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Combined inoculation of arbuscular mycorrhizal fungi and Azotobacter beneficial to *Proralea corylifolia* L.

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The effect of *Glomus macrocarpum*, *Glomus fasciculatum* and *Azotobactor Proralea corylifolia* L. was studied in sterilized soil. Compared to un-inoculated plants, chlorophyll content, height, weight, number and area of leaves of plant and weight were maximum in plants inoculated with *G. macrocarpum*, *G. fasciculatum* and *Azotobactor* or with *G. macrocarpum* and *G. fasciculatum* or with only *G. macrocarpum*, or *G. fasciculatum*. The levels of root colonization was higher in all AM inoculated plants. There was significant increases in (3.96%) and P (0.21%) in the plant treated with a combination of *Glomus macrocarpum*, *Glomus fasciculatum* and *Azotobactor*. The results clearly indicated that compared to individual inoculation, AM fungal species with *Azotobactor* used in combinations were more beneficial for much improved growth of onion.

Key word : *G. mosseae, G. fasciculatum, Proralea corylifolia*, Per cent colonization.

INTRODUCTION

Soil provides the matrix for the biological processes involved in nutrient cycling. Among the biological processes involved in the rhizoplane, the unique role of symbiotic bacteria and the AM fungi which ensure fixation and mobilization and availability to nitrogen and phosphorus to plants have been well recognized (Marchner, 1995). It is well-established fact that the AM always prefer certain host exhibiting maximum symbiotic response and increased the growth and yield of crop mainly through improved uptake of nutrients (Allen, 1991). Scanty information is available in wide variation among and within different species on AM fungi in their ability to promote plant growth (Read,1996). This led to the concept of host preference 'by AM fungi (Mosse, 1973). Hence it is always better to select an efficient AM fungus for a particular host-soil-climate combination to harness the maximum benefits. This study was aimed to find the response of *Proralea corylifolia* to inoculation of *Glomus macrocarpum*, *G. fasciculatum* and *Azotobacter* in unsterile soil either singly or in combinations.

MATERIALS AND METHODS

The investigation was carried out in sterilized soil of polyhouse during 2006 - 2007 using aromatic plant seedlings of *P. corylifolia*. The soil used for experiment contained organic carbon 0.86%; pH, 6.8; available, N 0.74%; P_2O_5, 2.8 kg/ha, Electrical conductivity (EC) was 0.17 ohm^{-1} and native AM spore population averaging 87 spores / 50 g soil. Seedlings, used in the experiment were grown on sterile soil and 30 seedlings were transplanted in earthen pots of 25 x 15 cm diameter. Soil-root-cultures of *Glomus macrocarpum* and *G. fasciculatum* were cultivated on maize roots using mixture of soil : sand : FYM (1:1:1). The cultures containing clamydospores (96-112 spores/50 g soil) and root segments of maize colonized by particular AM fungus were used as mycorrhizal inoculum. Application of AM inoculum was 129 g soil / plant when single species was used and 5g soil/plant when two species used in combination in soil with seedling roots. Azotobacter as per treatment was 10g/plants. The experiment was arranged in a completely randomized design with five replications. Observation such as chlorophyll content, plant height, number of leaves, size of stem, and fresh weight of bulb were recorded 30 and 60 days after planting. The fresh weight of plant and leaves was taken immediately after harvest. The dry weight was determined after drying the plant at 80^0 C for 48 hrs. The root samples of each treatment were hcollected, processed and stained in 0.05% cotton blue in lactophenol (Philips and Hayman, 1970). Per cent root colonization was calculated using the method of Giovanetti and Mosse (1980). The nitrogen and phosphorus from shoot and bulb of onion were determined following the method of Jackson (1973). Estimation of chlorophyll was carried out following the procedure of Arnon (1949).

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RESULTS AND DISCUSSION

There was more than 6 folds increase in total chlorophyll content,30 day after inoculation of plants with *G. macrocarpum, G. fasciculatum* and *Azotobacter chrococcum* compared to uninoculated plants.

