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Research Article

EFFECT OF INOCULATION SALT TOLERANT *Bradyrhizobium japonicum* AND PHOSPHATE SOLUBILIZING BACTERIA ON NUTRIENTS STATUS AND PROTEIN CONTENT OF SOYBEAN

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Abstract

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. The effect of salt tolerant *Bradyrhizobium japonicum* and phoshhate solubilizing bacteria on nutrient status and protein content of soybean was investigated in the present research. Highest protein content (41.80%) was recorded in the treatment T_8 (75% NPK + *Bradyrhizobium japonicum* + *Bacillus megaterium* + *Bacillus subtilis*). Less protein content (28.00%) was observed in the treatment T_4 (75% NPK + *Bacillus subtilis*). Maximum NPK uptake and available nutrients were recorded in the treatment T_8 (75% NPK + *Bradyrhizobium japonicum* + *Bradyrhizobium japonicum* + *Bacillus subtilis*). The treatments T_8 was on par with the control treatment T_1 (100% NPK). Lowest NPK uptake and available nutrients was recorded in the control treatment T_4 (75% NPK + *Bacillus subtilis*).

Keywords: Soybean, *Bradyrhizobium japonicum, Bacillus megaterium, Bacillus subtilis*, Nutrient status and Protein content.

Introduction

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 (Graham and Vance, 2008). Although, atmospheric N_2 is abundant, no eukaryotic organism is able to directly assimilate it, due to the strong triple bond linking the atoms (Brechenmacher *et al*., 2008; Oldroyd *et al*., 2011). However, when growing in nitrogen depleted soils, much of soybean's need for nitrogen can be obtained *via* biological nitrogen fixation (BNF) in root nodules, through the symbiotic association with bacteria, collectively called *Rhizobia*, belonging mainly to the species *Bradyrhizobium japonicum* and *Bradyrhizobium elkanii* (Hungria *et al*., 2006).

Nitrogen is one of the major important nutrient essential for plant growth. The economic and environment importance of legume crops is largely due to their ability to fix atmospheric dinitrogen in a symbiosis with specific bacteria (*Rhizobium* or *Bradyrhizobium* species). Like most legumes, soybeans performs N_2 fixation by establishing a symbiotic relationship with the rhizobia. *Bradyrhizobium japonicum* is a slow growing root nodule symbiont, which is widely used as an inoculant in soybean fields throughout the world. Generally, soybean inoculated with *Bradyrhizobium japonicum* forms highly effective nodules and frequently increased soybean yields, especially in fields where soybeans are cultivated for the first time (Caldwell and Vest, 1970; Sureshkumar *et al*., 2011).

The major problem of soybean inoculation is the existing indigenous strains in the field may often suppress the introduced inoculant strains applied to soybeans subsequently. Therefore, it is necessary that the highly effective introduced strain has also the capacity to compete with the resident ineffective rhizobia in the soil (Dowling and Broughton, 1986). Environmental factors such as temperature, moisture, acidity, salinity and several chemical component of the soil are the limiting factors of the legume-rhizobium symbosis. Both establishment and activity of the legume-*Rhizobium* symbosis are known to sensitive to drought stress (Kirda *et al.,* 1989). Numerous abiotic and biotic factors are known to influence the competitiveness of specific rhizobial inoculants (Bottomley, 1992; Turco and Sadowsky, 1995).

The beneficial effect of *Rhizobium* and *Bradyrhizobium* in legume in terms of biological nitrogen fixation has been a main focus in the recent past (Deshwal *et al*., 2003), as it is an important aspect of sustainable and environmental friendly food production and long term crop productivity. Soybean is a crop that is used for human food and livestock feed. Plant improvement generally seeks to increase the proportion of dry matter production that goes to seed production (Lukiwati and Simanungkalit, 2002). *Bradyrhizobium japonicum* strains form nitrogen fixing root nodule symbioses with soybean. Inoculation with highly effective *Bradyrhizobium japonicum*, a common practice in agricultural production (Catroux *et al.,* 2001), requires survival and establishment of inoculated rhizobia in the soil environment (Da and Deng, 2003). However, to initiate the soybean cultivation in other areas where the soil is sandy or sandy loam with low nutrients, it is essential to obtain some effective *Bradyrhizobium japonicum* strains having better adaptability to survive in such soil conditions.

Phosphorus is one of the most important macronutrient, most soil in India is low in available phosphorus. Numbers of chemical, physical and biological factors are related to availability of phosphorus in soil. The major problem in availability is its transformation from soluble form into insoluble (or) unavailable form (Saranraj *et al*., 2013; Usharani *et al*., 2013). Sorption (fixation), precipitation and immobilization are important transformation that curtail or restrict the P availability in soil either temporarily or permanently. Several phosphate solubilizing bacteria (PSB) particularly those belonging to the genera *Bacillus, Pseudomonas* and phosphate solubilizing fungi (PSF) belonging to the genera *Aspergillus* and *Penicillium* possess ability to convert insoluble phosphates into soluble forms by secreting organic acids, thereby lowering the pH and bringing about the solubilization of bound phosphates (Pal, 1998; 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

Estimation of Protein content

Bradford (1976) method was followed for estimation of proteins.

