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Mass production of VAM fungi using different substrates and hosts

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The influence of soil : farmyard manure, soil : flyash and soil : sand on the VAM fungi i.e. *Glomus mosseae, G. fasciculatum* and mixed inoculum (composite spores of *Glomus geosporum, G. reticulatum, Gigaspora gigantea, Sclerocystis coremoides, Acaulospora lacunosa* and *A. laevis*) were examined using different hosts maize, bajra and jowar. Among the substrates soil : farmyard manure gave best results of VAM mass production. Different hosts showed positive mycorrhizal associations in different substrates but the degree varied.

Key words : Mass production, VAM fungi, substrates, hosts

INTRODUCTION

VA mycorrhizal fungi have great potential for use as biofertilizers in agriculture, floriculture, horticulture and foresty (Singh, 2002). The inoculation of plants with efficient strains of VA fungal endophytes during their multiplication is a promising perspective for practical use. Considering the need of this low cost agricultural input in cultivated crops, agro forestry, and wasteland development programmes, the lack of massive inoculum is an obstacle coming in the way of large scale production of VA mycorrhizal fungi. Therefore, the standardization of large-scale production technology and distribution of variable inoculum are the demand of the time and the ultimate objective of the studies should include the use of mycorrhiza to the field or nursery level on the commercial scale. Achievement of goal of mass production of endomycorrhizal inoculmum on low cost basis will make the core of a network permitting transfer of this biofertilizers to farmers, foresters, researchers and extension workers at the nursery and the field level.

In the present study for mass production, two dominating VAM fungi i.e. *Glomus fasciculatum* and *G. mosseae* associated with teak and bamboo have been selected. Several fungal species within the inoculum could meet the requirement of the host in different soils within a large area (Sieverding, 1989). Therefore, besides, the two dominant VAM fungi, the other VAM fungi, *Glomus geosporum, G. reticulatum, Gigaspora gigantea, Sclerocystis coremoides, Acaulospora lacunosa* and *A. laevis*, isolated from the rhizosphere of the selected plants have also been taken for mass production as mixed inoculum. The host plants also may stimulate selectively or limit sporulation of certain VAM fungal species suggesting varied affinities between hosts and symbiont (Al. Raddad, 1995). Several workers have tried different hosts under different soil conditions for mass production of different VAM fungi (Babu *et al.*, 2001, Gill and Singh, 2001, Harikumar and Potty, 2002). In the present investigation three different hosts namely maize, bajra and jowar have been screened for mass production of the mentioned VAM spores.

Composition of potting mixture is a great importance of VAM colonization and sporulation (Mehrotra and Mehrotra, 1999). Present experiment has been conducted to select best host and substrate that would give maximum VAM fungal spore production in the least amount of time. Experiments have been conducted using three hosts, three substrates and three VAM fungal species.

MATERIALS AND METHODS

Selection of host : To select the most suitable host for maximum root colonization of VAM fungi three hosts, namely, maize, bajra and jowar were selected.

Selection of substrates : To find out the most suitable substrate for the mass production of *Glomus fasciculatum, G. mosseae* and mixed VAM inoculum three different substrates i.e. soil : farmyard manure (FY) (3 : 1 w/w), soil : flyash (FA) (5 : 1 w/w) and soil : sand (SS) (3 : 1 w/w) were selected. All the substrates were sterilized in an autoclave at 15 p.s.i. for 30 min.

VA endophytes : Dominant VAM fungi isolated from the rhizospheres of *Tectona grandis* and *Dendrocalamus strictus* were selected for mass production. These included *Glomus mosseae, G. fasciculatum* and all other isolated VAM fungi were grouped together as mixed VAM inoculum (*Glomus geosporum, G. reticulatum, Gigaspora gigantea, Sclerocystis coremoides, Acaulospora lacunosa* and *A. laevis*).