In inoculated plants with *G. fasciculatum* or with G. *macrocarpum* the total chlorophyll content increased. However, 60 day after inoculation the total chlorophyll content dropped gradually (Table 1) while control plants showed a continued small increase. Increase in chlorophyll content in VAM inoculated plants has been also reported by Devi and Reddy (2004) in groundnut and Shivaputra *et al*., (2004) in papaya. Plants treated with *G. fasciculatum* and Azotobacter in combination recorded rapid increase in dry weight from 30 to 60 d after inoculation. A similar increase in dry weghit was observed when inoculated with *G. macrocarpum* and *G. fasciculatum* or *G. fasciculatum* or Azotobacter (Table 1).

Significant increase was recorded in growth parameters *viz.* number of leaves, plant dry weight, stem diameter, fresh and dry weight of leaves (Table 2). The inoculation of *G. macrocarpum, G. fasciculatum* and *Azotobacter* increased the plant height, 55.35 cm after 30 d to 74.25 cm after 60 d. Similarly the same treatment, maximum number of functional leaves increased from 6 and 12 and leaf area from 51.20 to 90.64 cm^2. Similar observationswere recorded by Krishna and Bagyaraj (1982) in Lady's finger and Indi *et al.*, (1989) in brinjal recorded significant increase in height, number of leaves and overall growth of plants due to inoculation with AM fungi. It is evident from (Table 2) that root colonization 30 days after inoculation was higher in combined inoculation with *G. macrocarpum, G. fasciculatum* and *Azotobacter*. After 60 d, max. root colonization was found is this treatment. Clamydospore count in rhizosphere soil 60 d after inoculation was also the highest on inoculation of plants with *G. macrocarpum, G. fasciculatum* and *Azotobacter* (187 spore/50 g soil). G. *fasciculatum* and *G. macrocarpum* also showed high levels (147-161 spores/50 g soil). Inoculation with G. mosseae or *G. fasciculatum* alone was also effective (152-spore/50 g soil). This indicated that AM fungi used in inoculations were efficient in colonization as reported earlier in *Ablemoschus esculentus* (Krishna and Bagyaraj, 1982). In the present investigation, AM species were proved to be the best for the growth of *P. corylifolia* when used in combination. All AM inoculated plants showed significantly higher uptake of N and P over control (Table 2). Plants inoculated with G. *macrocarpum, G. fasciculatum* and *Azotobacter* recorded significantly after 30days the max. 0.13% P uptake (3.71% N in plant). *G. macrocarpum,* and *G. fasciculatum* and *G. fasciculatum, G. fasciculatum* and *G. macrocarpum* also showed high uptake of N and P. After 60 d of inoculation, the maximum N uptake was with *G. macrocarpum, G. fasciculatum* and *Azotobacter* was 3.86% N in plant. Plants inoculated with *G. macrocarpum, G. fasciculatum* and *Azotobacter* in combination showed a max. P uptake (0.18%). Inoculation with *G. macrocarpum* and *G. fasciculatum, G. fasciculatum* or *G. macrocarpum* also led increased P uptake.

The increased uptake of N and P could be attributed to the increased root absorption area induced by AM through an efficient symbiosis with the host and by assimilation and translocation of N (Yao and Li, 1999). It is well known fact that P uptake by plant is improved by AM association (Shivputra *et al*., 2004). Differential

Table 1 : Effect of AM fungi and *Azotobacter* inoculation showing chlorophyll content and fresh and dry weight of shoots *Proralea coryylifolia* L.

