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Co-inoculation of *Mesorhizobium* sp. and plant growth promoting rhizobacteria *Pseudomonas* sp. as bio-enhancer and bio-fertilizer in chickpea (*Cicer arietinum* L.)

Navprabhjot Kaur, Poonam Sharma* and Sunita Sharma

Department of Plant Breeding and Genetics,
Punjab Agricultural University, Ludhiana-141 004, India.
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ABSTRACT

The present investigation was undertaken to study the synergistic effect of recommended *Mesorhizobium* sp. (LGR -33) (*Meso*) and native potential PGPR *Pseudomonas* sp. (PGPR 2 and PGPR 3) along with reference strain *Pseudomonas diminuta* (LK884) on bio-enhancing activity, symbiotic parameters and grain yield in *desi* (PBG 1) and *kabuli* (BG 1053) under field conditions in chickpea during *rabi* 2009-2010. Significant improvement in growth and symbiotic parameters was observed with co-inoculation of *Mesorhizobium sp*. with native potential *Pseudomonas* sp. PGPR as compared to single inoculants of *Mesorhizobium* sp. in both varieties. Maximum improvement in symbiotic parameters was observed with co-inoculation of *Mesorhizobium* sp. and PGPR 3 followed by native PGPR 2 as compared to LK884 (reference). On an average, co-inoculation of *Mesorhizobium* sp. with PGPR 3 improved the yield by 7.0% *(desi)* and 5.3% *(kabuli)* over *Mesorhizobium* sp. alone. It appears that native potential *Pseudomonas* sp. PGPR can be explored as potent bio-enhancers and bio-fertilizers along with *Mesorhizobium* sp. in chickpea under low input technology programme of sustainable agriculture.

Key words: Chickpea, Co-inoculation, *Mesorhizobium* sp., *Pseudomonas.*

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the major *rabi* pulse crop and recognized as valuable source of protein (22%) in the developing countries. In India, it was grown on an area of 9.0 million hectare and had average yield of 841 $kgha^{-1}$ (Singh 2012). Moreover, chickpea being a leguminous crop, fixes atmospheric nitrogen (N) through symbiosis with an effective strain of *Mesorhizobium* (up to 141 $kgN^{-1}year$) (Singh 2007) and improves soil fertility as well as productivity of subsequent cereal crops and reduces dependence on soil N. Seed inoculation with appropriate rhizobia at sowing is a recommended agronomic practice in pulse production technology. The successful inoculation of legumes with rhizobia depends on the ability of introduced strain to compete with existing native population.

One of the major constraints in the success of legume inoculation is the poor survival and establishment of introduced rhizobia in the rhizosphere of inoculated seedling. The potential of introduced rhizobia to improve the productivity of legumes is often not realized because of competition from the native rhizobial population or due to unspecified type of antagonism that prevents root colonization by rhizobia strain (Hafeez *et al.* 2004). Although, competitive ability and effectiveness of rhizobia are genetic characters but soil biological environment also influences the competitive ability of introduced strain.

The nodulation process involves a signal exchange between the host and the bacterium. The early root colonizing microorganisms, in and around the growing roots of legumes, may interact with each other and with the plant resulting in symbiotic, associative, neturalistic or detrimental effects (Gulati *et al.* 2001). For their beneficial effects on plants, these bacteria have been termed as "Plant Growth Promoting Rhizobacteria" (PGPR). The PGPRs are defined by three intrinsic characters – they must be able to colonize root and survive and multiply in the micro-habits associated with the root surface in competition with other micro-biota at least for the time needed to express their plant promotion and protection activities and promote plant growth.

Co-inoculation of legumes with rhizobia and PGPR is even more effective for improving nodulation and growth of legume. PGPR are able to modify nodule formation and biological nitrogen fixation (BNF) when they are co-inoculated with rhizobia (Garcia *et al.* 2004). Several

*Corresponding author's e-mail: poonam1963in@yahoo.co.in.

mechanisms such as alteration in the composition of rhizosphere microorganisms, production of plant signaling compounds, bacteriocins, siderophores, plant growth hormones and improving availability of nutrients by rhizosphere microorganisms have been reported for synergism (Sivaramaiah *et al.* 2007).Synergistic effect of *P fluorescence* and *Bacillus* sp. with *Rhizobium* strains enhanced the nodulation, nodule weight, root length ,shoot biomass, total N content and thus promoting biological nitrogen fixation in chickpea(Yadav *et al.* 2010).Co-inoculation of legumes with PGPR and rhizobia has received increasing attention in recent years (Roopa *et al.* 2012) However, compatibility of these microorganisms needs to be evaluated because of the possibility of antagonistic interactions among them.

