The Bioscan 11(4): 2443-2447, 2016 (Supplement on Agronomy)
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.com

POTENTIAL USE OF *BRADYRHIZOBIUM JAPONICUM* AS PGPR FOR IMPROVE SOYBEAN YIELD AND SOIL NUTRIENTS IN A VERTISOL

F. C. AMULE[1], A. K. RAWAT[1] AND R. K. SAHU*[2]
[1]Dept. of Soil Science and Agril. Chemistry, JNKVV, Jabalpur .
[2]Deptt. Of Soil Science, College of Agriculture, Waraseoni, Balaghat, M.P.
e-mail: rakesh_sahujbp@yahoo.co.in

KEYWORDS
Potential
Bradyrhizobium
PGPR
Soil Nutrients

Received on :
11.08.2016

Accepted on :
26.10.2016

***Corresponding author**

ABSTRACT

The current study was conducted to assess the potential use of *Bradyrhizobium japonicum* as PGPR for improve soybean yield and soil nutrients in a Vertisol. The said experiment was the field testing part of research which was conducted during 2002-2007. The efficacy of 40 isolates were tested and also compared with the USDA isolates, FUI (Fertilized un inoculated control) and UFUI (Unfertilized un inoculated control) in field condition. Nitrogen content of soil is an important parameter for determining the nitrogen fixing ability of plants. The data showed that after harvest of soybean out of thirty six isolates only six isolates (BR_{11}, BR_{12}, BR_{16}, BR_{17}, BR_{21}, and BR_{35}) significantly improve the total and available N, P and K content in soil as compared to FUI it was maximum BR_{11} isolate 959, 275, 25 and 342 kg ha^{-1} respectively. The maximum production of soybean was recorded with BR_{11} (3350 kg ha^{-1}) followed by BR_{35} (3150 kg ha^{-1}). It was found that rest of the isolates except BR_{11}, BR_{12}, BR_{16}, BR_{17}, BR_{21}, and BR_{35} were statistically at par to FUI. Good relationships were obtained between addition BNF and harvest index R^2 0.74 (y=0.04x + 24.29). Similar correlation was found in case of addition BNF and nitrogen harvest index R^2 0.84 (y=0.09x + 37.06).

INTRODUCTION

Soybean has been recognized as one of the premier agricultural crops today, thus it is the best source of protein and oil and has now been recognized as a potential supplementary source of nutritious food (Wilcox and Shibles, 2001). Madhya Pradesh is known as soybean state of India and grown in about 1.68 million ha area in 2008-09 (Agricultural Statistics at a glance, 2010). Soybean was initially introduced in Madhya Pradesh in mid sixties, and due to absence of native effective rhizobial strains in most of the soils, it was inoculated with bradyrhizobial inoculants imported from USA. Later on, the indigenous biofertilizer production units came into existence in the state, which resulted in increased native Indian strains. Use of such inoculants leads to better establishment of native rhizobial population in the soil (Catroux *et al.*, 2001). The use of beneficial microorganisms has been proven to be an environmentally sound option to increase crop yields. Plant growth-promoting bacteria (PGPR) may facilitate plant growth either indirectly or directly. There are several ways in which plant growth promoting bacteria can directly facilitate plant growth. They may: fix atmospheric nitrogen and supply it to plants, although this is usually a minor component of the benefit that the bacterium provides to the plant; synthesize siderophores which can sequester iron from the soil and provide it to plant cells as a siderophore–iron complex which can be taken up; synthesize phytohormones such as auxins, cytokinins and gibberellins which can act to enhance or regulate various stages of plant growth; solubilize minerals such as phosphorus, making them more readily available for plant growth (Glick *et al.*, 2007 and Bashan and de-Bashan, 2010). Bacteria may directly affect plant growth and development by using anyone or more than one of these mechanisms. Since many plant growth-promoting bacteria possess several of these traits simultaneously, different mechanisms at various times during the life cycle of the plant can be used. However, the exact modes by which plant growth-promoting bacteria (PGPR) promote plant growth at a specific step in life cycle are not fully understood. Inoculation with PGPR can be used also to promote plant biocontrol (Bhattacharyya and Jha, 2012). The high temperature during summer, varying moisture status during monsoon, different cropping pattern and diverse soil types may be a reason for rhizobial biodiversity. In this study, screening approaches were employed to select the most potential effective rhizobial isolates and their impact on soil nutrients status.

