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Plant Host Selectivity for Multiplication of *Glomus mosseae* Spore

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Abstract: The study aimed to select plant host for multiplication of *Glomus mosseae* spores. Five plant species were used [(corn, (*Zea mays*) sorghum, (*Sorghum bicolor*) lentil, (*Lens culinaris*), barley, (*Hordeum vulgare*) and green bean, (*Phaseolus vulgaris*)]. Plants were inoculated with *Glomus mosseae* and grown for 75 days under glasshouse conditions. Mycorrhizal sporulation and colonization of all plant hosts were assessed at different sampling periods. At 75 days of growth the highest number of *Glomus mosseae* spores was found in mycorrhizosphere of corn plant (167 spore/10 g soil), while the lowest in the mycorrhizosphere of barley (35 spore/10 g soil). The highest percentage of root colonization was in corn (76%), while the lowest colonization was found in green bean (24%). Corn was the most suitable host for spore production of *Glomus mosseae* and to extensive root colonization. It was recorded that plants having more colonization percentage were able to produce more *Glomus mosseae* spores. The study indicated that different plant species significantly influenced the root spore production and root colonization percentage of *Glomus mosseae*.

Key words: *Glomus mosseae*, host, root colonization, spore number

INTRODUCTION

Among the symbiotic microorganisms, arbuscular mycorrhizal fungi (AMF) form mutual associations with more the 80% of plant species, improve mineral nutrition (Smith and Read, 1997) enhance resistance or tolerance to stress (Turnau and Haselwandter, 2002) and protection against pathogens (Azcon-Aguilar *et al*., 2002). AMF show strong impact on root morphogenesis and induced changes in root architecture (Berta *et al*., 2002). Vesicular arbuscular mycorrhizal (VAM) symbiosis are typically mutualistic as obligate symbionts, while AMF are believed to be dependent upon the host plant for fixing carbon (Douds and Millner, 1999). The relationship between mycorrhiza and plant is very widely spread among terrestrial vascular plants; around 80-90% of all such plants are mycorrhizas and at least 6000 fungal species from Zygo-Asco-to basidomomycotina were detected (Wilkinson, 2001).

This association is a mutually beneficial event, the plant supplies the fungus with carbon, while the fungus increase the ability of plant to uptake nutrients (mainly P) (Smith and Read, 1997). The most acceptable reason for the obligate biotrophy that, the fungi lost some of its carbon-fixing capabilities or the genetic machinery that supports them during the long evolution of its symbiotic relationship with the host. The fungi became completely dependent on the host plant for fixed carbon supply (Williams, 1992).

Homogenous spores are required for spore germination and storage research, the inability to axenically culture for arbuscular mycorrhizal fungi has made it difficult to produce inoculum on a commercial scale and has limited its use in large agricultural operations. Several methods have been developed to re-culture AMF including the classical soil-based system, *in vitro* root organ culture system and aeroponic and hydroponic systems (Douds, 1997). The application of AMF for glasshouse research depends on the development of AM fungi and production of spores, thus this lead to the importance of high AMF sporulations in pot culture methods (Douds and Schenck, 1990). Quantitative and qualitative of initial population of AMF depends on several factors which include cultivation practices used for plant growth, environmental conditions, type of substrate and host plant. The establishment of association between plant and AMF may be mediated by the interaction between plant environment and fungi (Carrenho *et al*., 2002). The production of inoculums is one of the hindrances in the large scale application of arbuscular mycorrhizal fungi (AMF) (Silva *et al*., 2002).

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The objectives of this study were to determinate the suitability of five hosts [(corn, (*Zea mays*), sorghum, (*Sorghum bicolor*), lentil, (*Lens culinaris*), barley, (*Hordeum vulgare*) and green bean, (*Phasiolus vulgaris*)] for the production of the most reliable, microorganisms free, high and good quantity of *Glomus mosseae* spores under glasshouse conditions for research purposes. Finally to provide information about the relationship between root colonization percentage and production of spores influenced by plant hosts used.