Starter inoculum production : Starter inoculum of each selected VAM fungi were raised by 'funnel technique' using sorghum as host. Spores were isolated from the rhizosphere of the selected plants by 'wet sieving and decanting technique' on Whatman filter paper. The spores were picked under stereobinocular microscope. These spores were surface disinfectd using 2% (w/v) Chloramine-T for 15 min and rinsed throughly in sterilized water. The viability of the spores were tested by thionine stain, and the viable spores turn dark blue on staining. Ten healthy and viable spores were selected and then introduced in funnels using sorghum as host. In the funnel technique 100 g of soil : sand (3: 1) substrate was taken. After 30 days the roots were studied for VAM root colonization and 10 g of soil samples were studied for VAM spore quantification. The colonized roots and soil samples containing the VAM spores were used for further mass inoculum production in pots using different substrates and hosts.

Filling of the pots : Pots selected for soil : farmyard manure substrate were filled with 1800 g of sterilized soil and 600 g of farmyard manure. Pots for soil : flayash were filled with 1800 g of sterilized soil and 360 g of flyash and for soil : sand substrate pots were filled with 1800 g of sterilized soil and 600 g of sterilized sand. The substrates were thoroughly mixed before adding the VAM inoculum.

VAM inoculation : After filling the post with different substrates a thin layer of 200 g of soil having approximately 150 VAM spores and colonized root pieces were added in each experimental pot.

Sowing of seeds : Twenty seeds of each selection host were surface sterilized with 10% solution of sodium hypochlorite for 1-2 min and then washed thoroughly with distilled water to remove sodium hypochlorite before sowing them. The seeds were sown approximately 2 cm below the soil in each pot. For each treatment with different host five replicates were taken. Plants were grown under natural illumination and watered with distilled water as required and also supplied with Hoagland's nutrient solution (100 ml/pot) after regular intervals of 15 days.

Records of the experiments : The plants were harvested after 30, 60, 90 and 120 days for studying the VAM root colonization and for spore quantification.

Estimation of VA mycorrhizal spore population : VAM spores were isolated from soil samples using 'wet sieving and decanting technique' (Gerdemann and Nicolson, 1963) and the quantification of VAM spores was done by 'grid-ling intersect method' (Adholeya and Gaur, 1994).

Estimation of VAM root colonization : Mycorrhizal root colonization of the different hosts was observed by 'rapid clearing and staining technique' (Phillips and Hayman, 1970).

Identification of VAM fungal spores : Intact VAM spores were examined and identified using manual of Schenck and Perez (1987), Morton and Benny (1990), and Mukerji (1996).

Statistical analysis : The data was statistically analyzed using analysis of variance with least significant difference (LSD) test used to determine significant differences.

RESULTS AND DISCUSSION

The mass production of the selected VAM fungi showed varying results with different hosts and substrates. In case of *Glomus fasciculatum* (Table 1) when maize was used as host after 30 days of inoculation, VAM mycelial growth was seen in all the three substrates used, however, arbuscules were observed more in case of soil : farmyard manure treatment. However, there were no vesicles seen after 30 days of inoculation in any of the substrates. VAM spore count was also observed more with soil : farmyard manure treatment followed by soil : flyash and simple soil : sand treatment. VAM mycelial growth, arbuscules, vesicles and spore count were found to be steadily increasing from 60 to 90 and 120 days. Soil : farmyard manure gave best results followed by soil : flyash and soil : sand treatment. The spore count was influenced by substrates and was observed statistically significant at P=0.05. In mass production of *G. fasciculatum* with bajra after 30 days of inoculation mycelial growth and arbuscules were seen in all the substrates, however, vesicles were observed only in soil : sand substrates. After 60 days of inoculation mycelial growth, arbuscules and vesicles showed a steady increase till 120 days in all the substrates. The spore counts as influenced by substrates was found significant at P=0.05. Mass production of *G. fasciculatum* with jowar showed same trend as with maize when substrates were used. In case of jowar mycelial growth, arbuscules, vesicles and spore count showed steady increase from 30, to 60, 90 and 120 days. VAM spore count was more in soil : farmyard manure followed by soil : flyash and simple soil : sand substrates and was observed statistically significant at P=0.05. When compare the three hosts for *G. fasciculatum* spore count, best results were seen with maize in soil : farmyard manure and soil : flyash treatment. In simple soil : sand treatment bajra gave best results of the three selected hosts.

