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Research Article

Co-Inoculation of salt tolerant *Bradyrhizobium japonicum* and phosphate solubilizing bacteria for the maximization of growth and yield of soybean

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Abstract

Soybean has significant agronomic and nutritional relevance because of the high concentrations of protein and oil in its grains. Concomitant with the high protein content, the legume shows a strong demand for nitrogen for optimal development and grain productivity. The co-inoculation effect of salt tolerant *Bradyrhizobium japonicum* and Phosphate solubilizing bacteria was studied in the present research for the maximization of growth and yield of Soybean. Maximum plant height was recorded during the harvest and highest plant height (62.99 cm) was recorded in the treatment T_8 (75% NPK + BR + BM + BS). Lowest plant height (41. 02 cm) was observed in the treatment T_2 (75% NPK + *Bradyrhizobium japonicum*). Maximum dry matter production was recorded during the harvest and more dry matter production (40.80 g plant^{-1}) was recorded in the treatment T_8. Less dry matter production 26.00 g plant^{-1}) was observed in the treatment T_4. The treatment with T_8 recorded the maximum of 67.00 pods plant^{-1} and test weight (100 seed wt) of 17.20 g, and grain yield of about 1340.00 kg ha^{-1}, and protein percentage of 41.80%.

Keywords: Soybean, *Bradyrhizobium japonicum*, *Bacillus megaterium, Bacillus subtilis*, Growth and Yield.

Introduction

Soybean (*Glycine max* L.) is one of the most important pulse crop in world. It being a two in one crop supplying about 43.3 per cent protein and 19.5 per cent oil is called as “Miracle bean”. Soybean is indigenous to China and was introduced in India in 1950’s (Caldwell, 1973; Sureshkumar *et al*., 2011). In India, the area and productivity have been rapidly increasing over the recent years. In Tamil Nadu, it is cultivated as an irrigated crop in an area of 31,000 hectares with the annual production crop of 8000 metric tonnes and has multiplicity of use as pulses, oil seeds, vegetarian meat, milk and also as an antibiotic. Soya protein is the only vegetable source of complete protein, of a quality comparable to meat and eggs, which contains all the essential amino acids required by human and animals. So, there is a pressing need to improve the yield of soybean in order to meet the protein malnutrition and the edible oil needs of our country.

Soybeans serve as one of the most valuable crops in the world, not only as an oil seed crop and feed for livestock and aquaculture, but also as a good source of protein for the human diet and as a biofuel feedstock. Soybean oil is used directly in food and preventing high blood pressure caused by arteriosclerosis. It also contains lot of the essential vitamins for the body. Soybean cultivation in India started in 1976. Soybean production in India has increased to about 966 ha. Yield levels have stabilized at about 2895 metric ton per hectare (Nandini, 2012).

The specific interaction between rhizobia and legume plants results in the most efficient form of biological nitrogen fixation, known as symbiotic nitrogen fixation, accounting for 60-80 % of total fixed nitrogen in nature. The rhizobium-host plant interaction leads to the formation of nodules, specialized structures generally found in roots, providing an ideal microenvironment to reduce gaseous nitrogen to ammonium. In this symbiotic interaction, the plant provides the carbon source for bacterial growth in exchange of the fixed nitrogen. The soil bacterium B*radyrhizobium japonicum* establishes symbiotic nitrogen fixation specifically with soybean (Alberton *et al*., 2006). This rod shaped Gram negative species produces abundant exopolysaccharides which dis-play specific functions as carbon source and protective barriers at the initial colonization steps during the bac-terium-host plant interactions, increasing bacterial sur-vival in the soil under adverse conditions. Two other *Bradyrhizobium* species, *Bradyrhizobium elkanii* and *Bradyrhizobium liaoningense* are capable to nodulate soybean. *B. japonicum* shows a slow growth in culture and has been extensively used to produce liquid and solid bioinoculants for application in seeds before sowing (Soto *et al*., 2013).