The present study was undertaken to evaluate the co-inoculation effect of *Mesorhizobium* sp. and native potential *Pseudomonas* sp. of PGPR along with reference strain *Pseudomonas diminuta* LK884 on growth, symbiotic parameters and yield in chickpea.

MATERIALS AND METHODS

Selected *Mesorhizobium* sp. (LGR 33**)** is recommended strain for chickpea isolated from variety GL 769. Out of 35 isolates of *Pseudomonas* sp. two PGPR strains native *viz.* PGPR 2 and PGPR 3 were selected due to their above mentioned beneficial traits along with reference PGPR *Pseudomonas diminiuta* (LK 884).

Field experiment: The present study was carried out at the Pulse Research Farm Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, during *rabi* 2009-10. Field experiment was conducted in Split Plot Design (SPD) with four replications. Seeds of chickpea *desi* PBG1and *kabuli* BG 1053 were procured from the Pulses Section, Department of Plant Breeding and Genetics, PAU, Ludhiana. Seed rate of 18-20 $kgha^{-1}$ was used for sowing. The chickpea varietiesPBG1and BG 1053 were sown on 15th November 2009 using 'kera' method at 30 cm row sp. acing, keeping a distance of about 10 cm between the seeds. Chickpea seeds of *desi* PBG1and *kabuli* BG 1053 varieties were inoculated with reference cultures of *Mesohizobium* sp. (LGR 33) as per treatment. Twenty g charcoal inoculant was used per kg of chickpea seeds for inoculation in monoculture treatment. In co-inoculation treatments, *Mesorhizobium* sp. and *Pseudomonas* sp. (PGPR) as charcoal inoculants were applied to chickpea seeds in ratio of 1:1. Before sowing, inoculated seeds were air dried at room temperature under shade and sown within two hours. The observations were recorded on germination count at 10 days after sowing (DAS). Observation for nodulation, nodule occupancy (LGR-33 resistant to streptomycin 15 µg ml^{-1}) (Chandra and Pareek 2002), number and dry weight of nodules were recorded at vegetative (65 DAS) and flowering stage (90 DAS). Leghaemoglobin content of nodules (Wilson and Reisenauer 1963), chlorophyll content of leaves (Witham *et al.* 1971), N content (McKenzie and Wallace 1954) and Phosphorous (P) content of shoot (Jackson 1973) were recorded at flowering stage. Crop was sickle harvested and dried plants and matured pods were threshed manually. Grain yield was recorded at the harvesting stage.

Analysis of data: The collected data were analyzed statistically using the CPCS 1 software developed by Department of statistics, Punjab Agricultural University, Ludhiana and Punjab, India. Differences among treatment and varieties were determined using comparison method at 5% level of significance.

RESULTS AND DISCUSSION

Bio-enhancer parameters: Data on emergence count (Table 1) revealed that differences due to various treatments in both the varieties of chickpea were found to be non-significant. However, all dual inoculation treatments improved emergence count significantly as compared to monoculture and control treatments. Germination in dual treatments was quite good and it varied from 91.0 % to 96.1 % in *desi* PBG1 and 91.3 to 94.9% in *kabuli* BG 1053. Similarly, Biswas (1998) and Ashrafuzzaman *et al.* (2009) also reported improvement in seed germination might be due to release of plant growth regulators which improve morphological characters of roots. These results suggest that increase in seed emergence in PGPR treatments might be due to induction of IAA production and phosphate solubilization. These results corroborated with the findings

Microbial Inoculation: For present study, following four inoculants were used.

