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EFFECT OF RHIZOSPHERIC BACTERIA AND RHIZOBIUM INOCULATION ON NODULATION, GROWTH AND YIELD OF CHICKPEA (*CICER ARIETINUM* L.)

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ABSTRACT

Field experiment during *Rabi* 2006-07 was conducted in sandy loam soil at Shamli to compare the efficiency of different rhizobacteria, alone and with *Rhizobium* on performance of chickpea (*Cicer arietinum* L.). Irrespective of rhizobacteria, *Rhizobium* sp. recorded numerical increases of 16.4 to 25.4 in nodule number and 21.7 to 44.4 % in nodule dry weight at different intervals. It also increased the grain and straw yields, by 10.8 and 14.4 %, respectively. Different rhizobacteria significantly influenced the nodule number, by 6.8 to 58.4%, nodule dry weight, by 8.24 to115.8% and plant dry weight, by 0.6 to30.4%, at different intervals. Grain and straw yields were 31 to 736 and 205 to 698 kg/ha higher following inoculation of different rhizobacteria. Rhizobacteria PUK-171 gave the highest number and dry weight of nodules at 60 days and grain and straw yield. It was also significantly superior to all the other treatments in grain yield production.

Key words : Chickpea, *Cicer arietinum* L., *Rhizobium*, Rhizospheric bacteria, Seed inoculation.

INTRODUCTION

Legumes roots are colonized by several rhizospheric microorganisms and these organisms have definite influence on the survival and nodulation ability of inoculated rhizobia (Dashti *et al*., 1998). Numerous rhizospheric microorganisms have been found to influence the nodulation and N_2 fixing capacity of rhizobia through synergism (Rautela *et al*., 2001; Gupta *et al*., 2003). However, compatibility of these microorganisms needs to be evaluated because of the possibility of antagonistic interactions among them. Co-inoculation of rhizobacteria with *Rhizobium* in pulse crops received much attention in recent years and their inoculation with rhizobia showed increased nodulation, N_2 fixation and yield in pot and field conditions through a variety of mechanism (Burdman *et al*., 1996; Khot *et al.*, 1996).

Chickpea is a major grain legume of India occupying 6.95 million ha area with a production of 5.47 m tones contributing 39.9% to the total production of pulses in the country. The average productivity of chickpea in India is about 815 kg/ha. Such low productivity of the chickpea may be attributed to several biotic and abiotic stresses associated with the crop including its cultivation on low fertile soils without adequate fertilizer and crop management. The crop has inherent capacity of nitrogen fixation symbiotically in association with *Rhizobium. Rhizobium* sp. nodulating Chickpea is highly specific in infectivity and do not show affinity with any member of

known cross inoculation group. Symbiotic nitrogen fixation with synergistic rhizobacteria (PGPR) appears to be a cost effective and eco-friendly technology of increasing the symbiotic N_2 - fixation, would lessen the need of fertilizer and decrease energy input. The present study was, therefore, undertaken to evaluate and compare the efficiency of different rhizobacteria, with and without *Rhizobium,* on nodulation and performance of chickpea under field conditions.

MATERIALS AND METHODS

Microbial cultures

Effective strains of *Rhizobium* sp. (strain LN 7007) was obtained from department of Microbiology, CCSHAU, Hisar and ten different rhizobacteia of urdbean and lentil (LK-373, LK-754, LK-786, LK-822, LK-884, PUK-46B6, PUK-171, PUK-791, CRB-2 and KB-133) were obtained from Department of Soil Science G. B. Pant University of Ag. & Tech. Pantnagar (Uttarakrand). The purity of the cultures was checked with routine microbiological techniques. The obtained *Rhizobium* 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.

Symbiotic efficiency of the rhizobacteria in terms of nodulation, growth and yield of chickpea was evaluated during *Rabi* 2006-07 at Shamli. The soil was sandy loam of pH 7.8 having 5.8 g/kg Organic C and 148.6, 16.4, 286.8 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 inoculant (s) of *Rhizobium* sp. and rhizobacteria @ 20 g inoculant /kg seed rate 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 40, 60, 80 and 100 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^o C to constant weight. Grain and straw yields were recorded at final harvest.