MATERIALS AND METHODS

Soil preparation: This study was conducted during June- September 2007 in glasshouse at Universiti Putra Malaysia, Malaysia. Top soil (Serdang series) was used for the reproduction of *Glomus mosseae*. The mixture volume was (2:1 v/v sand: soil). The soil was sieved using 0.5 mm mesh and sterilized twice using a dry oven at 121°C for 1 h two times. Chemical analysis of soil showed the following results pH, 6.00, 0.13% N, 0.023% P, 0.30% K, 0.063% Ca, 0.034% Mg, 0.063% S, 1.52% Fe, 0.0034% Mn, 0.0057% Zn, 0.00064% Mo, 0.0003% B, 0.0015% Cu (Sharifuddin, 1984). Plastic pots (20 cm diameter) were washed, dried and filled with the potting mixture. The soil was checked for microorganisms survival after sterilization using potato dextrose agar (PDA) media before used.

Primary inoculum: The inoculum was initially produced in pot culture using sorghum (*Sorghum bicolor*) as a host. The spore inoculum density of (100 spores/100 g dry soil was determined by wet sieving and decanting technique using different sieve sizes of 250, 106 and 45 μM (Mukerji *et al*., 2002).

The spores were collected in petri dish and counted under binocular-microscope (Gerdemann and Nicolson, 1963). Healthy and mature spores were selected for plant inoculation and 30 spores/100 g dry soil were poured and mixed well into the soil. Spores of *Glomus mosseae* (Nicolson and Gerdemann, 1963) were obtained originally from the laboratory of soil microbiology, Land Management Department, Faculty of Agriculture, Universiti Putra Malaysia, Malaysia.

Host selection and growth conditions: All plant hosts were selected based on their ability to be colonized by mycorrhizal fungi and non host specific (Simpson and Daft, 1990). The host plants used in this experiment were known to be good hosts and trap plant for mycorrhizal fungi for their characteristics such as, fast growth, tolerance to adverse conditions, produce more fine and hairy roots for sizeable sporulation and colonization, which allow the microbe to colonize the roots easily. Healthy seeds of [(corn, (*Zea mays)*, sorghum, (*Sorghum bicolor*), lentil, (*Lens culinaris*), barley, (*Hordeum vulgare*) and green bean, (*Phasiolus vulgaris*)] were disinfected with 0.5% sodium hypochlorite for 5 min and washed several times with sterilized distilled water. The seeds were planted directly in the pots under glasshouse conditions at 20-30°C day and night. Plants were weekly watered with Hoagland solution, modified by Vosatka and Gryndler (1999), as follows *Macronutrients* (KNO_3 = 240 mg, Ca $(NO_3)_2$ 4. H_2O = 295 mg, $MgSO_4.7H_2O$ = 720 mg, KH_2PO_4 = 12.2 mg, FeNaEDTA = 4.5 mg, NaCl =0.7 mg) *Micronutrients* $ZnSO_4.7H_2O$ = 0.75 mg, $CuSO_4.5H_2O$ = 0.001 mg, $MnCl_2.4H_2O$ = 0.75 mg, $NaMoO_4.2H_2O$ = 0.00017 mg, H_3BO_3 =1.5 mg. Distilled water was used for watering all plants as needed. At two weeks of growth the seedlings were thinned down to two plants per pot.

Mycorrhizal colonization: Root samples were collected at 25, 50 and 75 days of growth. The percentage of adventitious and lateral root colonization was evaluated microscopically after clearing root in 10% KOH and staining with 0.05% trypan blue in lactophenol (Phillips and Hayman, 1970). The following equation was used to calculate the percentage of root infection (Giovannetti and Mosse, 1980).

$$\text{Colonization (\%)} = (\text{Number of colonized segments} / \text{Total No. of segments examined}) \times 100$$

Spore population: Soil was collected at an interval of 25 days from all plant hosts for spore population determination. Spore population was counted under binocular-microscope (Gerdemann and Nicolson, 1963).

Data analysis: Treatments were arranged in Completely Randomized Design (CRD) with 5 treatments and four replicates (two plants/replicate). Data were subjected to an analysis of variance using SPSS 15.0 software (SPSS Inc. Chicago, USA).