MATERIALS AND METHODS

A field experiment was carried out at experimental station, Department of Soil Science and Agricultural Chemistry, JNKVV, Jabalpur during kharif 2009 -10. The presented research was a part of continue experiment since 2002-07 jointly by JNKVV, Jabalpur centre and IISS, Bhopal (M.P.) under All India Network Project on Soil Biodiversity and Biofertilizers. The best forty isolates were screened previously under laboratory and green house screenings at Jabalpur and Bhopal centers. The efficacy of these isolates further test under field conditions. Therefore, in the present investigation it is proposed to screen the symbiotic performance of 40 soybean rhizobial isolates under field conditions against USDA and JNKVV check in order to further shortlist their number and to get more efficient soybean isolates. The site is located at N - 23°12′46.6′′ and 079 °56′47.6′′ E with an altitude of 419 meter above mean sea level (MSL). The soil of the experimental site was Vertisols belongs to fine montmorillonite, Hypothermic family of *Typic Haplusterts* popularly known as "black cotton soil" with 56.8% clay, 25.3% sand and 17.9% silt, 7.02 pH, 0.22 dSm^{-1}EC, 6.9 g kg^{-1} soil organic carbon (OC), available nutrients 140 mg N, 12 mg P and 153 mg K kg^{-1} soil. The experiment was laid out in augmented RBD without replication. Soybean (JS – 9752) was grown in kharif season (June – October). Liquid formulations of all the isolates were prepared at Indian Institute of Soil Science, Bhopal using the respective liquid medium. Seed treated of particular isolates; total number of 36 isolates and JNKVV check were taken for seed treatment. The inoculants packets were supplied with gum Arabic for sticking as many cells as possible into the seeds and they were sown separately. All the packages of practices were followed as per recommendations to grow the crop.

Soil samples of 0 – 15 cm depth were collected from 8 spots in a zig-zag pattern and a initial composite sample was prepared after air drying and grinding for chemical analysis. Soil pH was determined in a 1:2.5 soil:water (s/w) suspension using KCl glass electrode pH meter and after setting down, the conductivity of supernatant liquid was determined by null method using wheat stone circuit through conductivity meter (Piper, 1967). Organic carbon was determined by oxidizing the organic matter using chromic acid and the excess of unreduced dichromate was back titrated with standard ferrous ammonium sulphate (Walkey and Black, 1934). Kjeldahl method was used to determine total nitrogen (Piper, 1967). Available nitrogen ($KMnO_4$ oxidizable N) was oxidized by potassium permanganate and released ammonia was absorbed in boric acid (Subbiah and Asija, 1956). Available phosphorus in soil was extracted by 0.5 M N_aHCO_3 having 8.5 pH using ascorbic acid as colour development agent (Adams, 1974). Ammonium acetate (1 N) was used for extracting available potassium and its estimation by flame photometer (Muhr *et al.*, 1963). Harvest index, nitrogen harvest index and additional BNF were computed by using formula. The data were statistically analyzed by using non replicated augmented randomized block design (Federer *et al.*, 1975). The fisher's least significance difference (L.S.D.) was used to compare treatment means at $p = 0.05$ level of significance (Steel *et al.*, 1997).

RESULTS AND DISCUSSION

Impact *Bradyrhizobium japonicum* as PGPR on soil nitrogen.