RESULTS AND DISCUSSION

Nodulation

Inoculated *Rhizobium* sp., did not influenced the number and dry weight of root nodules significantly, nevertheless, it formed 22.2, 24.8, 19.5 and 19.9% more nodules 27.4, 24.2, 27.8 and 43.3% more nodule dry weight than no *Rhizobium* treatment at 40, 60, 80 and 100 days after sowing (DAS). Such favourable effects of *Rhizobium* inoculation on nodulation in chickpea have also been reported earlier by (Gupta, 2006 and Khanna and Dudeja, 1997) 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 *Rhizobium* sp., gave increases in nodule number ranging from 8.1 to 40.0, 7.8 to 57.8, 32.2 to48.2 and 12.2 to 54.5% and nodule dry weight from 9.5 to89.5, 14.4 to 65.5, 45.4 to 85.4 and 30.2 to 120.8% at 40, 60, 80 and 100 DAS. However, only rhizobacteria LK-754, PUK-46B6, PUK-171, KB-133 recorded significant increases in both nodule number and nodule dry weight over no rhizobateria inoculation treatment at all the intervals. KB-133 and PUK-171 produced the highest number and dry weight of nodules at 40 and 60 DAS, respectively, but a definite trend in the nodulation due to different rhizobacteria was not be noticed at later intervals. It was probably due to initiation of nodule degeneration as indicated also with a decrease in nodule number and nodule dry weight at 80 DAS than at 60 DAS. It is interesting to note that CRB-2 recorded highest number and dry weights of nodules at 100 DAS possibly due to allowing more nodulation at later crop age by favouring native and inoculated rhizobia. The rhizobateria producing highest nodule number and nodule dry were significantly better than LK-373, LK-786, LK-884, PUK-791 and CRB-2 at 40 DAS and LK-822 and PUK-791 at 60 DAS. These rhizobacteria also recorded significantly more number and dry weight of nodules than application of 20 kg N + 40 Kg P_2O_5/ha. Such variation in the efficiency of rhizobacteria could be due to their different genetic make up and have also been reported by Chandra and Pareek (2002) in lentil and urdbean and Gupta *et al.* (2003) in mungbean. Interaction between inoculated *Rhizobium* sp. and rhizobacteria were non-significant in nodulation.

Plant dry matter

Significant improvement of 4.5 and 3.5% in plant dry matter due to *Rhizobium* sp. inoculation, averaged over different rhizobacteria treatment, was observed only at 40 and 60 DAS, respectively. However, its inoculation gave numerical increases of 3.5 and 5.0% in plant dry weight at 80 and 100 DAS, respectively. It could be because of better nodule efficiency and less plant dry

Table 1 : Effect of *Rhizobium* sp and rhizobacteria inoculation on nodulation at different days after sowing (DAS).

Treatments	Nodule number /plant				Nodule dry weight (mg/plant)			
	40 DAS	60 DAS	80 DAS	100 DAS	40 DAS	60 DAS	80 DAS	100 DAS
No *Rhizobium*	6.7	11.0	8.6	6.3	498	960	670	394
Rhizobium	8.4	14.1	10.4	7.5	628	1184	848	560
C.D. at 5 %	NS	NS	NS	NS	NS	NS	NS	NS
No Rhizobacteia	6.3	9.8	7.2	6.0	395	804	470	327
20 kg N + 40 Kg P_2O_5/ha	6.2	12.7	9.4	6.5	440	1110	765	314
LK-373	7.6	12.2	9.6	6.7	564	1038	862	465
LK-754	8.5	13.4	9.4	6.8	698	1170	764	530
LK-786	7.2	13.6	9.6	6.8	545	1149	772	470
LK-822	8.9	12.0	9.8	6.9	700	973	831	458
LK-884	7.8	12.6	10.2	6.0	556	1029	803	365
PUK-46B6	8.4	13.4	9.8	7.2	638	1120	778	512
PUK-171	8.2	15.5	10.4	7.9	610	1330	805	542
PUK-791	6.3	10.3	9.4	6.8	424	916	817	419
CRB-2	7.0	12.8	9.8	9.0	475	1072	800	720
KB-133	9.0	13.2	9.0	7.8	742	1148	672	591
C.D. at 5%	1.2	1.2	1.3	1.0	143	149	134	153

