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Research Article

Mass production of AM fungal inoculum by soil based pot culture

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Abstract

A mycorrhiza (fungus root) is a symbiotic association of a fungus and the roots of a vascular plant. In this association, the fungus colonizes the host plant's roots, either intracellular as in arbuscular mycorrhizal fungi or extracellularly as in ectomycorrhizal fungi. The AM fungi are not host specific, any plant species can be infected by an AM fungal species but the degree of AM infection and its effect can differ with different host endophytic combinations. Large quantities of the inoculum can be produced by pot culture technique. The present work examined the three plant species viz. maize (*Zea mays*, L.), sorghum (*Sorghum halepense*, L. Pers.) and paddy (*Oryza sativa*, L.) for selection of the suitable host for the mass production of AM spores and for mass production of consortium of AM fungi present in the rhizosphere soil. The present study reported that the AM fungi and root colonization in *Zea mays* was higher as 98±0.50% and was least in *Oryza sativa* as 58±1.00%. The maximum increase in shoot and root length was observed in AM fungi inoculated *Zea mays* (43.76% and 32.47%) as compared to control plants. However, it was 34.67% and 17.46% in AM fungi inoculated *Sorghum halepense* as compared to control. The minimum increase in shoot length was recorded as in AM fungi inoculated *Oryza sativa* (5.1%) than control. After 60th Day, the fresh and dry weight of host increased in AM fungi inoculated plants of *Zea mays* (25.33±0.42g and 6.15±0.14g per plant) than in the control plants (15.53±0.25g and 4.27±0.06g per plant). However, it was observed as 12.00±0.20g and 1.88±0.03g per plant in AM fungi inoculated and 9.63±0.15g and 1.8±0.01g per plant in control plant of *Sorghum halepense*. Comparatively, a little increase (1.22%) in dry weight was recorded in AM fungi inoculated plant of *Oryza sativa*. The AM fungal spores were isolated from the AM inoculated *Zea mays* soil and showed the presence of diversity of AM fungi belonging to *Glomus, Acaulospora, Gigaspora, Scutellospora* and *Sclerocystis*. The results evidenced that the rapid and efficient AM fungal root colonization and higher AM spores production was observed maximum in *Zea mays* compared to other host plants. Therefore, the *Zea mays* plant has been suggested as a suitable host for mass production of AM fungi under soil based pot culture.

Keywords: AM fungal spores, soil culture, Per cent root colonization and mass production.

Introduction

Arbuscular mycorrhizal (AM) fungi are associated with about 80% of the plant families in the world (Giovannetti and Sbrana, 1998).Most terrestrial plants associate with root colonizing mycorrhizal fungi, which improve the fitness of both the fungal and plant associates. Ubiquitous occurrence and importance of AM fungi for plant growth is now a well established fact. Distribution and abundance of AM fungi vary greatly among different sites i.e. natural and manmade ecosystems (Gianinazzi – Pearson *et al*., 1985; Chaurasia, 2001).

Natural soil offers consortium of indigenous mycorrhizal fungi and often used as source of inoculam. AM fungi can be produced on a large scale by pot culture technique. Since isolation and selection of AM species (effective for growth promotion) and rising of pure culture of these species is difficult, a suitable host is required to maintain the AM culture. The beneficial use of AM inoculum in agriculture and raising nurseries has been reported (Smith and Read, 1997; Muthukumar *et al*., 2001).

Chaurasia and Khare (2005) examined the four plant species viz. *Hordeum vulgare*, *Triticum aestivum*, *Phaseolus vulgaris* and *Phaseolus mungo* for mass production of consortium of AM fungi present in the rhizosphere soil. Such mass production of AM fungi was observed in terms of (%) AM colonization, AM consortia was recorded in terms of height and dry weight of inoculated and uninoculated plants. They observed that the *Hordeum vulgare* showed the highest colonization (92%) and 74 spores per 25 g soil.

The present study is focused on the mass production of AM fungi in three monocot plants. Here an attempt was made to mass produce the AM fungi in three different host plants viz. Maize, Sorghum and Paddy.

Materials and Methods

Selection of host

Three host plant species *viz.* maize (*Zea mays*, L.), sorghum (*Sorghum halepense*, L. Pers.) and paddy (*Oryza sativa*, L.) were examined for selection of the suitable host for the mass production of AM spores. All the plants were selected on the basis of their (i) sustainability to the climatic conditions of the area (ii) having thick root system for sizeable sporulation and colonization and (iii) annual growth habit.

Pot and potting mixture

Open pot culture was used for the mass production of AM fungi. A layer of 100g of AM spore soil samples (inoculum) was spread over pot mixture (sterilized soil and sand=1:3; about 3 kg) in earthen pots (20 cm height and 25 cm diameter). These pots were used for the experimentation. The pots without AM fungal inoculum was used as control.

