Indian Journal of Plant and Soil
Volume 1 Number 1, January - June 2014

Original Article

Inoculation Effect of *Mesorhizobium ciceri* and Rhizospheric Bacteria On Nodulation and Productivity of Chickpea (*Cicer arietinum* L.) and Soil Health

Parul Bhatt*, Ramesh Chandra**

Author's Affiliation: *Scientist-C, Forest Soil and Land Reclamation Division, Forest Research Institute, Dehradun, Uttarakhand, ** Professor and Head , Deptt. of Soil Science, G. B. P.U.A & T, Pantnagar, Uttarakhand, India.

Abstract

A field experiment was carried out at Pantnagar during 2005-06 to examine the interaction between 10 rhizospheric bacteria isolates with *Mesorhizobium ciceri* on nodulation, growth, yields and nutrient uptake by chickpea (*Cicer arietinum* L.). The experimental soil was sandy loam of pH 7.2 having 5.2 mg/kg Organic C, 140.2 kg/ha available N, 16.1 kg/ha available P and 282.5 kg/ha available K. The test crop variety was Pant G-186. Inoculated *Mesorhizobium* sp. alone, irrespective of rhizobacteria, increased the number and dry weight of root nodules numerically, by 23.2 and 23.1 % and plant dry weight significantly, by 3.2 % over uninoculated control at 60 DAS. It also gave numerical increases of 11.2 % and 13.0 % in grain and straw yields, 26.1 and 29.8 % in N uptake and 21.2 and 30.3 % in P uptake by grain and straw, respectively. Different rhizobacteria, irrespective of *Mesorhizobium* sp., gave increases of 77.2 to 58.7 % in nodule number and 13.3 to 65. % in nodule dry weight at 60 DAS, 20.0 to 57.7 % in grain yield, 12.9 to 44.1 % in straw yield, 17.8 to 85.4 % in N uptake by grain, 15.0 to 46.6 % in N uptake by straw, 5.5 to 63.8 % in P uptake by grain and 14.8 to 61.9 % in P uptake by straw over no rhizobacteria inoculation. All rhizospheric bacteria, except LK-754, LK-786, PUK-791 and KB-133 improved the grain and straw yields significantly. All rhizospheric bacteria, except LK-754, also recorded significantly more microbial biomass C, dehydrogenases activity and acid phospahatase activity in soil over no rhizobacteria inoculation. Interaction between the *Mesorhizobium* sp. and rhizobacteria with was not significant. PUK-171 was found to be the best for most plant growth and yield and soil health parameters.

Keyswords

Mesorhizobium; Rhizobacteria; Microbial biomass carbon.

Introduction

Chickpea is major pulse crop of India accounting for 35% area and 45% of total production of pulses. India also has the distinction of being the top producer of chickpea in the world accounting for 71.51 % of the global output. It has been an integral part of Indian agriculture since time immemorial because of its intrinsic ability of nitrogen fixation and adaptation to diverse agro- ecological conditions. The current productivity of chickpea of 943 kg/ ha in the country is relatively low because of its cultivation on marginal soils without adequate inputs management including plant nutrients. Being leguminous crop, chickpea has inherent capacity of atmospheric nitrogen fixation in association with rhizobia. Although, native soil rhizobia are capable of interacting and nodulating the chickpea to varying extent depending upon the genotypes, soil and crop management practices, there is need to develop an efficient symbiosis of host specific rhizobial isolates and

Corresponding Author: Parul Bhatt,
E-mail: parulbhatt29@gmail.com

also to develop isolates with superior nodulation competitiveness that can overcome the limitations of low nitrogen fixation, poor crop yield, and lower effectiveness under field conditions.[1]

The rhizospheric microorganisms not only influence the inoculated rhizobia adversely through saprophytic competition, but also help them in survival through synergistic interactions resulting in an increase in their nodulation efficiency. Co-inoculation of rhizobacteria with rhizobia have been found to increase nodulation, N_2 fixing efficiency, growth and yield of several pulse crops in laboratory and field conditions.[2-4] Several mechanisms such as alteration in the composition of rhizospheric microorganisms, production of plant signaling compounds, bacteriocins, siderophores, plant growth hormones and improving availability of nutrients by rhizospheric microorganisms have been reported for such synergism.[5] However, compatibility of these microorganisms needs to be evaluated because of the possibility of antagonistic interactions among them. Improving symbiotic N_2 fixation efficiency using synergistic rhizobacteria appears to be a cost effective and eco-friendly technology of increasing the pulse production, would lessen the need of fertilizer and decrease energy input. Keeping this in view, the present investigation was conducted to examine the effect of seed inoculation of rhizospheric bacteria and rhizobia in chickpea on root nodulation, plant dry matter, yields, nutrient uptake, residual soil nutrients and soil biological properties under field conditions.

