Biological Forum — An International Journal, **1**(2): 11-14(2009) **ISSN : 0975-1130**

Effect of *Mesorhizobium* and plant growth promoting rhizobacteria on nodulation and yields of chickpea

Jay Prakash Verma, Janardan Yadav* and Kavindra Nath Tiwari

Department of Botany, MMV, Banaras Hindu University, Varanasi INDIA
**Department of Soil Science and Agric. Chem., Institute of Agric. Sci., Banaras Hindu University, Varanasi INDIA*

ABSTRACT : A positive influence of plant growth promoting rhizobacteria (PGPR) and *Mesorhizobium* sp. strain BHURC03 (Accession No.GU124825) on nodulation, plant biomass and yield related parameter were recorded in both year of field experiment. The maximum significant increase in nodule number, dry weight of nodule, root and shoot were recorded in coinoculation of *Mesorhizobium* sp. and *Pseudomonas fluorescens* followed by co-inoculation of *Mesorhizobium* sp., *Azotobacter chroococcum* and *Bacillus megaterium* over uninoculated control in both year of field study while nitrogen and phosphorus content increase in nodules, grain and straw. The *Mesorhizobium* sp. and *P. fluorescens* have been shown maximum significant increase in all parameter due to higher nitrogen fixation by *Mesorhizobium* sp. and strong phosphate solubilizer, higher production of plant growth promoting hormones like indole acetic acid by *P. fluorescens*. Therefore, co-inoculation of *Mesorhizobium* sp. strain BHURC03 and *P. fluorescens* may be highly effective bioformulation for chickpea (*Cicer arietinum* L.) production.

Keywords : *Mesorhizobium* sp., Chickpea (*Cicer arietinum* L.), PGPR, *Pseudomonas fluorescens, Azotobacter chroococcum, Bacillus megaterium*

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a major grain legume crop. It is contributes to 38% of national pulse production in India. Chickpea can obtain a significant portion of its N requirement through symbiotic N_2 – fixation to give high grain yield when grown in association with effective and competitive *Rhizobium* strain (Kyei-Boahen *et al*., 2002). Due to excessive use of the chemical fertilizers and plant protection chemicals, the rhizosphere microflora has been greatly affected and in place of the beneficial associative bacteria, harmful types now predominate in the rhizosphere. Therefore, more attention being paid to the search for early root colonizers which directly or indirectly influence plant growth and productivity. Interactions between these PGPR with *Rhizobium* may be antagonistic or synergistic, and the beneficial effects of such interactions could be exploited for economic grain (Dubey, 1996, Glick, 1995). The role of symbiotic nitrogen fixing bacteria, plant growth promoting rhizobacteria (PGPR) and phosphate solubilizing microorganisms in crop productivity is well documented (Kennedy *et al*., 2004). Combined inoculation of *Rhizobium* with *Pseudomonas striata* or *Bacillus polymyxa* and with *Bacillus megaterium* have shown increased dry matter, grain yield and phosphorus uptake significantly over the uninoculated control in legumes (Elkoca *et al*., 2008; Yadegari *et al*., 2008). Hence in the present investigations, attempts have been made to evaluate the contribution of plant growth promoting rhizobacteria along with and *Mesorhizobium* sp. strain BHURC03 in terms of plant growth and yield of chickpea (*Cicer arietinum* L.).

MATERIAL AND METHOD

Culture, media and growth condition. The pure cultures of *Azotobacter chroococcum* strain MTCC-446, *Pseudomonas. fluorescens* strain MTCC-1748 and *Bacillus megaterium* strain MTCC-428 were obtained from MTCC (Microbial Technology Culture Collection), Institute of Microbial Technology, Chandigarh, Punjab. India. The bacterial strains of *A. chroococcum*, *P. fluorescens* and *B. megaterium* were maintained on nutrient agar medium. Indigenous *Mesorhizobium* sp. strain BHURC03 (Accession No.GU124825) was isolated from healthy root nodules of chickpea. It was identified by 16S rDNA gene sequencing and their accession number was obtained from GenBank, NCBI. It was maintained on yeast extract mannitol agar (YEMA) medium. The cultures were maintained by periodic transfer on their respective media and stored in the refrigerator for further studies.

Host seeds. Seeds of chickpea (*Cicer arietinum* L.) cultivar Radhey (common name: chana) were obtained from Indian Institute of Pulse Research (IIPR), Kalyanpur, Kanpur, Uttar Pradesh, India.