Biological N_2 fixation (BNF) and mineral soil or fertilizer N are the main sources of meeting the N requirement of high-yielding soybeans. At harvesting time of soybean, available N and P has been increased significantly under application of biofertlizer whether only bradyrhizobial inoculant or both bradyrhizobial inoculant and PSB fertilizer as compared to UFUI. Out of total isolates (36 Nos.) only six isolates were able to increase the total soil nitrogen content in post harvest samples statistically over FUI and these strains were BR_{11}, BR_{12}, BR_{16}, BR_{17}, BR_{21}, and BR_{35} while remaining strains were statistically identical to FUI (Table 1). BR_{11} followed by BR_{35} maintained their superiority among all the isolates. Available N, P and K content of soil was determined in the post harvest soil samples of 0 – 15 cm depth and it was found that strains BR_{11}, BR_{12}, BR_{16}, BR_{17} and BR_{35} increased the available soil nitrogen significantly over FUI and remaining strains were at par to it (Table 1). It was maximum with BR_{11} (275kg ha^{-1}) followed by BR_{35} and BR_{12} (274kg ha^{-1}). Hayat *et al.* (2004) also reported the similar finding. Tran Thi Ngoc Son *et al.* (2006) also reported that with the application of Bradyrhizobia

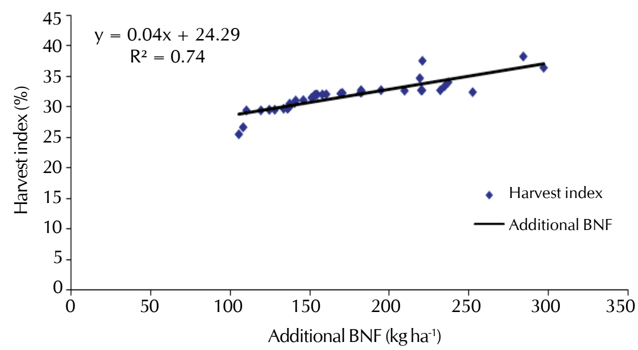

Figure 1: Correlation between additional BNF and harvest index

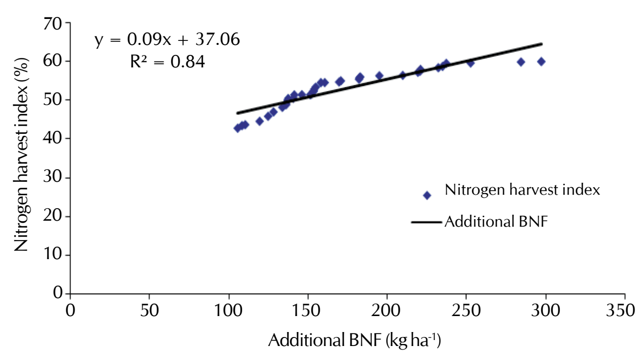

Figure 2: Correlation between additional BNF and nitrogen harvest index

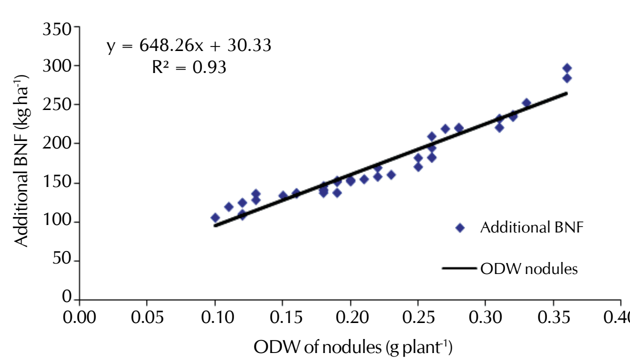

Figure 3: Correlation between Additional BNF and ODW of nodules

Table 1: Effect of seed inoculation with *B. japonicum* isolates on soil available N, P, K total N and yield (kg ha^{-1})

