Research Paper :

Interactions Between *Glomus fasciculatum* Fungi and *Rhizobium* on *Glycine max* Merr. (var DH-125)

RATNA AIRSANG AND H.C. LAKSHMAN

International Journal of Plant Protection, Vol. 2 No. 2 : 191-194 (October, 2009 to March, 2010)

See end of the article for authors' affiliations

Correspondence to :
RATNA AIRSANG
Department of Botany,
J.S.S. College,
Vidhyagiri,
DHARWAD
(KARNATAKA)
INDIA

SUMMARY

Interaction between *Glomus fascicultum* and *Rhizobium phaseoli* and their effects on *Glycine max* (var. DH-125), was studied in a phosphorus dicient sandy loam. The number, dry weight and nitrogen content of the root nodules in plants inoculated with *G. fasciculatum* plus *R. phaseoli* were significantly increased compared to uninoculated or with only *R. phaseoli*. *Rhizobium phaseoli* inoculation did not have a significant influence on sporulation of *G. fasciculatumin* in the rhizosphere soils. However, *Glycine max* plants inoculated with *G. fasciculatum* recovered increased phosphorus content, dry weight and grain yield than unioculated plants. Only *R. phaseoli* inoculation resulted in the increased nitrogen content of the plant and grain yield. Dual inoculation of both the symbionts significantly increased plant height, shoot dry weight and nitrogen content over single inoculation with either *G. fasciculatum* or *R. phaseoli*. These results suggest that arbuscular mycorrhizal (AM) fungi along with *R. phaseoli* can greatly increase nodulation and nitrogen fixation in *Glycine max*.

Key words : *Glomus fasciculatum*, Nitrogen fixation, Nodulation, Phosphorus content, *Rhizobium phaseoli*, *Glycine max*

Accepted :* *August, 2009

Glycine max. (var. DH-125) is an important leguminaceous oil yielding plant. Seeds yield very good edible oil. A study on interaction between AM fungus and *Rhizobium* had been carried out. In legume, *Rhizobium* symbiosis can provide an economic source of available nitrogen (Ahmad *et al.*, 1981; Saxena *et al.*, 2002). The use of *Arbuscular mycorrhizal* (AM) fungi can improve phosphorus uptake and ultimately plant growth and yield (Barea, and Azcon-Aguiar, 1983; Rodrigues *et al.*, 2003). Phosphorus deficiency is an important limiting factor in nitrogen fixation and legume production (Jacobson, 1985). In recent years, a number of studies conducted on interaction between *Arbuscular mycorrhizal* fungi and *Rhozobium* on legumes (Lakshman, 1999) have shown that the growth and yield of nodulating soybean increase after inoculation of *Glomus mossae* in sterilized soil. Inoculation on different crop plants with *Arbuscular mycorrhizal* fungi and *Rhizobium* was found to have synergistic beneficial effect (Hazarika *et al.*, 2000; Sampathkumar and Ganesh kumar, 2003). The present study was undertaken with the objective of assessing the response of *Glycine max* to dual inoculation with *Arbuscular mycorrhizal* fungus *Glomus fasciculatum* and *Rhizobium* inoculation in the earthen pots using sterilized soils.

