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Efficacy of Arbuscular Mycorrhizal Fungi for litchi [*Litchi chinensis* (Gaertn.) Sonn] *marcots* inoculation in nursery

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The pot experiments were conducted for the selection of efficient strain of AM fungi for establishment of marcots of litchi [*Litchi chinensis*(Gaertn.)Sonn]. Seven species of indigenous AM fungi were isolated from rhizospheric zone of litchi trees. Out of these, three species of AM fungi were selected on the basis of spore population and root colonization. Selected species were inoculated separately or in association with *Azospirillum brasilense* on two substrate mediums. Out of three selected fungi *S. pakistanica* along with *A. brasilense* was observed to have the best effect on plant growth and root colonization. The results clearly establish that inoculation of marcots with suitable AM fungi along with *A. brasilense* showed better survival and establishment of marcots in nursery. This finding can be used for expansion of litchi orchard even in non-traditional areas.

Key words: Arbuscular mycorrhizal fungi,*Azospirillum brasilense,* litchi, marcots

INTRODUCTION

Litchi [*Litchi chinensis* (Gaertn.) Sonn] plants are botanically member of family Sapindaceae and subfamily Nepheleae. Litchi fruit is a good source of sugar, ascorbic acid, carbohydrates, vitamin C, protein (0.8-0.9%) (Leung,1961), fats (0.03 to 0.5%) and mineral specially calcium, phosphorus and iron as well as it is economically, ecologically and environmentally viable fruits. They are excellent thrust quenchers and reported to be tonic for heart, brain and liver (Symal and Mishra,1984). Apart from fruit, other parts of plant e.g., leaves used for making poultice and flowers, bark and roots for making decoction for throat gargle (Maiti,1985). Presently in India an area of 0.05 million hectares is under litchi fruit cultivation with an annual production of 0.44 million tonnes. India is a second largest litchi producing country after China. In India 4,28,900 metric tonnes of litchi being produced that is grown in the state of Bihar, Tripura, West Bengal, Uttar Pradesh, Punjab and Haryana. The total production of litchi in India, 74% is contributed by Bihar. Productivity is highest in Bihar followed by West Bengal. Due to its major production, litchi is the livelihood security for millions of people as it provides both on-farm and off-farm employment. Small and marginal farmers get additional income from litchi plants in their homesteads.

Arbuscular mycorrhizal fungi play key role in establishment and survivability of juvenile plants (Miller and Jastrow,1992; Kranabetter and Wylie,1998), and also help in uptake of macro and micronutrients (Smith and Read,1997). AM fungi show a preferential colonization to hosts and thereby the extent to which a host is benefited depends on the fungal species involved in the symbiosis (Miller *et al.*,1987). Litchi is also a mycorrhizal dependent fruit tree (Coville,1912; Marloth,1947; Kadman and Slore,1974) and it shows luxuriant growth in presence of AM colonization in feeder roots. Thus, it is desirable to screen the efficient AM fungus for a particular variety of litchi plants in order to harness the maximum ben-

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efit from the fungus. There are reports to substantiate that these bio inoculants play vital role in the establishment of transplanted plants (Elo *et al.*, 2000 and Moreira-Souza *et al.*, 2003) and also provide ambient condition for seedling growth.

Hence, in the present investigation, pot experiments are conducted to evaluate the efficacy of selected AM fungi species separately and in combination with *Azospirillum brasilense* on growth and root colonization of marcots.

MATERIALS AND METHODS

This investigation was carried out under nursery conditions. The soil was collected from degraded field from depth of 0-15cm. The soil had pH=6.7, nitrogen=0.003%, phosphorus=0.002%, potassium=0.271%, calcium=0.041%, sodium=0.003%, organic carbon=0.13% and organic matter=0.224% and an indigenous AM mycorrhizal population was 10-30 spores/g soil. The vermicompost used in experiments had a pH=7.20, nitrogen=1.3%, phosphorus=0.69%, potassium=0.739%, calcium=0.654%, sodium=0.443%, organic carbon=11.1% and organic matter=19.14%.

