicolor* has root fresh weight 19.2 g higher and significantly different compared to varieties of Anjasmoro, significantly different from other host plants, except *Zea mays*. The type of host plants affects root dry weight. The heaviest root dry weight is *Sorghum bicolor*. Dry weight of Sorghum bicolor is 5,26 g heavier and significantly different compared to Glycine max varieties Anjasmoro and significantly different to others host plants (Table 3).

Table 2: List of AMF types which symbiosis with the host plant, result of soybean rhizosphere isolation of tidal area and trapping

Family/Genus/Species	Main characters of spore					
	Structure	Colour	Form	Size	wall thickness	Wall layer
Glomerales						
Glomeraceae						
Glomus macrocarpum	Aggregate	Brown	Roundish	101 – 132 x 97-128	7.12 – 18.05	1
Glomus fasciculatum	Aggregate	Yellow	Rounded	56 - 188 x 43 - 195	6 -15	1 – 2
Glomus clarum	Single	Yellow	Rounded	172 x 186	8	1
Glomus fecundisporum	Single	Yellow	Rounded	147 - 217 x 161 - 217	5 – 12	2
Septoglomus constrictum	Single	blackish brown	Rounded	268 - 255	12.06	1
Acaulosporaceae	Single		Rounded			
Acaulospora scrobiculata	Single	Clear	Rounded	131 - 128	5	2
Acaulospora tuberculata	Single	Tawny	Rounded	256 - 310	12	3

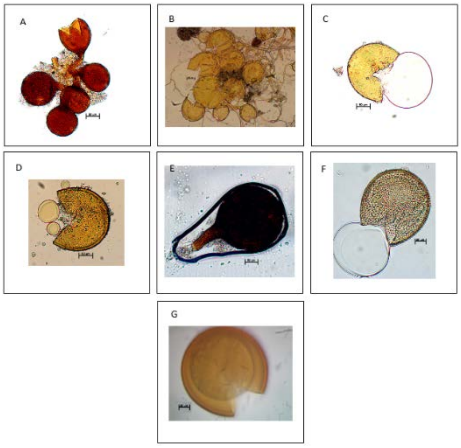

Figure 1: AMF Types associated with host plants, the result of soybean rhizosphere isolation and trapping : A. *Glomus macrocarpum*; B. *Glomus fasciculatum*; C. *Glomus Clarum*; D. *Glomus fecundisporum*; E. *Septoglomus constrictum*; F. *Acaulospora scrobiculata*; G. *Acaulospora tuberculata*.

Table 3: Roots colonization, inoculums weight, the dynamics of sporulation, wet and root dry weight at AMF trapping with different host plants

Treatment	Roots colonization (%)	Inoculums weight	Root fresh weight (g)	Root dry weight (g)
Zea mays	70.73 a	7.62 a	10.46 a	5.49 a
Pueraria javanica	17.93 b	0.47 c	2.23 c	1.41 cd
Sorghum bicolor	70.73 a	5.56 b	7.86 b	3.55 b
Glycine max varietas Tanggamus	14.77 b	0.34 c	2.62 c	1.78 c
Glycine max varietas Anjasmoro	16.47 b	0.16 c	0.94 c	0.68 d
Glycine max varietas Selamet	12.10 b	0.32 c	2.01 c	1.42 cd
Glycine max galur Pangrango Godek	10.40 b	0.27 c	2.36 c	1.71 c
Glycine max galur Sibayak Pangrango	22.30 b	0.57 c	2.59 c	1.63 c
Glycine max varietas Wilis	14.33 b	0.22 c	1.39 c	1.12 cd

Note: The numbers followed by the same lowercase showed no significant difference in the level of 5% of Tukey test

Species and varieties of host plants have different root wet weight. The heaviest roots wet weight is *Zea mays*, namely 9,52 g heavier and significantly different compared to *Glycine max* varieties Anjasmoro and significantly different from all other host plants. Root dry weight is influenced by the type of host plant. *Zea mays* is host plant with root dry weight 4.81 g heavier and significantly different compared to *Glycine max* varieties Anjasmoro and significantly different from the other host plants.

Inoculums weight is determined by types of species and varieties. The heaviest inoculums weight is from *Zea*

mays. Weights of *Zea mays* inoculums is 7,46 g heavier than *Glycine max* varieties Anjasmoro and significantly different to other host plants. The highest spores abundance, root colonization, inoculums weight, root wet and dry weight was found for *Zea mays* and significant difference to other host plants, followed by *Sorghum bicolor* were also significantly different with other host plants.

Type of host plants has varying levels of K root. The K root content of *Sorghum bicolor* is 1,25% larger than Glycine max strains Sibayak Pangrango and significantly different from all other host plants. K content is influenced by the type of host plant. The largest K content is *Sorghum bicolor*, that is 0.8% higher and significantly different compared with *Pueraria javanica*, significantly different to strain of Glycine max Pangrango Godek, but not significantly different from other host plants (Table 4).

