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GROWTH RESPONSE OF RUBBER (*HEVEA BRASILIENSIS* MUELL. ARG.) SEEDLINGS TO INOCULATION WITH ARBUSCULAR MYCORRHIZAL FUNGI AND OTHER BENEFICIAL MICROORGANISMS

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The effect of three arbuscular mycorrhizal fungi (AMF) on growth of rubber seedlings in polybags was studied. Among these the seedlings treated with *Glomus fasciculatum* had attained the highest growth rate in terms of girth and height followed by those treated with *Glomus mosseae* and *Gigaspora margarita.* The dual inoculation with *G. fasciculatum* and *G. mosseae* exhibited a synergistic effect on growth of plants. The high percentage of mycorrhizal root colonization, spore numbers and maximum plant biomass increase were recorded in the combined inoculation of *G. fasciculutum* and *G. mosseae* over the control. An increase in the total plant biomass over control was also observed in singly treated plants with *G. fasciculatum, G. mosseae* and *G. margarita*. In nursery, the girth and height of plants in combined inoculation with AMF and Phosphate solubilizing microorganisms (PSM) was significantly superior to rest of the treatments. The dual inoculation of seedlings with AMF + *Azotobacter* sp. also attained higher girth and height over the uninoculated plants. In single inoculation, plants with AMF attained the highest girth and height followed by PSM and *Azotobacter*. The present study shows the potential of AM fungi along with PSM or *Azotobacter* to improve the growth of rubber seedlings in the seedling nursery.

Keywords: AM fungi, Biomass, Growth, *Hevea brasiliensis*, Root colonization, Seedlings

INTRODUCTION

The presence of arbuscular mycorrhizal fungi (AMF) is highly essential for sustainable agriculture as they enhance the transportation of mineral nutrients to the plants and carbon compounds to the soil and its biota (Reid,1990). Earlier studies revealed that the AMF can improve the photosynthetic activity and water uptake of plants, reduce the susceptibility of plants to the pathogens and enhance the uptake of limited nutrients like phosphorus and zinc (Brown and Bethlenfalvay, 1988; Giovannetti, 1990; Iwano, 1993; Isabelle *et al*., 1998). The mycorrhizal fungi may be integrated in the disease management by producing mycorrhizal seedlings to prevent infection by pathogens (Mridha, 2003). AMF also provide a wider absorptive surface than root hairs and help in absorption of immobile ions in soils such as P, Cu and Zn

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(Munyanzia *et al.*, 1997). Plants inoculated with microbial consortia including AMF, *Azospirillum* sp., *Azotobacter* sp. and *Pseudomonas fluorescens* prompted successful establishment and reasonable growth after two years of inoculation (Singh and Jamaluddin, 2010). Many workers attributed the enhanced growth in mycorrhizal plants by the increased uptake of P from the low fertility soils (Bagyaraj, 1984; Hall, 1988; Youpensuk *et al*., 2004). Differential response of 13 fruit trees to AMF inoculation in terms of mycorrhizal formation, root colonization, relative mycorrhizal dependency and phosphorus concentrations in shoot tissues was observed by Ba *et al.* (2001).

The Para rubber, *Hevea brasiliensis* is a perennial crop which yields the natural rubber polymer. Rubber has been extensively cultivated in the North East India where the gestation period is almost 8 to 9 years to get crop return. Though much information is available on the importance of AMF in relation to growth and development of a variety of plant species, very little information is available with regard to rubber. Ikram *et al*. (1993) observed no relationship between the plant growth improvement and amount of root infection by AMF. However, it was confirmed that the P uptake by rubber in sterilized soils had improved in the presence of efficient AMF. Inoculation with selected AMF gave significant growth improvement of rubber seedlings when the level of rock phosphate was low (Joseph *et al*., 2002). Considering the role of soil and environment on host-AMF interaction, the present study was undertaken to understand the role of AMF on rubber in north east region.

MATERIALS AND METHODS

The experiment was carried out at the research farm of Rubber Research Institute of India at Sarutari, Kamrup, Assam. The pH of the soil ranged from 4.5 to 5.5. The polybags were filled with surface layer soil (0-15 cm depth) having indigenous AM propagules of 35 to 40 spores per 50 g soil.

The spores of three native AMF (*Glomus fasciculatum*, *Glomus mosseae*, *Gigaspora margarita*) were isolated following the method of Gerdemann and Nicolson (1963) and mass multiplied by inoculating single spore by funnel neck method using *Sorghum bicolor* as the host plant in sterilized soil (Nicolson,1967). The other beneficial organisms like phosphate solubilising microorganism (PSM) and *Azotobacter* were also isolated by dilution plate method usingPikovskaya's and Jensen's medium, respectively. Plant P content (%) was estimated following Tandon(1993).

