Environment & Ecology 35 (1) : 197—200, January—March 2016
Website: environmentandecology.com ISSN 0970-0420

Field Efficacy of Plant Growth Promoting Rhizobacteria Isolates and their Impact on Crop Growth, Nutrient Content and Production of Soybean in Vertisol

F. C. Amule, A. K. Rawat, B. K. Choudhary, R. K. Sahu

Received 5 April 2016; Accepted 11 May 2016; Published online 4 June 2016

Abstract Plant growth promoting rhizobacteria (PGPR) have gained worldwide importance and acceptance for agricultural benefits. This is due to the emerging demand for dependence of synthetic chemical products, to the growing necessity of sustainable agriculture within a holistic vision of development and to focalize environmental protection. With these views a field experiment was carried out during *kharif* of 2009-10 which was the part of continue experiment since 2007-12 with the objective to study the field efficacy of Plant Growth Promoting Rhizobacteria (PGPR) isolates and their impact on early vigor of crop growth, nutrient content and production of soybean in Vertisol. These isolates which were preliminary screened and short listed through laboratory and glass house techniques, initially a large number of isolates were isolated from different geographical ureas of MP. Efficacy of these isolates was also compared with the USDA isolates, FUI and UFUI. The treatments were laid out in Augmented RBD without replication. To study the field efficacy of PGPR isolates on soybean were observed on early growth parameters, nodulation, yield and NPK content in grain and straw. Outcome of the investigation revealed that PGPR isolates improved seed germination, seedling vigor, seedling emergence and Standing which ultimately reflected to seed yield. Study also suggests that simultaneous Screening of rhizobacteria for growth and yield promotion under field experiment is a good tool to select effective PGPR for biofertilizer development biotechnology. Results very well revealed that the inoculated PGPR isolates playd a synergistic role with native rhizobacteria for promoting of nodulation and biological nitrogen fixation. Among all the tested isolates P_{13} and P_{14} were found to be most effective in all respect.

Keywords Soybean, PCPR, Isolates, Efficacy, Vertisol.

F. C. Amule
Research Associate, Dept of Soil Science and Agricultural Chemistry, JNKVV, Jabalpur (MP), India
e-mail: amulejnkvv@gmail.com

A. K. Rawat
Professor, Dept. of Soil Science and Agricultural Chemistry, JNKVV, Jabalpur (MP), India
e-mail: rawat_ashok@rediffmail.com

B. K. Choudhary
Senior Research Fellow, Dept of Soil Science and Agricultural Chemistry, JNKVV, Jabalpur (MP), India

R. K. Sahu*
Assistant Professor, College of Agriculture Balaghat (MP), India
e-mail: rakesh_sahujbp@yahoo.co.in
*Correspondence

Introduction

Soybean [*Glycine max* (L.) Merrill] is one of th most important *kharif* oilseed crop of Madhya Pradesh. Besides high yield potential in terms of food components, Soybean is one of the most important sources of edible oil and protein in the world [1]. Soy products are the main ingredients in many meat and dairy substitutes. They are also used to make soy sauce, and the oil is used in many industrial applications. Besides having such dietary value, it is capable of fixing atmospheric nitrogen at the rate of 85-115 kg ha^{-1} $year^{-1}$ with symbiosis through *Rhizobium japonicum* bacteria. Rhizobacteria are root colonizing microorganisms which are known to be in constant communication with plants roots. An investigation on the role of root colonizing bacteria (rhizobacteria) that exert beneficial effect on plant development via direct or indirect mechanisms have been defined as plant growth promoting rhizobacteria (PGPR) [2]. On the basis of the results drawn from the earlier screening in growth chamber conditions in the laboratory for initial evaluation of their performance. All these strains were characterized for : seed germination, production of ammonia, production of Indole Acetic Acid (IAA), Phosphate solubilization in liquid medium, nitrogen fixation and productions of siderophores, antibiotics and antifungal metabolites. On the basis of laboratory screening 57 isolates (which were leveled as P_1-P_{57}) have been short listed for further testing of their efficiency under field conditions. In the state of Madhya Pradesh there is no availability of efficient PGPR isolates belonging to multiple locations which can form the basis for a more rational commercial projection of PGPR inoculants.

