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Effect of indiginous introduced arbuscular mycorrhizal fungi and *Rhizobium* on growth of *Pongamia pinnata* Vent.

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Indigenous arbuscular mycorrhizal (AM) fungus was isolated from rhizosphere soils of *Pongamia pinnata*. Young seedlings of *P. pinnata* inoculated with indigenous AMF *Glomous fasciculatum* showed significant growth and the plants introduced with *Glomous mosseae* did not show significant effect on growth. The dual inoculation with indigenous *Glomous fasciculatum* and introduced *Glmous mosseae* depicted growth response was much greater than the single inoculation. The tripartite system of indigenous and introduced VAM with *Rhizobium phaseoli* improved significant increase plant height, plant dry matter, nodulation number, N and P content of *Pongamia pinnata* over non-inoculated plants with either AMF or *Rhizobium* alone.

Key words : *Glomus fasciculatum, Glomus mosseae, Rhizobium phaseoli*.

INTRODUCTION

The arbuscular mycorrhizal (AM) fungi can improve plant growth through increased uptake of phosphorus, especially in soils of low fertility (Smith and Read, 1997). demonstrated that several legumes grow poorly and failed to nodulate in autoclaved soil unless they were mycorrhizal. This was probably due to phosphorus deficiency since an adequate phosphorus supply is important for satisfactory nodulation and nitrogen fixation (Bali *et al.*, 1987). Inoculation of crop plants with VA-mycorrhizal fungi and *Rhizobium* has been found to have synergistic beneficial effect on nodulation, nitrogen fixation and plant growth (Bhagyaraj *et al.*, 1984; Banwarilal *et al.*,1990; Lakshman, 1998). Most agricultural soils possess an indigenous VAM spore strains, the role of which in crop productivity has been examined in sufficient details (Tilak, 1993). Therefore, a suitable host endophyte combinations, however, required to obtain the better results. This object can be achieved through a better understanding of the effectiveness of VAM fungi with *Rhizobium*.

Pongamia pinnata is a deciduous trees that grows to about 15-20 meters in height. The tree is well suited to intense heat and sunlight withstanding temperatures slightly below 0^0C to 50^0C and annual rainfall of 5-25 cm and it grows well on sandy and volatile lime stone. Its oil is used for lubrication and indigenous medicine. Its roots make it valuable for checking erosion and stabilizing dunes.The present study was aimed for examining the role of indigenous VAM fungi on growth of *P. pinnata* and evaluating their interaction with introduced *Glomus mosseae, Glomus fasciculatum* and *Rhizobium phaseoli*.

MATERIALS AND METHODS

Field survey was carried in different locations of *Pongamia pinnata* growing in botanical garden of Karnatak University ,Dharwad. Minimum eight rhizoshperic soil samples and twenty five roots were collected from each individual plant. A summary of the analytical details of the botanical garden soil consist of; pH : 6.7, organic matter : 1.92(%), total nitrogen : 719 (ppm), total phosphorus :394(ppm) and total potassium : 663(ppm). Randomly selected root samples were cut into 1cm segments and cleared with 10% KOH and stained with 0.05% tryphan blue in lactophenol, following the procedure of Phillips and Hayman (1970) and percentage of root length by Giovanetti and Mosse (1980). The number of VAM spores per 50g. soil was calculated by adopting the procedure of wet-sieving and decanting technique (Gerdemann and Nicolson, 1963). Identification of VAM fungal species was done following the keys suggested by Schenek and Perez (1990).

