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Beneficial Effect of PSB and AM Fungus Inoculation on Growth and Nutrient (N, P, K) Uptake in *Glycine max* var. LSb1

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Abstract:

The influence of a phosphate solubilizing bacterium (Pseudomonas striata) and Arbuscular mycorrhizal (AM) fungus (Glomus mosseae) on the growth of Glycine max var. LSb1. revealed that G. mosseae and P. striata inoculated plants in sterilized soil produced significantly higher growth, dry matter, increase in nodule number, N, P and K uptake in shoot. A moderate or lower growth response was observed among the plants growth in unsterilized soil and either PSB or AM inoculation. On the contrary un-inoculated plants in sterilized garden soil do not showed better growth and total N, P and K uptake. Therefore, synergistic effect was recorded with increased plant dry matter, nodule number, N, P and K uptake in the plants treated with both the inoculants in sterilized soil.

Keywords: Glycine max, Glomus mosseae, Pseudomonas striata, sterile, unsterile soil

1. Introduction

Modern agriculture, which is characterized by intensive cultivation methods, is totally dependent on regular input of numerous types of inorganic fertilizers. The long term effects of such massive fertilization lead to affect on the environment and, it is in now a matter of serious concern. In recent days, researches on plant nutrition through PSB and AM fungi have amply demonstrated that these organisms play an important role in uptake of nutrient from the marginal soils. Research in the last three decades has established that dual inoculation of phosphate solubilizing bacteria and AM fungi stimulates plant growth (Subba rao, 1988). Phosphate solubilizing bacteria solubilize inosulable P and help plants to absorb and translocate more soluble phosphate (Azcon-Aguliar et al., 1986). It has been estimated that in some soil up to 75 per cent applied phosphatic fertilizers may be lost to the plant because of mineral phase precipitation (Kaulpunik and Douds, 2000). Many free living bacteria and fungi are being employed to enhance P use efficiency (Patel, 2002). Glycine max LSb1 grown in most of north Karnataka. It is an indigenous variety produced by University of agricultural sciences Dharwad-58003. Most of these varieties are being grown in nutrient deficient soils. AM fungi are inoculated on crop plants to study the possibility of saving phosphatic fertilizers and improving plant growth and yield (Jeffries, 1987). Phosphorus is usually considered to be the major problem when AM infection and responses are poor. Fertilization is often very important in such practical situations. High phosphorus levels in soil or P additions are known to reduce AM colonization of roots and sporulation. Several earlier studies indicated that addition of an excess of readily available P eliminates the beneficial mycorrhizal effect (Sreenivasa et al., 1993). At the same time, use of insoluble form of P had little or no effect when inoculated with AM fungi. The dual inoculation of Rhizobium and and PSB showed that the increased grain yield either with or without chemical fertilizers (Dhange and Kachhave, 2008). Airsang and Lakshman (2009) have observed that the significant increased yield when treated with Glomus fasciculatum and Rhizobium on Glycine max Merr. (var DH-125). Hence, in the present investigation, possible synergistic interactions between AM fungus, Glomus mosseae and a phosphate-solubilizing bacterium, Pseudomonas striata, at different proportions of available and unavailable form of P were studied in a vertisol. No research work was directed on *Glycine max* LSbl so far. Hence, the present study was carried out to evaluate the efficiency of phosphate solubilizing bacteria and Arbuscular-mycorrhizal fungus on the growth N, P and K uptake in soybean LSb1.

2. Materials and methods

The Green house experiments were undertaken in the Post graduate Department of Botany, Karnatak University, Dharwad-580003 India. *Pseudomonas striata*, a phosphate solubilizing bacterium, was procured from the Department of Agricultural Microbiology Department, U.A.S. Dharwad-580005. The AM fungus *Glomus mosseae* was multiplied with Sudan grass (*Sorghum*

vulgare var. *sudaneese*) as a host plant and maintained in a polyhouse, Department of Botany, Karnatak University, Dharwad-580003 India. Soil based AM inoculum was established and maintained in pot culture.