Phosphorus is an essential constituent of many organic components of biological importance. The deficiency of phosphorus impairs growth and reproductive process; phosphorus cannot be replaced by any another element in all respects and is uniquely associated with essential metabolites (Sadia Alam *et al*., 2002; Usharani *et al*., 2013). Phosphorus is required for proper growth development and maturity of field crops (Saranraj *et al*., 2013; Usharani *et al*., 2014).

Salinity became a serious problem for agriculture, all over the world. Salinity, water shortage and low water quality are the main problems for agriculture production. Under such circumstances, salt stress reduces the free energy of water in soils available to plants and results in negative water potential in soils. This drop in water potential is accompanied by specific ion toxicities, deficiencies, retardation of water uptake and nutritional imbalances in plants which affect enzymatic and physiological functions reducing growth and yield of crops. Soybean is a moderately salt tolerant crop and is being widely cultivated even in areas with salt influenced soils or irrigation water. Ion imbalances, shift in enzymatic reactions and biological processes caused by salinity may also affect the quality and flavor of the tomato fruits (Shanon, 2013; Kanchana *et al*., 2013).

Materials and Methods

Potting

The earthen pots (30 cm diameter) were used for conducting pot culture experiments. They were surface disinfected with copper sulphate solution (5%) and filled with unsterile and sterile soil as per the requirement of the experiment. The soil was disinfected by using 10% formaldehyde solution for 1-3 days.

Cultivars used

Soybean (*Glyline max* (L.) Merril.) Variety ADT -1 was used.

Treatment schedule

T_1- 100% NPK (Control)
T_2 – 75% NPK + *Bradyrhizobium japonicum* (BR)
T_3 – 75% NPK + *Bacillus megaterium* (BM)
T_4 – 75% NPK + *Bacillus subtilis* (BS)
T_5 – 75% NPK + BR + BM
T_6 – 75% NPK + BM + BS
T_7 – 75% NPK + BR + BS
T_8 – 75% NPK + BR + BM + BS

Determination of growth parameters

Plant height

Plant height was measured from the ground level to the tip of the primary branches were recorded on 30, 60 DAS and at harvest. The mean value was worked out and expressed in cm.

Plant dry matter production:

Five plants were selected randomly from each treatment on 30, 60 and 90 DAS were pulled out without damaging the roots and dried in shade and further oven dried at 60°C till it attained a constant weight.

Yield parameters

Determination of Pod Number

The number of pods per plant form each treatment were counted and recorded.

Pod yield

The harvested pods from each treatment were dried and yield was calculated in kg per hectare.

Seed Weight

The test weight of 100 seeds was recorded from each treatment and expressed in g.

Results and Discussion

The field experiment was conducted to study the effect of inoculation of salt tolerant BR-2 (*Bradyrhizobium japonicum*) and Phosphate solubilizing bacteria (*Bacillus megaterium* & *Bacillus subtilis*) on growth and yield parameters of Soybean (*Glycine max* L.) var ADT -1 grown under saline soil. The effect of inoculation of salt tolerant BR-2 (*Bradyrhizobium japonicum*) and Phosphate solubilizing bacteria (*Bacillus megaterium* & *Bacillus subtilis*) on individual, dual and consortium of microbial inoculants on plant height of soybean was measured. Maximum plant height was recorded during the harvest and highest plant height (62.99 cm) was recorded in the treatment T_8 (75% NPK + BR + BM + BS). The treatment T_8 was on par with the treatment T_1 (Control – 100 % NPK) (Plant height – 62.55 cm). Lowest plant height (41. 02 cm) was observed in the treatment T_2 (75% NPK + *Bradyrhizobium japonicum* [BR]) (Table – 1).

Table - 1: Effect of inoculation of Salt tolerant *Bradyrhizobium japonicum* and Phosphate solubilizing bacteria inoculum on the plant height Soybean (*Glycine max* L.) var ADT -1

Treatments	30 DAS	60 DAS	At harvest
T_1- 100% NPK (Control)	30.80	48.20	62.55
T_2 – 75% NPK + *Bradyrhizobium japonicum* (BR)	24.20	39.20	53.50
T_3 – 75% NPK + *Bacillus megaterium* (BM)	24.86	44.36	48.23
T_4 – 75% NPK + *Bacillus subtilis* (BS)	21.20	33.20	41. 02
T_5 – 75% NPK + BR + BM	30.30	47.26	61.78
T_6 – 75% NPK + BM + BS	30.40	47.50	61.85
T_7 – 75% NPK + BR + BS	27.90	27.90	57.80
T_8 – 75% NPK + BR + BM + BS	31.00	48.23	62.99
SE_D	0.34	0.38	0.49
CD (P = 0.05)	0.72	0.84	0.98