Surface sterilization of seeds

There will be enough number of microbes along with AM fungal populations in indigenous soil samples (inoculum) so there is no need of surface sterilization of seeds, but in order to decrease microbial contamination and to achieve healthy seedlings, seeds of maize, sorghum and paddy were surface sterilized with 0.01% mercuric chloride for 2 min. and washed several (3-4) times with sterilized water.

Sowing

Sterilized seeds of maize, sorghum and paddy were sown in each labelled Control-without AM inoculam and AM- inoculated pots respectively and water regularly for 60 Days.

Effectiveness of AM fungi

Effectiveness of consortium of AM fungi was determined by measuring plant height and weighing the fresh and dry weight after 60 Days of growth period.

Quantification of AM fungi
Assessment of fungal infection

Root samples and soil samples were collected at an interval of 15 Days. The root samples were cleared and stained using the improved procedure of Phillips and Hayman (1970). The root segments in 10% potassium hydroxide were incubated at 90^0 C in an oven for 2 h and washed well with distilled water. Then the segments were immersed in 30% hydrogen peroxide for 10-15 min for bleaching. They were thoroughly rinsed in water to remove hydrogen peroxide and acidified in 5N hydrochloric acid. They were stained by immersing for 30 min in 0.05 % trypan blue in lacto phenol and mounted. Then the root segments were squashed gently on slides containing few drops of acetic acid; glycerol (1:1 w/v) mixture and sealed the cover slips with nail polish. The slides were observed under microscope and recorded the arbuscular mycorrhizal infected root samples.

The per cent infection of AM for each plant species was measured by grid line intersect method (GioVannetti and Mosse,1980) which is based on the method of Newman (1966) and calculated by using the formula:

$$\% \text{ infection} = \frac{\text{Number of AM infected roots}}{\text{Total number of root bits examined}} \times 100$$

Isolation of AM spores from soil samples

The spore and sporocarps present in the root zone soil were isolated by the following decanting and wet-sieving technique of Gerdemann and Nicolson (1963).

Five grams of soil samples were suspended in water and were allowed to settle down for sometime. The suspension was passed through a series of sieves with 250, 206, 90 and 40 µm pore size. The spores in the soil suspension were collected.

The spores collected were placed on the filter paper and examined under a binocular microscope, transferred to a clean microscopic slide with the help of a fine needle and mounted in lacto phenol. Semi permanent slides were made by sealing the edges of the cover slip with nail polish. Microscopic observations were made under high magnification for qualitative and quantitative characters of spores.

Results and Discussion

Aeroponic inoculum production at large scale has been investigated by Souza *et al*., (1996), but has not reached commercialization. Pot-cultivation remains the preferred propagation technique, as it provides a convenient and relatively economic method to produce mycorrhizal inoculum on a large scale (Sahay *et al*., 1998). Generally, mycorrhizal fungi propagules, such as colonized roots, spores, and hyphae, are mixed with a growing substrate, and the pots are seeded and incubated under controlled conditions. Bioreactor assays with liquid AMF root-organ culture propagation (Jolicoeur *et al*., 1999) may eventually become suitable for commercialization for research needs.

The mass production of AM fungi using the different host plants showed significant response in morphological and biomass accumulation over the control plant. Results of growth performance of host after inoculum of consortium of AM fungi clearly indicated that AM inoculation increased the fresh and dry matter, shoot and root length and percent infection compared to control.

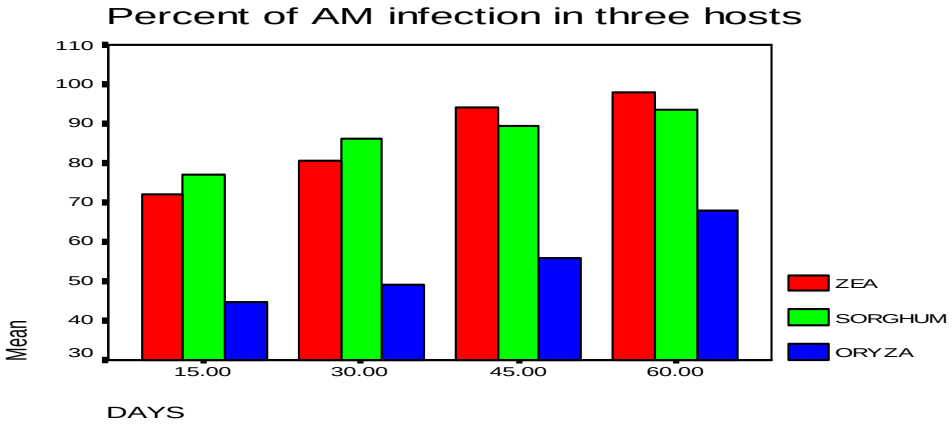
The current results showed that in *Zea mays* colonization of AM fungi was highest in all the growth periods. The AM fungi and root colonization in *Zea mays* was higher as 98±0.50% and was least in *Oryza sativa* as 58±1.00% (Table: 1 & Fig:1).The infectivity of the hypha network can be maintained in the absence of spores (Jasper *et al*., 1989; 1991). Simpson and Daft (1990) have reported that the growth stage and physiology of host plants have been postulated to influence spore production of endomycorrhizal fungi. The age of the crop and the harvest date greatly influence the size of the spore population and extent of root colonization of *Glomus mosseae* (Al-Raddad, 1991; Kapulnik and Koshnir, 1991).

