UNIVERSITI MALAYSIA TERENGGANU 11TH INTERNATIONAL ANNUAL SYMPOSIUM ON SUSTAINABILITY SCIENCE AND MANAGEMENT

UMTAS 2012
PROCEEDINGS

Greening the Technologies and Resources for Human Wellbeing

9 – 11th July 2012
Ri-Yaz Heritage Marina Resort & Spa,
Kuala Terengganu, Malaysia

Hosted by
Faculty of Agrotechnology and Food Science
and
Faculty of Fisheries and Aqua-Industries

Effect of Root Age on Successful Colonization of Arbuscular Mycorrhiza Fungi Colonization of Oil Palm Seedling

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Abstract

The area of oil palm plantation in Indonesia is increasing as its crude palm oil demand is also increase not only for vegetable oil but also for biofuel. In order to make the cultivation sustainable and echo friendly, the application of arbuscular mycorrhiza fungi (AMF) is strongly recommended as this fungi can help plant absorb more nutrient and water efficiently from the soil, hence the application of fertilizer can be reduced to at significant level. To get maximum benefit from the fungi, a proper application technique is needed. In these studies, we found that AMF (Glomus sp. and Enthropospora sp.) inoculum application to the germinated seed at the time of planting at prenursery failed to form symbiosis, no AMF infection was detected inside the root until 5 months after inoculation. In the next experiment, 10 fresh AMF spores (Glomus sp. Enthropospora sp. and Gigaspora sp.) were inoculated directly on the radicle of the germinated seed. We also found no infection of AMF until 3 month after inoculation. Another experiment were carried out by inoculating Glomus sp. and Gigaspora sp. to the one month old oil palm seedlings and the root infection was observed every two weeks until 8 weeks after AMF inoculation. The results showed that 6.7 % and 6.6% infection were detected 2 weeks after inoculation for Glomus sp and Gigaspora sp. respectively and the root infection increased by the time, 15.8% for Glomus sp. and 8.4% for Gigaspora sp. at 8 weeks after AMF inoculation.

Keywords: arbuscular mycorrhiza fungi, root age, oil palm seedling, root colonization

Introduction

The oil palm industry is one of the most important agricultural sectors in Indonesia. The country is one of the world largest producer and exporter of palm oil. In the Year 2010, 8.04 million hectares was under oil palm cultivation with a production of about 19.8 million tons of crude palm oil and the export value of oil palm product was USD 12.6 billion [1, 2]. The oil palm industry is also subjected to heavy usage of chemical fertilizers which gave negative impact to soil fertility and health. In order to make the cultivation sustainable and echo friendly, the application of arbuscular mycorrhiza fungi (AMF) is strongly recommended.

Arbuscular Mycorrhizal Fungi are ubiquitous symbionts formed in roots of a large majority of higher plants except those belonging to families Brassicaceae, Chenopodiaceae, Caryophyllaceae and Cyperaceae. The symbiosis is characterized by bidirectional movement of nutrients, where carbon flows to the fungus and inorganic nutrients move to the plant, thereby providing a critical linkage between the plant root and soil. Under diverse stress conditions the hyphae of the fungi exploit water and mineral from soils better than the roots and effectively transfer them to the plants. The hyphae are fine and highly branched able to intensively explore a soil volume, less efficiently explored by plant roots [3].

The importance of AMF symbiosis for plant mineral nutrition and water, and more generally for plant health, makes it one of the potentially more useful biological means of assuring plant production with a minimum input of chemicals such as fertilizer. More and more growers are now becoming aware of AMF as a new means of ensuring plant productivity. Furthermore, technology to produce AMF inoculums and apply it as biofertilizer also has been established. This situation, together with the present concern about man's negative impact on the environment, is creating the need and the conditions for introducing and rationally using AMF in plant production system.