MATERIALS AND METHODS

Seeds of *Glycine max* (var. DH-125) were surface sterilized in 2% sodium hypochlorite and germinated in sterile sand. Two weeks old seedlings were selected for uniformity and transplanted singly into 15 x 15 cm pots containing 2 kg sandy loam soil and pure sand with initial pH of 6.7, organic matter content of 1.4% and an available P content of 2.6 ppm extracted with NH_4 and HCL and air dried, pulverized, passed through a 4 mm sieve and sterilized with 2.5% methylbromide. There were four inoculation treatments: Uninoculated control; Inoculated with *R.phaseoli* (referred to as 'Rp'), Inoculated with *Arbuscular mycorrhizal* fungus *G.fasciculatum* ('Gf) and (4) inoculated with *R. phaseoli* and *G. fasciculatum* (Rp+Gf). *Rhizobium phaseoli* inoculation was done by treating *Glycine max* seeds with a peat based culture 10^{-8} ml^{-1} before sowing. Mycorrhizal inoculation was done by placing the seeds over a thin layer of mycorrhizal inoculum at the time of sowing 25g mycorrhizal inoculum consisted of chopped root bits and the soil from a pot culture of Sudan grass, (*Sorghum bicolour*) which was infected by *G. fasciculatum* and grown for 4 months. The inoculum contained hyphae, vesicles, 142 chlamydospores per 50g soil and arbuscules of *G fasciculatum.* There were three replications for each treatment and plants were watered on alternate days. At two intervals *i.e.*, after 40 and 80 days of sowing, plant height, and the number and dry weight of nodules, root and shoots were measured. Phosphorus content of the shoot was demined calorimetrically by the vanadomolybdate / phosphoric-yellow colour method (Jackson, 1973). Total nitrogen content was determined by the microkjeldahl method (Bremner, 1960). Percentage mycorrhizal infection of the roots was determined by the root slide technique (Nicolson, 1960) after clearing the roots with 10% KOH and stained with 0.05% trypan blue (Philips and Hayman, 1970). The numbers of *G. fasciculatum* spores in the soil surrounding the roots were determined by the wet sieving and decanting technique (Gerdemann and Nicoloson, 1963). The grain yield and 50 grain weight were also recorded at the time of harvest.

RESULTS AND DISCUSSION

Data on root nodule number, dry weight and nitrogen content of *C occidentalis* roots as influenced by *Rhizobium* and mycorrhizal inoculation are given in Table 1. There was no nodulation observed in uninoculated control plants. The number, size, dry weight and nitrogen content of nodules in plants inoculated with *G. fasciculatum* and *Rhizobium* were significantly greater than those of plants inoculated with only *Rhizobium*. The number of root nodules favourably increased at 80days than 40days after inoculation of Rp or *Rp+Gf* plants inoculated with Gf showed significantly greater mycorrhizal spores in the rhizosphere soils compared to the unioculated control plants and plants which received only *Rhizobium* (Table 2). These increased spore numbers were more striking when the plants were 80 days old than at 40 days. The percentage of mycorrhizal infection was higher in the plants which were inoculated with *G. fasciculatum.*

Plant height, dry weight of shots above ground and nitrogen and phosphorus contents of shoot steadily increased with dual inoculation. The dual inoculation of *Rhizobium* and *G. fasciculatum* produced significantly greater plant biomass production compared to uninoculated control. The grain yield of *Glycine max* treated with *R. phaseoli* alone or *R. phaseoli* and *G. fasciculatum* was significantly greater than of uninoculated control plants and plants inoculated with *Glomus* alone (Table 3). The 100 grain eight of *Glycine max* seeds was significantly greater in plants inoculated with *R. phaseoli* with or without *G.fasciculatum.* The N and P content of the above ground shoots at two different intervals are presented in Table 4. No significant difference could be seen in 40 days old plants. At 80 days, N and P content of the plants that received *G.. fasciculatum* plus *R.. phaseoli*, was significantly higher compared to the uninoculated control plants and plants which received only *G.fasciculatum* or *R. phaseoli.*

Table 2 : AM fungal spores in soil and per cent of mycorrhizal infection of *Glycine max* root nodules as influenced by inoculation with *Glomus fasciculatum* and *Rhizobium phaseoli*

Treatment	Number of nodules per plant		Dry wt. of nodule per plant (g)	
	40*	80*	40*	80*
Control	0d	0d	0d	0d
Glomus fasciculatum	175.66b	202.00b	58b	70b
Rhizobium phaseoli	65.66c	71.33c	50c	61c
Glomus + Rhizobium	204.33a	220.00a	75a	91a

40* = 40 days after sowing ; 80* = 80 days after sowing.
Significant at P=0.05 values not followed by identical letter in each vertical column are significantly different

