Seed Research, Vol. 45(2): 111-118, 2017

Interactive Effect of Phosphorus Fertilization and Rhizobial Inoculation on Symbiotic and Growth Potential of Selected Chickpea Cultivars

K SWARNALAKSHMI[1], S GOPALAKRISHNAN[2]*, M SINGH[3], V POONIYA[1], V SRINIVAS[2] AND SK CHATURVEDI[3]

[1]ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India
[2]International Crops Research Institute for the Semi-Arid-Tropics, Patancheru 502 324, India
[3]ICAR-Indian Institute of Pulses Research (IIPR), Kanpur 208 024, Uttar Pradesh, India
*s.gopalakrishnan@cgiar.org

ABSTRACT: The main aim of this study was to evaluate the effect of rhizobia and di-ammonium phosphate (DAP) on nodulation and plant growth-promotion (PGP) traits of chickpea cultivars under field conditions at IARI, New Delhi and ICRISAT, Patancheru during the *Rabi* season of 2014-15. Two chickpea cultivars, JAKI 9218 and Shubhra, were used in common by both the institutes, while the third cultivar varied; IARI used PUSA 372, whereas ICRISAT used JG11, popular variety of the respective locations. The seeds were subjected to four treatments, including T1 = positive control (only DAP); T2 = only rhizobia; T3 = rhizobia and DAP; and T4 = absolute control (no rhizobia and DAP). Manual planting was done in split plot design with three replications. Observations, including nodule number, nodule weight, shoot weight, root weight, pod weight, pod number, grain yield, stover yield, N and P uptake from shoots were recorded. Seed inoculation with rhizobia (T_2 and T_3) significantly enhanced nodulation and growth over un-inoculated controls (T_1 and T_4) at both IARI and ICRISAT locations. Among T_2 and T_3, nodule weight observed was more in T_3 than T_2 at both IARI and ICRISAT locations, except in case of one cultivar (Subhra) at ICRISAT where T_2 was significantly higher than T_3.Among the cultivars, Subhra was found to be more compatible to rhizobial strains used in this study than the other two cultivars at both the locations. At crop maturity stage, data were collected only at ICRISAT location due to hostile weather conditions at IARI. At ICRISAT, T_3 recorded significantly higher shoot weight (up to 25%), pod weight (up to 37%), pod number (up to 16%), grain yield (up to 25%), stover yield (up to 8%) and P uptake (up to 10%) over T_4, the absolute control. It can be concluded that rhizobial strains in combination with DAP supports not only nodulation potential of chickpea but also its PGP traits.

Keywords: P fertilization; Plant growth-promotion, Rhizobial inoculation; Symbiotic nitrogen fixation,

By the year 2050, world population is expected to increase 2.5 billion, which necessitates enhancing crop production to feed the population. In this context, nitrogen (N_2) can play a critical role for crop production. According to FAO (2000) estimate, N_2 fertilizer consumption will increase to 200 million tons by the year 2050. Legumes are known to fix atmospheric N_2 through symbiotic association with *Rhizobium* spp. Therefore, dependence of legumes on fertilizer N_2 is less compared to other crops. The symbiotic association between rhizobia and legumes, called symbiotic nitrogen fixation (SNF) provide large amounts of N_2 to the plant (N_2 fixed between 50 and 200 kg N ha^{-1} yr^{-1}). SNF is an energy intensive process that depends on supply of phosphorus (P) from soil or fertilizers. The P use-efficiency of applied phosphatic fertilizers varies between 5% and 10% and rest of the applied P is fixed in soil [1]. Phosphorus use for legume production is usually low (17%20%) due to adsorption and fixation of large proportion of applied phosphorus in soil. Legume root nodules

Received : November 2017 Revised : November 2017 Accepted : December 2017

are reported as P sinks,as P concentration was found higher in root nodules than other plant tissues. The reduced availability of P affects not only the nodulation but also plant growth in legumes. Nodule growth and number are more sensitive than shoot mass in response to phosphorus deficiency in soil [2]. Therefore, nodule productivity i.e. SNF process is highly sensitive to phosphorus limitation in soil. Phosphorus deficiency in pulse growing areas are widely reported and identified as a major limiting factor for N_2 fixation and productivity enhancement [3]. Therefore, phosphorus requirement for pulses is higher as compared to other crops.