Table 2 : Effect of *Rhizobium* sp and rhizobacteria inoculation on plant dry weight at different days after sowing (DAS) and yield.

Treatments	Plant dry weight (g/plant)				Yield (kg/ha)	
	40 DAS	60 DAS	80 DAS	100 DAS	Grain	Straw
No *Rhizobium*	2.36	3.82	6.28	8.80	1490	1803
Rhizobium	2.46	3.94	6.56	9.30	1668	2030
C.D. at 5 %	.078	.076	NS	NS	NS	NS
No Rhizobacteia	2.28	3.78	6.08	8.24	1290	1594
20 kg N + 40 Kg P_2O_5/ha	2.34	3.94	6.14	9.19	1454	1820
LK-373	2.38	3.78	6.08	8.80	1554	1900
LK-754	2.95	3.68	5.89	8.28	1320	1798
LK-786	2.18	3.62	6.04	8.99	1434	1578
LK-822	2.45	3.88	6.30	9.38	1608	1930
LK-884	2.94	4.05	7.34	9.50	1928	2150
PUK-46B6	2.38	3.78	6.38	9.20	1594	1998
PUK-171	2.84	4.17	7.38	9.90	2030	2298
PUK-791	2.48	4.03	6.27	9.28	1546	1815
CRB-2	2.52	3.88	6.86	9.30	1704	2238
KB-133	2.36	3.86	6.28	9.38	1470	1888
C.D. at 5 %	.194	.227	2.24	2.76	224	276

matter in early crop age. Inoculated *Rhizobium* sp. probably formed more proportion of nodules in initial period because of in close contact with the germinating roots following inoculation. It either did not proliferated in sufficient number in soil due to saprophytic competition with native rhizobia and other rhizospheric microorganisms. The nodules number also showed declining trend after 60 DAS as reported above. Inoculated rhizobacteria favourd the plant dry matter significantly and gave more plant dry weight 3.8 to 30.4% at 40 DAS, 0.8 to 12.4% at 60 DAS, 3.2 to 19.7% at 80 DAS and 7.5 to 20.5% at 100 DAS. Rhizobacteria PUK-171 recorded the highest plant dry weight at 40 and 100 DAS, while LK 754 and LK-884 recorded the maximum plant

dry matter at 40 and 100 DAS, respectively. PUK- 171 at 60 and 100 DAS was at par with LK-884 and PUK-791 at 60 DAS and significantly better than all the treatments at 100 DAS. Positive effects of rhizospheric bacteria on plant growth have also been reported by Tilak *et al*. (2006) and may be due to enhanced N_2 fixation, secretion of plant growth promotory substances, solubilization of P leading to its more availability, suppression of diseases etc. also reported by Arshad and Frankenberger (1998). Interaction between *Rhizobium* sp. and rhizobacteria were non-significant in plant dry matter.

Yield

Similar to the trend that observed in nodulation and plant dry matter, *Rhizobium* sp. inoculation did not favour the gain and straw yields significantly, however, gave 168 and 236 kg/ha more grain and straw than no *Rhizobium* sp. inoculation treatment, as reported earliar also by (Khurana and Sharma, 2000). This could be viewed in the light of earlier observations of marginal effect of *Rhizobium* sp. inoculation on nodulation and plant dry matter, that too in early crop age. The inoculated rhizobacteria recorded significant increases of 21.2 to 59.6% in grain yield and 13.25 to 45.6% in straw yield. PUK-171 by producing the highest grain and straw yields was significantly superior to all other treatments in grain and straw yield production. These results are in conformity with earlier reports by Khanna *et al*. (2006) in lentil who also reported variable and favourable effects of rhizobateria inoculation and could be due to enhancement in nodulation and N_2 fixation by native and inoculated *Rhizobium* sp. in presence of rhizobacteria. Interaction between inoculated Rhizobium and rhizobacteria were non-significant in grain yield also.