Seedlings were raised in earthen pots each pot measuring 15x20 (breadth length) filled with 3 Kg of sterilized and unsterilized garden soil. The used soil to the experiment was a sandy loam with a pH 6.8, EC 0.14 μ mhos/cm², organic carbon 0.38%, available N; 199 kg/ha, available K; 204 kg/ha and available P 4.6 kg/ha. Before sowing the seeds into experimental pots surface of Soybean seeds were sterilized in 2% Sodium hypochlorate, washed 1-2 times in sterile water. Seeds were pasted with peat based inoculum (0.10 mg/100g seeds) around the root system. Mycorrhiza colonized chopped root bits (5 g) and 5 g soil of the host plants which consisted of spores (approximately 158 / 250 g soil) and external hyphae was 10 g. dry inoculum singly or in combination was placed 3 cm below the soil of each experimental pots, before sowing the seeds. There were altogether 20 treatments including uninoculated control with 4 replications. Plants were watered on alternate days and harvested once 30 days. But here, 90 days harvest was given. The inoculation treatments were as follows.

- Un-inoculated control
- Pseudomonas striata alone
- Glomus mosseae alone
- P.striata + G. mosseae

Plants growth parameters: Plant height, dry weight of shoot and nodule number were recorded N, P and K uptake in shoots were determined. The per cent of AM fungal colonization of *Glycine max* LSb1 roots were estimated according to (Philips and Hayman, 1970). The extra metrical chalamydospores were isolated by adopting wet sieving and decanting technique outlined by (Gerdemann and Nicolson, 1963). Phosphorus content of shoots was estimated by vanadomolybdate phosphoric yellow colour method outlined by Jackson (1973). Nitrogen was determined Microkjeldahl method and potassium by the Flame photometer.

3. Results And Discussion

The data on growth, nodules number, biomass yield and N, P and K content in shoot and roots of both type experiments in both unsterilized and sterilized garden soil was given in (Table 1-2). Soybean plants inoculated with PSB phosphate solubilizing bacteria i.e Pseudomonas striata influenced greatly than AM fungus i.e. Glomus mosseae inoculation and no such improvement was observed non inoculated (control) plants. The two fold increase of plants height, tenfold increase of biomass yield, seven fold increase of nodule number and significantly increased N, P and K in shoot and roots was significant, when the soybean plants inoculated with the two micro organisms i.e PSB and AM fungus in unsterilized soil (Table 1). Experimental results clearly brought optimum growth biomass yield higher number nodules and per cent colonization under sterilized garden soil. Phosphate solubilizing bacteria is more influenced compared to AM fungus over the control plants. PSB plus AM fungus Pseudomonas striata with Glomus mosseae influenced very significantly increased fourfold growth, biomass yield, nodule number and nitrogen and phosphorous uptake and per cent root colonization (Table 2). Similarly, improvement of shoot dry weight and nitrogen content in sterilized and unsterilized soil are given (Fig 1). Specific rhizosphere micro organisms are important. That can play important role in promoting root growth and mycorrhizal development (Linderman et al., 1990; Lakshman, 1996). Soybean Glycine max LSb1 showed a positive response to combined inoculation of PSB plus AM fungus over the non inoculated control plants. These results are consistent with early workers contribution (Bagyaraj and Menge, 1978; Barea et al., 1983; Dileepkumar et al., 2001; Mohammad and Zaidi, 2007; Sabannavar and Lakshman, 2008). IAA and GA or cytokinins produced by bioinoculants have good impact on plant growth and useful for increasing vegetative growth and yield (Nirmalnath and Srinivas, 1992: Muthuraju et al., 2002: Eranna et al., 2002: Suman et al., 2003). It was at 90 days dual inoculation of PSB and AM fungus Pseudomonas striata plus Glomus mosseae has brought higher growth than that of single inoculation and over the non inoculated plants. Similar findings were also reported on Soybean and Lentil by (Azcon-Aguilar et al., 1986; Sattar and Gaur, 1989; Lakshman, 2010). The present investigation clearly demonstrated PSB Pseudomonas striata and Glomus mosseae AM fungus inoculation to Glycine max LSb1 in sterilized in more suitable in improving plants biomass yield, N, P and K uptake and nodule number and nitrogen fixation in roots. This indicates that the plants might need of AM fungal community associated with rhizobacteria. Synergistic positive interactions have more beneficial to leguminous plants. In conclusion, this study clearly brings out that the combined inoculation of G. mosseae and Psedomonas striata to Soybean LSb1 is better and obtain higher biomass yield.