Selection of indigenous AM fungi

The indigenous AM fungi were isolated from the different litchi orchards by Wet sieving and decantation method as outlined by Gerdemann and Nicolson(1963). Amongst all seven species of AM fungi viz., *Glomus fasciculatum, G. aggregatum, G. maculosum, G. multicauli, Sclerocystis pakistanica, Gigaspora* sp., and *Gigaspora margarita* were commonly found in the litchi orchard, which were identified by the help of Manual of Schenck and Perez (1989). These isolated fungi were multiplied using sterilized sand: soil mix (1:1) as the substrates of sorghum host plants. Three species of AM fungi were selected for further experiments due to their affinity toward the host plants. These plants were left for 60 days after that the sorghum harvested and substrate containing hyphae and root bits were air dried and used as inoculum for experiments.

Pot experiment

The experiments were conducted on two different potting medium. The first potting medium contained 3 kg non sterilized vermicompost and in second potting medium was 1 kg of degraded soil with 3 kg non sterilized vermicompost. The 25 g root inoculum of specific strain of AM fungi having a 75-85% root colonization and 25 g of sterilized carrier medium (Sterilized FYM: soil,1:1) of *A. brasilense* having a $3x10^8$ cell/g were used in experiment. The *Desi* marcots were planted in ready pots. The following sets of experiment were conducted under both the substrates. T1= Sp1 (*Glomus maculosum),* T2=Sp1 + *A. brasilense,* T3=Sp2 (*Glomus multicauli*), T4=Sp2 + *A. brasilense,* T5=Sp3 (*Sclerocystis pakistanica*), T6=Sp3 + *A. brasilense,* T7=Spmix (*G. maculosum, G. multicauli* and *Sclerocystis pakistanica*), T8=Spmix + *A. brasilense,*T9=Control set (3 kg non-sterilized garden soil). Each treatment (T) with five replications were maintained and watered according to requirement till one year.

The morphometric analysis was done in term of plant height, shoot length, diameter and new leaves emergence. Plant height was measured from soil to the growing tip of the plants. Shoot length was measured by this formula, Shoot length = Main stem + average of sub branches. Stem diameter was measured 1cm above from the soil surface using vernier calipers. The new born leaves were calculated from tips of plants. The feeder roots were collected from each pot and screened for root colonization by Phillips and Hayman's (1970) method.

RESULT AND DISCUSSION

Selection of indigenous AM Fungi: Performance of any plant depends on its relation with rhizosphere micro flora, host specificity, quantity and quality of native AMF population and PGPR (Howler *et al.*,1982; Vasanthakrishna *et al.*,1995). Therefore, before any recommendation it deems fit for the selection of an efficient and appropriate fungus for plant productivity (Singh and Tilak,1990; Bagyaraj and Verma,1995). The native flora may be more efficient than an introduced one because they can adapt themselves well with the known soil conditions. In this case it is essential to determine and identify native rhizosphere AMF population and their infectivity rate with litchi marcots.

The seven species of VAM genera viz., *Glomus fasciculatum, G. aggregatum, G. maculosum, G. multicauli, Sclerocystis pakistanica, Gigaspora sp.,* and *Gigaspora margarita* were isolated from rhizosphere soil of litchi plant. The findings clearly revealed that species of *Glomus* particularly *G.*

multicauli and *G. maculosum* were found to have greater affinity with host plants where %RC (root colonization) and SP (spore population) ranged between 94% - 99% and 30 - 32 spores/g soil respectively. Similar degree of roots infectivity was also observed in case of *Sclerocystis pakistanica.* On the basis of performance, these three species of AMF (*G. maculosum,G. multicauli* and *S. pakistanica*)were selected for further investigation.

It is also evident from the result (Table 1) that apart from better response of *S. pakistanica*. Sp1 and Sp2 responded differently either applied independently or in combination with *A. brasilense.* However, dual inoculation always enhanced the growth of marcots with few exceptions.

G. multicauli was also found to have a significant effect on the plant height, shoot length, stem diameter and new leaves emergence when inoculated with *A. brasilense*. This reflects that *A. brasilense* enhances the growth with any strain of AMF studied so far.

Table 1: Difference in growth parameters after one year in *Desi* var. treated with different strains of AMF under soil+vermicompost (1:3) and vermicompost medium.