Table 1 : Dry weight and nitrogen content of root nodules in *Glycine max* influenced by *Glomus fasciculatum* and *Rhizobium phaseoli.*

Treatment	Number of nodules per plant		Dry wt. of nodule per plant (g)		Nodule nitrogen per plant (mg)	
	40*	80*	40*	80*	40*	80*
Control	0d	0d	0d	0d	0d	0d
Glomus fasciculatum	4c	9c	0.03c	0.14c	1.12c	1.61c
Rhizobium phaseoli	12b	15b	0.13b	0.25b	2.14b	3.06b
Glomus + Rhizobium	24a	32a	0.24a	0.41a	3.28a	4.17a

40* = 40 days after sowing ; 80* = 80 days after sowing
Significant at P=0.05 values not followed by identical letter in each vertical column are significantly different

Improvement by inoculation with *Arbuscular mycorrhizal* fungi to the host plant and its growth and yield under green house conditions in soils in low phosphorus had helped to obtain better results (Mosse 1977; Smith *et al*.,1979). Similar results were obtained in the present work where in the sterilized soil used for pot experiments, had low phosphorus content. Manjunath *et*

Table 3 : Dry weight of shoot of the *Glycine max* as influenced by inoculation with *Glomus fasciculatum* and *Rhizobium phaseoli*

Treatment	Height of plant (cm)		Dry weight of shoot per plant (g^{-1})		Grain yield per pot (g^{-1})	50 grain wt (g^{-1})
	40*	80*	40*	80*	80*	80*
Control	9.72d	13.71d	0.55d	1.94d	2.44d	1.43d
Glomus fasciculatum	21.20c	32.25c	1.34c	2.75c	41.21c	14.80c
Rhizobium phaseoli	25.50c	44.20b	1.50b	2.92b	77.84b	17.54b
Glomus + Rhizobium	38.28c	52.84a	1.64a	4.73a	95.74a	19.36a

40* = 40 days after sowing ; 80* = 80 days after sowing

Significant at P=0.05, values not followed by identical letter in each vertical column are significantly different

Percentage infection values did not tend themselves to statistical analysis

Table 4 : Nitrogen and phosphorus content of the shoot of *Glycine max* root nodules as influenced by inoculation with *Glomus fasciculatum* and *Rhizobium phaseoli*

Treatment	Total nitrogen content per shoot (mg)		Total phosphorus content per shoot (mg)	
	40*	80*	40*	80*
Control	2.08d	2.17d	1.05d	1.11d
Glomus fasciculatum	38.13c	74.70c	4.57c	14.40c
Rhizobium phaseoli	42.04b	96.10b	4.71b	11.25b
Glomus + Rhizobium	47.15a	107.10a	5.01a	16.61a

40* = 40 days after sowing; 80* = 80 days after sowing

Significant at P=0.05 values not followed by identical letter in each vertical column are significantly different

al. (1984) and Thiagarajan (1991) worked on soybean and cowpea using unsterilized soil and concluded that the increased biomass production, nodulation, N and P uptake in mycorrhiza with *Rhizobium* inoculated plant was due to the presence of indigenous *Arbuscular mycorrhizal* fungi. The present investigation supports the work of Ross and Harper (1970) and Azcon and Rubio (1990) in demonstrating that two major elements, nitrogen and phosphorus, can be supplied to the host plant through symbiotic association. There is also growing evidence that certain endophytes preferentially associate with certain host plants (Mosse, 1977). The present investigations however do not concur with this finding. The *Arbuscular mycorrhizal* fungus is non-specific to host plants. Therefore inoculation of the introduced endophyte is more efficient in boosting the plant growth than the indigenous fungus, *G. fasciculatum* (Lakshman, 1999). In the present study, inoculation of *G.fasciculatum* to *Glycine max* results in an increase in plant height, dry weight total P content of shoot, N content in nodules and grain yield compared to uninoculated control plants.