Seed bacterization. The *Mesorhizobium* sp. strain BHURC03 was grown in YEM broth and *A. chroococcum*, *B. megaterium* and *P. fluorescens* were grown in Nutrient broth by incubation for 120 rpm at 28 ± 2°C for 48. Healthy seeds weighed for each plot of 5 m^2 (@ 100 kg ha^{-1} were separately inoculated as per treatments in plastic bags with 5 ml of 7 days old broth cultures grown in specific media of respective inoculants (mixed in 1:1 ratio for combined treatments) along with 1ml of 1% (w/v) sticker solution of gum acacia to ensure bacterial population in the range of

10^7 to 10^8 colony forming unit (CFU) seed^{-1}. After drying for one hour in shade, uninoculated seeds were sown first followed by inoculated seeds just to avoid contamination.

Field Experiments. The field experiments were set up in the first week of October 2006 (first-year trial) and 2007 (second-year trial) at Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi Uttar Pradesh, India. The field experiments were conducted with 5 treatments and 4 replication of indigenous *Mesorhizobium* sp. strain BHURC03 and appropriate synergestic combination of plant growth promoting rhizobacteria (PGPR) as (*A. chroococcum P. fluorescens* and *B. megaterium*) and one uninoculated control. The plot size was 4 × 2.5m and spacing 25 × 25cm between row and 10 × 10 cm between plants. The physico-chemical properties of the initial soil of experimental field was sandy clay loam in texture with 40.83% water holding capacity, neutral in reaction (pH 7.25) and electronic conductivity (dSm^{-1}) 0.155. The content of organic carbon (0.778%) (Walkley and Black, 1934); available N (213.24 kg ha^{-1}) (Subbiah and Asija, 1956); P_2O_5 (27.22 kg ha^{-1}) (Olsen's, 1954) and K_2O (254.76 kg ha^{-1}) (Jackson, 1967); respectively were estimated in soil. The microbial population of total bacteria, fungi and actinomycetes (4.5×10^{-8}, 3.1×10^{-8} and 3.4×10^{-8} CFU g^{-1}soil), respectively (Aneja, 2003) were found in soil.

Assessment of nodules number, dry weight of root and shoot, yield and harvest index. For assessment of root shoot dry weight, ten plants were randomly harvested at their peak flowering time (80 days after sowing) from inoculated and control plots and washed several time with running water. First roots were cut off, and root and shoots were dried at 70°C for 72 h. separately, for each plant. For nodules number, another ten representative plants were harvested from control and inoculated plots. The nodules were separated and counted from each plant, and dry weight was recorded after drying the nodules at 70°C for 72 h. Towards the end of the growth period, another ten representative plants were randomly harvested from control and inoculated plots to determine yield parameters. The plants were dried at 70°C for 72 h and weighted for calculating the biological yield. Grain weight (economic yield) was also recorded at the time of senescence. Harvest index was calculated according to the formula: harvest index = economic yield × 100 / biological yield.

Determination of nitrogen and phosphorus in nodules. For determination of nitrogen and phosphorus in nodules, ten representative plants from control and inoculated plots at flowering time were taken. For determination of nitrogen and phosphorus in grain and straw, ten representative plants from control and inoculated plots at harvesting time were taken. The dry nodules (0.2g) sample was digested in 10 ml of 4.1 ratio of HNO_3: $HClO_4$ for total P (Vanadomoly-bdophosphoric acid yellow color methods), in 10 ml diacid mixture of 9:1 ratio of H_2SO_4: $HClO_4$ for the analysis of total N (Nessler's reagent method).

Statistical analysis. The experiment was arranged in a randomized block design and was replicated four times. Statistical analysis was conducted using one-way analysis of variance (ANOVA). Comparisons of mean were performed by the least significant deferent (LSD) test at $p \leq 0.05$ by using SPSS software version 12.0.