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Treatments	Plant height (cm)	Shoot dry weight (g/pl.)	% AMF Root coloniz ation	Spore count/ 50g soil	Nodule number/ plant	Root Dry weight (g/plant)	P uptake in shoot (ppm)	N uptake in root (ppm)	K uptake in shoot (ppm)
Un-inoculated (control)	12.6a	2.3a	14.6b	14.2a	0.4a	0.62b	23	33b	49a
PSB	26.8b	7.2b	31.3b	29.2b	3.4b	0.78c	51c	67c	132b
AMF	21.3d	6.4a	42.2d	186.7c	3.1c	1.99b	73b	98d	141d
PSB+AMF	32.5c	9.7c	51.6e	201.4d	8.4e	2.84d	89a	124b	148c

 Table 1: Effect of PSB and AM fungus inoculation of growth, dry matter, nodule number and P uptake of 90 days old Glycine max

 LSb1. in unsterilized garden soil.

• Mean values followed by the same letter within a column do not differ significantly at p=0.05 by one way ANOVA

Treatments	Plant height (cm)	Shoot dry weight (g/pl.)	% AMF Root colonizati on	Spore count/ 50g soil	Nodule number/ plant	Root Dry weight (g/plant)	P uptake in shoot (ppm)	N uptake in root (ppm)	K uptake in shoot (ppm)
Un-inoculated (control)	19.7a	3.4a	11.2	11.0b	0.51d	0.89c	34b	43a	51a
PSB	38.2c	12.5c	27.9a	33.23b	4.3b	1.92b	96a	102c	133d
AMF	35.8d	10.4d	54.3d	52.57a	2.9b	2.11e	98e	123b	142c
PSB+AMF	49.1b	14.8e	74.8d	128.5d	11.3c	3.75d	109b	134d	157b

 Table 2: Effect of PSB and AM fungus inoculation of growth, dry matter, and nodule number and P uptake of 90 days old
 Glycine max LSb1. in sterilized garden soil.

• Mean values followed by the same letter within a column do not differ significantly at p = 0.05 by one way ANOVA.

5. References

- 1. Airsang, R. V. and Lakshman, H. C. (2009). Interactions Between Glomus fasciculatum Fungi and Rhizobium on Glycine max Merr.(var DH-125). International Journal of Plant Protection, Vol.2 (2) : 191-194.
- Azcon-Aguilar., Gianinazzi-Pearson, V., Fardean, J. C. and Gianinazzi, S. (1986). Effect of vesicular–arbuscular mycorrhizal fungi and phosphate solubilizing bacteria on growth and nutrition of soybean in a neutral calcarious soil amended with ³²P⁴⁵ Ca-tricalcium phosphate. Pl.Soil, 93:3-15.
- 3. Barea, J. M., Bonis, A. F and Olivares, J. (1983). Interaction between Azospirillum and VA mycorrhiza and their effects on growth and nutrition of maize and rye grass. Soil Biol Biochem, 15:705-709.
- 4. Bagyaraj, D. J., and Menge, J. A. (1978). Interaction between VA-mycorrhizae Azotobacter and their effect on rhizosphere microflora and plant growth. New Phytologist, 80:567-573.
- 5. Dhange S. J. and Kachhave, K. G. (2008). Effect of dual inoculation of Rhizobium and PSB on Yield contributing characters and seed yield of Soybean. Maharashtra agric. Univ., 33(2): 209-211.
- 6. Dileep-kummar, B.S., Berggren, I and Martensson, A.M. (2001). Potential for improving pea production by coinoculation with fluorescent Psedomonas and Rhizobium. Plant and soil, 229: 25-34.
- 7. Eranna, N., Farooqi, A. A., Bagyaraj, D. J. and Suresh, C. K. (2002). Influence of Glomus fassciculatum and plant growth promoting rhizo microorganisms on growth and biomass of periwinkle. J. Soil Biology and Ecology, 22: 22-26.
- 8. Gerdemann, J. W., and Nicolson, T. H. (1963). Spores of mycorrhizal endogene species extracted from the soil by wet sieving and decanting. Trans.Br.Mycol.Soc. 46: 235-244.
- 9. Jackson, M. L. (1973). Soil chemical analysis.Prentice Hall of India (P) Ltd., New Delhi.
- 10. Jeffries, P. (1987). Use of mycorrhiza in agriculture. Crit. Rev. Biotechnol. 5: 319-357.
- 11. Sreenivasa, M. N., Krishnaraj, P. U. Gangadhara, G. A. and Manjunathaiah, H. M. (1993). Response of chilli (Capsicum annuum L.) to the inoculation of an efficient vesicular–arbuscular mycorrhizal fungus. Scientia Horticultural, 53: 45-52.