The soil used in pot experiments was a phosphorus deficient 2.5 ppm available extracted with NH_4F+HCL sandy loam, pH 6.8. Two-day-old seedlings were transplanted in pots containing 6 kg soil, which was sterilized in 5% methyl bromide. Four triplicate pots were

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maintained in Glass house at 25-27° C and watered on alternate day. *Rhizobium* inoculation was done by treating *Pongamia pinnata* seeds with a peat based culture before sowing. Mycorrhizal mixed inoculum of 100g (introduced/ indigenous) was applied to the planting hole in the corresponding pot consisting of spores, hyphae and infected fragments, thoroughly homogenized and divided into similar aliquots. The inoculum approximately AMF spores 218/ 50g soil. There were five inoculation treatments : A Non-mycorrhizal (control),Inoculated with *G.mosseae* introduced AMF, Inoculated with *G.fasciculatum* indigenous AMF, Inoculated with *G.mosseae* + *Rhizobium* and Inoculated with *G.fasciculatum*+*Rhizobium*

Plants were harvested at two intervals, i.e., 60 and 120 days after sowing. The number and dry weight of nodules and dry weight of shoots were taken. Fresh and dry weight of shoots and leaves were recorded at the time of harvest. Phosphorus content of the shoot was determined colorimetrically by the vanadomolybdate/phosphoric-yellow colour method. Total nitrogen determinations were made by the microkjeldahl method (Bremner,1960).

Results and Discussion

VA-mycorrhizal fungi were isolated and identified, in different locations of plants grown locality. The number of nodules each plant was recorded. Fourteen VAM spores were isolated and their related fungi were identified. The most dominated genera and species were listed against each site (Table1). Important VAM-spores were i.e. *Glomus mosseae, Gigaspora margrita, Acaulospora aurigloba, Scutellospora geospora, Glomus velum, Glomus monosporum, Glomus constrietum, Glomus aggregatum Glomus macrocarpum, Gigaspora globosa, Glomus citricolum, Glomus fasciculatum, Gigaspora margarita, Sclerocystis clavispora.* The percentage of mycorrhizal colonization varied in different sites. The intensity of mycorrhizal colonization in the sites was not much as that observed under Glass house. This may be due to the pattern of root growth in the sites which might have easily outgrown with different mycorrhizal fungi. Plants

Table 1 : VAM fungi associated with *Pongamia pinnata* growing in Botanical garden of Karnatak University, Dharwad

Sites	pH	Nodulation number / plant	Category of colonization range
			Recorded AM fungal spores / 25g soil
L_1	6.8	0.3	* [a] *G. mosseae, Gigaspora margrita, Acaulospora aurigloba, Scutellospora geos*
L_2	6.9	11.4	[b] *G. mosseae, Glomus velum, Glomus monosporum*
L_3	6.4	13.1	[a] *Glomus constrietum, Glomus aggregatum, Scutellospora geos*
L_4	7.3	7.4	[a] *G. mosseae, Glomus aggregatum, Scutellospora geospora*
L_5	7.2	9.2	[c] *G. mosseae, Glomus margrita, Acaulospora denticulata*
L_6	7.4	5.4	[b] *G. macrocarpum, Gigaspora globosa, Glomus citricolum*
L_7	6.6	12.5	[a] *Scutellospora reticulata, Acaulospora constrictum, G. mosseae, G. Velum*
L_8	6.8	10.3	[b] *Gigaspora reticulatum, Glomus microcarpum, Gigaspora reticulatum*
L_9	6.9	8.4	[a] *G. mosseae, Glomus fascilatum*
L_{10}	7.3	6.1	[a] *Glomus fascilatum, Gigaspora margarita, Sclerocystis clavispora*
L_{11}	7.1	6.5	[a] *G. mosseae, Glomus aggregatum, Acaulospora aurigloba*
L_{12}	6.7	12.4	[a] *G. mosseae, Sclerocystis clavispora, Glomus aggrigatum*
CD at 0.05%	4.05	4.05	

* Colonization range :
a) 0% - 25% b) 25% - 50% c) 50% - 75%

L_1 = Shalmala Hostel, L_2 = Kanavi, L_3 = Hosamani,
L_4 = Itagigudda, L_5 = Ravali, L_6 = Bindhura,
L_7 = Roghani, L_8 = Londala, L_9 = Suhara,
L_{10} = Madhura, L_{11} = Krishna L_{12} = Kariamma.