RESULT AND DISCUSSION

The three PGPR isolates (*A. chroococcum*, *P. fluorescens* and *B. megaterium*) did not antagonize *Mesorhizobium* sp. strain BHURC03 when grown together on plates. The *Mesorhizobium* sp. strain BHURC03 interacted differentially with PGPR isolates and showed significant variation in nodulation, dry weight of nodules, root and shoot, grain and straw yield of chickpea (Table 1,2). Seed inoculation of *Mesorhizobium* sp. strain BHURC03 produced significant 24.39% nodule number in first year and 14.89% nodule number in second year of field experiment. uninoculated control. Dual inoculation of seed with *Mesorhizobium* sp. and *P. fluorescens*, *B. megaterium* and *A. chroococcum*, produced 58.54, 51.21 and 36.59%, respectively, more nodule number in first year of analysis and 51.06, 40.43 and 27.65%, respectively, more nodule in second year of analysis than uninoculated control. Similarly combined inoculation of *Rhizobium* and PSB in chickpea has been reported to enhance in nodulation, plant growth, yield and nutrient uptake (Rudresh *et. al.*, 2005). The maximum significant increased nodule dry weight 48.85 and 42.42%, nitrogen 45.65 and 52.31% and phosphorus in nodules 13.8 and 17.93% in the first and second year study, respectively, while dry weight of root increased 24.81 and 36.22% in combination of *Mesorhizobium* sp. and *P. fluorescens* followed by combination of *Mesorhizobium* sp., *B. megaterium* and *A. chroococcum* over uninoculated control. Similarly, shoot dry weight was increased significant, 84.47 and 76.56% (Table 1,2).

Except for harvest index, the higher grain yield 21.05 and 25.74% and straw yield 28.51 and 31.15% in both year of field study, respectively was increased significantly in co-inoculation of *Mesorhizobium* sp. with *P. fluorescens* followed by *Mesorhizobium* sp. with *B. megaterium* and *A. chroococcum* over uninoculated control (Table 2). Wani *et. al.*, (2007) have been reported the synergistic effect of nitrogen fixing and phosphate-solubilizing rhizobacteria on plant growth, yield, grain protein, and nutrient uptake of chickpea plants. Effects of coinoculations with *Pseudomonas jessenii* PS06 (a phosphate-solubilizing bacteria) and *Mesorhizobium ciceri* C-2/2 strains enhanced the growth and seed yield of chickpea under greenhouse and field conditions (Valverde *et. al.*, 2006) Phosphate solubilizing bacteria are also known to increased phosphorus uptake resulting in better growth and higher yield of crop plants (Bajpai and Sundara Roa, 1971; Gaur *et. al.*, 1980; Alagawadi

and Gaur, 1988). Co-inoculation studies with PGPR and *Rhizobium*/*Bradyrhizobium* spp. have been shown to increased root and shoot biomass, nodule dry matter, nitrogenase activity, N_2-fixation, and grain yield in chickpea (Sindu et al., 2002; Zaidi et al., 2003; Gull et al., 2004) and various legume such as green gram (Sindhu et al., 1999) and pigeonpea (Tilak et al., 2006). Furthermore, combined inoculations with N_2-fixing and P-solubilizing bacteria were more effective than single inoculation possibly by providing a more balanced nutrition for plants (Belimov et al., 1995).

Table 1 : Effect of plant growth promoting rhizobacteria and *Mesorhizobium* inoculation on nodule related parameters of chickpea in field experiments of two consecutive years.

Year 2006-2007				
Treatment	**Nodule Number Plant^{-1}**	**Nodule dry weight (g) Plant^{-1}**	**Nitrogen % in Nodule**	**Phosphorus % in Nodule**
Control	41 ± 2.641^{a*}	0.131 ± 0.007^{a}	3.57 ± 0.445^{a}	0.152 ± 0.003^{a}
Mesorhizobium sp.	51 ± 1.552^{b}	0.161 ± 0.011^{ab}	4.17 ± 0.481^{a}	0.159 ± 0.001^{b}
Mesorhizobium sp. +*A. chroococcum*	56 ± 4.171^{c}	0.177 ± 0.038^{ab}	4.20 ± 0.287^{a}	0.161 ± 0.002^{c}
Mesorhizobium sp. +*P. fluorescens*	65 ± 9.632^{d}	0.195 ± 0.027^{b}	5.20 ± 0.459^{b}	0.179 ± 0.004^{d}
Mesorhizobium sp. +*B. megaterium*	62 ± 1.612^{c}	0.172 ± 0.034^{b}	4.27 ± 0.725^{a}	0.165 ± 0.002^{b}
Year 2007-2008				
Control	47 ± 4.576^{a}	0.132 ± 0.013^{a}	3.02 ± 0.037^{a}	0.145 ± 0.004^{a}
Mesorhizobium sp.	54 ± 0.524^{b}	0.166 ± 0.019^{b}	3.17 ± 0.411^{a}	0.160 ± 0.023^{a}
Mesorhizobium sp. +*A. chroococcum*	60 ± 5.823^{c}	0.165 ± 0.005^{b}	4.07 ± 0.573^{b}	0.156 ± 0.003^{a}
Mesorhizobium sp. +*P. fluorescens*	71 ± 3.614^{d}	0.188 ± 0.009^{c}	4.60 ± 0.794^{c}	0.171 ± 0.001^{b}
Mesorhizobium sp. +*B. megaterium*	66 ± 1.614^{e}	0.181 ± 0.006^{c}	3.84 ± 0.483^{b}	0.158 ± 0.023^{a}

*Values are the mean ± SD, Mean values in each column with the same superscript (s) do not differ significantly by LSD (P = 0.05).