In mass production of *Glomus mosseae* (Table 2) with maize, bajra and jowar it was observed that soil : farmyed manure was best substrate followed by soil : flyash and simple soil : sand substrate when mycorrhizal root colonization and spore count were considered. However, in soil : farmyard substrate vesicles appeared after 60 days of inoculation. VAM spore count in all the three selected hosts in different substrates showed increasing trend from 30 to 60, 90 and 120 days of inoculation. However, the extent of spore production, in all the three hosts was different. The spore count of *G. mosseae* in maize were found significant at P=0.05. The spore count in bajra along substrates was sig-

Table 1 : Effect of various substrates and hosts on spore count and root colonization by *G. fasciculatum* inoculum

Host	Maize								Bajra								Jowar							
Days	30		60		90		120		30		60		90		120		30		60		90		120	
Sub.	M	SC	M	SC	M	SC	M	SC	M	SC	M	SC	M	SC	M	SC	M	SC	M	SC	M	SC	M	SC
FA	71	25	95	31a	99	46	99	60	85	27	85	29	88	58a	42	54a	87	20a	90	24a	91	31a	99	55a
5:1	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	0.4	2.8	2.1	0.6	0.4	0.7	0.2	0.9	2.3	0.9	2.3	2.2	0.7	4.6	0.9	1.7	2.8	2.2	0.7	1.1	0.7	0.7	0.4	1.9
FY	86	43	75	55	85	64	94	85	98	42	98	48	98	61a	57	77	75	35	91	50	93	66	99	71
3:1	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	2.5	1.5	2.3	0.9	4.6	1.2	2.1	2.3	0.2	1.2	0.2	0.9	0.5	1.7	1.4	0.7	2.3	2.0	1.3	0.4	2.7	0.7	0.5	0.7
SS	70	17	85	26a	98	32	98	47	98	22	98	24	99	58a	31	53a	76	18a	90	26a	89	32a	99	51a
3:1	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	0.2	1.2	2.0	2.5	1.2	1.1	0.2	0.9	0.4	1.1	0.2	1.6	0.2	3.6	0.4	2.5	2.7	0.9	0.4	0.4	0.2	2.3	0.2	0.7
LSD 0.05	—	8.4	—	7.6	—	4.5	—	6.6	—	4.8	—	5.6	—	4.3	—	7.9	—	6.9	—	3.3	—	6.4	—	5.2

M : % mycorrhizal rot colonization. SC : Mycorrhizal spore count (10 g of soil). FA : Soil : Flyash. FY : Soil : Farmyard manure, SS : Soil : Sand
Mean of three replicates, ± Standard error. The mean in each column followed by same letter don't differe significantly.

Table 2 : Effect of various substrates and hosts on spore count and root colonization by *G. mosseae* inoculum

Host	Maize								Bajra								Jowar							
Days	30		60		90		120		30		60		90		120		30		60		90		120	
Sub.	M	SC	M	SC	M	SC	M	SC	M	SC	M	SC	M	SC	M	SC	M	SC	M	SC	M	SC	M	SC
FA	99	35	100	40	100	43a	100	75	89	34	90	50	100	60	100	80	50	32	82	35ab	100	55	100	63a
5:1	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	0.2	2.8	0	2.0	0	0.4	0	1.5	1.7	0.9	0.9	0.2	0	1.4	0	0.9	2.3	1.2	1.7	1.6	0	0.9	0	0.7
FY	72	48	91	63	100	84	100	113	98	43	99	71	100	72	100	90	83	44	99	45a	99	64	100	73
3:1	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±		±				±	±	±
	1.1	0.4	1.3	1.2	0	2.4	0	1.1	0.4	1.5	0.2	0.4	0	0.4	0	1.2	1.5	1.9	0.4	2.3	0.2	2.3	0	0.9
SS	91	24	98	33	99	42a	100	62	56	21	84	35	96.6	51	99	65	71	20	84	28b	93	31	99	65a
3:1	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	0.4	1.3	0.7	0.7	0.4	1.1	0	0.9	0.4	0.9	0.7	1.2	1.65	0.4	0.4	0.7	0.7	2.3	0.9	3.4	2.3	0.4	0.2	0.9
LSD 0.05	—	8.6	—	3.7	—	6.0	—	5.2	—	3.8	—	3.8	—	3.4	—	5.0	—	8.7	—	11.6	—	3.4	—	3.5