RESULTS AND DISCUSSION

The sporulation of AMF was affected by plant hosts. Spore number increased with increased plant growth period. Significant differences in spore number were observed on different host at 50 days of growth. Corn plant produced the highest number of spores (Table 1). At the end of this experiment, barley plant was unable to produce a significant number of spores followed by lentil

Table 1: Effect of different plant host on *Glomus mosseae* spores at different harvest times

	Harvest period (spore/10 g soil) (days)		
Host	25	50	75
Lentil	18.4a	65.4bc	52.0cd
Corn	13.8a	109.0a	167.00a
Sorghum	17.0a	73.0bc	111.80b
Greenbean	17.4a	57.8bc	64.00c
Barley	19.2a	38.6c	35.20d

Means within columns followed by the same letters are not significantly different at p≤0.05 level using Tukey post Hok test

Table 2: Effect of *Glomus mosseae* on root colonization assessment of different plant host at different harvests times

	Colonization (%)		
	Assessment period (days)		
Host	25	50	75
Lentil	0a	6ab	28c
Corn	0a	18a	76a
Sorghum	12b	18a	50b
Greenbean	0a	0b	30c
Barely	0a	4b	30c

Means within columns followed by the same letters are not significantly different at p≤0.05 level using Tukey Post Hok test

plant and both were not varied statistically. At 75 days corn produced the highest number of spores and it was varied compared to other hosts. Spore number counted in the soil of sorghum (167 spore/10 g soil) followed by sorghum (111 spores/ 10 g soil). Spore number were low in greenbean followed by lentil and barley. Increasing plant growth period did not increase their spore multiplication (Table 1).

Root colonization varied between different host plant affected by host plant. All hosts were not colonized by *Glomus mosseae* at 25 days (first harvest) in. At 50 days (second harvest) all plants-except- greenbean were colonized by *Glomus mosseae* (Table 2). High root colonization was observed after 75 days (3rd harvest) of corn plant growth (76%) by *G. mosseae* significantly compared to all treatments, followed by sorghum (50%) (Table 2).

There was a negligible relationship between spore number and the percentage of root colonization at 25 days. There was positive relationship between spore number and root colonization at 50 days. After 75 days of plant growth the spore number decreased but the root colonization percentage was increased linearly. Strong relationship between spore number and root colonization percentage was observed in corn, sorghum and greenbean during experiment period (Fig. 1).

This study showed that spores number in the mycorrhizosphere at 25 days after application were less than the original inoculum in all treatments, this could be due to the dispersal of spores in soil after watering plant host. The differences in spore production in the different plant species could be due to the characteristics of plant hosts which varies in their ability to adapt to the growth conditions like soil temperature, soil pH, soil moisture, soil fertility, soil microorganisms interactions, light conditions and others (Mukerji *et al*., 2002). Some plants are more susceptible than others in relation to development of the symbiosis (Smith and Read, 1997).

Corn plant grew faster and more rigid in growth which providing suitable conditions for higher sporulation. The stability of host growth and plant size are considered as important factors for *Glomus mosseae* production. Corn plant was more stable in growth and the root size was bigger, which often have more extensive root system than smaller hosts. The current results were similar with that obtained by Sinegani and Sharifi (2007) and Saif and Khan (1997) who detected higher AMF spores in the rihzosphere of corn (*Zeay maze*) as compared to other trap species used. They also confirm that the host type is the most important factor for spore production and multiplication as well as growth period in some host is critical for more colonization and spore production.

This study showed that the lowest spore numbers (35 spore/10 g soil) was counted in barley. The results contracted with the results of Al-Raddad (1995) who documented that barley plant was the best host for *Glomus mosseae* mass production. Similarly, Chaurasia and Knare (2005), found that barely plant showed the highest colonization (92%) and the highest spore production (74 spore/10 g dry soil). The differences in sporulation may be due to the differences in environmental conditions, study area and soil type. The initial inoculums used is also very important for increasing the colonization percentage, as more inoculums resulted in more chance to produce high number of AM fungi spores. The current finding was strongly related to the results reported by Dabire *et al.* (2007) they found that AMF *Glomus intraradices* inoculum density was positively related to the sorghum plan growth. Another biological considration in the production of inoculum is the host plant upon which the fungus will grow (Ryan and Graham, 2002).