AM	Chl a	Chl b	Total chl	Chl a	Chl b	Total chl	Shoot fresh wt (g)		Shoot dry wt (g)	
	30 DAP			60 DAP			30 DAP		60 DAP	
Control	0.170	0.126	0.113	0.213	0.088	0.311	3.11	11.13	0.74	1.32
Gm	0.236	0.129	0.381	0.260	0.128	0.380	9.69	24.17	0.76	1.32
Gf	0.317	0.281	0.587	0.243	0.098	0.342	13.74	32.68	1.23	3.52
Gm+Gf	0.269	0.19	0.439	0.252	0.147	0.386	8.01	41.12	0.92	4.63
Gm+Gf+B.P	0.371	0.414	0.782	0.244	0.173	0.423	14.25	71.03	1.24	7.24
CD(*P=0.05*)	0.07	0.12	0.23	0.012	0.033	0.046	3.02	27.62	0.25	2.72

Gb - *Glomus macrocarpum,* Gf = *Glomus fasciulatum,* B.P=*Azotobactor chrococcum*

Table 2 : Effect of AM fungi and *Azotobacter* inoculation on leaves, plant, VAM colonization N and P status in *Proralea coryylifolia.*

AM	Leaf (no)		Leaf area		Plant height (cm)		VAM colonization (%)		N (%) in shoot/plant		P (%)in shoot/plant	
	(40 d)	(80 d)	(40 d)	(80 d)	(40 d)	(80 d)	(40 d)	(80 d)	(40 d)	(80 d)	(40 d)	(80 d)
Control	3	6	30.4	35.7	49.11	46.21	-	-	2.05	3.11	0.06	0.08
Gm	5	10	28.01	51.20	44.52	59.30	49.10	73.10	3.42	3.72	0.08	0.11
Gf	5	10	34.8	77.00	51.4	71.14	61.0	62.50	3.55	3.80	0.12	0.12
Gm+Gf	7	10	30.15	84.32	46.32	74.10	61.0	78.4	3.68	4.62	0.13	0.15
Gm+Gf+B.P	7	12	35.12	91.64	55.35	74.25	67.3	84.22	3.71	3.86	0.13	0.18
CD(*P=0.05*)	1	12	3.18	22.15	5.05	13.10	31.06	41.03	0.71	0.82	0.02	0.02

Gm - *Glomus macrocarpum,* Gf = *Glomus fasciulatum,* B.P=*Azotobactor*

Table 3 : Effect of AM fungi inoculation on leaf weight and stem size of *Proralea corylifolia*

Treatment	Leaf fresh wt (g)		Leaf dry wt (g)		Stem size (cm)	
	(30 d)	(60 d)	(30 d)	(60 d)	(30 d)	(60 d)
Control	1.0	2.9	0.189	0.431	0.6	1.13
Gm	1.3	4.10	0.381	0.473	0.8	1.64
Gf	2.7	6.62	0.302	0.442	0.8	1.72
Gm+Gf	2.1	7.72	0.576	0.761	0.9	1.78
Gm+Gf+B.P	2.6	8.44	0.605	0.891	0.9	1.84
CD at 0.05%	0.88	2.45	0.158	0.210	0.14	0.23

Gm - *Glomus macrocarpum,* Gf = *Glomus fasciulatum,* B.P=*Azotobactor*

ability of inoculated AM fungi stimulating P uptake was also reported in different crops *viz*. lady's finger (Krishna and Bagyaraj 1982) and garlic (Wani and Konde 1998). There were significant differences in stem dimater, fresh and dry weight of leaves following inoculations with AM fungi (Table 3). Inoculations with any AM in general increased bulb wt and size and the best treatment was inoculation made with *G. macrocarpum, G. fasciculatum* and *Azotobacter* in combination. Similar studies have been conducted earlier by Lakshman, (2000). Ramananda and Sreenivasa (2000) reported that the fresh and dry wt of *Proralea corylifolia* inoculated with different AM fungi increased significantly. An increase in stem diamater is attributed to the increased dry matter accumulation. In conclusion, the results confirm that appropriate strains of AMF and *Azotobacter* inoculum would help to increase biomass production and nutrient uptake in *Proralea corylifolia* plants.

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