Materials and Methods

Microbial Cultures

Effective strains of *Mesohizobium* sp. *Cicer* (strain LN 7007) was obtained from department of Microbiology, CCSHAU, Hisar and 10 rhizobacteia LK-786 (*Kurthia* sp.), LK-884 (*Pseudomonas* sp.), PUK-46B6 (*Pseudomonas diminuta)*, PUK-171(*Klebsiella* sp.), CRB-2 (*Pseudomonas* sp.), KB-133 (*Pseudomonas* sp.), LK-822, LK-373, LK-754, PUK-791 (unidentified) were obtained from Department of Soil Science of the University. The purity of the cultures was checked with routine microbiological techniques. The obtained *Mesorhizobium* sp. was multiplied in YEM broth for 4 days and rhizobacteria in succinate broth for 2 days and mixed with sterilized charcoal, neutralized with 12.5 % $CaCO_3$, in 1:2 ratio separately to prepare their carrier based inoculants.

Field Study

The efficiency of the rhizobacteria in terms of nodulation, growth and yield of chickpea and soil health was evaluated in a field study during *Rabi* 2005-06 at Crop Research Centre of the G. B. Pant University of Agriculture and Technology, Pantnagar. The soil was sandy loam of pH 7.2 having 5.2 g/kg Organic C and 140.2, 16.1, 282.5 kg/ha available N, P and K, respectively. Treatments consisting of inoculation with 10 rhizobacteria, alone and with *Rhizobium* sp., along with 20 Kg N + 40 Kg P_2O_5/ha and an uninoculated control were laid out following two factorial experiment in Randomized Block Design in plots of 2.4 m × 3.0 m size in 3 replications. Chickpea seed (cv. Pant G 186) was treated with the required inoculants of *Mesohizobium* sp. and rhizobacteria @ 20 g inoculant /kg seed at the time of sowing. Crop was raised as per the recommended agronomic practices. Five plants from the each plot were randomly uprooted along with a soil core at 60 days after sowing (DAS). Soil cores with plants were placed in sieve and roots were washed off with water jet to remove the adhering soil. Nodules were removed from the roots and counted. Dry weights of nodules and plants were determined after drying in hot air oven at 70 °C to constant weight. Grain and straw yields were recorded at final harvest. N and P concentrations in grain and straw were determined after grinding the samples to 40 mesh. N contents was determined by micro-Kjeldahl method and P after wet digestion in tri-acid mixture (HNO_3 : H_2SO_4 : $HClO_4$ in 9:4:1 ratio) by vanadomolybdophoshporic yellow colour methods [6] and N and P uptake by grain and straw were calculated.

Soil Studies

Soil samples were collected, in duplicate, from individual plot after harvesting the crop. One soil sample of each plot was air-dried, processed to pass through 2 mm sieve and analysed for available N (0.32% alkaline $KMnO_4$ oxidizable) and available P (0.5 M $NaHCO_3$ extractable) following the methods described by Page.[6] Another soil sample was stored at low temperature in a deep freeze and used for estimation of different soil biological properties. Microbial biomass carbon in soil samples was estimated by chloroform fumigation extraction method of Jenkinson and Powlson[7] using Kc value of 0.45.[8] Soil dehydrogenase activity was estimated by reduction of 2,3,5 triphenyl tetrazolium chloride to triphenyl formazan (TPF) by the methods of Tabatabai [9] and acid and alkaline phosphatase activities by incubating with buffered p-nitrophenyl phosphate following method of Tabatabai and Bremner.[10] The treatments were compared using the F-test by calculating the critical difference at 5% level of significance.

Results and Discussion

Nodulation

Seed inoculation with *Mesohizobium* sp., irrespective of rhizobacteria, gave numerical increases of 21.6 and 23.1 % in number and dry weight of root nodules over no inoculation treatment at 60 DAS, respectively (Table 1). Such favourable effects of *Rhizobium* inoculation on nodulation in chickpea have also been reported by Gupta [11] and may be due to either presence of sufficient native rhizobia nodulating the crop or presence of large but ineffective population that gave strong competition to the inoculated rhizobia in root colonization and infection. Different rhizobacteria, irrespective of *Mesorhizobium* sp., showed increases ranging from 7.2 to 58.7% in nodule number and 13.3 to 65.6 % in nodule dry weight over no rhizobacteria treatment. All rhizobacteria, except LK-822, PUK-791, indicated significant increases in both nodule number and nodule dry weight over no rhizobateria inoculation treatment. PUK-171 by producing the highest number and dry weight of nodules was significantly superior to all other rhizobacteria and fertilizer treatment. Such variation in the efficiency of rhizobacteria have also been reported by Chandra and Pareek [12] in lentil and urdbean and Gupta [11] in mungbean due their different genetic make up and biochemical functions.