The effectiveness of AMF inoculum depends on the colonization potential of the inoculum and the AMF species, proper placement, proper timing, age of the roots and susceptibility of crops to AMF colonization [4, 5]. The age and condition of plant roots have a major effect on initial infection and rapid colonization by AMF inoculum [6]. The purpose of this study was to examine the effect of the root age on AMF colonization of oil palm seedling.

Materials and Methods

The present investigation consisted of mentioned below three experiments. The experiments were conducted in polybags containing 3kg sterilized Ultisol top soil unless stated otherwise. The soil was autoclaved twice at 121^0C for 45 min with 5day interval.

Experiment 1

Arbuscular mycorrhizal fungi used in this study were previously isolated *Glomus* sp. (isolate MV-1 collected from rhizosphere of oil palm, West Sumatera, Indonesia, 2008) and *Entrophospora* sp. (Isolate MV-2 collected from rhizosphere of oil palm, Lampung, Indonesia, 2009). The AMF spore were obtained by trap culture method and was pure cultured on Setaria for inoculum production of each species. Pure culture of each species was raised in 5kg polybags filled with sterilized sand.

Forty uniform and healthy DxP pregerminated oil palm seeds were selected for this experiment. Each Seed was treated with following mentioned treatments i.e. control or uninoculated seed (T1), inoculated with 300 spores of *Glomus* sp. (T2), inoculated with 300 spores of *Entrophospora* sp. (T3), and inoculated with 300 spores of mixed *Glomus* sp. and *Entrophospora* sp. (T4). Each treatment was applied to ten pregerminated seeds giving a total of 40 pre-germinated seed. The AMF inoculum containing 300 spores (sand base inoculum) were applied to the pre-germinated seed at the time of sowning by placing the inoculum as a layer 2 cm below the seed. Each treatment was replicated 10 times resulting in 40 (10x4) polybags receiving appropriate treatments. The 40 planted polybags were placed in green house in a completely randomized design for 12 weeks. Each poly bag was watered daily and received the same amount of fertilizer.

At the end of experiment, the plants from each polybag was uprooted carefully and roots were was washed free of adhering soil particles. Data on seedling fresh weight, dry weight (oven drying at 70 ^{o}C), and AMF root colonization [7] were recorded.

Experiment 2

In this experiment three AMF species isolated previously were used i.e. *Glomus* sp., *Entrophospora* sp. (as in experiment 1), and *Gigaspora* sp. (isolate MV-8 collected from rhizosphere of *Jatropha* sp., East Java, Indonesia, 2009). At this study, ten fresh and viable spores of each AMF species were inoculated to the radicle of DxP pre-germinated oil palm seed. The spores were placed directly on the surface of radicle. Inoculation was done by using spore forcep under stereo microscope. Inoculated seeds were then planted into a polybag containing 3 kg of sterilized Ultisol top soil. Tweleve pre-germinated seeds were inoculated with each AMF species giving a total of 36 pre-germinated seed. The seedlings were watered daily and received the same amount of fertilizer. The destructive sampling was done at 2, 4, 6, and 8 weeks after inoculation. At every sampling time, three seedlings of each AMF treatment were sampled to check AMF root colonization.

Experiment 3

In this experiment, pre-germinated seeds were raised in sand bed for one month. The one month old seedlings were then transferred to the polybag containing 3 kg sterilized Ultisol top soil. At the time of transplanting half of the total raised seedlings were inoculated with *Glomus* sp. (isolate MV-1 collected from rhizosphere of oil palm, West Sumatera, Indonesia, 2008) and the remaining half with *Gigospora* sp. (isolate MV-8 collected from rhizosphere of *Jatropha* sp., East Java, Indonesia, 2009). Each AMF species was inoculated by placing AMF inoculum (sand base inoculum) containing 300 spores as a layer 2 cm below the seedling as well as around the roots to ensure better infection in

e-ISBN 978-967-5366-93-2

appropriate polybag. The destructive sampling was done at 2, 4, 6, and 8 weeks after inoculation. At every sampling time, three seedlings of each AMF treatment were sampled to check AMF root colonization. A total 24 seedlings were used in this study.