M : % mycorrhizal rot colonization, SC : Mycorrhizal spore count (10 g of soil), FA : Soil : Flyash, FY : Soil : Farmyard manure, SS : Soil : Sand
Mean of three replicates, ± Standard error, The mean in each column followed by same letter don't differe significantly.

nificant at P=0.05. In jowar spore count in different substrates was found significant at P=0.05 but there was not much significant difference in spore count between soil : flyash and soil : sand substrates. In soil : flyash treatment bajra showed maximum spore counts, maize was best host for soil : farmyard manure substrate, jowar and bajra were found suitable host for simple soil : sand treatment.

Mass production of mixed VAM inoculum (Table 3) that contained mixed spores of *Glomus geosporum, G. reticulatum, Gigaspora gigantea, Sclerocystis coremoides, Acaulospora lacunosa* and *A. laevis.* In maize mycelial growth, arbuscules and vesicles were found in all the substrates after 30 days of inoculation, however, in soil : sand substrate vesicles were observed after 60 days of inoculation. In bajra mycelial growth and arbuscule were observed after 30 days of inoculation and vesicles in all the substrates were seen after 60 days of inoculation. In jowar mycelial growth, arbuscules and vesicles were seen in all the substrates after 30 days of inoculation but in soil : sand substrates vesicles were seen after 60 days of inoculation. All three hosts and substrates showed increasing trend in case of mycorrhizal root colonization and spore count after 30, 60, 90 and 120 days. Here also the data were found statistically signifi-

Table 3 : Effect of various substrates and hosts on spore count and root colonization by Mixed VAM inoculum

Host	Maize								Bajra								Jowar							
Days	30		60		90		120		30		60		90		120		30		60		90		120	
Sub.	M	SC	M	SC	M	SC	M	SC	M	SC	M	SC	M	SC	M	SC	M	SC	M	SC	M	SC	M	SC
FA	62	38	94	85a	96	86	99	85a	68	36a	99	51	99	67	99	73a	88	41a	95	82a	95	90a	98	94a
5:1	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	1.2	1.3	2.3	0.4	1.6	0.7	0.2	2	1.4	1.9	0.4	1.5	0.4	0.9	0.2	1.9	3.1	0.4	2.3	1.1	1.4	0.7	0.7	0.7
FY	82	60	96	91a	97	93	99	108	91	41a	99	62	98	80	99	102	85	55	96	83a	96	95a	98	103
3:1	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	1.2	0.7	0.2	0.7	0.9	0.9	0.2	3.8	3.1	1.5	0.4	1.1	0.2	0.4	0.2	3.0	1.1	1.9	0.7	2.0	0.9	0.2	0.5	2.8
SS	59	22	89	65	96	82	99	89a	21	25	96	43	97	56	99	65a	78	37a	93	48	96	70	98	91a
3:1	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	0.7	1.1	0.4	2.5	1.4	1.1	0.4	0.4	0.7	2.1	0.4	2.3	0.5	1.8	0.2	2.3	1.2	3.5	1.9	3.6	1.5	2.3	1.2	0.9
LSD 0.05	—	4.7	—	6.7	—	3.6	—	11.1	—	7.9	—	7.3	—	5.3	—	10.5	—	10.7	—	10.5	—	6.0	—	7.6

M : % mycorrhizal rot colonization, SC : Mycorrhizal spore count (10 g of soil), FA : Soil : Flyash, FY : Soil : Farmyard manure, SS : Soil : Sand
Mean of three replicates, ± Standard error, The mean in each column followed by same letter don't differe significantly.

cant at P=0.05. Jowar was best host for mass production of mixed VAM inoculum in soil : flyash and simple soil : sand substrate and maize was the best host for maximum VAM spore production in soil : farmyard substrate.

