INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 5, No. 12, p. 116-122, 2014

RESEARCH PAPER OPEN ACCESS

Isolation of indigenous arbuscular mycorrhizal fungi and selection of host plant for inoculum production

R. Abdullahi[1], S. Lihan[1], R. Edward[2]

[1]*Department of Molecular Biology, Universiti Malaysia Sarawak,Kota Samarahan, Sarawak, Malaysia*

[2]*Department of Plant Science and Environmental Ecology, Universiti Malaysia Sarawak, Kota Samarahan, Sarawak, Malaysia*

Key words: Indigenous AMF, *Glomus mossea, Glomus geosporum*, *Glomus etunicatum*, host plant.

http://dx.doi.org/10.12692/ijb/5.12.116-122 Article published on December 15, 2014

Abstract

The objective of this study was to select a suitable host plant for mass production of indigenous arbuscular mycorrhizal (AM) fungi. Lemongrass and onion were compared for mass multiplication of *Glomus* species *viz*; *Gl. mossea, Gl. geosporum* and *Gl. etunicatum*. Spore count ranges from 17.67 (*Gl. etunicatum*) to 26.33 (*Gl. mossea*) g^{-1} soil under onion and lemongrass respectively. There was no significant difference (0.05) between *Gl. Mossea* and *Gl. Geosporum* in onion. Similarly, no significant difference was observed between *Gl. Geosporum* and *Gl.etunicatum* in lemongrass. *Gl.mossea* recorded the highest spore number followed by *Gl.geosporum* in both plant species. Root colonization % ranges from 67.33% (*Gl.mossea*) in onion to 80% (*Gl.geosporum*) in lemongrass. Colonization % of *Gl.mossea* and *Gl.geosporum* were statistically similar under individual plant species. Despite the lowest spore counts recorded by *Gl.etunicatum*, % root colonization was significantly (0.05) higher compared to *Gl.mossea* and *Gl.geosporum* in onion. Lemongrass recorded the highest average mean (77.33%) of root colonization % and spore counts (23.44) compared to onion (68.44%, 19.67). The study showed that AMF-plant interaction was host preference. Lemongrass favored the mass multiplication of *Gl. mossea*, *Gl.geosporum* and *Gl.etunicatum* thus, was the most suitable host plant compared to onion for inoculum production.

* **Corresponding Author:** R. Abdullahi ✉ rakiyaa6@gmail.com

Introduction

The negative impacts of chemical fertilizer on food quality, environment, and higher costs of crop production demand the search for substitute. Contrary to chemical fertilizer, arbuscular mycorrhizal fungi (AMF) are less expensive and could promote eco-friendly agriculture. Its application has been revealed to improve food quality and safety, lessen production costs, and reduce environmental hazards. AM fungi could serve as tool to sustainable crop production especially, in low-input farming (Ryan and Graham, 2002; Gosling *et al.,* 2006). Their role in cycling of nutrient, increase in efficiency use of fertilizers, enhancing plant growth and yield of crops (Harrier and Watson, 2004;Tiwari *et al*., 2003) created ground for AM fungal application as biofertilizer (Schwartz *et al*., 2006). Arbuscular mycorrhizas occur naturally in soil and form the oldest group of organisms that live symbiotically with nearly 90% of higher plant (Blackwell, 2000; Redecker *et al.,* 2000; Brundrett, 1993; Wang and Qiu, 2006). They are the most common endomycorrhizas (Brundrett *et al*., 1996), formed from phylum belonging to Glomeromycota (Schüβler *et al*., 2001), having nine genera; *Glomus*, *Sclerocystis*, *Acaulospora*, *Gigaspora*, *Paraglomus*, *Entrophospora*, *Scutellospora,Geosiphon* and *Archaeospora* (White *et al.,* 1990) with more than 150 described species (Schüβler *et al*., 2001; Smith and Read, 1997). Abundance of AM fungi and their interactions with plant are basic indicators of plant health and soil fertility. From such symbiosis, there are benefits to plant, fungus and soil. Plant benefits from improved soil nutrients uptake, disease resistance, drought, and heavy metals; the fungi obtained fixed carbon from host plant, and ecosystem benefits improved soil structure and stable soil aggregate (Rillig and Mummey, 2006). It has been shown that applications of AM fungi could play major role in sustainable agriculture and ecosystems due to, improved crop yields under low soil nutrients condition and reclamation of degraded soil.

