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Growth response and nitrogen fixation of *Phaseolus lunatus* (Lima bean) with the inoculation of AM fungi and *Rhizobium*

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ABSTRACT

The indigenous arbuscular myeorrhizal (VAM) fungi was recovered from soils at the crop research centre. University of Agriculture Sciences, Dharwad. Plants were able to promote growth of *Phaseolus lunatus*. Introduced *Glomus mosseae* were not showed significant effect on growth. The dual inoculation with indigenous *Glomus fasciculatum* depicted a growth response was much grater gain with single inoculation. The tripartite system, indigenous AMF and *Rhizobium phaseoil* improved plant growth and also resulted in increased plant height, plant dry matter, modulation number. N and P content of lima bean that in non-inoculated plants with either AM-*mycorrhiza* or *Rhizobium* alone.

Key words : *Indigenous AM fungi, Glomus fasciculalum, G. mosseae. Rhizobium, Phaseolus lunatus*

INTRODUCTION

Research in the last three decades has established that *Arbuscular mycorrhizal* (AM) fungi can improve plant growth through increased uptake of phosphorus, especially in soil of low fertility (Harley and Smith, 1983), Asai (1944) 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 are important for satisfactory nodulation and nitrogen fixation (Subbarao *et al.*, 1986), Ross and Harper (1984), showed that the growth and yield of nodulating soybean increased after inoculation with *Glomus mosseae* in fumigated sil. Inoculation of crop plants with *Arbuscular mycorrhizal* fungi and *Rhizobium* was found to has synergistic beneficial effect on nodulation, nitrogen fixation and plant growth (Cruz *et al.*, 1988; Lakshman, 1998). Most agricultural soils possess an indigenous VAM spore strains, the role of which in crop productivity has been examined in sufficient details (Mathew and Johri, 1989). 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 AM fungi. *Phaseolus lunatus* Linn (Lima bean) is a herbaceous annual legume, rich in protein mineral and carbohydrates, it is primary grown for green pods which is cooked as vegetables, as dry beans it is a pulse. The present study was aimed at examination the role of indigenous AM fungi on growth of *Phaseolus lunatus* and evaluating their interaction with introduced *Glomus mosseae Glomus fasciculatum* and *Rhizobium phaseoli*.

MATERIALS AND METHODS

Field survey work were carried in different locations of lima bean grown centers of University of Agricultural Sciences, Dharwad. The geographical location of Dharwad is between 15"20 to $15^0$28 north latitude and 75" to 75"70 east longitude. Minimum eight rhizoshperic soil samples and twenty five roots were collected from each individual plants growing in the fields. A summary of the analytical details of the field soil consist of pH : 6.8. organic matter: 1.72 (%), total nitrogen : 810 (ppm), total phosphorus : 491 (ppm) and total potassium : 563 (ppm). Randomly selected root samples were cut into lcm segments and cleared with 10 % KOH and strained with 0.05 % tryphan blue in lactophenol. Following the procedure of Phillips and Hayman (1970). The percentage of root length by Giovanetti and Mosse (1980). The number of AM spores per 50g soil was calculated by adapting the procedure of wet-sieving and decanting technique Gerdemann and Nicolson (1963). Identification of AM fungal species was done following the keys suggested by (Morton, 1988; Schenck and Perez, 1990).

The soil used in pot experiments was a phosphorus deficient 2.4 ppm available extract with NH_4F + HCl sandy loam. pH 6.8. Two day old seedlings were transplanted in pots containing 5km soil sterilized in 5 % methyl bromide. Four triplicate pots were maintained in glass house at 25-27^0C temperature, watered alternatively. *Rhizobium* inoculation was done by treating the field bean seeds with a peat based culture before sowing. Mycorrhizal inoculum (introduced/indigenous) was applied to the planting hole in the corresponding pot and it consisted of spores, hyphae and infected fragments thoroughly homogenized and was divided into similar

aliquots. The inoculum containing mycorrhizal spores *Glomus mosseae* and *Glomus fasciculatum* approximately 108/50kg soil. There were five inoculation treatments : Non-mycorrhizal (control), Inoculated with *G. mosseae* introduced VAM, Inoculated with *G. fasciculatum* indigenous VAM, Inoculated with *G. mosseae + rhizobium)* and Inoculated with *G. fasciculalum + rhizobium).* At two intervals, *i.e.*, 30 and 60 days after sowing observation on the number and dry weight of nodules and dry weight of shoots were taken, bean yield and 100-seeds weight were recorded at the time of harvest. Phosphorus content of the shoot was determined colorimetrically by the vanadomolybdate/ phosphoric-yellow colour method cut lined by Jackson (1973). Total nitrogen determinations were made by the microkjeldahl method (Bermner, 1960). Percentage of mycorrhizal colonization of roots, clearing of roots and the number of *G. fasciculatum* spores in the surrounding the roots were determined according to the procedures as already mentioned earlier.

RESULTS AND DISCUSSION

Different *Arbuscular mycorrhizal* spores were isolated and identified, in different field bean grown sites. The number of nodules per each plant was recorded. The results showed the alkaline soil pH 7.6 don't influence much in the production of nodules, though there was main mycorrhizal spores associated in their rhizoshperic soils (Table 1).