Rubber seeds were surface sterilized with 0.1% $HgCl_2$ solution and kept on the steam sterilized sand bed for germination. The seedlings were transplanted into polybags (65x35 cm) @ one seedling per bag. Ten gram soil based AMF containing 40-50 infective propagules per gram soil along with infected roots of host plants were added to the planting hole. Completely randomized design (CRD) was adopted for laying out the experiment. The treatments for inoculating rubber seedlings in polybags were: T1 = *G. fasciculatum* alone, T2 = *G.mosseae* alone, T3 = *G. margarita* alone, T4 = *G.fasciculatum* + *G. mosseae*, T5 = G.*fasciculatum* + *G. margarita*, T6 = Uninoculated control. Each treatment was replicated three times with 10 polybag plants per replication. In another study

conducted in nursery, six treatments with three replicated beds having 40 seedlings in each bed were maintained. The germinated rubber seeds were planted directly in the nursery beds and inoculated singly with AMF, PSM, *Azotobacter* sp. and in combination of these species. The treatments in seedling nursery were: T1 = AMF alone, T2 = PSM alone, T3 = *Azotobacter* sp. alone, T4 = AMF + PSM, T5 = AMF + *Azotobacter* sp., T6 = Uninoculated control. Both the experiments were repeated for two consecutive years (2008 – 09 and 2009 – 10). Univariate analysis was done using IRRI Cropstat statistical package.

The girth of rubber seedlings at 10 cm above the soil surface and the height up to the tip of the plants were measured at monthly intervals. The plants were uprooted fifteen months after planting. The plant biomass was determined by drying the plant at 70 °C in a hot air oven to a constant weight. The per cent mycorrhizal colonization of feeder roots was estimated following Philips and Hayman (1970). Soil samples of each treatment from polythene bags and nursery were estimated for the mycorrhizal spore population (Gerdemann and Nicolson, 1963).

RESULTS AND DISCUSSION

Polybag experiment

Rubber seedlings inoculated with AM fungi had higher girth and height than uninoculated control plants (Table 1). The polybag plants inoculated with *G. fasciculatum* alone, attained the higher growth in terms of girth (5.25 cm and 7.36 cm) and height (93.55 cm and 101.25 cm) during 2008-09 and 2009-10 study period, respectively as compared to uninoculated control plants. This was followed by the seedlings inoculated with *G. mosseae* and *G. margarita*, respectively. This implies that the exogenously introduced AMF species stimulated the growth of rubber seedlings. The role of AM fungi in plant growth and development and its association with *H. brasiliensis* has already been established (Ikram *et al.*, 1996). A similar observation has also been recorded by Ambika *et al*. (1994) while studying the role of AMF on the growth mulberry plants. Mridha (2003) reported that arbuscular mycorrhizae can profitably be introduced with the existing forestry system as well as agricultural and horticultural crops for better nutrient utilization, crop productivity and for

Table 1. **Girth and height of rubber seedlings treated with AMF in polybags (after 15 months)**

Treatment	2008-09		2009-10	
	Girth (cm)	Height (cm)	Girth (cm)	Height (cm)
G. fasciculatum	5.25	93.55	7.36	101.25
G. mosseae	5.12	86.77	6.82	98.45
G. margarita	4.65	79.85	5.49	81.25
G. fasciculatum + *G. mosseae*	5.56	122.82	8.60	133.57
G. fasciculatum + *G. margarita*	4.75	107.45	7.63	115.35
Control	3.42	79.88	5.27	71.75
CD P = 0.05)	1.22	27.76	2.09	36.47

reducing the inputs of chemical fertilizers. The dual inoculation of rubber seedlings with *G. fasciculatum* and *G. mosseae* had further stimulated the girth (5.56 cm and 8.60 cm) and height (122.82 cm and 133.57 cm) of the seedlings during the two year study period as compared to single inoculation. The total biomass of *H. brasiliensis* seedlings inoculated with AMF alone and in combination had increased corresponding to control plants. After 15 months of inoculation, the per cent increase in total biomass due to *G. fasciculatum* alone was 84.4% and 156.2%, respectively during 2008-09 and 2009-10 over uninoculated control plants. Dual inoculation with *G. fasciculatum* and *G. mosseae* further enhanced the total biomass by 109.8% and 182.3% over uninoculated control plants (Table 2). Chulan and Martin (1992) also found that inoculation with mixed species of AM fungi resulted in higher dry matter, yield and stem diameter of cocoa plants. The study also revealed that the application of AMF exogenously to the rubber plants in polybags improved the P content of plant biomass. Dual AMF inoculation with *G. fasciculatum* and *G. mosseae* was superior over all the other treatments with high phosphorus content. Among AMF inoculants *G. fasciculatum* alone was performing better over *G. mosseae* and *G. margarita* with higher P percentage (Table 2).