In the present study there were various effects of the three hosts on spore production by different VAM fungal species on the three substrates used. Among the three hosts screened for mass production of the selected VAM fungi, maize and bajra were suitable for all the VAM fungal species after 90 days. The VAM fungus-plant symbiosis is a complex system and the extent of endophyte-host interaction depends on type of root system and supply of carbohydrates to the fungal partners (Struble and Skipper, 1988).

The maximum spore production (per 10 g of soil) of *Glomus fasciculatum* and mixed VAM inocula showed influence of host plant on these two fungal species. *G. mosseae* did not show much difference in spore production in the different hosts used. These results are consistent with findings of Barbara *et al.*(1986) who also reported that *G. fasciculatum* is more influenced by the type of host used while *G. mosseae* appear to be less affected by host plant.

When the spore producing capacity of the three VAM fungi was analyzed, differences among fungi were evident and mixed VAM inoculum consistently produced higher number of spores in all the three host plant species used. When the spore producing capacity of the three hosts were analyzed, maize gave the maximum VAM spores irrespective of the inoculum used. Maize has been reported as best host due to its finer and fibrous root system which provides the favorable conditions with more entry points for the VAM fungus to cause maximum root colonization (Al. Raddad, 1995).

VAM spore number and root colonization were low in the beginning of the growing season of the hosts. This may be due to the presence of small roots at this stage and less entry parts for the fungus (Van Duin *et al.* 1989). Spore numbers tend to increase with age of the corp near the harvest time. This is similar to findings of Al-Raddad (1995), Gill and Singh (2001). Another reason for maximum VAM root colonization at the harvest time may be that at this time plants are larger and larger plants often have more extensive root systems than smaller plants and thus allowing greater mycorrhizal colonization and sporulation (Mago and Mukerji, 1994).

In the root colonization arbuscules were quickly produced and extensive vesicles were formed only at the end of the growing season. Allen (1983) and Van Duin *et al.* (1989) also reported arbuscules formation at the beginning of the growing season. Arbuscules are formed at the beginning of the growing season may be to make possible the additional nutrient exchange between symbiont and host.

When the substrates were compared, in all the cases soil : farmyard manure combination gave best results. Harnikumar and Bagyaraj (1989), Panja and Chaudhuri (1999), EL-Din *et al.* (2000), Joner (2000), Upadhyay (2004) also reported that farmyard manure enhance the VAM spore count. Mycorrhizal fungi compete successfully for P during mineralization of organic matter in soils (Joner and Jakobsen, 1994) that may be responsible for maximum mycorrhizal associations. The VAM fungi survive saprophyticaly in organic particles (Warner 1984), which favours further colonization during the vegetation period (Schonbeck, 1984). In the present investigation 600 g of farmyard manure, sand and 360 g of flyash was taken at this ratio of different substrates maximum mycorrhizal root colonization was seen. Flyash application in soil at low level appears to be helpful for root symbiont and consequently may enhance plant growth and their productivity. Thus selection of substrates is important from the point of view its suitability as material enhancing growth of the host plants in mass production process and as carrier in effective delivery of VAM spores and colonized root segment upto user's end.

Different species of VA mycorrhizal fungi differ in their ability to improve plant growth. Therefore, for inoculation of VAM species to the respective plant the dominant VAM species must be isolated and mass produced on suitable host and substrate to get large VAM inoculum for further inoculations. The significance of this impact of flyash which is now being abundantly produced by thermal power plants

and the disposal of which poses an environmental problem can be explored further and the results may be useful in agriculture.

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