Table: 1Percent of AM fungal infection in *Zea mays, Sorghum halepense* and *Oryza sativa*

Host Plant	Period (days)			
	15	30	45	60
Zea mays	$72.00^{a} \pm 1.00$	$80.67^{b} \pm 1.53$	$94.00^{c} \pm 0.50$	$98.00^{d} \pm 0.50$
Sorghum halepense	$70.00^{a} \pm 2.00$	$86.17^{b} \pm 1.04$	$89.50^{c} \pm 0.50$	$93.67^{d} \pm 0.76$
Oryza sativa	$44.67^{a} \pm 1.53$	$49.00^{b} \pm 1.00$	$55.83^{c} \pm 0.76$	$58.00^{d} \pm 1.00$

Values are mean of five replicates ± SD;The mean difference is significant at the 0.05

Fig: 1 Percentage of Arbuscular Mycorrhizal infection in *Oryza sativa, Sorghum halepense* and *Zea mays*



The maximum increase in shoot and root length was observed in AM fungi inoculated *Zea mays* (43.76% and 32.47%) as compared to control plants (Table:2). However, it was 34.67% and 17.46% in AM fungi inoculated *Sorghum halepense* as compared to control. The minimum increase in shoot length was recorded in AM fungi inoculated *Oryza sativa* (5.1%) than control. After 60th Day, the fresh and dry weight of host increased in AM fungi inoculated plants of *Zea mays* (25.33±0.42g and 6.15±0.14g per plant) than in the control plants (15.53±0.25g and 4.27±0.06g per plant). However, it was observed as 12.00±0.20g and 1.88±0.03g per plant in AM fungi inoculated and 9.63±0.15g and 1.8±0.01g per plant in control plant of *Sorghum halepense*. Comparatively, a little increase (1.22%) in dry weight was recorded in AM fungi inoculated plant of *Oryza sativa* (Table: 3).

Table: 2 Effectiveness of AM fungi on the shoot and root length of host plant

Host Plant	Shoot Length(cm)			Root length(cm)		
	C	I	% increase	C	I	% increase
Zea mays	47.67 ± 1.04	68.53 ± 0.47	43.76	15.00 ± 0.10	19.87 ± 0.32	32.47
Sorghum halepense	31.93 ± 0.40	43.00 ± 1.00	34.67	12.77 ± 0.25	15.00 ± 0.44	17.46
Oryza sativa	21.00 ± 0.05	22.07 ± 0.21	05.10	8.10 ± 0.10	9.27 ± 0.25	14.44

Values are mean of five replicates ± SD; C-Control; I- AM fungi inoculated

Table: 3 Effectiveness of AM fungi on the fresh and dry weight of host plant

Host Plant	Fresh weight(g)/plant			Dry weight(g)/plant		
	C	I	% increase	C	I	% increase
Zea mays	15.53 ± 0.25	25.33 ± 0.42	63.10	4.27 ± 0.06	6.15 ± 0.14	44.03
Sorghum halepense	9.63 ± 0.15	12.00 ± 0.20	24.61	1.80 ± 0.01	1.88 ± 0.03	4.44
Oryza sativa	3.37 ± 0.32	4.10 ± 0.46	11.72	0.82 ± 0.01	0.83 ± 0.01	1.22

Values are mean of five replicates ± SD; C - Control; I - AM fungi inoculated

At the end of the mass production, the AM spores were isolated from the AM inoculated *Zea mays* soil and showed the presence of species of AM fungi such as *Glomus*, *Acaulospora*, *Gigaspora*, *Scutellospora* and *Sclerocystis*. This is evident from the pot experimental results, rapid AM colonization and higher spores production was observed in *Zea mays*. Therefore, the heavily (AM fungi) infected root biomass of *Zea mays* served as an inoculum for further mass culture technique.

Conclusion

The AM fungi were mass produced by soil based pot culture technique. Since isolation and selection of AM species (effective for growth promotion) and rising of pure culture of these species is difficult, a suitable host is required to maintain the AM culture. The effectiveness of indigenous AM fungi on growth of different hosts like maize, sorghum and paddy were studied and found that the maize was a suitable host for mass production of AM fungi.

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