inoculated with *Glomus fasciculatum* plus *Rhizobium phaseoli* showed a significantly greater plant height (five and half fold increase). Mycorrhizal colonization and spore number was higher as compared to the non-mycorrhizal plants (Table 2). The increased plant height was more striking at 120 days old than 60 days. The shoots, leaves and dry weight of roots was also greater in plants inoculated with indigenous G. *fasciculatum* plus *Rhizobium phaseoli* (Table 3) and the similarly also the number of nodules per plant, nodule dry weight and nitrogen content in the nodules (Table 4). Dual inoculation with indigenous *G. fasciculatum* and *Rhizobium* gave very significantly increased biomass production compared to non-mycorrhizal plants or plants inoculated, with introduced *G. mosseae* or indigenous *G. fasciculatum* alone. Dry weight of leaves, shoots and roots were significantly greater in plants inoculated with *Rhizobium* plus *G. fasciculatum.* Nitrogen and phosphorus contents of the shoot at two different intervals are presented in Table 5. In 120 days old plants, the nitrogen and phosphorus contents of the plant which received indigenous *G. fasciculatum* plus *Rhizobium phaseoli* were significantly higher compared to the plants inoculated with *G.mosseae* plus *Rhizobium phaseoli* and non-mycorrihizal (control) plants.

Improvement by inoculation with VA-mycorrhiza in phosphate deficient soil under Glass house experiments on legumes have been obtained by earlier workers (Crush 1974; Azcon *et al.,* 1979; Banwarilal *et al.,* 1990; Singh, 2000). The present studies results suggest that soil conditions, that favour the development of *Rhizobium* also increase the AMF colonization and spore population. This may reflect a correlation between VA-mycorrhizae and *Rhizobium.* The intensity of mycorrhizal colonization varied from 25 to 100% shown (Table 1) compared to the glass house experiments. This may be due to the pattern of root growth in Botanical garden sites, which might have easily out-grown associated with different endophyte (Lindermann, 1988; Azcon and Rabio, 1990) worked on *Medicago sativa* and concluded, that indigenous and introduced endophytes co-operate together to assist the growth of this legume. This is probably due to, experiments might not be conducted in sterlized soil, so the native endophytes in unsterlized soil could be deducted from the calculation of growth. However, the experimental data of the present study suggests that

Table 2 : Plant height, per cent AMF colonization and spore number as influenced by introduced *G. mosseae* indigenous *Glomus fasciculatum* and *Rhizobium phaseoli* on *Pongamia pinnata.*

Treatment	Plant height (cm)		% VAM colonization		VAM spores/50 g soil	
	60 days	120 days	60 days	120 days	60 days	120 days
NM	12.9±1.0	19.2±1.1				
G. mosseae	15.7±0.0	24.4±0.1	41.2±4.3	46.2±5.0	61.3±1.0	64.0±2.4
G. fasciculatum	23.2±1.4	28.2±2.2	52.3±2.1	54.5±2.0	67.2±2.2	71.3±1.0
G.m+Rhizobium	27.2±5.0	37.6±3.2	53.2±5.1	57.7±5.2	68.5±3.1	69.3±4.2
G.f+Rhizobium	42.3±1.2	63.1±3.0	68.6±2.3	64.1±3.2	94.4±4.1	98.2±2.0
C.D. at 0.05%	7.0±1.0	11.0±1.0	21.0±0.0	23.2±0.0	14.0±0.0	16.2±1.0

Table 3 : Leaf, shoot and root dry weight as influenced by inoculation with introduced *G. mosseae*, indigenous *Glomus fasciculatum* and *Rhizobium phaseoli* on *Pongamia pinnata*.