Effect of inoculation salt tolerant *Bradyrhizobium* and Phosphate solubilizing bacteria on Available nutrients

Available Nitrogen

The available nitrogen content of soil samples was estimated by the alkaline permanganate ($KMnO_4$) method suggested by Subbiah and Asija (1956) and expressed in per cent. The total nitrogen uptake was worked out by multiplying the nitrogen content (%) with dry matter production (DMP) of that treatment and computed and expressed in kg ha^{-1}.

Available phosphorus

The available phosphorus content of soil samples was estimated by the method described by Olsen *et al*. (1954) and expressed in kg ha^{-1}.

Available potassium

The available potassium content of soil samples was estimated by the method suggested by Stanford and English (1949) and expressed in kg ha^{-1}.

Effect of inoculation salt tolerant *Bradyrhizobium* and Phosphate solubilizing bacteria on Nutrients uptake

The oven dried plant samples were cut into small pieces and powdered in a Willey mill. The powdered material was used for the chemicals analysis. In groundnut, the plant samples were analyzed for the nutrient contents in the above ground portion and pod (Kernel and shell). The nutrient uptake in kg ha^{-1} was worked out by multiplying the nutrient content with the weight of the above ground dry matter. Uptake in the shell and kernel was worked out separately and summed up to arrive at nutrient uptake by crop.

Nitrogen uptake (kg ha^{-1})

The nitrogen content of plant samples was estimated by the Microkjeldahl's method suggested by Yoshida *et al.,* (1976) and expressed in per cent. The total nitrogen uptake was worked out by multiplying the nitrogen content (%) with DMP of that treatment, computed and expressed in kg ha^{-1}.

Phosphorus uptake (kg ha^{-1})

Phosphorus content in plant samples was determined by triple acid digestion method (Jackson, 1973) using photoelectric colorimeter. From the standard curve drawn, the P content in plant was calculated. The total phosphorous uptake was calculated by multiplying the crop biomass with phosphorous content of crop and expressed in kg ha^{-1}.

Potassium uptake (kg ha^{-1})

The potassium content was estimated by triple acid digestion method (Jackson, 1973) using flame photometer. The potassium content of the plant sample was calculated from the standard curve drawn. The uptake was computed by multiplying the potassium content (%) with the dry matter production (DMP) of that treatment and computed in terms of kg ha^{-1}.

Results and Discussion

The effect of individual, dual and consortium of microbial inoculants on protein content was investigated. Maximum protein content was recorded during the harvest and more protein content (41.80%) 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) (41%). Less protein content (28.00%) was observed in the treatment T_4 (75% NPK + *Bacillus subtilis* [BS]) (Table – 1). The findings of Capuno *et al.* (1980) are almost similar to the present study who found that biofertilizers increased the protein content of soybean.

Shaarawi *et al.* (2011) stated that inoculation with *B. japonicum* tended to compensate the adverse effect of applying lower doses of mineral nitrogen. The combinations between the inoculant and the lower doses tended to enhance most growth and yield characters compared to the lower doses applied alone. The beneficial effect of *B. japonicum* on total dry weight of shoots and pods and seed yield was increased by decreasing the applied level of mineral nitrogen, reached the maximum with the lowest rate. The combination between 20kg MN/fed with the higher rate of *B. japonicum* induced the highest increase in seed yield/fed. (19.2 and 31 %) over that of the highest rate of mineral nitrogen alone in the two successive seasons, respectively.

Nitrogen is an element essential for the support of all forms of life. It is found in amino acids and proteins, and many other organic compounds are derived from the nitrogen fixation process (Pham and Burgess, 1993). Rhizospheric microorganisms can interact positively in promoting plant growth, as well as Nitrogen and Phosphorous uptake (Mehrvarz *et al.,* 2008; Sivasakthi *et al*., 2013; Sivasakthivelan *et al*., 2013; Kanchana *et al*., 2013; Usharani *et al*., 2014).

The effect of individual, dual and consortium of microbial inoculants on nutrients uptake (N, P & K) was determined. Among the treatments tested, maximum NPK uptake was recorded in the treatment T_8 (75% NPK + BR + BM + BS) (145.16 kg ha^{-1}, 17.23 kg ha^{-1} and 116.65 kg ha). The treatments T_8 was on par with the control treatment T_1 (100% NPK) (144.90 kg ha^{-1}, 17.18 kg ha^{-1} and 116.15 kg ha^{-1}). The minimum NPK uptake was recorded in T_4 – 75% NPK + *Bacillus subtilis* (BS) (106.8 kg ha^{-1}, 11.11 kg ha^{-1} and 91.11 kg ha^{-1}) (Table – 2).