Results and Discussions

Experiment 1

Successful inoculation of AMF on oil palm has been reported by some researchers [8, 9, 10]. AMF inoculums was applied to one month old seedling or at the time of transplanting from pre-nursery to main .This practice need more AMF inoculum to ensure better contact of the inoculum with the root system to get better infection.
In experiment 1, AMF inoculum was applied to the pre-germinated seed of oil palm with aim to minimize the dosage of AMF inoculum used. However, the results obtained showed that no AMF root colonization was detected in the roots of three months old oil palm seedling. In line with AMF root colonization, no significant differences were detected in fresh and dry weight of shoot and root as a result of AMF treatment (Table 1).

Table 1: Fresh and dry weight of shoot and root of oil palm seedlings and percent AMF root colonization at 3 months after planting

Treatment	Fresh Weight		Dry Weight		% AMF Root Colonization
	Shoot	Root	Shoot	Root	
	……. g ……				
Control	3.7 a	1.05 a	1.3 a	0.22 a	0
Glomus sp.	3.6 a	0.93 a	1.3 a	0.23 a	0
Entrophospora sp.	4.3 a	1.05 a	1.4 a	0.24 a	0
Glomus sp. + *Entrophospora* sp.	3.7 a	1.01 a	1.3 a	0.23 a	0
BNT 5%	0.8	0.20	0.1	0.02	

Experiment 2

To ensure AMF spores were in good contact with the root/radicle of pre-germinated oil palm seeds, in this study AMF spores were placed directly on the radicle of pre-germinated seeds. Results from the present study strongly support results obtained from the experiment 1. Results obtained showed that no root was colonized by AMF at every sampling time i.e. 2, 4, 6, and 8 weeks after inoculation (Table 2).

Table 2: Percentage of AMF root colonization in oil palm seedlings at 2, 4, 6, and 8 weeks after inoculating with ten of AMF spores.

Treatment	% AMF Root Colonization			
	2 Weeks	4 Weeks	6 Weeks	8 weeks
		…… % ……		
Glomus sp.	0	0	0	0
Entrophospora sp.	0	0	0	0
Gigaspora sp.	0	0	0	0

e-ISBN 978-967-5366-93-2

Experiment 3

Results obtained from this study showed that AMF isolates used could form symbiosis with oil palm seedling. No AMF colonization detected in experiment 1 and 2 could be attributed to the time of AMF inoculation or correlated with the condition of root system. In this study, AMF colonization were detected as early as 2 weeks after inoculation both for *Glomus* sp. (6.7%) and *Gigaspora* sp. (6.6%) and the percentage of root colonization increased by the time of sampling, 15.8% for Glomus sp. and 8.4% for Gigaspora sp. at 8 weeks after inoculation (Table 3). However, the AMF colonization was only observed in the secondary and tertiary roots of the seedling, no infection was detected in primary root.

Table 3: Percentage of AMF root colonization in oil palm seedlings at 2, 4, 6, and 8 weeks after inoculating with AMF inoculums containing 300 spores

Treatment	% AMF Root Colonization			
	2 Weeks	4 Weeks	6 Weeks	8 weeks
		…….…… % ……..		
Glomus sp.	6.7	8.4	10.2	15.8
Gigaspora sp.	6.6	7.9	8.3	8.4

