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Impact of endomycorrhizal fungi and other bioinoculants on growth enhancement of *Glycine max* (L.) Merrill

Alpa, Neetu, Anju Tanwar, Ashok Aggarwal* and K.K. Gupta[1]

Department of Botany, Kurukshetra University, Kurukshetra-136119 (Haryana), INDIA

[1]Department of Botany and Microbiology, Gurukula Kangri University, Haridwar-249404 (Uttarakhand), INDIA

*Corresponding author. E-mail-aggarwal_vibha@rediffmail.com

Abstract: In the present investigation, the contributions of two indigenous arbuscular mycorrhizal fungi (*Glomus mosseae* and *Acaulospora laevis*), along *with Trichoderma viride* and *Bradyrhizobium japonicum* on growth parameters of Soybean, *Glycine max* (L.) Merrill were investigated. The results obtained indicated the dependence of soybean on mycorrhizal symbiosis. The different growth parameters increased significantly after 120 days of inoculation in comparison to control. Among all the growth parameters studied, plant height (162±3.34), fresh shoot weight (31.26±1.45), dry shoot weight (3.52±0.05), fresh root weight (4.07±0.56), dry root weight (1.03±0.03), root length (49.0±4.47) and leaf area (32.58±1.70) were highest in the combination of *G. mosseae* + *A. laevis* + *T. viride* + *B. japonicum* but arbuscular mycorrhizal (AM) spore number (95.2±3.19) and percent mycorrhizal root colonization (93.26±3.96) were maximum in single inoculation of *G. mosseae*. Second most effective results were observed in the plants treated with *G. mosseae* alone. Thus the presence of arbuscular mycorrhizal fungi (AMF) and other bioinoculants in rhizosphere of soybean had positive effect on the different growth parameters.

Keywords: *Bradyrhizobium japonicum*, *Glycine max*, P-uptake, Symbiosis, *Trichoderma viride*

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is a globally important oilseed crop and source of high quality protein for human consumption, used as fodder for animal and is also important in improved crop rotation system (Manyong *et al*., 1996, Carsky *et al*., 1997). It is being reported medically that by its regular consumption, helps to reduce cardiac disorders due to presence of abundant poly unsaturated fatty acid in Soya oil. Soybean being the richest, cheapest and easiest source of best quality proteins and fats and having a vast multiplicity of uses as food and industrial products is sometimes called a wonder crop. A plant with such a vast importance commercially, demands increase in productivity. This can be achieved either by the addition of inorganic nutrients or by improving the agricultural practices. Among several treatments, the use of arbuscular mycorrhizal fungi (AMF) is gaining more attention because it enhances growth parameters resulting in higher yield and is eco- friendly. AMF is known to affect positively plant establishment and survival, enhance plant nutrient uptake, improve soil structure and reduce the negative effects of various biotic and abiotic stresses (Vessey, 2003; Riaz *et al.*, 2007 and Manoharachary *et al*., 2009). Arbuscular mycorrhizal fungi have mutualistic symbiosis with most vascular plant and these fungi are ubiquitous on terrestrial ecosystem (Allen, 1991; Smith and Read, 1997). Potential use of AM fungi in agriculture has received much attention to reduce the use of chemical fertilizers and pesticide in the past decades (Sharma *et al*., 1997; Harrier and Watson, 2004). Furthermore, legumes like soybean can form tripartite symbiotic association with nodule –inducing rhizobia and AM fungi simultaneously, which may benefit both P and N efficiency (Lisette *et al*., 2003). In contrast, none or negative responses to co-inoculation have been reported in pea (Blilou *et al*., 1999). To explain the contrary reports of effect of co-inoculation with rhizobia, in the present investigation an attempt has been made to see the effect of dominant AM fungi along or in various combinations with other bioinoculants (*Trichoderma viride* and *Bradyrhizobium japonicum*) on the various morphological parameters with a view to find out the best combination that has the capability of increasing plant growth and plant biomass of soybean.

MATERIALS AND METHODS

Collection of soil sample: Composite soil sample from rhizospheric soil of soybean was collected from Botanical Garden, Kurukshetra University, Kurukshetra and kept in sterilized polythene bags at 10°C till further processing.