Plant Dry Matter

Averaged over different rhizobacteria treatments, *Mesohizobium* sp., showed significant improvement in plant dry matter of 3.2 % over no inoculation at 60 DAS. (Table 1). The results corroborates with findings of Gupta who also found significant improvement in chickpea plant dry matter due to *Mesohizobium* sp. inoculation.[13] The inoculated rhizobacteria also favoured the plant dry matter registering significant increases of 7.5, 11.2 and 7.2 % with LK-82, PUK-171 and PUK-791, highest being with PUK-171. Similar positive effects of rhizospheric bacteria on plant growth have also been reported by Gupta [11] in chickpea and Tilak *et al.*[4] in pigeonpea due to enhanced N_2 fixation, secretion of plant growth promotory substances, solubilization of P leading to its more availability, suppression of diseases etc.

Productivity

Inoculation of *Mesohizobium* sp. indicated non-significant increases of 167 and 234 kg/ ha in grain and straw yields over no inoculation treatment (Table 1). This could be viewed in the light of earlier observations of marginal effect of *Mesohizobium* sp. inoculation on nodulation. However, the inoculated rhizobacteria recorded significant increases of 20.0 to 57.7 % in grain yield and 12.9 to 44.1 % in straw yield, irrespective of *Mesorhizobium* sp. inoculation. PUK-171 by producing the highest grain and straw yields was significantly superior to all other treatments in grain and straw yield production. The results are in agreement with reports of Chandra and Pareek [12] in urdbean and mungbean and Khanna *et al* [14] in lentil. It could be attributed to enhancement in nodulation and N_2 fixation.Interactions between inoculated

Table 1: Effect of *Mesorhizobium* sp. and rhizobacteria inoculation on chickpea root nodulation and plant dry matter at 60 DAS and productivity

Treatment	Nodule number /plant	Nodule dry weight (mg/plant)	Plant dry weight (g/plant)	Yield (kg/ha)	
				Grain	Straw
No *Mesorhizobium* sp.	11.2	958	3.80	1493	1800
Mesorhizobium sp.	13.8	1180	3.92	1660	2034
C.D. at 5 %	NS	NS	0.08	NS	NS
No Rhizobacteia	9.7	803	3.75	1292	1591
20 kg N + 40 kg P_2O_5/ha	12.6	1108	3.94	1451	1822
LK-373	12.0	1037	3.75	1551	1906
LK-754	13.4	1169	3.69	1322	1798
LK-786	13.4	1148	3.61	1433	1573
LK-822	11.8	974	3.86	1603	1936
LK-884	12.7	1030	4.03	1924	2152
PUK-46B6	13.3	1121	3.77	1598	1998
PUK-171	15.4	1330	4.17	2038	2294
PUK-791	10.4	915	4.02	1545	1814
CRB-2	12.6	1073	3.87	1700	2236
KB-133	13.0	1141	3.85	1468	1878
C.D. at 5 %	1.2	149	0.23	224	276

Table 2: Effect of *Mesorhizobium* sp. and rhizobacteria inoculation on nutrient uptake by chickpea

Treatment	**N uptake (kg/ha)**		**P uptake (kg/ha)**	
	Grain	Straw	Grain	Straw
No *Mesorhizobium* sp.	53.1	13.2	5.99	4.29
Mesorhizobium sp.	67.0	17.1	7.26	5.59
C.D. at 5 %	NS	NS	NS	NS
No Rhizobacteia	46.7	13.3	5.26	3.84
20 kg N + 40 kg P_2O_5/ha	57.9	13.5	5.74	4.13
LK-373	58.3	16.7	6.38	4.41
LK-754	50.5	16.3	5.55	4.73
LK-786	50.7	13.3	5.91	4.11
LK-822	62.6	13.8	6.88	4.74
LK-884	66.5	17.8	8.50	6.22
PUK-46B6	57.9	15.3	6.54	5.24
PUK-171	86.6	18.7	9.09	6.11
PUK-791	55.9	12.9	6.48	4.96
CRB-2	63.8	16.3	6.87	5.93
KB-133	60.4	15.7	6.28	4.69
C.D. at 5 %	10.9	2.7	0.92	0.79

Mesohizobium sp. and rhizobacteria were non-significant for all the studied parameters.