Isolate No.	Ava. N (kg ha^{-1})	Ava. P (kg ha^{-1})	Ava. K (kg ha^{-1})	Total N (kg ha^{-1})	Grain yield (kg ha^{-1})
BR_1	213a	14a	289a	925a	2400a
BR_2	247a	18a	320a	948a	2700a
BR_3	228a	17a	311a	940a	2550a
BR_4	253a	21b	330b	954a	2700a
BR_5	207a	13a	270a	910a	2250a
BR_6	208a	13a	272a	915a	2250a
BR_7	227a	17a	310a	940a	2550a
BR_8	248a	19a	325a	950a	2700a
BR_9	210a	14a	277a	918a	2250a
BR_{10}	225a	16a	301a	935a	2475a
BR_{11}	275b	25b	342b	959b	3350b
BR_{12}	274b	22b	336b	957b	2850b
BR_{13}	207a	13a	241a	900a	2100a
BR_{14}	247a	19a	324a	949a	2700a
BR_{15}	249a	20a	329b	953a	2700a
BR_{16}	273b	22b	335b	957b	2850b
BR_{17}	273b	22b	332b	957b	2850b
BR_{18}	225a	16a	300a	934a	2400a
BR_{19}	226a	17a	307a	938a	2475a
BR_{20}	220a	15a	300a	930a	2400a
BR_{21}	272b	22b	332b	957b	2850b
BR_{22}	255a	21b	330b	956a	2700a
BR_{23}	226a	16a	306a	936a	2550a
BR_{24}	210a	13a	272a	917a	2175a
BR_{25}	249a	20b	329b	953a	2700a
BR_{26}	231a	18a	314a	944a	2550a
BR_{27}	243a	19a	320a	947a	2625a
BR_{28}	248a	19a	326a	951a	2700a
BR_{29}	210a	14a	275a	918a	2250a
BR_{30}	239a	18a	315a	946a	2625a
BR_{31}	213a	14a	287a	921a	2325a
BR_{32}	211a	14a	282a	921a	2250a
BR_{33}	218a	15a	297a	930a	2400a
BR_{34}	262a	21b	331b	956a	2775a
BR_{35}	274b	24b	339b	958b	3150b
BR_{36}	214a	15a	297a	928a	2475a
BR_{37}	211a	14a	281a	919a	2250a
BR_{38}	233a	18a	315a	945a	2625a
BR_{39}	214a	15a	290a	927a	2400a
BR_{40}	231a	17a	311a	943a	2550a
JNKVV Check	213	17	305	923	2500
FUI control	207	13	241	900	2100
SEd (±)	28.6	2.8	39.6	26.1	322.7
LSD (p=0.05)	62.4	6.1	86.3	56.8	703.1

Means followed by dissimilar letter (s) in a column are significantly different from each other at p = 0.05 according to Fischer least significance difference (LSD)

and phosphate solubilizing bacteria can enhance soil available nitrogen increased from 3.17 % to 28.35 % and available phosphate increased from 14.6% to 59.7%.

Impact *Bradyrhizobium japonicum* as PGPR on available soil phosphorus

Phosphorus is essential for plant growth as it stimulates growth of young plants, giving them a good and vigorous start (Tucker, 1999). It is among the important elements needed for crop growth and production in many soils. The available phosphorus content of soil was increased significantly over FUI by only two strains *i.e.* BR_{11} and BR_{35}, rest of the strains were statistically non significant over FUI (Table 1). Maximum available P was noticed with BR_{11} (25 kg ha^{-1}) follow by BR_{35} (24 kg/ha^{-1}). It is abundantly available in soils in both organic and inorganic forms. Plants are unable to utilized phosphate because 95-99% phosphate present in the insoluble, immobilized, and precipitated form (Pandey and Maheshwari, 2007). Plants absorb phosphate only in two soluble forms, the monobasic ($H_2PO_4^-$) and the diabasic (HPO_4^{-2}) ions (Bhattacharyya and Jha, 2012). Microorganisms enhance the phosphorus availability to plants by mineralizing organic phosphorus in soil and by solubilizing precipitated phosphates (Chen *et al.*, 2006).