Isolation of dominant AM fungi from soil samples: Isolation of dominant AM spores were done by using 'Wet Sieving and Decanting Technique' of Gerdemann and Nicolson (1963). Spores were then picked by hypodermic needle under stereobinocular microscope.

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Mass multiplication of AM spores: Dominant AM spores *Glomus mosseae* (Nicol. and Gerd.) and *Acaulospora laevis* (Gerd. and Trappe) were mass multiplied by using wheat as host plant (Tanwar *et al.*, 2010).

Mass culture of *T. viride*: *T. viride* was isolated from the soil and then further mass produced by using wheat bran and saw dust medium which was prepared by using wheat bran, saw dust and distilled water in the ratio of 3:1:4.

Mass culture of *B. japonicum:* *B. japonicum* was grown on nutrient broth medium for 24 hrs for proper growth of bacteria.

Preparation of pot mixture: Surface sterilized seeds of soybean were grown in earthen pots (25×25 cm) under polyhouse conditions. To each pot 10 percent of inoculum of each AM fungi (*G. mosseae* and *A. laevis*) was added alone and in combination with *T. viride*, *B. japonicum* and then the effect of these bioinoculants were recorded on different growth parameters of soybean after 120 days of inoculation. Different treatments used during the present investigation were as follows:

1. Control (without any bioinoculant)
2. *Glomus mosseae* (G)
3. *Acaulospora laevis* (A)
4. *Trichoderma viride* (T)
5. *Bradyrhizobium japonicum* (B)
6. *G. mosseae* + *A. laevis* (G + A)
7. *G. mosseae* + *T. viride* (G + T)
8. *G. mosseae* + *B. japonicum* (G + B)
9. *A. laevis* + *T. viride* (A + T)
10. *A. laevis* + *B. japonicum* (A + B)
11. *T. viride* + *B. japonicum* (T + B)
12. *G. mosseae* + *A. laevis* + *T. viride* (G + A + T)
13. *G. mosseae* + *T. viride*+ *B. japonicum* (G + T + B)
14. *G. mosseae* + *A. laevis* + *B. japonicum* (G + A + B)
15. *A. laevis* + *T. viride* + *B. japonicum* (A + T + B)
16. *G. mosseae* + *A. laevis* + *T. viride* + *B. japonicum* (G + A + T + B)

Five replicates of each treatment were taken.

Quantification of AM spores: It was done by Adholeya and Gaur 'Grid Line Intersect Method' (1994). Spores were counted under stereo binocular microscope by using a counter.

Identification of AM fungi: For identification of AM spores, the keys of Walker (1983), Scheneck and Perez (1990), Morton and Benny (1990), Mukerji (1996), Sharma *et al.* (2008), Tanwar *et al.* (2008) and Sharma *et al.* (2009) were followed.

Mycorrhizal root colonization and growth parameters: After 120 days roots were uprooted, washed, blotted dry for determination of fresh root weight and mycorrhizal root colonization and then oven dried for root dry weight and P content estimation. Mycorrhizal root colonization was studied by 'Rapid Clearing and Staining Method' of Phillips and Hayman (1970). The percentage mycorrhizal root colonization was calculated by following formula:

Percentage root colonization = Number of root segments colonized / Total number root segments studied X 100

Statistical analysis: All results were analyzed using analysis of variance (ANOVA), followed by post hoc test through computer software SPSS 11.5 version. Means were ranked at P d"0.005 level of significance using Duncan's Multiple Range Test for comparison.