The present high-input farming system has negatively affected the population and beneficial activities of AMF (Ezawa *et al*., 2000; Jansa *et al*., 2002). This in certain cases; necessitate the application of mycorrhizal fungal inoculum. Interestingly, most commercial AM fungal inoculant (Biofertilizer) are non-native in origin. Attention needs to be given about the possibility of impoverish indigenous species and unknown negative ecological consequences from spreading of invasive species (Schwartz *et al*., 2009). The basis for AMF inoculum production is dependent on suitable plant host (Sahay *et al*.,., 1998; Becard and Piche, 1992). AM fungi were considered not host specific in colonizing mycorrhizal host plant (Klironomos, 2000; Smith and Read, 2002). However, current study suggested some taxa to be host preference (Helgason *et al.,* 2002; Vandenkoornhuyse *et al*. 2003; Husband *et al*. 2002). Previous work in study area have focused on isolation and identification of AM fungi associated with forest tree species (Chubo *et al*., 2009; Ong *et al*., 2012) but less research work has been conducted on host plant selection involving different AM fungi species. Tahat *et al*., (2008) worked on host plant selectivity for mass propagation of *Glomus mossea*. Hence it was pertinent to determine suitable host plant for mass propagation of dominants indigenous AM fungi species. In this study, we have isolated and identified population of native AM fungi associated with four plant species; *Carica papaya* (pawpaw), *Musa* specie (banana), *manihot esculenta* (cassava), and *Zea mays* (maize) in different agricultural soils. The objective was to determine a suitable host plant for mass production of dominants native AM fungi for inoculum production. Achieving this goal could develop new perspectives for the development of adapted inocula suitable for field application.

Materials and methods

Experimental site

The experiment was conducted at the Forestry Research Garden green house (East Campus), University Malaysia Sarawak, Kota Samarahan. Sarawak is in the tropical rainforest of Southeast Asia. Located at (0°50' and 5° N and 109°36' and 115°40'E). Having average rainfall of 247 days per annum with mean annual precipitation between 2,500 and 5,000,

and a monthly minimum rainfall recorded around June or July but exceeded 100 mm (Andriesse, 1968). The temperatures ranges between 23 °C (73 °F) and 33°C (91° F) in the early hours of the morning and during mid-afternoon respectively with heat index reaching 42 °C (108 °F) during dry season due to humidity reaching to about 85%.

Isolation and identification of AM fungi

Rhizospheric soils and roots (0-15 cm depth) were sampled under actively growing; pawpaw, banana, cassava and maize plants in some farms around Kota Samarahan for isolation of AM spore. Sampled soils were transported to the laboratory in a polythene bag. Stone debris, root fragments >2 mm and any visible animal materials were handpicked. The soil samples were spread for air-drying in the laboratory and later passed through 2 mm sieve. AM spores were isolated using wet sieving-and-decanting techniques (Gerdemann and Nicolson, 1963). The spores were examined and sorted according to morphological characteristics (spore size, colour, shape and sub-tending hypha) thereafter mounted onto slides for identification. Spores were identified to specie level according to Schenck and Perez (1990) manual. Spore density was expressed in number g^{-1} soil.

AM fungi species

Dominants indigenous AM fungi spores associated with all plant species were found to be *Glomus* species (*Gl. mossea, Gl. geosporum* and *Gl. etunicatum*). Identified spores were surface-sterilized by exposing them to 2% (w/v) chloramine T and 2% (w/v) streptomycin sulphate, allowing contact for 10 minutes.

Host plant for Mass production of AMF

Lemongrass (*Cymbopogon citratus*) and onion (*Alium cepa*) seedlings were used for mass production of AM fungi spores. Both crops were reported as good host plant for AM fungi due to their fast growth; produce numerous fine and hairy roots for abundant sporulation.

Growing substrate

Sand: soil (1:2) was used as growing substrate. Soil was air dried, passed through 2 mm sieve and sterilized at 121°C for 2 h in a dry oven to kill all microorganisms, including AM fungi.

Experimental design and plant maintenance

The experiment was laid in 2×3 factorial with three replications in a randomized complete block design (2 plant host and 3 AMF species). Ten and fourteen days old lemongrass and onion seedlings that showed no mycorrhizal association were sampled from University Malaysia Sarawak, Research Nursery. Twenty matured, viable and sterilized AMF spores of *Gl. mossea, Gl. geosporum* and *Gl. etunicatum* were placed directly to roots of seedlings in 15 cm ×20 cm (diameter and height) individual plastic pots containing 3 kg of the growing substrate. Plants were nourished with Hoagland's nutrient solution without P (Hoagland and Arnon, 1950) at 100 ml pot^{-1} weekly, grown for 3 months on the culture in green house facility of Forest Research Unit, Universiti Malaysia Sarawak. The average temperature in the green house day/night cycles was 32/25°C with natural light intersection. Plants were watered when necessary using tap water.