- 12. Kaulpunik, Y and Douds, D. Jr. (2000). Arbuscular mycorrhizas: Physiology and function. Kluwer Academic Publishers. Pp-65.
- 13. Lakshman, H. C. (1996). Possible role of weeds in maintaining VA-mycorrhizal colonization of rice soils. J. World weeds. 3: 53-56.
- 14. Lakshman, H. C., Hiremath, S. G., Muthuchelian, K. and Jayashankara, M. (2010). Mass culture and molecular approaches of Arbuscular mycorrhizal fungi and its role in biotechnology. In: Genetically modified organisms and biosafety. (Eds.) K. Muthuchelian and S. Kannaiyan, Associated Publishing Company, New Delhi. 73-92.
- 15. Linderman, R. G. (2000). Effects of mycorrhizas on plant tolerance to diseases. In Arbuscular Mycorrhizas: Physiology and Function. Kapulnik, Y., and Douds, D.D.J. (eds). Dordrecht, the Netherlands: Kluwer Academic Publishers, pp. 345–365.
- 16. Mohammad, S. H and Zaidi, A. (2007). Synergistic effects of the inoculation with plant growth-promoting rhizobacteria and an Arbuscular mycorrhizal fungus on the performance of wheat. Turk.J.Agric.For, 31:355-362.
- 17. Muthuraju, R., Boby, V. U., Suvarna, V. C. and Jayasheela, N. (2002). Interactive effect of Glomus mosseae, Psedomonas fluroscens and Azospirillum brasilense on growth and yield of Potato. J. Soil Biology and Ecology, 22:8-15.
- Nirmalnath, P. J. and Sreenivasa, M. N. (1992). Effect of inoculation of VA-mycorrhiza and or Phosphate solubilizing bacteria on rhizosphere microflora of Sunflower. Bacteria, Fungi and Actinomycetes. Journal of Ectotoxicology and Environmental Monitoring, 2:243-249.
- 19. Phillips, J. M. and Hayman, D. S. (1970). Improved procedures for clearing roots and staining parasitic and vesiculararbuscular mycorrhizal fungi for rapid assessment of infection. Transactions British Mycological Society, 55: 158-161.
- 20. Sabannavar, S. J and Lakshman, H. C. (2008). Interaction between Azotobacter, Pseudomonas and Arbuscular mycorrhizal fungi on two varieties of Sesamum indicum L.J. Agronomy and crop Science, pp 1-9.
- 21. Sattar, M. A and Gaur, A. C. (1989). Effect of Vesicular-arbuscular mycorrhiza and phosphate dissolving microorganisms on the yield and phosphate uptake of Lentil. Lens esculenta Moench. Thai. J. Agri. Sci., 22: 129-136.
- 22. Subba Roa, N. S. (1988). Biofertilizers in agariculture. Oxford IBH publishing Co.Pot.Ltd.pp.206.
- 23. Sumana, D. A., Bagyaraj, D. J. and Arpana, J. (2003). Interaction between Glomus mosseae, Azotobacter chroococcum and Bacillus coagulans and their influence on growth and nutrition of Neem, J. Soil Biology and Ecology, 23: 80-86