RESULTS AND DISCUSSION

Effect on height: In the present investigation it was found that soybean plant growth was significantly affected by AM fungi and other bioinoculants (*T. viride* and *B. japonicum*). It is evident from table-1 that inoculated or treated plants showed significant increment in morphological parameters in comparison to the control. After 120 days, change in morphological parameters was maximum in the combination i.e. *G. mosseae* + *A. laevis* + *T. viride* + *B. japonicum* followed by single inoculation of *G. mosseae* and then followed by triple combination i.e *G. mossseae* + *A. laevis* + *T. viride* respectively. According to the results on the growth attributes shown in Table 1, *G. mosseae* was found to be the most effective bioinoculant among all the bioinoculants used. It is evident from Table 1 that inoculated or treated plants showed significant increase in height in comparison to the control. After 120 days, change in height was maximum in the combination i.e., *G. mosseae* + *A. laevis* + *T. viride* + *B. japonicum* (162±3.34) followed by single inoculation of *G. mosseae* (151±2.30) and triple inoculation of *G. mosseae* + *A. laevis* + *T. viride* (143±4.92) respectively and lowest in control (91±8.40). The results of all present investigation are in close conformity with the finding of Hernandez and Cuevas (2003). AM fungi also have been reported to be involved in improvement of plant growth by enhancing accumulation of plant nutrients through greater soil exploration by mycorrhizal hyphae as reported by Dugassa *et al.* (1996). The other possible mechanism can be that AM fungi also improve health by activating resistance mechanisms and inducing tolerance against some pathogenic microorganism. Similarly, significant increases in the height of soybean plants were observed throughout the growth period when plants were inoculated with AM fungi (Mali *et al.*, 2009).

Effect on root and shoot fresh and dry weight: Table 1 showed that biomass of all the inoculated plants of soybean increased significantly in terms of fresh and dry shoot weight after 120 days of inoculation. Maximum increase in shoot fresh and dry weight was recorded in the combination with *G. mosseae* + *A. laevis* + *T. viride* + *B. japonicum* (31.26±1.45, 3.52±0.05) and lowest in control (10.28±1.47, 1.03±0.02). Second most effective results were observed in the plants treated with *G. mosseae*

Table 1. Interaction of AMF, *T. viride* and *B. japonicum* on different growth parameters of soybean after 120 days.

S. No.	Treatments	Plant height (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)	Leaf area	Root length (cm)	% Root colonization	AM spore number per 10 g of soil
1	Control	91.0±8.40^{f}	10.28±1.47^{f}	1.03±0.02^{f}	0.65±0.10^{f}	0.15±0.03^{f}	19.42±1.47^{f}	15.0±6.08^{f}	39.93±5.11^{f}	13.6±4.21^{f}
2	G	151.0±2.30^{b}	29.34±1.73^{b}	3.42±0.03^{ab}	3.86±0.51^{ab}	0.99±0.07^{b}	30.34±2.08^{b}	48.0±7.21^{ab}	93.26±3.96a	95.2±3.19^{a}
3	A	106±4.18^{e}	21.08±1.92^{d}	2.38±0.05^{c}	1.96±0.34^{d}	0.55±0.03^{de}	25.16±1.77^{cd}	30.6±3.04^{de}	60.65±4.15^{d}	56.0±5.43^{d}
4	T	134±3.39^{cd}	20.58±1.96^{de}	1.97±0.02^{d}	1.71±0.23^{de}	0.50±0.04^{de}	27.18±2.70^{c}	38.0±5.74^{cd}	77.35±1.79^{c}	71.4±4.27^{c}
5	B	098±5.06^{ef}	20.10±2.39^{de}	1.75±0.04^{e}	1.62±0.14^{de}	0.44±0.03^{e}	22.32±3.04^{ef}	32.2±4.76^{de}	44.42±2.92^{e}	23.0±3.03^{ef}
6	G + A	137±4.32^{cd}	25.68±1.31^{c}	2.90±0.04^{bc}	3.04±0.32^{b}	0.97±0.03^{bc}	29.16±2.87^{bc}	44.4±2.30^{b}	87.44±2.61^{b}	82.4±2.40^{b}
7	G + T	132±4.35^{cd}	16.36±1.66^{e}	1.58±0.06^{e}	1.40±0.41^{e}	0.29±0.04^{ef}	27.64±2.38^{c}	40.4±4.77^{c}	78.64±5.18^{c}	72.2±2.68^{c}
8	G + B	102±6.58^{ef}	24.44±2.05^{c}	2.53±0.04^{c}	2.48±0.36^{c}	0.86±0.04^{d}	22.50±2.08^{ef}	39.0±3.16^{c}	45.61±6.96^{e}	38.2±4.96^{e}
9	A + T	134±3.70^{cd}	26.64±1.71^{c}	2.63±0.04^{c}	2.90±0.41^{bc}	0.89±0.03^{cd}	26.38±2.00^{cd}	37.0±3.39^{cd}	76.35±2.84^{c}	68.2±5.76^{cd}
10	A + B	131±3.84^{cd}	20.72±2.14^{c}	1.93±0.05^{de}	1.60±0.12^{de}	0.49±0.03^{e}	25.64±1.23^{cd}	26.0±2.91^{e}	68.29±4.07^{cd}	57.0±4.69^{d}
11	T + B	127±3.53^{d}	21.74±1.21^{d}	2.40±0.03^{c}	2.25±0.16^{c}	0.83±0.03^{d}	23.36±1.43^{de}	35.6±2.40^{d}	48.42±4.20^{de}	42.0±5.91^{de}
12	G + A + T	143±4.92^{c}	28.20±1.71^{b}	3.29±0.06^{b}	3.48±0.33^{ab}	0.91±0.04^{c}	29.30±4.01^{bc}	47.2±4.14^{ab}	89.97±6.24^{b}	83.8±4.43^{b}
13	G + T + B	135±2.38^{cd}	25.88±2.18^{c}	2.89±0.51^{bc}	2.97±0.31^{bc}	0.92±0.04^{c}	28.22±2.51^{bc}	43.0±4.30^{bc}	84.62±3.34^{bc}	78.2±4.14^{bc}
14	G + B + A	111±2.40^{de}	23.84±1.35^{cd}	2.46±0.04^{c}	2.30±0.28^{c}	0.85±0.04^{d}	28.00±2.90^{bc}	33.0±4.84^{d}	50.30±3.92^{de}	48.4±5.02^{de}
15	A + T + B	118±2.07^{de}	25.28±2.12^{c}	2.76±0.02^{bc}	3.07±0.36^{b}	0.95±0.01^{bc}	24.70±1.22^{d}	42.6±3.36^{bc}	53.22±5.03^{de}	52.4±5.22^{d}
16	G + A + T + B	162±3.34^{a}	31.26±1.45^{a}	3.52±0.05^{a}	4.07±0.56^{a}	1.03±0.03^{a}	32.58±1.70^{a}	49.0±4.47^{a}	90.90±5.52^{ab}	92.0±3.80^{ab}
	L.S.D($P \leq 0.05$)	5.556	2.286	0.058	0.425	0.051	3.165	5.547	5.621	5.509
	ANOVA($F_{15,32}$)	100.645	39.898	1112.149	39.57	237.510	9.777	20.573	90.681	149.972