Kumar *et al.* (1999) reported a significant increase in sorghum plant height by inoculation of different bacterial strains in combination with *Glomus fasciculatum*. Similar results that plant height increases with *Glomus fasciculatum, Bacillus megaterium* var *phosphaticum* and *Azospirillum brasilense* were reported by Maqsood *et al.* (2001); Ayub *et al.* (2002) and Sharar *et al.* (2003). Burd *et al.* (2000) reported that plant growth promoting rhizobacteria might enhance plant height and productivity by synthesizing phytohormones, increasing the local availability of nutrients, facilitating the uptake of nutrients by the plants decreasing heavy metal toxicity in the plants antagonizing plant pathogens (Usharani *et al*., 2014).

The effect of individual, dual and consortium of microbial inoculants on dry matter production was investigated and the observations recorded on dry matter production at 30 DAS, 60 DAS. Maximum dry matter production was recorded during the harvest and more dry matter production (40.80 g plant^{-1}) was recorded in the treatment T_8 (75% NPK + BR + BM + BS). The treatment T_8 was on par with the treatment T_1 (Control - 100% NPK) (Dry matter production – 39. g plant^{-1-}). Less dry matter production 26.00 g plant^{-1}) was observed in the treatment T_4 (75% NPK + *Bacillus subtilis* [BS]) (Table – 2).

Table – 2: Effect of inoculation of salt tolerant *Bradyrhizobium japonicum* and Phosphate solubilizing bacteria inoculums on the dry matter of Soybean (*Glycine max* L.) var ADT-1

Treatments	Dry weight (g plant^{-1})		
	30 DAS	60 DAS	At harvest
T_1- 100% NPK (Control)	8.58	26.28	39.60
T_2 – 75% NPK + *Bradyrhizobium japonicum* (BR)	5.25	19.00	30.25
T_3 – 75% NPK + *Bacillus megaterium* (BM)	5.75	21.20	32.25
T_4 – 75% NPK + *Bacillus subtilis* (BS)	4.40	15.00	26.00
T_5 – 75% NPK + BR + BM	7.20	24.48	37.48
T_6 – 75% NPK + BM + BS	8.58	26.28	39.00
T_7 – 75% NPK + BR + BS	6.00	21.00	32.00
T_8 – 75% NPK + BR + BM + BS	8.90	27.50	40.80
SE_D	0.19	0.34	0.40
CD (P = 0.05)	0.42	0.76	0.84

Egamberdiyeva *et al.* (2004) reported the effect of *Bradyrhizobium* spp. strains on dry matter yield, nodulation and seed yield of soybean varieties grown in N-deficient soil in pot and field experiments. They noticed the significant effects on growth, nodule number and yield of soybean were obtained after inoculation with *Bradhyrhizobium* spp. strains (Sivasakthi *et al*., 2013; Kanchana *et al*., 2013).

Appuna *et al.* (2008) studied the symbiotic interactive effect of different *Bradhyrhizobium japonicum* strains with 6 soybean cultivars. Plants inoculated with strain ASRO11 produced higher plant dry matter accumulation and seed yield over all other cultivars (Sivasakthivelan *et al*., 2013; Sivasakthi *et al*., 2014; Kanchana *et al*., 2014).

The effect of individual, dual and consortium of microbial inoculants on dry matter production was tested. The treatment with T_8 – 75% NPK + BR + BM + BS recorded the maximum of 67.00 pods plant^{-1} and test weight (100 seed wt) of 17.20 g, and grain yield of about 1340.00 kg ha^{-1}, and protein percentage of 41.80%. The treatment T_8 was on par with the treatment T_1 (Control - 100% NPK) (Table – 3). The results of the present research are also in agreement with those of Maqsood *et al.* (2001), Ali *et al.* (2002), Younas (2002), Sharar *et al.* (2003), Rasheed *et al.* (2004) and Oktem *et al.* (2005).