	Soil : Vermicompost (1:3)				Vermicompost			
Treatments (T)	Pl. ht. (cm.)	Sh.lt (cm.)	Diam. (cm.)	No. of lf.	Pl. ht. (cm.)	Sh.lt (cm.)	Diam. (cm.)	No. of lf.
Sp1	6.50 ± 0.18 (18.8)	0.80 ± 0.17 (11.8)	0.01 ± 0.05 (0.80)	025 (250)	5.00 ± 0.09 (20.0)	1.10 ± 0.17 (13.4)	0.03 ±0.06 (2.36)	013 (130)
Sp1+A.b	8.00 ± 0.16 (24.2)	1.90 ± 0.08 (31.6)	0.04 ± 0.06 (2.85)	074 (740)	8.00 ± 0.12 (26.6)	2.10 ± 0.14 (26.3)	0.04 ±0.08 (3.00)	031 (310)
Sp2	27.7 ± 0.08 (83.9)	1.00 ± 0.21 (13.8)	0.10 ± 0.08 (9.00)	066 (660)	4.10 ± 0.10 (17.4)	1.20 ± 0.09 (14.8)	0.10 ±0.02 (9.00)	023 (230)
Sp2+A.b	32.0 ± 0.06 (91.4)	0.30 ± 0.10 (4.28)	0.03 ± 0.09 (1.92)	070 (700)	7.50 ± 0.15 (34.9)	1.60 ± 0.16 (16.3)	0.11 ±0.08 (12.3)	059 (590)
Sp3	31.5 ± 0.10 (81.8)	1.60 ± 0.12 (25.0)	0.04 ± 0.05 (2.38)	061 (610)	7.00 ± 0.9 (26.9)	1.20 ± 0.23 (12.9)	0.06 ±0.02 (6.31)	049 (490)
Sp3+A.b	42.8 ± 0.09 (133)	1.80 ±0.07 (32.7)	0.09 ± 0.02 (8.10)	075 (750)	31.5 ± 0.20 (128)	4.60 ± 0.05 (43.3)	0.08 ±0.04 (7.61)	063 (630)
Spmix	18.5 ± 0.12 (48.0)	1.40 ± 0.17 (19.7)	0.04 ±0.05 (3.50)	071 (710)	14.9 ±0.22 (53.0)	2.20 ± 0.12 (27.5)	0.03 ±0.09 (2.36)	023 (230)
Spmix+A.b	8.8 ± 0.21 (25.1)	4.00 ± 0.05 (68.9)	0.05 ± 0.03 (3.00)	025 (250)	24.0 ± 0.4 (88.8)	4.10 ± 0.06 (41.8)	0.04 ±0.05 (2.91)	080 (800)
Control	3.10 ± 0.18 (9.50)	0.20 ± 0.22 (3.27)	0.00 (0.00)	030 (300)	4.20 ± 0.15 (16.3)	1.00 ±0.20 (12.5)	0.02 ±0.04 (1.50)	008 (80)

(±): Indicate the Standard Errors, the values in parenthesis indicate the percent increase. (Pl. ht. =Plant height, Sh.lt= Shoot Length, Diam.= Diameter, No. of lf. = Number of leaves)

Effect of individual AMF on the growth of marcots

The maximum per cent increase in plant height (132%), diameter (8.1%) and number of new leaves emergence (750%) were recorded in T6 treatment, however, maximum increase in shoot length (68.9%) was recorded in T8 treatment in soil+vermicompost substrate whereas, in case of vermicompost substrate only *S. pakistanica* significantly enhanced the plant height(112.23%), shoot length(30.8%) and diameter(6.05%) against control, moreover the Sp mix+*A. brasilense* showed better impact on the number of leaves emergences.

The comparative study revealed that the impact of different growth parameters on *Desi* marcots, illustrate that the *S. pakistanica* strain of AM fungus was found the best and efficient strain for growth and survivability of marcots (*Desi).*

Root colonization

Mixture of the three strains of AM fungi (Spmix) separately and in combination with *A. brasilense*

showed maximum per cent of root colonization i.e. 78% and 76% respectively on both substrates. It was followed by Sp3+*A. brasilense* in vermicompost substrate only. Soil + vermicompost medium showed better colonization in all the treatments except Sp3+*A. brasilense.*

However, no definite correlation could be established between growth parameters and root colonization.

Desi variety of litchi marcot showed varied responses to different AMF strains with or without *A. brasilense* under two different potting materials. *S. pakistanica* with *A. brasilense* conferred maximum growth benefits compared to other bioinoculants used under study henceforth this combination might be considered as the best for survivability and growth of marcots under nursery conditions.

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