Oil palm is known to respond to AMF inoculation [8, 9, and 10], even this plant is mycorrhizal in nature [5]. To get the maximum benefit from these fungi, proper inoculation technique is needed. Base on the results of present studies, when AFM inoculum was inoculated to the radicle of pregerminated seed (experiment 1 and 2), no AMF colonization was detected in all roots system (primary root, secondary root, and tertiary root) of the seedling until 3 months after inoculation. However, when AMF inoculum was applied to the one month old seedling (at this stage, the seedling already have primary roots, secondary roots, and tertiary roots), AMF colonization were detected as early as 2 weeks after inoculation in the secondary roots and tertiary roots, but no colonization were observed in primary root (experiment 3). Until 8 weeks after inoculation, AMF colonization only detected from secondary roots, and tertiary roots. This suggested that primary root of the oil palm seedling was not susceptible for AMF colonization; entry by the AMF was actually inhibited in this part of the root. Afek et al. [11] found that extensive AMF colonization took place in 3- and 30-day-old onion seedlings, whereas 10- and 17-day-old seedlings were far less responsive to AMF colonization. They suggested that this phenomenon due to the unique rooting habit of onion, whereby new flushes of roots are periodically produced at the base of the bulb and will pass through AMF inoculums. These new roots are young and have a high degree of susceptibility. According to Hepper [6], in normal root systems, the young, potentially colonizable roots are produced as branches from other roots. He also found that the most proximal part of the main root of leek and clover remained non-mycorrhizal even when the seedling roots grew continuously through heavily infested soil. He suggested that a concentration gradient of a compound, such as a plant growth hormone, diffusing from the shoot into the root might be sufficient to prevent AMF infection. In the present investigations, radicle of pre-germinated seed and primary root (the grown radicle) of oil palm was not colonized by the AMF species tested. This fact could be attributed to the age of the root or the plant growth hormone contain inside the root.

References

[1] Direktorat Jenderal Perkebunan. Luas Areal dan Produksi Perkebunan Seluruh Indonesia Menurut Pengusahaan. http://ditjenbun.deptan.go.id/cigraph/index.php/viewstat/komoditiutama/8-KelapaSawit
[2] Information on http;//www.ditjenbun.deptan.go.id/index.php/component/content/article/36-news/203-ekspor-kelapa-sawit-terus-naik.html.
[3] S. E. Simth and D. J. Read: *Mycorrhizal Symbiosis* (Academic Press, New York 2008).

[4] M. Brundrett, N. Bougher, B. Dell, T. Grove, and N. Malajczuk: *Working with Mycorrhizas in Forestry and Agriculture* (Pirie Printers, Canberra 1996).

[5] E. Sieverding: *Vesicular Arbuscular Mycorrhiza Management in Tropical Agrosystems* (Deutsche Gesellschaft fur Technische Zusammenarbeit (GTZ) Gmbh., Eschborn 1991).

[6] C. M. Hepper: *Influence of age of roots on the pattern of vesicular-arbuscular mycorrhizal infection in leek and clover* . New Phytologist Vol. 101 (1985), p. 685-693.

[7] M. Giovannetti and B. Mosse: *An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots.* New Phytologist Vol. 84 (1980), p. 489-500.

[8] C. Schultz, G. Ginting, A. M. Moawad, and P. L. G. Vlek: *Influence of (vesicular-) arbuscular mycorrhiza on survival and development of micropropagated oil palms during acclimatization.* In Proceedings of the Deutscher Tropentag, (1999).

[9] S. Sundram: *Growth effects by arbuscular mycorrhiza fungi on oil palm (Elaeis guineensis* Jacq.*) seedlings.* Journal of Oil Palm Research Vol. 22 (2010), p. 796-802.

[10] H. Widiastuti, N. Sukarno, L. K. Darusman, D. H. Goenadi, S. Smith, and E. Guhardja: *Penggunaan spora cendawan mikoriza arbuskular sebagai inokulum untuk meningkatkan pertumbuhan dan serapan hara bibit kelapa sawit.* Menara Perkebunan Vol. 73 (2005), p. 26-34.

[11] U. Afek, E. Rinaldelli, J. A. Menge, E. L. V. Jonhson, and E. Pond: *Mycorrhizal species, root age, and position of mycorrhizal inoculums influence colonization of cotton, onion, and pepper seedlings.* Journal of the American Society for Horticultural Science Vol. 115 No. 6 (1990), p. 938-942.