The better response of *Glycine max* to *G. fasciculatum* inoculation could be attributed to the presence of *Glycine max* specific rhizobial strain inoculation, in the sterilized soil in pot experiments. The increased plant growth in 40 days was due to *Rhizobium* inoculation alone when compared to uninoculated control plants and was not statically significant. While the grain yield at 80 days harvest was significantly greater suggesting that the crop derived greater benefit from fixed atmospheric nitrogen in later stages of plant growth. Smith and Daft(1977) and Tilak(1993) highlighted that nodule activity of soybean reaches maximum during pod filling and does not decline until pod filling is completed. A similar process may be expected in *Glycine max* of the present work. This investigation shows clearly that *Arbuscular mycorrhizal* fungus (*G. fasciculatum)* can greatly increase nodulation and N fixation on *Glycine max* plants growth in pots inoculated with *R. phaseoli*. Similar results were obtained for beans in pot experiments which were conducted in fumigated soil, with double inoculation of endomycorrhizal fungus plus *Rhizobium* (Lakshman, 2001; Sampath Kumar and Ganeshkumar, 2003). While the principal effect of mycorrhiza on nodulation was probably phosphate mediated (Asini *et al.*, 1980), there may be secondary effects, possibly of a hormonal nature (Mosse, 1977; Andrade *et al.*, 1995).

It is a common practice to grow nodulated plants on poor agricultural soils to increase soil fertility and effective strains of Rhizobia are often used for treating seeds. The present study therefore suggests that an efficiently introduced *Arbuscular mycorrhizal* fungus can contribute to the efficiency of such a system, especially in P deficient soils, even though indigenous (native) endophytes may be present.

Authors' affiliations:

H.C. LAKSHMAN, Post Graduate Department and Research in Botany (Microbiology Lab), Karnataka University, DHARWAD (KARNATAKA) INDIA

REFERENCES

Ahmad, M.H, Eagleshman, A.R.J., Hassouna (1981). Examining the pot trial for inoculants use with cowpeas in West African soils. *Tropical Soils*, **58** : 325-335.

Andrade, G., Azcon, R. and Bethlenfalvay, G.J. (1995). A *Rhizobacterium* modifies plant and soil responses to the mycorrhizal fungus *Gloumus mossae*. *Applied Soil Ecology*, **2** : 195-202.

Asin, Gianinazzi-Pearson, V. and Gianinazzi, S. (1980). Influence of increasing soil phosphorus levels, an interaction between vesicular-arbuscular mycorrhizas and Rhizobium in soybean. *Canadian J. Bot.*, **58** : 2200-2206.

Azcon, R. and Rubio, R. (1990). Interaction between different VA mycorrhizal fungi *Rhizobium* strains on growth and nutrition of *Medicago sativa*. *Agric. Ecol. & Environ.*, **29** : 5-9.

Barea, J.M. and Azcon-Aguilar, C. (1983). Mycorrhizas and their significance in nodulating nitrogen fixing plants. *Advances in Agron.*, **36** : 1-56.

Bremner, J.M. (1960). Determination of nitrogen in soil by Kjeldahl method. *J. Agric. Sci.*, **55**:11.

Freire, J.J.R. (1984). Important limiting factors in soil for the Rhizobium legume sysbiosis. In : *Biological Nitrogen Fixation and Its Ecological Basis*. Ed. by M. Alexander Plenum press New York, pp 75-98.

Gerdemann, J.W. and Nicolson, T.H. (1963). Spores of mycorrhizal, Endogone species extracted from soil by wet sieving an decanting. *Trans. Br. Mycol. Soc.*, **46** : 235-244.

Hayman, D.S. (1986). Mycorrhiza of nitrogen fixing legumes. *Mikcen*, **1**: 319-357.

Hazarka, D. K., Dubey, L.N. and Phookan, A.K. (2000). Effects of vesicular mycorrhizal fungi and *Rhizobium* on growth and yield of green gram (*Vigna radiata* L). *J. Mycol. & Pathol.*, **30**(3): 424-426.