Treatment	Leaf dry weight (cm)		Shoot dry weight/ plant(g)		Root dry weight / plant(g)	
	60 days	120 days	60 days	120 days	60 days	120 days
NM	2.4±0.3	2.9±1.0	4.2±3.0	7.91±4.0	0.7±0.0	0.9±1.0
G. mosseae	3.6±1.1	3.9±1.1	9.4±1.0	12.6±4.3	0.9±3.2	1.1±0.2
G. fasciculatum	4.8±0.0	5.6±2.0	10.1±2.2	15.2±2.1	1.5±3.0	2.2±2.0
G.m+Rhizobium	6.3±1.2	10.3±5.2	13.5±3.0	17.2±4.1	2.6±0.5	3.0±1.0
G.f+Rhizobium	11.0±0.0	14.6±2.4	18.2±3.3	31.1±5.2	2.8±3.2	3.3±0.4
C.D. at 0.05%	1.0±0.0	1.1±0.0	2.2±0.1	2.5±1.0	NS	0.6±0.0

Table 4 : Number, dry wieght and nitrogen content of root nodule as influenced by inoculation with introduced *G. mosseae*, indigenous *Glomus fasciculatum* and *Rhizobium phaseoli* on *Pongamia pinnata*.

Treatment	Nodule number / plant		Nodule dry weight/ plant(g)		Nodule nitrogen/ plant(mg)	
	60 days	120 days	60 days	120 days	60 days	120 days
NM						
G. mosseae	2.1±0.0	2.4±0.0	0.9±0.0	0.93±0.0	ND	2.12
G. fasciculatum	2.3±0.0	2.7±1.0	0.96±0.0	1.0±0.1	1.81	3.24
G.m+Rhizobium	7.1±0.0	9.0±0.0	1.87±2.0	3.3±1.1	3.6	5.71
G.f+Rhizobium	8.3±1.2	14.5±4.1	2.1±2.2	3.7±1.2	8.69	12.14
C.D. at 0.05%	NS	1.0±0.0	NS	0.1±0.0	NS	0.9±0.0

Table 5 : Nitrogen and phosphorus content of shoot as influenced by inoculation with introduced *G. mosseae*, indigenous *Glomus fasciculatum* and *Rhizobium phaseoli* on *Pongamia pinnata*

Treatment	Total nitrogen content / shoot (mg)		Total phosphorus content shoot (mg)	
	60 days	120 days	60 days	120 days
NM	ND	10.64	ND	9.33
G. mosseae	14.31	17.21	8.25	13.72
G. fasciculatum	21.51	31.10	11.89	13.81
G.m+Rhizobium	38.42	9.72	15.30	17.33
G.f+Rhizobium	52.20	103.32	26.37	42.64
C.D. at 0.05%	0.00	0.12	0.04	0.06

NS : Not significant,ND = Not detected,

G.m=*Glomus mosseae*, G.f= *Glomus fasciculatum*.

indigenous mycorrhiza *(G. fasciculatum)* influence much favourable mycorrhizal colonization, number of spores and nodulation. But, the best response was obtained with combined or dual inoculation of indigenous *G. fasciculatum* + *Rhizobium phaseoli* than introduced *G. mosseae* + *Rhizobium phaseoli*. The results are consistent with other workers (Grandison and Cooper, 1986; Kumar, 1998). Nodulation by *Rhizobium* species depends on the adequate mycorrhization and it is obvious that the introduction of efficient strain (indigenous mycorrhiza) that may co-operate with introduced VA-mycorrhizal fungi, might lead to the improvement of soils after several harvests.

Pongamia pinnata inoculation with VA-mycorrhizal fungi in phosphorus deficient soils was most successful, as it not only improved the plant growth and nutrition, but also enhanced the activity of *Rhizobium* applied as inoculant. VA-mycorrhizal fungi too, not penetrated the nodule tissue directly and therefore, influence of the rhizobial activity through altered root or rhizosphere environment. Sangiga *et al.*, (1989) and Tilak (1993) have emphasized that mycorrhiza of nitrogen fixing legumes should be taken into account .

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