Strains	Beneficial Traits					
	IAA(µg ml^{-1}**)**	**P-solubilization** (mg100 ml^{-1})	**HCN**	**NH_3**	**Catalase**	**Siderophore**
Meso	43.185	4.40	+	+	+	_
PGPR 2	62.38	13.15	+	+	+	+
PGPR 3	66.79	13.45	+	+	+	+
LK 884reference	43.185	6.40	+	+	+	_

of Sivaramaiah *et al.* (2007) who also found stimulatory effect on seedling emergence at 10 days with two rhizobacteria *Bacillus* strains CBS 127 and CBS 155 in chickpea. The same results were reported by Kumar *et al.* (2000) in which seed bacterization with both fluorescent *Pseudomonas* strains and *Rhizobium* and their combinations (co-inoculations) brought distinct crop enhancement in pea.

The results summarized in Table 1 depicted the effect of *Mesorhizobium* sp. alone or in dual inoculation with different PGPRs on plant height. On the basis of data collected at vegetative stage 65 DAS in both the varieties of chickpea: *desi* PBG1 and *kabuli* BG 1053 non-significant difference for plant height was observed between dual treatments and *Mesorhizobium* sp. alone. Maximum height was recorded with Meso+ PGPR 3 in both varieties (20.4 cm in *desi* PBG 1 and 21.9 cm in *kabuli* BG1053) followed by Meso + PGPR 2 and Meso+ LK884. This investigation has been found coherent between the result of Sivaramaiah *et al.* (2007) who reported the enhancement in root and shoot length in agar plates at 10 days with *Bacillus* strains in chickpea. Improvement in plant height with PGPR or *Mesorhizobium* sp alone or dual over control plants in both varieties of chickpea could be attributed to presence of phytohormone (IAA) which function as signal molecule in the regulation of plant development.

Co-inoculation of *Mesorhizobium* sp. with different PGPR showed non-significant differences for chlorophyll content as compared to *Mesorhizobium* sp. alone (Table 1). Significantly higher chlorophyll content was observed in Meso + PGPR 3 in both the varieties (1.69mg g^{-1} fresh weight of leaves in *desi* PBG 1 and 1.86 mg g^{-1} fresh weight of leaves in *kabuli* BG 1053) followed by Meso+PGPR 2 (1.61 and 1.75 mg g^{-1} fresh wt. of leaves in PBG 1 and BG 1053 respectively). *Rhizobium* inoculation significantly increased the chlorophyll content as compared to control. Similarly, Ladha *et al.* (1998) observed the improvement in chlorophyll content may be due to increased N uptake by a larger root surface areas associated with additional root hairs and lateral root development and/or to BNF, either directly by the inoculant strains or indirectly by stimulating BNF activity of the associated rhizosphere community. Amir *et al.* (2001) reported the beneficial bio-enhancing effect of rhizobacteria through higher photosynthetic activity and more nutrients (P, K, Ca and Mg) uptake in oil palm seedlings. A similar finding of improvement in leaf sugar content of rice and sugarbeet was documented by co-inoculation of N- fixing and P solubilizing bacteria by Afzal and Bano (2008) and Sahin *et al.* (2004). Sarna *et al.* (2008) observed the effecting symbiotic N_2 fixation as another possible reason by which PGPR may influence chickpea performance. Sarig *et al.* (1990) reported PGPRs strains like pseudomonads also delayed leaf senescence there by bio-enhancing photosynthesis. Zhang *et al.* (1996) reported *Serratia* as PGPR that also induced the increase in soybean photosynthesis prior to onset of N_2 fixation.

Symbiotic parameters: At 65 DAS the highest occupancy of inoculants *Meso*rhizobium sp. in nodules of 38.7% was recorded in BG 1053 with combined inoculation of *Mesorhizobium* sp. with PGPR 3 than *Mesorhizobium* sp. alone at the same interval (Table 2). Inoculation of *Mesorhizobium* sp. occupied 28.0% nodules in BG 1053. In *desi* PBG-1, the highest occupancy of *Mesorhizobium*

TABLE 1: Effect of co-inoculation of *Mesorhizobium sp.* with *Pseudomonas* sp.(PGPR) on growth parameters in chickpea.