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

Treatment	Protein (%)
T_1- 100% NPK (Control)	41.00
T_2 – 75% NPK + *Bradyrhizobium japonicum* (BR)	33.00
T_3 – 75% NPK + *Bacillus megaterium* (BM)	35.00
T_4 – 75% NPK + *Bacillus subtilis* (BS)	28.00
T_5 – 75% NPK + BR + BM	38.40
T_6 – 75% NPK + BM + BS	40.00
T_7 – 75% NPK + BR + BS	34.75
T_8 – 75% NPK + BR + BM + BS	41.80
SE_D	1.65
CD (P=0.05)	3.24

Table - 2: Effect of inoculation of salt tolerant *Bradyrhizobium japonicum* and Phosphate solubilizing bacteria inoculums on nutrient uptake

Treatments	Nitrogen (kg ha^{-1})	Phosphorous (kg ha^{-1})	Potassium (kg ha^{-1})
T_1- 100% NPK (Control)	144.90	17.18	116.15
T_2 – 75% NPK + *Bradyrhizobium japonicum* (BR)	112.59	12.20	84.29
T_3 – 75% NPK + *Bacillus megaterium* (BM)	119.34	13.31	91.11
T_4 – 75% NPK + *Bacillus subtilis* (BS)	106.78	11.11	75.87
T_5 – 75% NPK + BR + BM	132.81	15.35	104.72
T_6 – 75% NPK + BM + BS	135.43	16.21	105.84
T_7 – 75% NPK + BR + BS	126.08	14.34	97.91
T_8 – 75% NPK + BR + BM + BS	145.16	17.23	116.65
SE_D	5.04	0.80	5.17
CD (P = 0.05)	10.08	1.60	10.34

Matiru and Dakora (2004) used light, scanning, and transmission electron microscopy to show that roots of sorghum and millet landraces from Africa were easily infected by rhizobial isolates from five unrelated legume genera. With sorghum, in particular, plant growth and phosphorus (P) uptake were significantly increased by rhizobial inoculation, suggesting that filed selection of suitable rhizobial cereal combination could increase yields and produce fodder for livestock production (Sivasakthi *et al*., 2014; Kanchana *et al*., 2014).

The effect of individual, dual and consortium of microbial inoculants on available nutrients (N, P & K) was estimated. Among the treatments, the treatment T_8 (75% NPK + BR + BM + BS) showed more available nutrients (194.70 kg ha^{-1}, 18.32 kg ha^{-1} and 186.42 kg ha^{-1}). The treatment T_8 was on par with T_1 (100% NPK) (192.25 kg ha^{-1}, 18.16 kg ha^{-1} and 183.52 kg ha^{-1}). The minimum available nutrients (NPK) were recorded in T_4 – 75% NPK + *Bacillus subtilis* (BS), (159.35 kg ha^{-1}, 17.12 kg ha^{-1} and 168.14kg ha^{-1}) (Table – 3).

Nitrogen is one of the major important nutrients essential for plant growth. The economic and environment importance of legume crops is largely due to their ability to fix atmospheric dinitrogen in a symbiosis with specific bacteria (*Rhizobium* or *Bradyrhizobium* species). Like most legumes, soybeans performs N_2 fixation by establishing a symbiotic relationship with the *Rhizobium*. *Bradyrhizobium japonicum* is a slow growing root nodule symbiont, which is widely used as an inoculant in soybean fields throughout the world. Generally, soybean inoculated with *Bradyrhizobium japonicum* forms highly effective nodules and frequently increased soybean yields, especially in fields where soybeans are cultivated for the first time (Caldwell and Vest, 1970).

Table - 3: Effect of individual, dual and consortium of microbial inoculants on available nutrients

Treatments	Nitrogen (kg ha^{-1})	Phosphorous (kg ha^{-1})	Potassium (kg ha^{-1})
T_1- 100% NPK (Control)	192.25	18.16	183.52
T_2 – 75% NPK + *Bradyrhizobium japonicum* (BR)	162.78	17.46	170.90
T_3 – 75% NPK + *Bacillus megaterium* (BM)	168.01	17.63	173.79
T_4 – 75% NPK + *Bacillus subtilis* (BS)	159.35	17.12	168.14
T_5 – 75% NPK + BR + BM	180.27	18.02	170.92
T_6 – 75% NPK + BM + BS	185.37	18.42	179.57
T_7 – 75% NPK + BR + BS	175.14	17.80	176.67
T_8 – 75% NPK + BR + BM + BS	194.70	18.32	186.42
SE_D	4.64	0.15	2.30
CD (P = 0.05)	9.28	0.30	4.60

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