Tabel 4: Content of N root, P root, K root, N, P and K plant of host in AMF *trapping*

Treatment	Variable					
	N root (%)	P root (%)	K root (%)	N plant (%)	P plant (%)	K plant (%)
Zea mays	0.50 g	0.25a	2.13 ab	0.66 f	0.33a	1.78 ab
Pueraria javanica	2.14 a	0.37a	1.74 bcd	1.60 e	0.33a	1.33 b
Sorghum bicolor	0.56 f	0.33 a	2.57 a	0.61 f	0.39 a	2.13 a
Glycine max varietas Tanggamus	2.06 b	0.30 a	1.81 bcd	2.61 b	0.40 a	1.59 ab
Glycine max varietas Anjasmoro	2.08 b	0.29 a	1.65 bcd	2.95 a	0.43 a	1.58 ab
Glycine max varietas Selamet	1.66 d	0.32 a	1.51 cd	2.46 c	0.43 a	1.56 ab
Glycine max galur Pangrango Godek	1.50 e	0.32 a	1.47 cd	2.54 bc	0.41 a	1.51 b
Glycine max galur Sibayak Pangrango	1.91 c	0.35 a	1.32 d	2.07 d	0.43 a	1.58 ab
Glycine max varietas Wilis	2.03 b	0.34 a	1.91 bc	2.74 ab	0.43 a	1.78 ab

Note: The numbers followed by the same lowercase showed no significant difference in the level of 5% of Tukey test

Type of host Plants have varying levels of N roots. The root N content of *Pueraria javanica* is 1.64% higher than Zea mays and significantly different to all other host plants. N content was affected by the type of host plant. The highest N content is Glycine max varieties Anjasmoro. N content of Anjasmoro is 2,34% higher and significantly different compared to *Sorghum bicolor* and other host plants, except Wilis. The type of host plant does not affect the levels of P roots and plants.

Host plants are of legumes group, namely *Pueraria javanica* and *Glycine max* produce higher N levels and uptake than graminae groups such as *Zea mays* and *Sorghum bicolor*. This is reasonable because legumes host

has the ability to symbiosis with rhizobium bacteria to fix N from the air. Therefore, the potential use of rizobakteria as inoculants has received much attention from soil microbiologists and plant diseases experts, due to the nature of this rhizobacteria is very aggressive for root colonizing in replacing microorganisms that can cause disease in plants [8]. The relationship between plants and microorganisms occur in the rhizosphere. Microorganisms can survive from substrate released by plants through roots or dead plants, stimulate nutrients from the roots [9], and produce compounds that accelerate growth [10].

Legumes such as beans and soybeans have roots nodules contain bacteria that able to fix aerial nitrogen to replace soil nitrogen that has been uptake by plant. Symbiosis between plants and bacteria is mutually beneficial to both parties. Bacteria get energy-rich nutrients from the host plant, while the host plants get nitrogen compounds from bacteria.

This study shows that Zea mays has advantages on several parameters such as abundance of spores, root colonization, inoculums weight, root fresh weight and root dry weight followed by *Sorghum bicolor* were also significantly different with other host plants. The result were in line with [11] those of that the host plant of the group Graminae, namely *Zea mays* and *Sorghum bicolor*, was more suitable for AMF production because it had high percent of root infection and number of spores than Leguminosae, namely *Centrosema pubescens* (CP) and *Calopogonium mucunoides* (CM). The number of spores produced by group of graminae is higher than class of Leguminosae although the degree of infection is similar. Graminae group has numerous roots and extensive root system.

This research found that the highest number of spores was shown by *Zea mays*, followed by *Sorghum bicolor* that has significant difference with other host plants, because *Zea mays* and *Sorghum bicolor* have many roots and extensive root system. This is confirmed by [12], that requirements in selection of host plant for AMF production is the plants can grow quickly and produces lots of roots. According to [13], lots of host plants roots with high level of AMF root infection is an indicator for good sources of AMF inoculums. In this research, *Zea mays* as a host produce a lot of spores and have many AMF species than *Sorghum bicolor*. Therefore, from practical standpoint, the land that once AMF has been inoculated can be inserted corn within 2-4 m to keep the number and type of AMF contained in the soil.

In contrast, [14] found that *Pueraria javanica* produce larger degree of root infection (91.50%) compared to *Zea mays* and *Glycine max*. This difference may relate to differences of trapping environment eg humidity and temperature that greatly affect host plant adaptation.

Sporulation dynamics are influenced by the type of host plant. *Zea mays* and *Sorghum bicolor* show increasing sporulation dynamics, while other host plants had the increase of sporulation until the age of 80 days after planting, then decreased (Table 5).