Materials and Methods

The experiment was laid out during 2009-10 at research station, Depertment of Soil Science, INKVV, Jabalpur in Central India. The soil of the experimental site (23^0 10´´ N latitude and 79^0 57´´ E longitudes at 393 meters above the mean sea level) was *Vertisols* during *kharif* 2009-10 and research was the part of continue experiment since 2007 to 2012. Fourteen districts of Madhya Pradesh [6] isolates were isolated from soybean fields and previously screened in all respect. Out of these 161isolates, best 57 were evaluated under field conditions and their performance was compared with JNKVV strains as check (standard check) using augmented randomized block design (non replicated). Overall the isolates performance was compared with fertilized uninoculated control. On the basis of laboratory and green house screenings at Jabalpur and IISS Bhopal 57 isolates were short-listed to further test their efficiency under field conditions. The inoculation of soybean seeds was carried out with a mixture of rhizobacteria strains obtained as described above (final culture density - 64 × 10^6 CFU/ml). The soybeans seeds inoculated or uninoculated (UI) with plant growth promoting rhizobacteria were planted. All the isolates were provided with recommended dose of NPK (20:80:20 kg ha^{-1}) along with fertilized uninoculated control where the conditions wre same except seed inoculation. The soybean variety JS - 9752 was sown in last week of June 2009 and harvested at second week of October 2009. The screen out isolates were purified by streaking on methyl red agar media for isolation of gram positive bacteria Crystal violet agar media for isolation of gram negative bacteria king's B media for isolation of *Psedumortas,* N free Jensen' media for isolation of *Azotobacter*, Pikovaskya media for isolation of phosphate solubilizing bacteria, Rojo congo media for isolation of *Azospirillium* and transferring the single colony on the respective slants. 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 were done and sown separately. All the packages of practices were followed as per recommendations to grow the crop. The plants were growing in ecological conditions, without using any organic fertilzers and pesticides. From the next day of sowing critical observations were made on seed germination and plot wise visual rating (%) was done at 4^{th} and 5th day of sowing. Plant height was measured at 6^{th}, 13^{th} and 20^{th} day of sowing. For the purpose plant height of five plants /plot was measured randomly. These plants were tagged initially and same plants were used to measure the plant height at all 3 intervals. Nodulation studies were done at 45 days of sowing. After counting, the nodules were detached from the roots and were kept in small paper bags and were oven dried in hot air oven at 60^0C for 3-4 days till constant weight to record their oven dried weight. After harvesting, the plant sample and beans were collected and further analyzed for different parameters for N by micro-Kjeldhal method. Phosphorus content was determined by vanadomolybdo phosphoric acid yellow color method and K content by using flance photometer as. The data were statistically analyzed by using non replicatd augmented randomized block design therefore, whole the experimental area is divided in to N plots (where N is equal in the number of test strains (V) + number of checks (C) which are standard strains of known performance) repeated b times, i.e. N=V+bC; and total number of strains, e = V+C. The strains all the N plots are allotted randomly for all V and C, the later repeated b times.