From the present data, the extensive root colonization of plant host resulted in healthy plant growth and more root system development. These data are in line with data documented by Scheloske *et al.* (2004) who reported that mycorrhizal root showed a high degree of mycorrhizal colonization of 60-70% and formed approximately 25% more dry weight compared to non-mycorrhizal control.

In the present study, low phosphorus amount in the soil, root colonization and sporulation were strongly correlated positively between some species used. The data documented by Schroeder and Janos (2005) was not

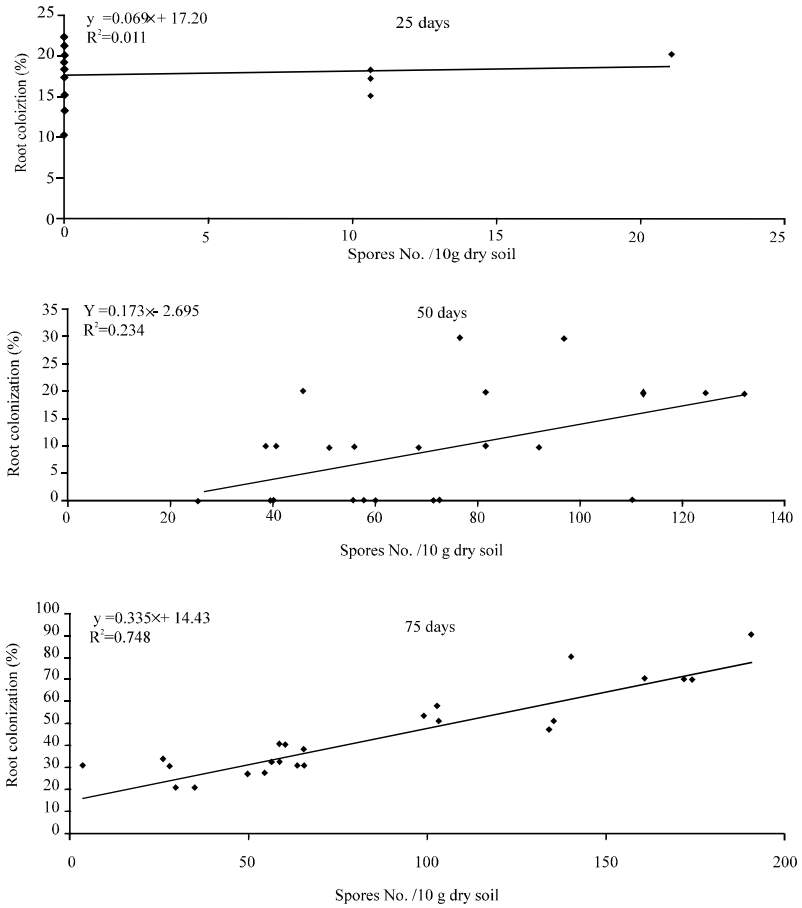

Fig. 1: The relationship between spore number and the percentage of root colonization in different plant hosts within different interval time (25, 50 and 70 days)

compatible with present investigation, they found that AM, phosphorus and inoculums density interacted significantly to modify growth of *Capsicum annuum* and *Capsicum pepo*, they also found that increased density and phosphorus diminished beneficial of AM.

Sorghum is commonly used for the propagation of AMF spores and present data show that the use of corn and sorghum in spore mass production in pots made a greater collection of species used, but the use of leguminous (lentil, green bean) enhance less number of spores in contract with that obtained by Carrenho *et al.* (2002), he showed that peanut gave the highest root colonization and spores number, while sorghum was the lowest in both parameters considered.

CONCLUSION

Corn was the most suitable host for the sporulation of *Glomus mosseae* under glasshouse conditions. Corn roots were more colonized by *Glomus mosseae* compared to other hosts. Lintel, greenbean, barely plant showed low AMF sporulation and colonization. Several important factors affecting the multiplications of AMF and increase root colonization include, plant host species, environmental conditions, soil type, nutrient regime, inoculums amounts. The pot trap technique is a trusted method for mass production of AMF in glasshouse conditions, if good host selected. Finally mass production of AMF varies greatly on root structure and habitat of host plant.

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