Table – 3: Effect of inoculation of salt tolerant *Bradyrhizobium japonicum* and Phosphate solubilizing bacteria on the yield parameters of Soybean (*Glycine max* L.) var ADT -1

Treatment	No. of pods/ plant
T_1- 100% NPK (Control)	66.00
T_2 – 75% NPK + *Bradyrhizobium japonicum* (BR)	60.00
T_3 – 75% NPK + *Bacillus megaterium* (BM)	62.00
T_4 – 75% NPK + *Bacillus subtilis* (BS)	55.00
T_5 – 75% NPK + BR + BM	64.00
T_6 – 75% NPK + BM + BS	64.50
T_7 – 75% NPK + BR + BS	63.00
T_8 – 75% NPK + BR + BM + BS	67.00
SE_D	1.34
CD (P=0.05)	2.68

Table – 4: Effect of inoculation of salt tolerant *Bradyrhizobium japonicum* and Phosphate solubilizing bacteria on the Test weight of Soybean (*Glycine max* L.) var ADT -1

Treatment	Test weight (g)
T_1- 100% NPK (Control)	16.50
T_2 – 75% NPK + *Bradyrhizobium japonicum* (BR)	11.00
T_3 – 75% NPK + *Bacillus megaterium* (BM)	13.00
T_4 – 75% NPK + *Bacillus subtilis* (BS)	19.00
T_5 – 75% NPK + BR + BM	14.80
T_6 – 75% NPK + BM + BS	15.65
T_7 – 75% NPK + BR + BS	12.50
T_8 – 75% NPK + BR + BM + BS	17.20
SE_D	0.94
CD (P=0.05)	1.88

Table – 5: Effect of inoculation of salt tolerant *Bradyrhizobium japonicum* and Phosphate solubilizing bacteria inoculums on Grain yield of Soybean (*Glycine max* L.) var ADT -1

Treatment	Grain yield (kg ha^{-1})
T_1- 100% NPK (Control)	1330.00
T_2 – 75% NPK + *Bradyrhizobium japonicum* (BR)	1000.00
T_3 – 75% NPK + *Bacillus megaterium* (BM)	1140.00
T_4 – 75% NPK + *Bacillus subtilis* (BS)	700.00
T_5 – 75% NPK + BR + BM	1200.00
T_6 – 75% NPK + BM + BS	1280.00
T_7 – 75% NPK + BR + BS	1100.00
T_8 – 75% NPK + BR + BM + BS	1340.00
SE_D	13.26
CD (P=0.05)	26.52

Seed inoculation of *Bradhyrhizobium* increased the number of pods $plant^{-1}$, number of grain pod^{-1} and 1000 seed weight in soybean (Sable and Khuspe, 1997). Lal *et al.* (1983) reported that *Bradyrhizobium japonicum* seed inoculation increased the soybean seed yield by 11.8% over uninoculated control and improved fertility status of soil.

Ibrahim *et al.* (2011) carried out an experiment to investigate the effect of Bradyrhizobium inoculation and chicken manure or sulphur fertilization on growth, nodulation and yield of soybean. The results showed that inoculation, chicken manure, sulphur and their interaction significantly improved the dry weight of shoots and roots, nodulation, yield and yield components.

Agraw (2012) conducted an experiment to study the effects of co-inoculation of *Bradyrhizobium japonicum* (TAL-378 and TAL-379) and phosphate-solubilizing bacteria (PSB) (*Pseudomonas* spp.), and conventional farmers' fertilizer level (combined and individual application of 46N kg ha-1 and 46 P_2O_5 kg ha-1) on nodulation, seed yield and yield components of soybean (*Glycine max* L.). Analyses of variance indicated that most of the parameters measured were significantly (P>0.05) affected by the treatments. Accordingly, dual inoculation with TAL-378 and PSB significantly increased plant height at harvest, number of nodules per plant, nodule volume per plant, nodule fresh weight per plant, and shoot height at late flowering and early pod setting compared to the other treatments.

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