Treatments	Emergence count (%)			Plant height (cm)			Chlorophyll content (mg g^{-1} fresh weight of leaves)		
	10 DAS			65 DAS			90 DAS		
	PBG1	BG 1053	Mean	PBG 1	BG 1053	Mean	PBG 1	BG 1053	Mean
Control	86.6	86.4	86.5	16.1	18.2	17.15	16.1	18.2	17.15
Meso	88.9	89.0	88.9	18.4	19.3	18.85	18.4	19.3	18.85
Meso+PGPR2	94.0	93.3	92.6	19.6	20.2	19.9	19.6	20.2	19.9
Meso+PGPR3	96.1	94.9	95.5	20.4	21.9	21.15	20.4	21.9	21.15
Meso+LK884	91.0	91.3	91.3	19.3	19.8	19.55	19.3	19.8	19.55
PGPR 2	89.7	90.1	89.9	19.1	19.0	19.05	19.1	19.0	19.05
PGPR 3	91.1	91.7	91.4	20.2	19.3	19.7	20.2	19.3	19.7
LK884	87.7	87.8	87.7	19.0	19.4	19.2	19.0	19.4	19.2
Mean	90.6	90.6		19.0	19.6		19.0	19.6	
CD 5%									
Vsariety (V):		NS			NS			NS	
Treatment(T)		5.06			NS			0.31	
V×T		NS			NS			NS	

NS – Non significant

TABLE 2: Effect of co-inoculation of *Mesorhizobium sp.* with different *Pseudomonas* sp. (PGPR) on nodule occupancy in chickpea

Treatments	Nodule occupancy (%)					
	65 DAS			90 DAS		
	PBG 1	BG 1053	Mean	PBG 1	BG 1053	Mean
Meso	23.7	28.0	25.8	30.7	28.3	29.5
Meso +PGPR 2	32.0	34.3	33.1	40.7	35.3	38.0
Meso+ PGPR 3	35.3	38.7	37.0	42.3	39.9	41.1
Meso+LK884	28.0	30.7	29.3	32.3	34.3	33.3
PGPR 2	16.0	17.3	16.6	19.3	16.9	18.1
PGPR 3	17.5	18.5	18.0	20.0	18.7	19.3
LK884	15.3	16.7	16.0	18.8	16.7	17.7
Mean	23.97	26.31		29.15	27.15	
CD 5%						
Variety (V):		4.7			NS	
Treatment(T)		2.7			3.1	
V×T		3.8			4.5	

NS – Non significant

sp. was recorded in 35.3% of nodules in co-inoculation of Meso + PGPR 3 over *Mesorhizobium* sp. alone (23.7% of nodules occupancy) followed by Meso + PGPR 2 in both *desi* PBG 1 (32.0%) and *kabuli* BG 1053 (34.3%). Inoculation of chickpea with PGPRs alone resulted into nodule occupancy ranged between 15.3% and 18.5% in both varieties of chickpea. Dual inoculation of *Mesorhizobium* sp. with different PGPRs gave significantly more nodule occupancy than *Mesorhizobium* sp. alone at 65 DAS. Difference for nodule occupancy in both varieties was non-significant.

At 90 DAS the highest nodule occupancy of *Mesorhizobium* sp. was recorded 42.3% with PGPR 3 in *desi* PBG 1 and 39.9% in *kabuli* BG 1053 in nodules of co-inoculated Meso + PGPR 2 (40.7% in *desi* PBG 1 and 35.3% in *kabuli* BG 1053). At 90 DAS over Meso alone (30.7% in *desi* PBG-1 and 28.3% in *kabuli* BG 1053) and treatment of PGPRs alone ranged between 16.7% and 20.0% in both varieties. Dual inoculation of Meso with PGPR 2 and PGPR 3 was significant over *Mesorhizobium* sp. in PBG 1 where as there was significant difference exists between all dual inoculations and *Mesorhizobium* sp. (alone) for nodule occupancy (Table 2). Interaction between varieties and treatment was found to be significant. Kumar and Chandra (2008) also reported the influence of PGPR and PSB on *Rhizobium leguminosarum* bv. *viciae* strain competition and symbiotic performance in lentil. In the present study, co-inoculation of native isolates of PGPR (PGPR 2 and PGPR 3) with *Mesorhizobium* sp. revealed better nodule occupancy as compared to reference PGPR (LK884) which might be due to siderophores production and release of growth hormone (IAA) by inoculants. These results are in close agreement with Pamar and Dadarwal (1999) that also correlated improvement in the nodule occupancy due to siderophores production and release of growth hormones of rhizobia in rhizosphere of chickpea. This enhancement in nodule occupancy might be due to well adaption of native rhizobacteria in their niche and plant growth promotional activities.