G- *Glomus mosseae*, A-*Acaulospora laevis*, T- *Trichoderma viride*, B- *Bradyrhizobium japonicum*, ± Standard deviation, *Mean difference is significant at 0.5 levels,Mean value followed by different alphabet/s within a column do not differ significantly over one other at P<0.05(Duncan's Multiple Range Test).

(29.34±1.73).This confirms the earlier findings of Shanmugaiah *et al.* (2009) that fresh and dry weight in sesame plant was significantly increased by *G. mosseae* inoculation in oil seed crops. EL-Ghandour *et al.* (1996) reported the same result that root and shoot dry mass was significantly higher with dual inoculation (VAM + *Rhizobium*) in comparison to single treatment. The dry matter yield of *Centrosema pubescens* and *Pueraria phaseoloides* inoculated with *Glomus* sp. increased many folds as compared to control (Lukiwati *et al.*, 1994) Earlier studies have also shown that inoculation of sesame plant with mycorrhizal fungi results in higher fresh and dry shoot weight (Sabannavar and Lakshman, 2009). According to the results shown in Table-1 root biomass (fresh and dry) has been found to increase significantly irrespective of treatments over control. After 120 days, the increase in root biomass was observed maximum with *G. mosseae + A. laevis + T. viride + B. japonicum* (4.07±0.56, 1.03±0.03) followed by *G. mosseae* (3.86±0.51, 0.99±0.07) and lowest in control (0.65±0.10, 0.15±0.03). The results of the present study indicate that inoculation of soil with AMF alone or in combination markedly improved the dry matter in soybean in comparison to control. This increase in root and shoot weight may be due to more absorption of nutrients via an increase in root surface area. Soybean inoculated plants with AMF grew markedly better than non-mycorrhizal plant.