In India, chickpea (*Cicer arietinum* L.) is an important cool (*rabi*) season pulse cropcultivated around 9.92 million hectare [4] land under rainfed agriculture on low fertility soils, having low carbon content and multiple nutrient deficiency. Chickpea productivity at farmer's field is very low; around 50 to 60 per cent of the potential yield of the high yielding chickpea varieties (2.0 t/ha) released over the past two decades, which is primarily attributed to various biotic and abiotic stresses. To bridge large yield gaps in potential and average grain yield of newly developed high yielding varieties of chickpea, a number of production technologies including inoculation with efficient strains of rhizobia and other plant growth-promoting (PGP) bacteria to improve N_2 fixation in chickpea has been suggested [5]. Any chickpea cultivars efficient in P-acquisition from soil and its utilization will support high symbiotic potential and N_2 fixation. There is reports of genetic differences in phosphorus-acquisition efficiency (PAE) amongst legume species, including chickpea. Preliminary work done at Indian Institute of Pulses Research (IIPR), Kanpur, India also showed that chickpea cultivars vary in their P-acquisition efficiency (PAE), which may offer the possibility of breeding more phosphorus-acquisition efficient chickpea cultivars. Chickpea roots excrete organic acids which dissolve insoluble P compounds and make them available for plant uptake [6]. The potential for genetic variation in chickpea for N_2 fixation and PAE is therefore, worth exploring. This manuscript, therefore, aims to evaluate the interactive effect of P fertilization and rhizobial inoculation on symbiotic potential of three chickpea cultivars under two different agro-climatic regions of India.

MATERIALS AND METHODS

A field experiment was performed to evaluate the effect of inoculants and P fertilization on nodulation, plant growth and nutrient uptake on selected chickpea cultivars at Indian Agricultural Research Institute (IARI), New Delhi and International Crops Research for the Semi-Arid Tropics (ICRISAT), Patancheru during *rabi* 2014-15 season. The experiment consisted of four treatments (T_1 - positive control i.e., 100% recommended dose of fertilizer [RDF]-100 kg di-ammonium phosphate [DAP]ha^{-1}; T_2 - mixed strains of chickpea rhizobia -IC-59 + IC-76; T_3 -mixed strains of IC-59 + IC-76 + 100% RDF; and T_4-absolute control - without rhizobia and DAP). Three chickpea cultivars were studied at each location. Cultivar, JAKI 9218 (C_1; low P-use efficient [PUE]) and Shubhra (C_2; high PUE) were common in both locations and the third cultivar (C3) represented predominant variety of respective location, PUSA 372 for IARI and JG 11 for ICRISAT. Thus, twelve treatment combinations with three replications were laid out in split plot design with the plot size of 10 m^2. Physico-chemical properties of soil (pH, EC, % organic C, available N, available P, soil type and annual rainfall)at both locations were recorded in the beginning of the experiment (Table 1). The previous crop in the experimental site was maize during *Kharif* season at both the locations. DAP was applied in respective plot just before sowing. The seeds were treated with the peat based rhizobial inoculants of IC-59 and IC-76 (containing 10^8 CFU

Table 1. Physico-chemical properties of soils at IARI, New Delhi and ICRISAT, Patancheru

Soil properties	IARI, New Delhi	ICRISAT, Patancheru
pH	8.5	8.1
EC (dSm^{-1})	0.70	1.44
Organic carbon (%)	0.5	0.5
Available N (kg/ha)	60.2	24.7
Available –P (kg/ha)	12.0	8.6
Soil type	Sandy-loam	Vertisols
Annual rainfall (mm)	650%720	700%800

g^{-1}) as per the standard protocols and sown immediately in rows 30 cm apart at a depth of 4-5 cm to achieve an estimated plant population of at least 25 plants m^{-2}. No pesticide was sprayed during the cropping period, as no serious insect pest attacks or phytopathogens were observed. Weeding was done 20 days after sowing. At vegetative stage, 35% 40 days after sowing (DAS), observations on the nodule number, nodule weight, shoot weight and root weight were recorded. At flower initiation stage, 60% 75 DAS, observations were made on shoot weight, N uptake and P uptake. The yield attributes at IARI, New Delhi could not be recorded due to hostile weather conditions during reproductive stage. The experiment at ICRISAT, Patancheru was harvested manually in February 2015 and observations on shoot weight, pod weight, pod number, 100 seed weight, stover yield and grain yield were noted. Total N and P uptake from the shoot samples were also analyzed as per the protocols of [7] and [8], respectively. The data recorded on various parameters were subjected to analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) to assess the significance of the treatments. Each treatment was replicated thrice and mean values were separated according to LSD test at $P < 0.05$.