Impact *Bradyrhizobium japonicum* as PGPR on available soil potassium

Potassium (K) is the third major essential macronutrient for plant growth. The concentrations of soluble potassium in the soil are usually very low and more than 90% of potassium in the soil exists in the form of insoluble rocks and silicate minerals (Parmar and Sindhu, 2013). Plant growth promoting rhizobacteria are able to solubilize potassium rock through production and secretion of organic acids (Han and Lee, 2006). More number of strains was able to increase the soil available potassium content as compared to available nitrogen and phosphorus. BR_4, BR_{11}, BR_{12}, BR_{15}, BR_{16}, BR_{17}, BR_{21}, BR_{22}, BR_{25}, BR_{34}, BR_{35} strains were significantly better to FUI. It was maximum with BR_{11}(342 kg/ha^{-1}) followed by BR_{35}(339 kg/ha^{-1}).The potassium availability in soil also increased with the application of Bradyrhizobium along with inorganic fertilizer (Dhage and Kachhave, 2008). However, Katkar *et al.* (2002) reported that the use of organic sources either alone or in combination with chemical fertilizer recorded higher available potassium in soil. The bioavailability of P and K in the soils increased with inoculation of PGPR or with combined inoculation and rock materials has been reported by many researchers (Lin *et al.*, 2002), which may lead to increased P uptake and plant growth (Wahid and Mehana, 2000)

Impact *Bradyrhizobium japonicum* as PGPR on Soybean production

The minimum grain yield was recorded with fertilized uninoculated control (2100 kg ha^{-1}) while maximum yield was recorded with BR_{11} (3350 kg ha^{-1}) followed by BR_{35} (3150 kg ha^{-1}). All the isolates yielded numerically better seed yield as compared to FUI. On comparing the statistical resemblance it was found that rest of the isolates except BR_{11}, BR_{12}, BR_{16}, BR_{17}, BR_{21}, and BR_{35} were statistically at par to FUI. These isolates were numerically better to USDA and JNKVV strains. BR_{11} and BR_{35} proved statistically superior to USDA and JNKVV checks (Table 1). Isolate BR_{11} gave the maximum grain yield (Amule *et al.*, 2015 and Prashad *et al.*, 2014) and no other strain was found to be at par to it. The role of Rhizobacteria as PGPR in plant growth promotion is well documented and the increase in shoot and root length under control conditions might be due to different plant growth promoting activities like synthesis of phytohormones (2013, Zahir *et al.*, 2003) and nutrient availability (Peralta *et al.*, 2013). The (Zahir *et al.*, 2003) study by Hussain *et al.* (2012) showed that inoculation with *Rhizobium* increased nitrogen fixation in mungbean crop. Bambara and Ndakidemi (2010) found that *Rhizobium* inoculation of *P. vulgarish* had a significant influence on the amount of fixed N. Relative to the un-inoculated treatments, inoculation significantly increased the N fixed in different tissues of the plant such as roots, shoots, pods and whole plants of *P. vulgarish* grown both in the greenhouse and in the field. These positive results are a result of improved N nutrition thus plant growth and good performance in terms of yield was significantly achieved through the simple symbiotic relationship between the legume plant and the rhizobia.

Correlation between additional BNF and nitrogen harvest index

The physiological ability of crop plant to content proportion of dry matter into economic yield is measured in terms of harvest index. The higher the harvests index the more productive efficiency of a crop vice versa. The correlation between oven dried weight of nodules and nitrogen harvest index (Fig. 1) by crop. Since the value of R^2 is found to be 0.84 which suggests that 84% variability in Additional BNF can be explained due to nitrogen harvest index while remaining 16% variability may be due to other factors. Rawat (2002) observed that biological nitrogen fixation (BNF) makes a very important and crucial contribution to the nitrogen economy of soybean based cropping system. A larger N harvest index is expected in soybeans compared to other legumes, since a great amount of N is mobilized to the grain in relation to that remaining in the residues (Lawn, 1989). Mean nitrogen harvest index (NHI) was 0.73 with an IQR of 0.64–0.82 (Salvagiotti *et al.*, 2008). Similar results was reported by values Ayaz *et al.* (2004) in chickpea and lupine which had a lower N content in both in seeds and residues, but greater NHI was reported in peas, which have a lower N concentration in seeds and a greater N content in the residues. Variation in yields and NHI greatly impacted the amount of N left in crop residues and hence N availability and N management in subsequent crops (Bundy *et al.*, 1993).

Correlation between additional BNF and Soybean harvest index

The correlation between oven dried weight of nodules and harvest index (Fig. 2) by crop. Since the value of R^2 is found to be 0.74 which suggests that 74% variability in Additional BNF can be explained due to harvest index while remaining 26% variability may be due to other factors.

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