Effect on root length: As Table 1 shows that inoculating soybean with AM fungi significantly increased the root length after 120 days of inoculation. In the present study, it was found that a synergistic effect was observed and maximum root length increment was observed in plants treated with *G. mosseae + A. laevis + T. viride + B. japonicum* (49±4.47), followed by *G. mosseae* (48±7.21) and triple inoculation *G. mosseae + A. laevis +T. viride* respectively and lowest in control (15±6.08). The present results are in agreement with findings of Shanmugaiah *et al.* (2009). Torrisi *et al.* (1999) observed an increase in the root length of cotton plants when soil was inoculated with an isolate of *G. mosseae*. It can be said that as a result of AMF treatment root elongation was observed which ultimately absorb more of the nutrients especially away from the P depletion zone and resulted in the better growth of the plant in comparison to the non- mycorrhizal plants.

Effect on leaf area: The results indicate that leaf area was greatly enhanced in inoculated plants in comparison to control. Highest increment in leaf area activity was observed in the plants inoculated with *G. mosseae + A. laevis + T. viride + B. japonicum* (32.58±1.70) and lowest in control (19.42±1.47). Second highest results were obtained in the single inoculation of *G. mosseae* (30.34±2.08) followed by the triple combination *G. mosseae + A. laevis + T. viride* (29.30±4.01) respectively. The results indicate that inoculation with mycorrhizal fungi alone or in combination with other bioinoculants significantly increase leaf area of soybean. The leaf area enhancement is due to potential of the arbuscular mycorrhizal fungi to improve water and phosphorus uptake as suggested by Harley and Smith (1983) and Sieverding (1991). Similar results were found in the case of corn (Kothari *et al.*, 1990), lettuce (Tobar *et al.*, 1994) and maize (Subramanian *et al.*, 1995). The increase of the leaf area with AM inoculation would be beneficial by maintaining a higher photosynthetic rate (Auge *et al.*, 1987; Panwar, 1993).

Effect on root colonization and AM spore number: Percent mycorrhizal root colonization and AM spore number also increased in all AM treated plants over control (Table 1). After 120 days of inoculation, percent mycorrhizal root colonization (93.26±3.96) and AM spore number (95.2±3.19) were highest in plants treated with *G. mosseae*. Second most effective results were found in the combination *G. mosseae + A. laevis + T. viride + B. japonicum* (90.90±5.52, 92.0±3.80) and lowest in the control (39.93±5.11, 13.6±4.21). It was found that root colonization and AM spore number were greatly enhanced synergistically by interaction of AM fungi, *B. japonicum* and *T. viride*. *T. viride is* highly effective in root colonization by producing secondary metabolites and these metabolites enhance AMF growth and thus mycorrhizal spore number and colonization (Mangla *et al*., 2010). It was observed that inoculation of plants with AM fungi alone or in different combinations showed significant increase in mycorrhizal root colonization. According to Asimi *et al.* (1980), the mycorrhizal fungi and nodule symbiosis bacteria act synergistically, both in root colonization rate and on mineral nutrition uptake and growth of the plant. A significant promoting effect on mycorrhizal colonization density and frequency was observed in soybean plant when inoculated with AMF (Jeong *et al.*, 2006; Wang *et al.*, 2011). The results of the present investigation indicate that colonization with more number of AM fungal species i.e. *G. mosseae* increase the percentage of mycorrhizal root colonization. Similarly AM inoculated plants exhibited higher percent root colonization by in Sunflower plant (Soleimanzadeh, 2010). Mycorrhizal plants grow better than non- mycorrhizal plants due to the difference in the root colonization as arbuscules and vesicles of colonized root played an important role in P transfer.

Conclusion

The current study showed that inoculation with plant growth promoting microorganisms (*G. mosseae*, *A. laevis*, *T. viride* and *B. japonicum*) enhanced the overall growth performance of soybean plants grown under polyhouse conditions. AM inoculation resulted in an increase in mycorrhizal spore number as well as root colonization, which causes significant increase in the other morphological parameters also after 120 days of inoculation. This increment could be attributed to the enhanced uptake of nutrients, better water absorption, increased surface area of roots and also secretion of some enzymes by inoculated microorganisms. *G. mosseae + A. laevis + T. viride + B. japonicum* proved to be the best combination for inoculating soybean seedlings under polyhouse condition to get maximum biomass. This combination can be tested further in the field conditions at seed sowing stage and can be recommended to farmers after proper confirmation.

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