Table 2 : Effect of plant growth promoting rhizobacteria and *Mesorhizobium* inoculation on biomass production and yield-related parameters of chickpea in field experiments of two consecutive years.

Year 2006-2007	**Biomass production (g dry weight plant^{-1})**		**Yield (qha^{-1})**		**Harvesting Index**
Treatment	**Root**	**Shoot**	**Grain**	**Straw**	
Control	0.14 ± 0.021^{a*}	1.15 ± 0.13^{a}	22.80 ± 1.26^{a}	13.50 ± 0.79^{a}	59.21 ± 2.65^{a}
Mesorhizobium sp.	0.15 ± 0.006^{a}	1.44 ± 0.31^{a}	24.91 ± 0.83^{b}	13.80 ± 0.35^{a}	57.41 ± 3.32^{a}
Mesorhizobium sp. +*A. chroococcum*	0.16 ± 0.006^{b}	1.91 ± 0.07^{b}	27.16 ± 0.94^{c}	15.50 ± 0.75^{b}	57.07 ± 3.24^{a}
Mesorhizobium sp. +*P. fluorescens*	0.17 ± 0.004^{b}	2.11 ± 0.03^{b}	27.60 ± 0.18^{d}	17.35 ± 0.65^{d}	59.24 ± 4.23^{a}
Mesorhizobium sp. +*B. megaterium*	0.17 ± 0.009^{b}	2.05 ± 0.05^{b}	27.41 ± 0.94^{d}	16.68 ± 0.59^{c}	57.21 ± 1.24^{a}
Year 2007-2008					
Control	0.13 ± 0.003^{a}	1.14 ± 0.098^{a}	21.60 ± 1.09^{a}	13.03 ± 0.06^{a}	60.32 ± 3.21^{a}
Mesorhizobium sp.	0.141 ± 0.016^{b}	1.33 ± 0.097^{b}	24.08 ± 0.81^{b}	15.03 ± 0.42^{bc}	62.42 ± 4.32^{a}
Mesorhizobium sp. +*A. chroococcum*	0.152 ± 0.019^{b}	1.84 ± 0.115^{d}	25.94 ± 1.52^{c}	15.14 ± 1.71^{bc}	58.37 ± 3.85^{a}
Mesorhizobium sp. +*P. fluorescens*	0.173 ± 0.001^{c}	2.01 ± 0.023^{c}	27.16 ± 0.85^{c}	17.09 ± 1.51^{c}	62.92 ± 1.25^{a}
Mesorhizobium sp. +*B. megaterium*	0.163 ± 0.008^{c}	1.95 ± 0.117^{cd}	26.21 ± 0.87^{c}	16.52 ± 1.91^{c}	63.03 ± 4.18^{a}

*Values are the mean ± SD, Mean values in each column with the same superscript (s) do not differ significantly by LSD (P = 0.05).

CONCLUSION

Present investigation of two year field experiment data, we have been found highly effective synergistic combination of *Mesorhizobium* sp. strain BHURC03 and *Pseudomonas fluorescens* for better growth and yield of chickpea production. PGPR in combination with other inoculants, if studied further at the farmer's field, could be an alternative to chemical fertilizer to promote plant growth in chickpea and other field crops.

ACKNOWLEDGEMENT

We thank the Centre of Organic Farming, Ministry of Agriculture, Government of India, Ghaziabad, for providing a project fund to carry out this research work at Agricultural Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India.

REFERENCE

Aneja, K.R. (2003). Experiments in Microbiology, Plant pathology and Biotechnology, fourth ed. New Age International Publishers, Daryaganj, New Delhi.

Alagawadi, A.R. and Gaur, A.C. (1988). Associative effect of *Rhizobium* and phosphate-solubilizing bacteria on the yield and nutrient uptake of chickpea. *Plant and Soil*, **105:** 241-246.

Belimov, A.A., Kojemiakov, P.A. and Chuvarliyeva, C.V. (1995). Interaction between barley and mixed cultures of nitrogen fixing and phosphate-solubilizing bacteria. *Plant and Soil* **17:** 29-37.