Fourteen AM spores were isolated and identified. The most dominated genera and species were listed against each sites. Important AM spores were *Glomus mosseae, Gigaspora margrita, Acaulospora aurigloba, Sculellospora geospora, Glomus valum, Glomus monosporum, Glomus conslrielum. Glomus aggregatum, Glomus macrocarpum, Gigaspora globosa, Glomus cilricolum, Glomus fasciculalum, Gigaspora margarita, Sclerocystis clavispora.* The percentage of mycorrhizal colonization were varied in different sites. And thus the intensity of mycorrhizal colonization in the field (sites) was not much as that observed under glass house. This may be due to the pattern of root growth in the field, which might have easily outgrown-with different mycorrhizal fungi. Plants inoculated with *Glomus fasciculatum; in addition, Rhizobium phaseoli* showed a significantly greater plant height (six and half fold). Per cent mycorrhizal colonization and spore number was higher compare to the non-mycorrhizal plants, and the plants received only introduced *G. mosseae* plus *Rhizobium phaseoli* (Table 2). These increased plant height were more striking when the plants were 60 days old than 30 days. The dry weight of shoots, bean yield and seed weight was also greater in plants inoculated with indigenous *G. fasciculatum* plus *Rhizobium phaseoli* (Table 3). Number of nodules per plant, nodule dry weight and nitrogen content in the nodules (Table 4). The differences in dry weight of the shoots were not significant in 30 days old plants, dual inoculation with indigenous *G. fasciculalum* and *Rhizobium* produced were significantly increased biomass production compared to non-mycorrhizal plants and plants inoculated with introduced *G. mosseae,* indigenous *G. fasciculatum* alone. The 100 bean weight of field bean seeds, was significantly greater in plants inoculated with *Rhizobium* plus *G. fasciculatum.* Nitrogen and phosphorus content of the shoot at two different intervals are presented in (Table 5). No significant differences could be seen in 30

Table 1 : AM fungi associated with lima bean grown in the field of University of Agricultural Sciences, Dharwad

Sites	pH	Nodulation number/ plant	Category of colonization range recorded VAM fungi / 25g soil
1.1	6.8	89.3	*G. mosseae, Gigaspora margrita, Acaulospora aurigloba, Sculellospora, geospora*
1.2	6.9	11.4	*Glomus mosseae, Glomus velum, Glomus monosporum*
1.3	6.4	13.1	*Glomus constritum, Glomus aggregatum, Scutellospora geospora*
1.4	7.3	07 4	*Glomus mosseae, Glomus aggregatum, Scutellospora geospora*
1.5	7.2	09.2	*Glomus mosseae, Glomus margarita, Acaulospora denticulata*
l.6	7.4	05.4	*Glomus macrocarpum, Gigaspora globosa, Glomus citricolum*
1.7	6.6	12.5	*Scutellospora reliculatum, Acaulospora conistrictuu G. mosseae, G. velum*
1.8	6.8	10.3	*Gigaspora reticulatum, Glomus microcarpum, Gigaspora reticulatum.*
1.9	6.9	08.4	*Glomus mosseae, Glomus fasciculatum*
1.10	7.3	06.1	*Glomus fasciculatum, Gigaspora margarita, Sclerocystis clavispora*
1 .11	7.1	06.5	*Glomus mosseae, Glomus aggregatum, Acaulospora aurigloba*
1.12	6.7	12.4	*Glomus mosseae, Sclerocyslis clavispora, Glomus aggrigatum*
C.D. (P=0.05)	5.1	6.53	

Colonization range : a:0%-25 %, b:25 %-50%, c:50% - 75%. d:75% - 100 %.

Table 2 : Plant height, shoot dry weight, per cent AMF colonization and spore number as influenced by introduced G. *mosseae* / indigenous *Glomous fasciculatum* and *Rhizohium* phaseoli on lima bean

Treatments	Plant height (cm)		% VAM colonization		VAM spores / 50g soil	
	30	60	30	60	30	60
NM	7.9±1.0	10.2±1.1				
G. mosseae	13.7±0.0	18.4±0.1	44.2±4.3	45.2±5.0	64.3±1.0	69.0±2.4
G. fascieulalum	24.1±1.0	26.2±2.0	58.3±2.1	61.5±2.0	67.2±2.2	71.3±1.0
*G.*M+ *Rhizobium*	27.2±5.0	38.6±3.2	59.2±5.1	61.7±5.2	68.5±3.1	72.3±4.2
G.F.+*Rhizobium*	42.3±1.2	64.1±3.0	72.6±2.3	76.1±3.2	101±4.1	105±2.0
C.D. (P=0.05)	7.3	10.2	14.1	14.7	21.2	24.0

Table 3 : Shoot dry weight yield and 100 seed weight as influenced by inoculation with introduced *G. mosseae* indigenous G. *fascilatum and* Rhizohium *phaseoli* on lima bean