This study indicates that even AMF can be symbiotic with almost all crops, but AMF has its own peculiarities to symbiosis with the host plant, so the spores can grow well. There were only few spores obtained on land to be planted with soybeans, but after trapping the number and type of spores more diverse.

AMF symbiosis with plants can widen the interactions range of species from mutualism to parasitism on different environmental conditions [15]. AMF can be classified as a parasite if the amount of carbohydrates incurred by plants has greater value than nutrients available to plants from AMF. The condition can occur at high available P so that absorption of nutrients directly through root hairs is greater than the absorption of nutrients through the AMF. Complexity of AMF association requires description of parameters that affect the functioning of the AMF, such as symbiont morphology and physiology as well as biotic and abiotic factors in the rhizosphere level, communities and ecosystems. The description is important for AMF management in the system of agriculture, forestry and land restoration [15].

Table 5: Sporulation dynamics of Arbuscular Mycorrhizal Fungi at 70, 80 and 90 HST on AMF trapping using different host plants

No.	Type of Host Plant	Number of AMF spores at days after planting		
		70 HST	80 HST	90 HST
1	*Zea mays*	149	113.33	203.33
2	*Pueraria javanica*	7	9	2
3	*Sorghum bicolor*	21	55	168.67
4	*Glycine max* varietas Tanggamus	5	4	0.33
5	*Glycine max* varietas Anjasmoro	6	29.00	0.33
6	*Glycine max* varietas Selamet	2	6.67	1.33
7	*Glycine max* galur Pangrango Godek	3	5.67	0.33
8	*Glycine max* galur Sibayak Pangrango	0	1.67	1
9	*Glycine max* varietas Wilis	6	2.67	0.33

Note: The numbers followed by the same lowercase showed no significant difference in the level of 5% of Tukey test

AMF is able to grow in a wide range of natural conditions [16]. Reference [17] states that although every plant is able to be used as AMF host but there are some plants that are specific as AMF host. This can be seen in root colonization response from maximum infection. According to [2], selection of host plant for AMF production in pot culture has considerable influence on AMF spores formation and root infection. The same was stated by [18] that the AMF species, host plant, planting media, and environmental conditions all affect the time of sporulation. According [19], AMF infection on host plants is also determined by the root exudates, such as sugars, organic acids, and amino acids as food sources for AMF. Each plant releases different root exudates so that AMF's response to the host plant is different.

AMF was known able to grow and develop in less favorable environment for the growth of other soil microbes [20]. Its presence can provide positive benefits for the infected host plants in the provision of water and nutrients, especially P for plant growth [21]. Reference [22] states the level of root infection does not determined by the number of spores, but by the ability of AMF and the plant roots response to the ongoing infection process. Reference [23] stated that root infection level by AMF influenced by the level of host

sensitivity, climate and soil. Reference [24] stated that crops and soil pH affects the number of spores on rhizosfer. [4] stated that AMF live in symbiosis with responsive host plant and has many roots.

Furthermore [12] states that the number of spores does not directly correlate with the number of colonies that formed in the roots; low spore production can be formed although the percentage of infected root is high. This is because AMF species have different ability to improve nutrients absorption for plant growth. AMF species may have different ability to form hyphae in the soil, both the distribution and quality of the hyphae. This can cause the hyphae growing inside the roots have better development than outside roots. If this happens then the number of spores will be less, because parts of hyphae growing outside the roots will absorb nutrients then produce spores.

Low spore production may occur despite the high percentage of infected root. It can be caused due to photosynthate used by AMF just enough for the development of hyphae. In other words, high percent of root infection is not accompanied by high formation of spores. Other possibilities are the spores germinate again. Reference [18] stated that the spore formation is a dynamic process so that some spores are formed and others germinate at the same time.

In general, the expected AMF production is high percent of root infection and large number of spores. The number of spores may increase due to the aging of plant roots. Reference [12] showed that the development of spores usually occurs because reaction to the roots growth, then the production of the spores is higher when plant approached to mature and even when approaching older at the time of harvest.

4. Conclusion

There are some conclusions that can be drawn from this research, among others:

1) There are two genus of arbuscular mycorrhizal fungi identified from soybean rhizosphere of tidal areas, Simpang Village, District of Berbak, East Tanjung Jabung namely Acaulospora and Glomus.
2) After trapping Genus Acaulospora consisted of two species, namely Acaulospora scrobiculata and Acaulospora tuberculata, while Glomus has five species, namely Glomus macrocarpum, Glomus fasciculatum, Glomus Clarum, Glomus fecundisporum and Septoglomus constrictum.
3) AMF trapping process showed that corn is the best host plant with the highest spore abundance variables and two types of AMF spores ie Glomus Clarum and Acaulospora tuberculata, the highest colonization, the largest inoculums weight, the highest wet and dry root weight.

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