Co-inoculation with Meso + PGPR 3 registered significantly higher number of nodules (27.3 NN plant^{-1} in *desi* PBG 1 and 25.6 NN plant^{-1} in *kabuli* BG 1053) followed by Meso + PGPR 2 treatment as compared to *Mesorhizobium* sp. alone (19.0 in *desi* PBG 1 and 20.3 in *kabuli* BG 1053) at 65 DAS (Figure 1). At 90 DAS, nodule number was again increased in all the dual treatments ranged between 51.0-70.5 NNplant^{-1}. Number of nodules in dual inoculation of Meso + PGPR 2 and Meso + PGPR 3 differed significantly in comparison to *Mesorhizobium* sp. alone treatment (Figure1). The number of nodules was increased by dual inoculation at both the stages over *Mesorhizobium* sp alone. The data was supported by Kumar and Chandra (2008) that dual inoculation of *Rhizobium* sp. + *Pseudomonas diminuta* and *Rhizobium* sp. + LK884 produced more nodule number at different intervals which was statistically comparable to *Rhizobium* alone. Similar trend was observed by Sivaramaiah *et al.* (2007) that co-inoculation of *Bacillus* strain with *Mesorhizobium* sp.

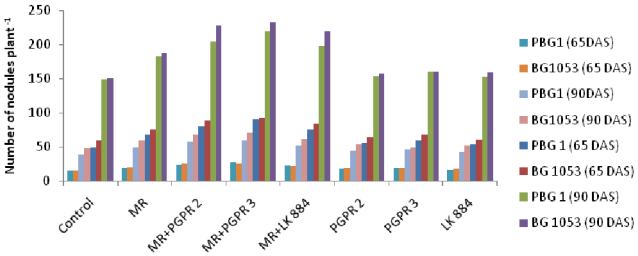

FIG 1: Effect of Co-inoculation of *Mesorhizobium sp.* with *Pseudomonas* sp. (PGPR) on number of nodules in chickpea

Ca181 resulted in increased nodule number at 60 and 80 day of plant growth under chillum jar conditions. Pramar and Dadarwal (1999) also reported rhizobacteria enhancing the production of flavonoid like compound or phytoalexins in roots of several crop plants enhancing nodulation. Similarly, Halverson and Handelsman (1991) observed that seed treatment with *Bacillus cereus* UW85 increased nodulation of soybean in three field seasons. Earlier Sarna *et al.* (2008) found that the dual inoculation of *Rhizobium* + PGPR was better for nodulation than *Rhizobium* + *Azotobacter* and *Azotobacter* + PGPR in chickpea. Varieties differed non-significantly for number of nodules; however variety BG 1053 registered numerically higher nodulation as compared to PBG 1. Variation in nodulation due to different host genotype for nodulation with *Rhizobium*, PGPR and *Azotobacter* inoculation has been reported by Sarna *et al.* (2008) which might be due to their inherent capability of N_2 fixation. Interaction between variety and treatment was found to be non-significant.

Maximum enhancement of nodule dry weight was recorded in both varieties at both stages with co-inoculation of Meso + PGPR 3 (ranged between 90.3 to 234.0 mg/plant) followed by Meso + PGPR 2 (ranged between 80.3 mgplant^{-1} to 229.0 mgplant^{-1}) as compared to Meso + LK884 co-inoculation (75.5 to 220.2 mg/plant) (Figure 2). Difference for nodule dry weight between dual inoculation of Meso and native PGPR (PGPR 2 and PGPR 3) was significant in *kabuli* BG 1053 and *desi* PBG 1, in comparison to Meso + Reference PGPR (LK844) at both vegetative and flowering stage. Sharma and Khurana (2007) reported that the native PGPR favored *Mesorhizobium* inoculation to form more nodules either by favoring it's survival in the rhizosphere or the synthesis of plant growth regulators which result in more root hair development and leading to more infection. Kumar and Chandra (2008) also reported improvement in nodule dry weight at different intervals being maximum at 70 DAS in dual inoculation of *Rhizobium* sp. + LK786 and *Rhizobium* sp. + *Pseudomonas* sp. as compared to *Rhizobium* alone inoculation in lentil.