Treatments	Shoot weight plant (g)		Bean yield/plant (g)		100 seed weight (dry)	
	30	60	30	60	30	60
NM	9.8±0.0	1.98±1.0	5.2±3.0	5.91±4.0	1.3±0.0	2.2±1.0
G. mosseae	2.1±1.0	3.3±1.1	42.6+4.3	42.6±4.3	8.5±3.2	14.2±2.4
G. fascieulalum	3.0±0.0	5.8±2.0	32.1±2.2	58.2±2.1	11.5±7.0	21.2±5.2
G.m+ Rhizobium	7.3±1.2	8.3±5.2	73.5±3.0	76.2±4.1	28.6±5.5	29.0±1.0
G.f.+Rhizobium	10.0±0.0	17.6±2.4	126.2±3.3	132.1±5.2	38.1±3.2	44.5±5.1
C.D. (P=0.05)	4.3	5.3	11.3	13.1	5.5	14.2

Table 4 : Number, dry weight and nitrogen content root nodule as influenced by inoculation with introduced G. *mosseae* / G. *fascilatum and* Rhizohium *phaseoli* on lima bean

Treatments	Nodule number/plant		Nodule dry weight/ plant(g)		Nodule nitrogen / plant (mg)	
	30	60	30	60	30	60
NM						
G. mosseae		3.1±0.0		0.92+0.0		2.12
G. fascieulalum	1.3±0.0	4.2±3.0	0.76±0.0	1.6+2.2	1.81	3.24
G.m+ Rhizobium	2.1±0.0	9.7±5.0	0.87±0.0	3.9+3.0	3.6	5.71
G.f.+Rhizobium	6.3±1.2	17.5±4.1	3.1±2.2	5.8+1.4	8.69	12.14
C.D. (P=0.05)	3.0	5.2	0.12	0.30	0.06	0.06

Table 5 : Nitrogen and phosphorus content shoot as influenced by inoculation with introduced *G. mossae/indigenous* G. *fasciculatum and* Rhizobium *phaseoli* on lima bean

Treatments	Total content/shoot (mg)		Total phosphorus content/shoot (mg)	
	30**	60*	30** days	60+days
NM	07.30	10.64	2.16	09.33
G. mosseae	14.31	17.21	8.25	13.72
G. fasciculatum	21.51	31.10	11.89	11.84
G.m. + Rhizobium	41.42	59.72	15.30	17.33
G.f. + Rhizobium	52.20	151.32	26.37	41.64
C.D. (P=0.05)	00.00	00.12	00.07	00.07

days old-plants. In 60 days old plants the nitrogen and phosphorus content of the plant which received indigenous *G. fasciculatum* plus *Rhizobium phaseoli* were significantly higher compared to these plants, inoculated with *G. mosseae* plus *Rhizobium phaseoli*, than non-mycorrihizal (control) plants, and the plants which received only introduced *G. mosseae* or *G. fasciculatum* plus *Rhizobium phaseoli.* Improvement by inoculation with VA-mycorrhiza in phosphate deficient soil under glass house experiments as legumes obtained by earlier workers (Banwarilal *et al.,* 1990; Singh, 2000). The present study results suggest that soil conditions that favour the development of *Rhizobium* also increase the AM colonization and spore population. This may reflect a correlation between arbuscular 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 the field sites, which might have easily out-grown associated with different endophytes, Barea, Escadero and Azcon Gdeaguilar (1980) worked on *Medicago sativa* and they concluded, the indigenous and introduced endophytes co-operate together to assist the growth of legumes. This was probably due to, the experiments that might have not been conducted in sterlized soil, so the native endophytes in unsterlized soil could be deduced from calculation of growth. However, the experimental data of the present study suggests that indigenous mycorrhiza *(G. fasciculatum)* influence much favourable mycorrliizal 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*, as increased nodule number, dry weight of shoots, increased bean yield and significant increase on total uptake of P and N by the host plants (Table 3 and 5). Such a significant increase was compared with non-mycorrhizal plants and the plants inoculated with *G. mosseae* or *G. fasciculatum* alone. These results are consistent with other workers (Shivprasad and Rai, 1991; Lakshman, 1999). Table - 4 showed the gradual increased mycorrliizal colonization in those plants inoculated with *G. mosseae* (introduced) and *G. fasciculatum* (indigenous) irrespective *of .Rhizobium* inoculation. It is clear that 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 *Arbuscular mycorrhizal* fungi, might lead to the improvement of soils after several harvest. Manjunath *et al.* (1984) have reported a closely similar growth response in *Leucaena laucocephala* when native mycorrhizal fungi were inoculated with *Rhizobium phaseoli* on mungbean plants.

Hence, it can be concluded that lima bean inoculation with *Arbuscular mycorrhizal* fungi in phosphorus deficient soils was most successful, since it not only improved plant growth and nutrition, but also enhanced the activity of *Rhizobium* applied as inoculant. It is also interested that *Arbuscular mycorrhizal* fungi not penetrate the nodule tissue directly and therefore influence of the rhizobial activity through altered root or rhizosphere environment (Hayman 1986; Gerdmann. 1990) has emphasized that their studies dealing with mycorrhizas of nitrogen fixing legumes should take into account the total dynamics of the system, so that practical applications can be reached.

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