Percentage root colonization

At harvest, plant shoots were cut-off and pot substrate from individual pots turned onto clean tray. Roots were investigated for infection following the method described by Phillip and Hayman, (1970). Percent root colonization was calculated using the formula below;

$$\% \text{ colonization} = \frac{\text{No. of colonized root}}{\text{Total root No.}} \times 100$$

Analysis of variance

Data were subjected to one-way ANOVA using SPSS 19 ver. Difference between treatment means were compared using Fisher's LSD for post-hoc comparisons ($p<0.05$).

Results and discussion

The result of this study showed that lemongrass and onion were both colonized by AM fungi. However, the

AMF-plant interaction was host preference.

Table 1. AMF spores g^{-1} soil as affected by different plant specie.

Mycorrhiza	Lemongrass	Onion
Gl. mossea	26.33^{a}	20.33^{a}
Gl. geosporum	23.33^{b}	21.00^{a}
Gl. etunicatum	20.67^{b}	17.67^{b}
LSD (0.05)	2.96	2.48

Values are means of three replicates. Different superscript indicate significance (5%) between treatments

Spore density (Table I) and root colonization % (Table II) varied significantly between the plant species. Spore count ranges from 17.67 (*Gl. etunicatum*) to 26.33 (*Gl. mossea*) g^{-1} soil under onion and lemongrass respectively. There was no significant difference (0.05) between *Gl. Mossea* and *Gl. Geosporum* under onion. Similarly, no significant difference was observed between *Gl. Geosporum* and *Gl.etunicatum* in lemongrass. *Gl.mossea* recorded the highest spore number followed by *Gl.geosporum* in both plant species. Spore densities of the three indigenous *Glomus* spp. were significantly higher under lemongrass compared to onion.

Table 2. Root colonization % for *Gl. mossea*, *Gl. geosporum* and *Gl. Etunicatum* under different plant specie.

Mycorrhiza	Lemongrass	Onion
Gl. mossea	79.67^{a}	67.33^{a}
Gl. geosporum	80.00^{a}	67.67^{a}
Gl. etunicatum	72.33^{b}	71.33^{b}
LSD (0.05)	4.33	1.64

Values are means of three replicates. Different superscript indicate significance (5%) between treatments

Root colonization % ranges from 67.33% (*Gl.mossea*) to 80% (*Gl.geosporum*) under onion and lemongrass respectively. Colonization % of *Gl.mossea* and *Gl.geosporum* were statistically similar under individual plant species. % root colonization by *Gl.etunicatum* was significantly (0.05) higher compared to *Gl.mossea* and *Gl.geosporum* in onion. Lemongrass recorded the highest average mean (77.33%) root colonization % compared to onion (68.44%). A positive correlation (0.01) was observed between spore density and root colonization %.

In the present study, it is evident that lemongrass and onion were both colonized by AM fungi and served as good host plant for mass multiplication of *Gl.mossea*, *Gl.geosporum* and *Gl.etunicatum* due to environmental adaptability. Significant differences in spore density and root colonization % were observed between lemongrass and onion. This could be attributed to different root exudates from both plant species. Plant root exudates, light distribution, soil moisture, growing substrate, host plant physiology (Simpson and Daft, 1990) and soil temperature could equally affect sporulation and colonization potential of the symbionts (Marshner and Timonen, 2004; Douds and Nagahashi, 2000; Akiyama *et al*., 2005). Likewise, different AMF species exhibit different preference in colonizing plant host (Ortas, 2008, 2009; Ortas and Varma, 2007).

As observed in this study lemongrass recorded the highest spore counts and % root colonization for *Gl.mossea*, *Gl.geosporum* and *Gl.etunicatum*. The fact of host preference by some AM fungi has clearly been documented (Li *et al*., 2010; Danny and Brenda, 2005). Further evidence is the fact that all three *Glomus* species used in this study formed the best AMF-plant interaction with lemongrass compared to onion. This corroborated with findings by Kaushish *et al*., (2009), in their study using different growing substrate, they revealed lemongrass as most suitable trap plant compared to onion and *Sebania aculeate* for mass multiplication of *Gl. mossea*. Sonika *et al*., (2013) also reported lemongrass to have recorded the highest spore counts and root colonization % compared to onion and lilly grass for mass production of *Acaulospora laevis*.

Conclusion

In conclusion to this study, lemongrass was the most suitable host plant for mass multiplication of *Gl.mossea*, *Gl.geosporum* and *Gl.etunicatum* compared to onion for inoculum production.

Acknowledgement

Weacknowledge the support by FRGS/SG05(02)/1070/2013(16) for funding this work.

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