Results and Discussion

Plant growth promoting rhizobacteria stimulate plant growth by producing phytohormone which enhance the groth and physiological activities of the host plant. The results suggested that the inoculation of PGPR increased the seedling emergence significantly and it was found that a 4th day of sowing about 6% germination was observed in the fertilized uninoculated plots and the isolates $P_{6.}$ P_{26} and P_{56} were also similar to fertilized uninoculated control on the other hand about 96% germination was recorded with isolate no. P_{14}. Out of total 57 isolates 16 were statistically at par to FUI while 96% seed germination was recorded where seed was inoculated with P_{14} isolate followed by 86% and 82% due to P_{13} and P_{17} isolates respectively. Same trend was observed at 5th day of sowing. The rice seedling emergence was positively influenced by the application of different PGPR inoculation [3]. They also revealed that the differences accelerated with time and clear differences were visible at 48 h after inoculation which may be due to fast multiplication of these PGPR's in the spermosphere (region surrounding the seed) in response to seed exudates. Plant growth promoting effects of PGPR strains towards germination rate and improved seedling emergence in different crops [4]. Similar improvement of seed germination by rhizobacteria has been reported in sorghum, pearl millet, wheat and sunflower {5, 6]. Soybean plant height was measured at 6th, 13th and 20 th day of sowing and it was observed that significant responses were more at 6th days as compared to 13th and 20th day (Fig. 2.). It was observed at 6th days of sowing but with advancement of plant age the responses were not prominent. Maximum plant height was recorded with P_{14} (5.42 cm) followed by P_{13} (5.40 cm), P_{19} (5.30 cm), P_{45} (5.10 cm) and P_3 (5.02 cm). While at 13th days of sowing only five isolates i.e. P_{13}, P_{14}, P_{17}, P_{19} and P_{42} were significantly better over FUI and rest of the isolates were statistically at par to FUI. The inoculation of PGPB (plant growth promoting bacteria) significantly increased the plumule (shoot) length. The PGPR inoculation of *A. brasilense* and *P. stutzeri* either alone or in combination with half dose of chemical fertilizers was highly effective in improving root morphology and growth [7].

The nodulation at 45th days of soing most of the PGPR isolates significantly increased the nodule occupancy (no. of nodules/plant) over FUI but the there was no significant increase innodular over dried mass. Biological yield at 45 DAS was also increased with some of the PGPR isolates but the nodule nitrogen content was significantly increased due to five isolates only i.e. P_{13}, P_{14}, P_{17}, P_{19}, and P_{32}. The mung bean inoculating by *Rhizobium* + PGPR + PSB significantly higher nodules/plant, ODW of nodules/plant and grain yield was achieved [8].This kind of effect was previously observed and the experiments demonstrate that Bacillus sp. strains enhance soybean nodulation and growth under low temperature stress [9].

It was observed that nitrogen, phosphorus and potassium content in soybean by inoculation of PGPR isolates at 45 DAS, only 17 strains could increase the plants nitrogen content significantly over FUI while phosphorus content was increased by 34 strains and only two strains P_{13} and P_{14} were able to increase potassium content significantly over FUI. Strains P_{14} remained on top for increasing maximum nitrogen (4.31%), phosphorus (0.095%) and potassium (0.60%) contents in plant followed by P_{13} and P_{19} (Table 1). In case of nitrogen content only two isolates P_{13} and P_{18} were statistical identical to P_{14} which increased maximum nitrogen content in plant while for phosphorus content the statistical identity of P_{14} was with P_{13}, P_{16}, P_{19}, P_{21}, P_{22}, P_{24}, P_{32}, P_{33}, P_{40}, P_{45}, P_{51} and P_{53}. Potassium content due to all isolates including FUI was statistically identical. The inoculation with PGPR increased overall plant growth, which leads to increased nitrogen demand, which in turn leads to increased in nodule number and / or size [10].

Out of 57 isolates, 45 isolates increased the seed yield (Table 1) significantly over FUI and it ranged from 1950 to 3750 kg ha^{-1} while rest of the 12 isolates were statistically at par to FUI. The isolate P_{56} was the least effective (1950 kg ha^{-1}) while P_{13} and P_{14} strains were highly effective (3750 kg ha^{-1}) and they increased the seed yield by 92%. The inoculated PGPR isolates played a synergistic role with native rhizobacteria for promotion of nodulation and biological nitrogen fixation. Similarly the grain nitrogen was increased with 18 strains only and out of these successful isolates P_{13} and P_{14} each gave the highest grain nitrogen (7.06%). On considering the total nitrogen uptake by crop it was found that 28 strains significantly enhanced the total nitrogen uptake by crop over FUI. Maximum nitrogen uptake (443 kg ha^{-1}) was recorded due to P_{13} strain followed by P_{14}, P_{19}, P_{17} and P_{25} and all these strains were at statistically at par (Table 2). The fixed N as a percentage of total plant N, and protein yield were also increased with PGPR inoculation. Many bacteria have the ability to release phosphorus from organic and inorganic sources and it is believed that PGPR release organic acids that are able to convert the phosphate into soluble forms [11].