RESULTS AND DISCUSSION

Low P availability negatively affects not only above- and below-ground plant growth but also SNF [9]. It is a well-known fact that sufficient numbers of natural compatible rhizobia are also essential to improve SNF. Further, it is widely known that the host cultivars also vary in their potential for SNF. Hence, the current investigation was aimed to evaluate the interactive effect of P fertilization and rhizobial inoculation on symbiotic potential of three chickpea cultivars under two different agro-climatic regions of India.

In the present study, the physiochemical properties of soils collected from the experimental fields of IARI as well as ICRISAT showed that not only the soil types are different (sandy-loam for IARI and Vertisols for ICRISAT) but also pH, EC, organic carbon, available N and available P of soils and rainfall pattern (Table 1). Under such different conditions, selection of the chickpea cultivars and rhizobia that enhances nodulation potential has major implications on SNF in chickpea.

At vegetative stage, inoculation of rhizobia either alone (T_2) or combined with application of DAP (T_3) significantly improved both number (up to 57% for IARI and 100% for ICRISAT, respectively) and weight (up to 54% for IARI and 100% for ICRISAT, respectively) of nodules in chickpea. Application of chemical fertilizer alone (T_1-only DAP) or absolute control (T_4) recorded significantly lower nodule number $plant^{-1}$ and nodule weight $plant^{-1}$ at both IARI and ICRISAT locations. At IARI, among the cultivars studied, the local best cultivar (PUSA 372) recorded better nodulation potential. More numbers of nodules $plant^{-1}$ were observed in the cultivar PUSA 372 with inoculation (C_3T_2 and C_3T_3), whereas interactive effect was not significant at ICRISAT. Similarly, the nodule weight was observed to be higher in PUSA 372 with rhizobial inoculation (C_3T_2 and C_3T_3) at IARI, and cultivar Shubhra with rhizobial inoculation at ICRISAT. Between the two P use efficient cultivars, Shubhra performed better at IARI, New Delhi conditions, whereas JAKI 9218 performed better at ICRISAT, Patancheru. ICRISAT soils contained lesser nodules as compared to IARI soils; this was true in all the treatments including both controls. Among the three chickpea cultivars used in this study, Subhra was found to be more compatible to IC-59 and IC-76 strains of rhizobia than the other two cultivars, JAKI 9218 and PUSA 372/JG 11 and this was found true at both the locations (Table 2). The shoot and root weight of chickpea was also showed similar trend at vegetative stage at both the locations. Rhizobia inoculation significantly improved shoot weight (T_2 and T_3). Root weight was higher in T_3 at IARI and non-significant at ICRISAT. Total plant weight (shoot and root) was higher in PUSA 372 (local best) at IARI, whereas the cultivar Shubhra performed better under ICRISAT conditions. At IARI, the interactive study showed better performance with the local best cultivar (PUSA 372) when inoculated with rhizobia and DAP (T_3), followed by rhizobia alone in PUSA 372 (T_2). However, interactive effect of cultivar and treatment was non-significant at ICRISAT (Table 3).