The AM fungi differ in their efficiency as they show "host preference rather than host specificity" (Vasanthakrishna *et al*., 1995) and hence the selection of efficient AMF has been stressed for inoculating different mycotrophic plants (Bagyaraj and Varma, 1995). In the present study each AMF had responded differently in promoting plant growth as reported earlier (Rajan *et al*., 2000; Joseph *et al*., 2002). The mycorrhizal spore numbers in the rhizosphere soils and the percentage root colonization of the polybag plants were increased after 15 months of inoculation. The highest spore number per gram soil (7.24 and 6.73) was observed in the mixed inoculation of plants with *G. fasciculatum* and *G. mosseae* during 2008-09 and 2009-10, respectively (Table 3). This was followed by the dual inoculation of plants with *G. fasciculatum* and G. *margarita*.

Table 2. **Biomass and P content of polybag plants (after 15 months)**

Treatment	Biomass (g/plant)		P (%)	
	2008-09	2009-10	2008-09	2009-10
G. fasciculatum	45.0 (84.4)	55.1 (156.2)	0.24	0.22
G. mosseae	47.1 (93.0)	45.4 (111.2)	0.22	0.20
G. margarita	38.8 (59.0)	33.3 (54.8)	0.21	0.17
G. fasciculatum + *G. mosseae*	51.2(109.8)	60.7 (182.3)	0.25	0.25
G. fasciculatum + *G. margarita*	50.6(107.3)	57.1 (165.5)	0.21	0.21
Control	24.4	21.5	0.14	0.16
SE	1.32	1.50	0.0043	0.0044
CD (P=0.05)	4.15	4.73	0.013	0.013

Percentage increase in biomass is given in parenthesis

In the single AMF inoculated plants, *G. fasciculatum* was having the highest number of spores per gram soil followed by *G. mosseae* and *G. margarita,* respectively. The percentage root colonization was also increased with the increased age of the treated plants with AM fungi. The highest root colonization was observed in dual inoculation with *G. fasciculatum* and *G. mosseae* followed by *G. fasciculatum* and *G. margarita*. Awasthi *et al*. (1996) also observed the highest root colonization and spore numbers in the dual inoculated plants. Plants inoculated with *G. fasciculatum* alone had recorded 85.5% and 74% root colonisation followed by *G. mosseae* (71.2% and 70.3%). The minimum root colonisation was observed in the plants treated with *G. margarita* (Table 3). Songachen and Kayong (2011) studied the diversity of AM fungi under a pine forest and observed 66 to 71% AMF colonization and 319 to 661 AMF spore density in 25g rhizosphere soils. However, Isabelle *et al.* (1999) did not find any correlation between AM spore numbers and colonization of AM roots. The endogone spore population and the root colonization were also recorded even in the uninoculated control plants from the native indigenous endophytes already present in the soil.

Table 3. **Mycorrhizal root colonisation and soil spore numbers in polybag plants (after 15 months)**

Treatment	Root colonisation (%)		Spore no(per g soil)	
	2008-09	2009-10	2008-09	2009-10
G. fasciculatum	85.5	74.0	6.72	5.44
G.mosseae	71.2	70.3	6.61	4.82
G. margarita	67.8	53.3	4.78	3.61
G. fasciculatum + *G. mosseae*	80.6	87.6	7.24	6.73
G. fasciculatum + *G. margarita*	69.7	73.0	7.10	5.99
Control	35.8	37.6	4.15	3.04
SE	3.02	2.30	0.23	0.20
CD (P=0.05)	9.52	7.26	0.72	0.65

Table 4. **Girth and height of rubber seedlings treated with beneficial organisms in nursery (after 15 months)**

Treatment	2008-09		2009-10	
	Girth (cm)	Height (cm)	Girth (cm)	Height (cm)
AMF	7.14	122.33	7.35	127.45
PSM	6.20	115.75	6.18	119.45
Azotobacter	4.74	106.42	5.79	107.90
AMF + PSM	7.65	136.45	8.73	151.63
AMF+ *Azotobacter*	7.50	128.70	7.66	131.55
Control	4.34	90.50	4.74	105.46
CD (P=0.05)	2.33	26.81	2.34	27.75