On the basis of data obtained through experimentation harvest index, nitrogen harvest index and additional BNF was worked out by considering grain and straw yields, nitrogen uptake by grain and the total nitrogen uptake by crop over FUI respectively. On considering the harvest index and NHI percentages it was found that none of the isolates were significantly better over FUI and these were maximum with isolates no. P_{14} followed by P_{13}. On the other hand additional BNF was significantly better due to most of the strains over FUI except P_{6}, P_{18}, P_{26}, P_{27}, P_{30}, P_{44}, P_{46}, and P_{56}.Maximum additional BNF was recorded with P_{25} very closely followed by P_{26} and the same treand was with HI and NHI. Similarly the Co-inoculant of *P.mucilaginosus* 3016 and *B. japonicum* 5136 could significantly increase soybean harvest index, yield and nodule occupancy, and reduce the empty pods per plant while increase the enzyme activities. However, the Nitrogen treatment had more HI to control treatment [12]. Probably one of reasons for decreasing on HI in application of biofertilizer (Nitroxin and *Azotobacter*) is due to further increase in biological yield [13].

References

1. Li HY, Zhu YM, Chen Q, Conner RL, Ding XD, Li J (2004) Production of transgenic soybean plants with two anti-fungal protein genes via Agrobacterium and particle bombardment. Biologia Plantarum 48 : 367—374.
2. Nelson LM (2004) Plant growth promoting rhizobacteria (PGPR) : Prospects for new inoculants. Online. Crop Management doi : 10.1094/Cm-2004-0301-05-RV.
3. Min MAB, Shamsuddin ZH (2009) Enhanced emergence and vigor seedling production of rice through growth promoting bacterial inoculation. Res J Sed Sci 2 : 96—104.
4. Gholami A, Shahsavani S, Nezarat S (2009) The effect of plant growth promoting Rhizobacteria (PGPR) on germination, seedling growth and yield of maize, World Acad Sci, Engg Technol, pp 49.
5. Niranjan SR, Shetty NP, Shetty HS (2004) Seed biopriming with pseudomonas fluorescence isolates enhances growth of pearl millet plant sand induces resistance against downy mildew. J pest Manag 50 : 41—48.
6. Shaukat K, Affrasayab S, Hasnain S (2006) Growth responses of *Triticum aestivum* to plant growth promoting. rhizobacteria used as a biofertilizer. Res J Microbiol 4 : 330—338.
7. Nosheen A, Bano A, Ullah F, Farooq U, Yasmin H, Hussain I (2011) Effect of plant growth promoting rhizobacteria on root morphology of Safflower *(Carthamus tinctorius* L.). Afr J Biotechnol 10 : 12669—12679.
8. Bansal RK (2009) Synergistic effect of Rhizobium, PSB and PGPR on nodulation and grain yield of mungbean. J Food Leg 22 : 20—23.
9. Bai Y, Zhou X, Smith DL (2003) Enhanced soybean plant growth resulting from coinoculation of Bacillus strains with *Bradyrhizobium japonicum*. Crop Sci 43 : 1774—1781.
10. Verma JP, Yadav J, Tiwari KN (2010) Application of Rhizobium sp. BHURC01 and plant growth promoting rhizobactria on nodulation, plant biomass and yields of Chickpea (*Cicer arietinum* L.). Int J Agric Res 5 : 148—156.
11. Chen YP, Rekha PD, Arun AB, Shen FT, Lai WA, Young CC (2006) Phosphate solubilizing bacteria from subtropical soil and their tricalciumphosfate solubilizing abilities. Appl Soil Ecol 34 : 33—41.
12. LIU Li, Ming-chao MA, Xin JIANG, Da-wei GUAN, Binghai DU, Feng-Ming CAO, Jun LI (2015) Effect of *Rhizobia* and PGPR co-inoculant on soybean characteristicsand soil enzyme activities. J Pl Nutr and Fertil 21 : 664—654.
13. Bahamin S (2011) Effect of biofertilizers manure, and chemicals on yield and quality of sunflower. Agroecol Master's thesis. Facul Agric Univ Birjand.