Jackson, M.L. (1973). *Soil Chemical Analysis*. Prentice-Hall of India (Pvt) Ltd., New Delhi, 498pp.

Jacobson, I. (1985). The role of phosphorus in nitrogen fixation by young pea plants (*Pisum sativum*). *Physiol. Pl.*, **64** : 190-196.

Joshi, S.G. (2000). *Medicinal plants*. Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi, Calcutta, 495pp.

Lakshman, H.C. (1999). Dual inoculation of Vesicular *Arbuscular mycorrhizal* fungi and *Rhizobium* is beneficial to *Ptericarpus marsupium* Roxb. *J. Environ. & Conservation*, **5** (2) : 133-136.

Lakshman, H.C. (2001). Effect of introduced *Glomus fasciculatum* on field bean (*Dolchos* lablab L). *Indian J. Environ. & Eco Planning*, **113** : 11-17.

Manjunath, A., Bagyaraj., D.J. and Gopalgowda, H.H. (1984). Dual inoculation of VAM and *Rhizobium* is beneficial to *Leucaena*. *Pl. & Soil*, **78** : 445-449.

Mosse, B. (1977b). The role mycoorhiza in legume nutrition on marginal soils. In : *Exploiting the Legume-Rhizobium Symbiosis in Tropical Agriculture* (Eds. J.M. Vincent, A.S. Whitney and J. Bose), pp.275-292. College of Tropical Agriculture, University of Hawaii, U.S.A., Miscellaneous Publication No. 145.

Nicoloson, T.H. (1960). Mycorrhiza in *Gramineae* II Development in different habitats particularly sand dunes. *Trans. Br. Mycol. Soc.* **43** : 132.

Phillips, J. M. and Hayman, D.S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular *Arbuscular mycorrhizal* fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.*, **53** : 158-161.

Rodrigues, L.A., Martin, M.A. and Salomao, M.S. (2003). Use of mycorrhizas and *Rhizobium* in intercropping use and phosphate-fractions. *Revista Brasileira de Eiencia Do Solo*, **27** (4) : 593-599.

Ross, P. and harper, J.A. (1970). Effect of *Endogone* mycorrhiza on soybean yields. *Phytopathol.*, **60** : 1552-1556.

Sampathkumar, G. and Ganeshkumar, A. (2003). Effect of AM fungi and *Rhizobium* on growth and nutrition of *Vigna mungo* and *Vigna ungniculata Mycorrhiza News*, **14** (4) : 15-18.

Saxena,A.K. Shende, R. and Tilak, K.V.B.R. (2002). Interaction of Arbuscular mycorrhiza with Nitrogen Fixing Bacteria. In : *Rhizosphere and Soils*. Ed. A.K. Sharma and B.N.Johri. Oxford IBH Publishing Co. (Pvt) Ltd. New Delhi. 311pp.

Smith,E., Nicholas, D.J.D. and Smith, F.A. (1979). Effect of early mycorrhizal infection nodulation and nitrogen fixation in *Trifolium subterraneum* L. *Australian J. Pl. Physiol.*, **65**: 305-315.

Smith,S.E. and Daft, M.J. (1977). Interaction between growth phosphate content and nitrogen fixation in mycorrhizal and non-mycorrhizal *Medicago sativa*. *Australian J. Pl. Physiol.*, **64** : 403-413.

Thiagarajan, T.R., Ames, R.N. and Ahmad, M.H. (1991). Response of cowpea (*Vigna ungniculata*) to inoculation with co-selected vesicular-*Arbuscular mycorrhizal* fungi and *Rhizoibum* strains in field trails. *Canadian J. Microbiol.*, **38** : 573-576.

Tilak, K.V.B.R. (1993). Associative effects of vesicular-arbuscular with nitrogen fixers. *Proc.* Indian *National Sci. Acad.*, **59**: 325-332.

****