Nutrient Uptake

Averaged over different inoculated rhizobacteria, *Mesohizobium* sp. inoculation recorded only numerical increases of 26.2 and 29.5 % in N uptake and 21.2 and 30.3 % in P uptake by grain and straw, respectively. The rhizobacteria registered increases from 17.8 to 85.4 % and 15.0 to 46.6 % in N uptake and 5.5 to 63.8 % and 14.8 to 61.9 % in P uptake by grain and straw, respectively, over no rhizobacteria inoculation. Rhizobacteia LK 884, PUK-171 and CRB-2 indicated significant increases in N uptake by both grain and straw. Similarly, LK-822, LK 884, PUK-46B6, PUK-171 and PUK-791 recorded significantly more P uptake by both grain and straw. The highest N and P accumulation in grain and P accumulation in straw was obtained with PUK-171. Such

Table 3: Effect of *Mesorhizobium* sp. and rhizobacteria inoculation on soil properties after chickpea harvesting

Treatment	Available nutrients (kg/ha)		Microbial biomass C (μg/g soil)	DHA (μg TPF/g soil/24 h)	Phosphomonoesterase activity (μg PNP / g soil/h)	
	N	P			acid	alkaline
No *Mesorhizobium* sp.	215.0	20.5	317.1	115.2	145.2	34.9
Mesorhizobium sp.	230.8	20.7	359.7	122.2	155.0	42.7
C.D. at 5 %	NS	NS	NS	4.9	NS	NS
No Rhizobacteia	159.9	16.2	263.6	96.7	128.6	32.3
20 kg N + 40 kg P_2O_5/ha	271.9	20.7	306.1	112.6	146.1	34.4
LK-373	183.7	20.0	321.0	112.3	146.8	37.4
LK-754	192.8	19.9	294.0	102.5	129.9	30.4
LK-786	213.6	20.8	325.1	102.5	148.9	31.6
LK-822	233.2	21.1	350.3	121.6	154.0	34.8
LK-884	298.3	21.4	417.9	155.5	171.4	57.3
PUK-46B6	204.9	21.1	339.4	122.1	146.7	36.9
PUK-171	269.7	21.7	429.8	137.4	176.3	64.1
PUK-791	201.3	21.5	326.3	118.4	147.3	33.1
CRB-2	242.5	21.4	365.7	129.3	158.1	40.3
KB-133	203.3	21.1	321.5	112.2	147.3	32.6
C.D. at 5 %	18.2	1.88	50.4	12.1	3.1	2.9

DHA, Dehydrogenase activity

variable effects of rhizospheric bacteria in N and P accumulation by crops have also been reported by Gupta *et al* [3] in greengram and attributed to their positive effects on nodulation and N_2 fixation and P solubilization in soil. *Mesorhizobium* sp. did not show significant interaction with rhizospheric bacteria in N and P uptake by chickpea.

Soil Properties

Available N and P in soil after crop harvesting improved numerically due to *Mesorhizobium* sp. inoculation, irrespective of rhizospheric bacteria inoculation, which could be explained in view of non-significant improvement in nodulation by *Mesorhizobium* sp. inoculation (Table 3). All the inoculated rhizospheric bacteria resulted in significantly more available N, by 14.8 to 86.6 %, and available P, by 22.8 to 33.9 %, over no rhizobacteria inoculation. The highest available N in soil was recorded with LK-884, which was significantly better than all other rhizobacteria. The highest available P in soil was found with PUK-171, however, it was statistically comparable to all other rhizospheric bacteria. The increase in available N in soil may be attributed to improvement in nodulation and N_2 fixation following rhizobacteria inoculation due to their synergistic interaction with native as well as inoculated rhizobia nodulating chickpea [10] while an increase in available P may be due to P solubilization activity of the inoculated rhizobacteria. Soil microbial biomass C after crop harvesting reflected the trend that observed in soil available N. All the rhizospheric bacteria, except LK-754, registered significantly more microbial biomass C in soil of 12.8 to 63.1 % than no rhizobacteria inoculation, highest being with PUK 171. The latter rhizobacteria was statistically comparable to LK-884, which gave maximum available N in soil. Microbial biomass is most labile pool of soil N and has positive correlation with available N in soil.[15] The differences in soil microbial biomass C under different treatments could be due to variation in crop growth, biomass production and rhizodeposition. A part of crop biomass returns to soil through leaf fall, influences availability of organic substrates for microorganisms causing variations in microbial biomass. The activity of dehdrogenase enzyme in soil represents the total metabolic activity of soil. The various rhizospheric bacteria, except LK-754, gave significantly more DHA than no inoculation. It may be viewed in the light of microbial biomass C in soil. The different rhizobacteria, except LK-754, recoded significantly more acid phosphatase activities than no inoculation, but only LK 373, LK-884, PUK-46B6, PUK-171 and CRB-2 registered significantly more alkaline phosphatases activities in soil. Phosphates activities in soil are related with P minerlization and such variations due to inoculation of rhizobacteria have been reported earlier.[16] None of the studied soil parameter showed significant interaction between inoculated *Mesorhizobium* sp. and rhizospheric bacteria.