Dual inoculation of *Mesorhizobium* sp. with PGPR 2, PGPR 3 and LK884 showed significant improvement in leghaemoglobin content over *Mesorhizobium* sp. alone in both the varieties. Maximum content of leghaemoglobin was observed in co-inoculations of *Mesorhizobium* sp. with PGPR 3 (5.39mg/g fresh weight of nodules in *desi* PBG 1 and 6.18 mg/g fresh weight of nodules in *kabuli* BG 1053) (Figure3). Similarly a positive correlation between leghaemoglobin content and N_2 fixation was reported by Singh and Hiremath (1990). Higher nodulation and N_2 fixation increased the occupancy of effective nodules which might have increased the leghaemoglobin content. Production of leghaemoglobin occurs only under symbiotic relationship between effective strains of rhizobia and specific plant as nodules produced by ineffective strain do not have pink colour and may contain only one tenth leghaemoglobin content of normal pink nodules produce by effective strains. Our data also supported with studies of Mahmoud and Abd-Alla (2001) where microbial siderophores may be involved in biosynthesis of leghaemoglobin by facilitating the uptake of iron, a constituent of key proteins such as nitrogenase and leghaemoglobin content from environment and helps in its enhancement on dual inoculation of *Mesorhizobium* with PGPRs in chickpea.

Nutrient uptake: Considerable variation for nitrogen content of shoot was observed in different treatments. Data revealed the significant increase in nitrogen content with co-inoculation of *Mesorhizobium* sp. with PGPR 2 and PGPR 3 in *desi* PBG 1 over *Mesorhizobium* sp. alone and un-inoculated control where as Meso+LK884 showed non-significant increase in dual inoculation over *Mesorhizobium* sp. but significant increase over un inoculated control (Table 3). The difference for nitrogen content in dual treatment over *Mesorhizobium* sp. alone was significant in *kabuli* BG 1053. Increase in phosphorous content in both the chickpea varieties was observed with co-inoculation of Meso+PGPR 3 (0.212% in *desi* PBG 1 and 0.169% in *kabuli* BG 1053) followed by Meso+PGPR 2 and Meso+LK884. Difference for phosphorous content was significant in all the dual inoculated

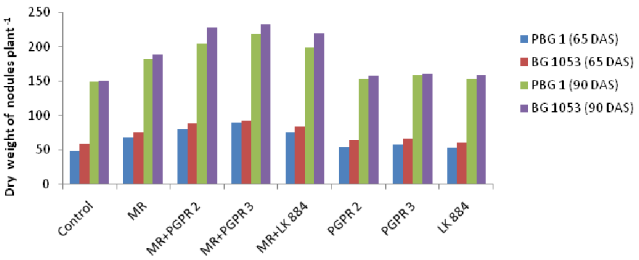

FIG 2: Effect of Co-inoculation of *Mesorhizobium sp.* with *Pseudomonas* sp. (PGPR) on dry weight of nodules in chickpea

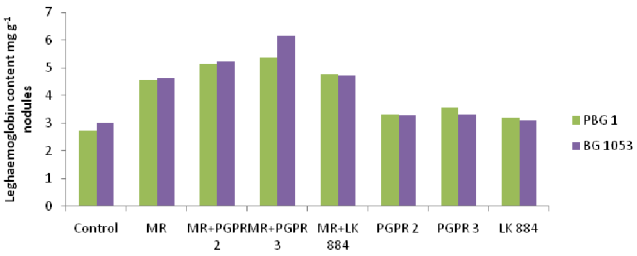

FIG 3: Effect of Co-inoculation of *Mesorhizobium sp.* with *Pseudomonas* sp. (PGPR) on leghaemoglobin content of nodules in chickpea at 90 DAS

TABLE 3: Effect of co-inoculation of *Mesorhizobium* sp. with *Pseudomonas* sp. (PGPR) on N&P content of shoot and grain yield in chickpea at harvesting stage.