It could be concluded that rhizospheric bacteria had potential to enhance the nodulation and yield of chickpea in field conditions, however their effects varied with the used bacteria. Further, it is necessary to identify the rhzobacteria having synergistic interactions with *Rhizobium* sp. for harnessing their benefits in co-inoculation.

REFERENCES

Abd-Alla M. H. and Omar S. A. (2001). Survival of rhizobia / bradyrhizobia and a rock phosphate solubilizing fungus *Aspergillus niger* on various carriers from some agro-industrial wastes and their effects on nodulation and growth of faba bean and soybean. *J. Plant Nutr.*, **24** : 261-272.

Arshad M. and Frankenberger W. T. Jr. (1998). Plant growth-regulating substances in the rhizosphere: microbial production and function. *Advances in Agronomy*, **62** : 145-151.

Burdman S., Volpin H., Kigel J., Kaplunic Y and Okon Y. (1996). Promotion of nod gene inducers and nodulation in common bean (*Phasiolus vulgaris*) root inoculated with *Azospirillum brasilance* cd. *Appied and Environmental Microbiology*, **62** : 3030-3033.

Chandra R. and Pareek R. P. (2002). Effect of Rhizobacteria in urdbean and lentil. *Indian Journal of Pulses Research*, **15** : 152-155.

Dashti N., Zhang F., Hynes R. and Smith D. L. (1998). Plant growth promoting rhizobacteria accelerate nodulation and increase nitrogen fixations activity by field grown soybean [*Glycine max (*L) merr.] under short season conditions. *Plant and Soil*, **200** : 205-213.

Gupta A., Saxena A. K., Murali G. and Tilak K.V. B. R. (2003). 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)*, **80** : 28-35.

Gupta S. C. (2006). Effect of combined inoculation on nodulation nutrient uptake and yield of chickpea in Vertisol. *Journal of Indian Society of Soil Science*, **54** : 251-254.

Khanna V., Sharma P. and Sekhen H. S. (2006). Effect of rhizobium inoculation and PGPR on nodulation and grain yield in lentil (*Lens culinaris* L.). *Environment and Ecology*, **245** (Special 1) : 224-226.

Khot G. G., Tauro P. and Dadarwal K. R. (1996). Rhizobacteria from chickpea (*Cicer arietinum* L.) rhizosphere effective in wilt control and promote nodulation. *Indian Journal of. Microbioogy*, **36** : 217-222.

Khurana A. L. and Dudeja S. S. (1997). *Biological Nitrogen Fixation Technology for Pulses Production in India*. Indian Institute of Pulses Research, Kanpur, Pp 1-18.

Khurana A. S. and Sharma Poonam (2000). Effect of dual inoculation of phosphate solubilizing bacteria, *Bradyrhizobium* sp. (*Cicer*) and phosphorus on nitrogen fixation and yield of chickpea. *Indian Journal of Pulses Research*, **13** : 66-67.

Pareek R. P. and Chandra R. (2003). Chickpea: Microbiology and Nitrogen Fixtion. In: *Chickpea Research in India* (M. Ali, Shiv Kumar and N. B.Singh eds.). I.I.P.R., Kanpur, pp. 167-193.

Rautela L. S., Chandra R. and Pareek R. P. (2001). Enhancing *Rhizobium* inoculum efficiency in blackgram (*Vigna mungo* L.) by co-inoculation of *Azotobacter chroococcum* and *Bacillus* sp. *Indian Journal of Pulses Research*, **14** : 133-137.

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**(1) : 67-71.