It could be concluded that rhizospheric bacteria had varying potential to enhance the nodulation and productivity of chickpea. Among different rhizobacteria, PUK-171 was found most efficient in improving yields of chickpea and soil health. Further, it is necessary to identify the rhzobacteria having synergistic interactions with *Mesohizobium ciceri* for harnessing their benefits in co-inoculation.

References

1. Dudeja SS, Singh NP, Poonam Sharma, Gupta SC, Ramesh Chandra, Bansi Dhar, Bansal RK, Brahmaprakash GP, Potdukhe SR, Gundappagol, RC, Gaikawad BG and Nagaraj KS. Biofertilizer Technology and Pulse Production. In: *Bioaugmentation, Biostimulation and Biocontrol*. (A Singh *et al* Eds.). *Berling Heidelberg: Springer-Verlag; 2011, 43-63.*
2. Prasad H, Chandra R, Pareek RP and Kumar N. Synergism among phosphate solubilizing bacteria, rhizobacteria and *Rhizobium* in urd bean. *Indian Journal of Pulses Research. 2002; 15: 131-135.*
3. Gupta A, Saxena AK, Murali G and Tilak KVBR. Effect of co-inoculation of plant growth promoting rhizobacteria and *Bradyrhizobium* sp. (*Vigna*) on growth and yield of green gram (*Vigna radiata* (L.) *Wilczek*). *Tropical Agriculture (Trinand). 2003; 80: 28-35.*
4. Tilak KVBR, Ranganayaki, N and Manoharachari C. Synergistic effect of plant growth promoting rhizobacteria and *Rhizobium* on nodulation and nitrogen fixation by pigeonpea (*Cajanus cajan*). *European Journal of soil Science. 2006; 57: 67-71.*
5. Kloepper JW. A review of mechanisms for plant growth Promotion by PGPR. 6th International PGPR Workshop, 5-10 October 2003. *Calicut, India: 2003; 81-92.*
6. Page AL. Methods of soil analysis. Part 2, Chemical and microbiological properties. 2nd ed. *Madison (WI): ASA and SSSA; 1982*.
7. Jenkinson DS and Powlson DS. The effect of biological treatments on metabolism in soil. V. A method for measuring soil biomass. *Soil Biology and Biochemistry. 1976; 8: 209–213.*
8. Vance ED, Brookes PC, and Jenkinson DS. An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry. 1987; 17: 703–707.*
9. Tabatabai M A. Soil enzymes. In: Weaver RW, Angele S, Bottomley P, Bezdick D, Smith S, Tabatabai A, Wollum A, editors. Methods on soil analysis. Part 2. Microbiological and biochemical properties. SSSA Book Series No. 5. *Madison (WI): SSSA; 1994, 775–833.*
10. Tabatabai MA and Bremner JM. Use of P-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biology and Biochem*istry. *1969; 1: 301-307.*
11. Gupta SC. Effect of combined inoculation on nodulation nutrient uptake and yield of chickpea in Vertisol. *Journal of Indian Society Soil Science. 2006; 54: 251-254.*
12. Chandra R and Pareek RP. Effect of Rhizobacteria in urdbean and lentil. *Indian Journal of Pulses Research.* 2002; *15: 152-155.*
13. Gupta SC. Evaluation of liquid and carrier based *Rhizobium* inoculants in chickpea. *Indian Journal of Pulses Research. 2005; 18: 40-42.*
14. Khanna V, Sharma P and Sekhen HS. Effect of rhizobium inoculation and PGPR on nodulation and grain yield in lentil (*Lens culinaris* L.). *Environment and Ecology. 2006; 245(Special 1): 224-226.*
15. Nannipieri P, Ascher J, Cecherini MT, Landi L, Pietramellara G and Renella G. Microbial diversity and soil functions. *European Journal of Soil Science. 2003; 54: 655-670.*
16. Ramesh A, Billore SD, Joshi OP, Bhatia VS and Bundela VPS. Phosphatase activity, phosphorous utilization efficiency and depletion of phosphate fractions in rhizosphere of soybean (*Glycine max*) genotypes. *Indian Journal of Agricultural Science. 2004; 74: 150-152.*