Bajpai, P.D. and Sundara Rao, W.V.B. (1971). Phosphate solubilizing bacteria. *Soil Science and Plant Nurition*, **17:** 46-52.

Dubey, S.K. (1996). Combined effect of *Bradyrhizobium japonicum* and phosphate–solubilizing *Pseudomonas striata* on nodulation, yield attributes and yield of rainfed soyabean (*Glycine max*) under different sources of phosphorus in Vertisols. *Indian Journal of Microbiology* **33:** 61-65.

Elkoca, E., Kantar, F. and Sahin, F. (2008). Influence of nitrogen fixing and phosphate solubilizing bacteria on nodulation, plant growth and yield of chickpea. *Journal of Plant Nutrition.* **33:** 157-171.

Gull, M., Hafeez, F.Y., Saleem, M. and Malik, K.A. (2004) Phosphorus uptake and growth promotion of chickpea by co-inoculation of mineral phosphate solubilizing bacteria and a mixed rhizobial culture. *Australian Journal of Experimental Agriculture*. **44:** 623-628.

Glick, B.R. (1995). The enhancement of plant growth by free living bacteria. *Canadian Journal of Microbiology.* **41:** 109-117.

Jackson, M.L. (1967). In: *Soil Chemical Analysis*, Prentice Hall of India Pvt. Ltd., New Delhi, 664.

Kennedy, I.R., Choudhury, A.T.M.A. and Kecskes, M.L. (2004). Non-symbiotic bacterial diazotrophs in crop-farming systems: Can their potential for plant growth promotion be better exploited. *Soil Biology and Biochemistry* **36:** 1229-1244.

Kyei–Boahen, S., Walley, F.L. and Slinkard, A.E. (2002). Evaluation of Rhizobial inoculation methods for chickpea. *Agronomy Journal*, **94:** 851-859.

Olsen, S.R., Cole, C.V., Watanabe, F.S. and Dean, L.A. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. *USDA circular No*. 939, *Washington* DC.

Rudresh, D.L., Shivaprakash, M.K. and Prasad, R.D. (2005). Effect of combined application of *Rhizobium*, phosphate solubilizing bacterium and *Trichoderma* spp. on growth, nutrient uptake and yield of chickpea (*Cicer aritenium* L.). *Applied Soil Ecology*, **28:** 139-146.

Sindhu, S.S., Suneja, S., Goel, A.K., Parmar, N. and Dadarwal, K.R. (2002). Plant growth promoting effects of *Pseudomonas* sp. on coinoculation with *Mesorhizobium* sp. *cicer* strain under sterile and "wilt sick" soil conditions. *Applied Soil Ecology*, **19:** 57-64.

Sindhu, S.S., Gupta, S.K. and Dadarwal, K.R. (1999). Antagonistic effect of *Pseudomonas* spp. on pathogenic fungi and enhancement of plant growth in green gram (*Vigna radiata*). *Biological Fertility of Soils*, **29:** 62-68.

Subbiah, B.V. and Asija, G.L. (1956). A rapid procedure for estimation of available N in soils. *Current Sciences*. **25:** 149-153.

Tilak, K.V.B.R., Ranganayaki, N. and Manoharachari, C. (2006). Synergistic effects of plant growth promoting rhizobacteria and *Rhizobium* on nodulation and nitrogen fixation by pigeonpea (*Cajanus cajan*). *European Journal of Soil Science*, **57:** 67-71.

Valverde, A., Burgos, A., Fiscella, T., Rivas, R., Velaz quez, E., Rodriguez-Barrueco, C., Cervantes, E., Chamber, M., Igual, Jose-Mariano., (2006). Differential effects of coinoculations with *Pseudomonas jessenii* PS06 (a phosphate-solubilizing bacteria) and *Mesorhizobium ciceri* C-2/2 strains on the growth and seed yield of chickpea under greenhouse and field conditions. *Plant and Soil*. **287:** 43-50.

Wani, P.A., Khan, M.S. and Zaidi, A. (2007). Synergistic effect of the inoculation with nitrogen-fixing and phosphate-solubilizing rhizobacteria on performance of field-grown chickpea. *Journal of Plant Nutrition and Soil Science*, **170:** 283-287.

Walkley, A. and Black, I.A. (1934). An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Science*, **37:** 29-38.

Zaidi, A., Khan, M.S. and Amil, M.(2003). Interactive effect of rhizotrophic microorganisms on yield and nutrient uptake of chickpea (*Cicer arietinum* L.). *Europian Journal of Agronomy*, **19:** 15-21.