Seedling nursery experiment

In the nursery experiment the dual inoculation of seedlings with AMF and phosphate solubilizing microorganisms had attained the highest girth and height during the two year study period. This was followed by the seedlings treated with AMF and *Azotobacter* (Table 4). Among the single inoculated seedlings those with AMF alone attained the highest girth and height followed by those treated with PSM and *Azotobacter*during the years under observation. Saleh and Nokhal (1988) had also reported the beneficial effects of dual inoculation and opined that the presence of AMF might have influenced root development, which facilitated the bacterial colonization. Similarly, Zaghoul *et al*. (1996) had reported that the importance of dual inoculation with *G. mosseae* and *Azospirillum brasilense* in increasing the uptake of nutrients in wheat which in turn improves the growth of plants. High plant growth achieved due to dual inoculation of AMF and *Azotobacter* compared to single inoculation with AMF has also been reported by other workers (Bagyaraj and Menge, 1978; Gururaj, 1983). The dual inoculation with AMF and PSM (98.2% and 156.2%) as well as AMF and *Azotobacter* (90.5% and 137.8%) also increased the total plant dry weight over the control seedlings during the two year period. The single inoculated seedlings with AMF, PSM and *Azotobacter* also attained the significantly higher total biomass over uninoculated control plants (Table 5). Umakanth and Bagyaraj (1998) and Bagyaraj and Menge (1978) also reported higher dry weight of plants inoculated with both *Azotobacter* sp. and *G. fasciculatum* as compared to single inoculation with either of the organisms. Edathil and Udaiyan (1996) while studying the interaction of four AMF species observed that percentage increase of biomass compared to control was more in the treatments of quadruple combination than in triple or dual combination treatment. Ikram *et al.* (1992) observed that AM increased the stem diameter, shoot dry weight and plant height only in the treatments where P is not applied and noted 70% more increase in shoot dry weight due to AM as compared to uninoculated control plants.In the nursery, dual inoculated seedlings with AMF and PSM was found to be superior in P over the plants inoculated with AMF and *Azotobacter*.

Table 5. **Biomass and P content of nursery plants (after 15 months)**

Treatment	Biomass(g/plant)		P (%)	
	2008-09	2009-10	2008-09	2009-10
AMF	51.5 (79.4)	65.6 (115.7)	0.24	0.24
PSM	40.4 (40.7)	39.5 (29.9)	0.24	0.25
Azotobacter	39.1 (36.2)	36.5 (20.0)	0.15	0.14
AMF + PSM	56.9 (98.2)	77.9 (156.2)	0.29	0.28
AMF + *Azotobacter*	54.7 (90.5)	72.3 (137.8)	0.24	0.23
Control	28.7	30.4	0.16	0.17
SE	1.51	0.89	0.0061	0.0065
CD (P=0.05)	4.75	2.79	0.019	0.020

Percentage increase in biomass is given in parenthesis

Table 6. **Mycorrhizal root colonisation and endogone spore numbers in nursery plants (after 15 months)**

Treatment	Root colonisation (%)		Spore no (per g soil)	
	2008-09	2009-10	2008-09	2009-10
AMF	75.20	88.00	7.33	6.02
PSM	70.80	71.30	6.14	4.94
Azotobacter	68.60	72.00	5.48	4.06
AMF + PSM	79.00	84.30	7.82	6.40
AMF + *Azotobacter*	77.50	81.00	7.40	6.10
Control	38.50	47.00	5.27	3.72
SE	2.11	3.21	0.14	0.15
CD (P=0.05)	6.67	10.12	0.46	0.49

In single inoculated plants, AMF and PSM were on par with higher P over *Azotobacter* and control plants (Table 5). The *Azotobacter* produces growth regulators which enhance the root growth resulting in more root area for AMF to colonize and there by enhancing plant growth (Umakanth and Bagyaraj, 1998). High percentage of root colonization was observed in nursery plants where AMF and PSMwas inoculated singly and also in dual inoculum with AMF + PSM and AMF + *Azotobacter.* However, not much variation was observed in the spore numbers of the seedlings inoculated singly with *Azotobacter* (Table 6). Veeraswamy *et al.* (1992) observed more spore production and increased root colonization with dual inoculation. Awasthi *et al*. (1996) observed that the increased root colonization may be due to the activity of *Azotobacter* that has favored the spore germination by production of growth promoting substances in the rhizosphere.

The present study clearly revealed that the *H. brasiliensis* plants are heavily mycorrhizal in natural conditions. Inoculation with AM fungi and other beneficial microorganisms improve the growth of rubber seedlings. Out of the three AM fungi, *G. fasciculatum* was the best inoculum for rubber seedlings followed by *G. mosseae* and *G. margarita*. The mixed inoculation with *G. fasciculatum and G. mosseae* further enhanced the growth of plants in polybags, followed by *G. fasciculatum* and *G. margarita*. Inoculation with nursery beds with AMF and PSM also enhanced the growth of rubber seedling, closely followed by AMF and *Azotobacter* sp. The growth of rubber seedlings was always higher in the mixed as compared to single inoculation. Thus treatment in nursery beds with mixed inoculation of two AM fungi or AMF and PSM or AMF and *Azotobacter* shows the possibility of getting better growth in rubber seedlings.

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