Treatments	N Content (%)			P Content (%)			Grain Yield (kg ha^{-1})		
	PBG 1	BG 1053	Mean	PBG 1	BG 1053	Mean	PBG 1	BG 1053	Mean
Control	0.91	1.37	1.14	0.168	0.128	0.148	2236	1567	1901
Meso	1.90	1.78	1.84	0.182	0.149	0.165	2438	1718	2078
Meso+PGPR 2	2.41	2.30	2.35	0.205	0.158	0.181	2585	1785	2185
Meso+PGPR 3	2.70	2.58	2.63	0.212	0.169	0.190	2610	1810	2210
Meso+LK884	1.96	2.28	2.12	0.198	0.152	0.175	2508	1755	2131
PGPR 2	1.51	1.59	1.55	0.180	0.144	0.162	2310	1590	1950
PGPR 3	1.71	1.95	1.83	0.180	0.146	0.163	2355	1602	1978
LK884	1.24	1.46	1.35	0.175	0.140	0.157	2300	1585	1942
Mean	1.79	1.91		0.187	0.148		2417	1676	
CD 5%									
Variety (V):		NS			0.014			354.2	
Treatment(T)		0.34			0.005			NS	
V×T		NS			0.007			NS	

NS – Non significant

treatments in *desi* PBG 1 where as *kabuli* BG 1053, the difference was significant for Meso+PGPR 2 and Meso+PGPR 3 (Table 3).

Enhanced N and P content of plant due to co-inoculation of PGPR and rhizobia has been reported by various researchers in soybean, chickpea and lentil respectively (Garcia *et al* 2004, Sarna *et al.* 2008, Qureshi *et al.* 2009, Sharma and Khurana 2007, Lata and Tilak 2000, Sharma *et al.* 2007). Similarly, Parmar and Dadarwal (1999) also reported enhancement in the total plant nitrogen in chickpea due to dual inoculation of Meso +PGPRs may be attributed to production of siderophores which increase flavonoid like compounds. Rai and Hunt (1993) obtained similar results who concluded that *Azospirillum* inoculation in maize increased the N_2 fixation rate along with mineral nutrient contents and plant growth. Khan *et al.* (2006) also reported that co-inoculation resulted in more N_2 fixation and P solubilization due to release of protons by Rhizobium during biological nitrogen fixation results into lowering of soil pH and by producing organic acids. These results are in close agreement with the findings of Barea *et al.* (2005) who demonstrated that the interactive effect of rhizobia and rhizobacteria mediated the number of soil processes and thus enhanced the availability of nutrients.

Grain yield: The grain yield recorded at harvesting stage revealed that *Mesorhizobium* sp. alone significantly increased the grain yield by 9.0 to 9.6 % in PBG 1 and BG 1053, respectively compared to un-inoculated control (Table 3). Such an increase may be attributed due to the presence of either low and /or ineffective population of native rhizobia in the soil as reported by Khurana and Sharma (2000). In the present study, association of PGPR *Mesorhizobium* sp. promoted chickpea growth and yield as compared to their individual inoculations. On the basis of overall mean, increase in yield due to dual inoculation was in the range of 2.5-6.3% over *Mesorhizobium* alone application. It is highly likely that PGPR with IAA production and phosphate solubilization activity might have improved colonization niches through root proliferation to introduced *Mesorhizobium* in the rhizosphere of chickpea by reflecting better nodulation and yield as reported by Qureshi *et al.* (2009).

Similar to our work, various researchers have reported the synergistic effects of phytohormone producing PGPR and *Rhizobium* on nodulation and yield of legume crops (Bansal 2009, Tilak *et al.* 2005, Sharma and Khurana 2007, Sarna *et al.* 2008). Varieties differed significantly for grain yield. However, the interaction between varieties and treatment was non-significant. Perret *et al.* (2000) discussed the role of signal exchange between host plant and specific rhizobial species in nodule formation. Inoculation with free living diazotrophs increased the signal exchange between host legumes and resulting in more N_2 fixing sites and ultimately higher nutrient concentration and yield of legume as reported by Parmar and Dadarwal (1999) and Qureshi *et al.* (2009). It thus appears that the dual inoculation of *Mesorhizobium* sp. with native PGPR (*Pseudomonas* sp.) are effective as bio-enhancer and bio-fertilizer to enhance plant growth, symbiotic efficiency, nutrient uptake and yield in chickpea.

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