Table 2. Response of chickpea cultivars to rhizobial inoculation and P fertilization on nodulation potential at vegetative stage

	Nodule number (plant^{-1})								Nodule weight (mg plant^{-1})							
	IARI				ICRISAT				IARI				ICRISAT			
Treatment	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean
T_1	6.33^{d}	4.67^{d}	6.67^{d}	5.89^{c}	0.17	0.17	0.17	0.17^{c}	17.00^{g}	22.67^{ef}	25.67^{e}	21.78^{d}	2.0^{g}	1.0^{h}	1.0^{h}	1.33^{d}
T_2	7.67^{d}	11.33^{cd}	29.67^{a}	16.22^{a}	3.50	3.20	1.83	2.84^{b}	25.33^{e}	41.33^{b}	56.00^{a}	40.89^{a}	14.3^{e}	24.7^{a}	16.3^{d}	18.43^{b}
T_3	8.00^{d}	15.33^{bc}	20.67^{b}	14.67^{a}	6.33	3.30	7.40	5.68^{a}	21.67^{f}	36.67^{c}	52.67^{a}	37.00^{b}	20.0^{c}	14.3^{e}	23.0^{b}	19.10^{a}
T_4	5.33^{d}	6.67^{d}	20.33^{b}	20.78^{b}	1.23	0.00	0.30	0.51^{c}	19.67^{fg}	29.33^{d}	26.00^{de}	25.00^{c}	4.0^{f}	0.0^{i}	1.0^{h}	1.67^{c}
Mean	6.83^{b}	9.50^{ba}	19.34^{a}		2.81	1.67	2.43		20.92^{c}	32.50^{b}	40.09^{a}		10.08^{ab}	10.00^{b}	10.33^{a}	
	C	3.23			NS				1.69				0.28			
CD	T	3.73			1.62				1.95				0.32			
(p=0.05)																
	CxT	6.47			NS				3.37				0.56			

T_1 - Positive control (100 % DAP); T_2 - Rhizobia (IC-76+IC-59); T_3 –Rhizobia(IC-76+IC-59) + DAP; T_4 -Absolute control (No rhizobia and DAP). C_1- JAKI 9218; C_2- Shubhra; C_3- PUSA 372 at IARI; JG 11 at ICRISAT

Table 3. Response of chickpea cultivars to rhizobial inoculation and P fertilization on plant growth at vegetative stage

	Shoot dry weight (mg plant^{-1})								Root dry weight (mg plant^{-1})							
	IARI				ICRISAT				IARI				ICRISAT			
Treatment	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean
T_1	280.0^{h}	918.3^{d}	1020.0^{c}	739.4^{d}	1583.3^{e}	2483.3^{bc}	2300.0^{cd}	2122.0^{a}	40.0^{h}	167.7^{abc}	163.3^{bc}	123.3^{b}	150.0	214.7	198.3	187.7
T_2	656.7^{f}	1083.3^{b}	1091.7^{b}	943.9^{a}	1683.3^{e}	2700.0^{b}	2183.3^{d}	2188.7^{a}	88.3^{fg}	171.7^{abc}	176.7^{ab}	145.7^{a}	183.0	240.0	151.0	191.3
T_3	456.7^{g}	855.0^{e}	1330.0^{a}	880.6^{b}	1617.7^{e}	2900.0^{a}	2150.0^{d}	2222.3^{a}	61.7^{gh}	108.3^{ef}	191.7^{a}	120.7^{b}	163.3	252.3	205.0	206.7
T_4	501.7^{g}	915.0^{d}	1105.0^{b}	840.6^{c}	1467.7^{e}	2067.7^{d}	2116.7^{d}	1883.7^{b}	76.7^{g}	130.0^{de}	148.3^{cd}	118.3^{b}	188.0	202.3	203.7	198.0
Mean	473.8^{c}	942.9^{b}	1136.7^{a}		1587.5^{c}	2537.5^{a}	2187.5^{b}		66.7^{c}	144.3^{b}	170.0^{a}		171.0^{b}	227.2^{a}	189.5^{b}	
	C	23.83			118.33				12.77				27.81			
CD	T	27.52			136.63				14.75				NS			
(p=0.05)																
	CxT	47.66			236.66				25.55				NS			

T_1 - Positive control (100 % DAP); T_2 - Rhizobia (IC-76+IC-59); T_3 - Rhizobia (IC-76+IC-59) + DAP; T_4 -Absolute control (No rhizobia and DAP). C_1- JAKI 9218; C_2- Shubhra; C_3- PUSA 372 at IARI; JG 11 at ICRISAT

At flower initiation stage, the shoot dry weight was found higher in treatment involving rhizobial inoculation at both locations. The cultivar, Shubhra recorded higher shoot dry weight at both locations. The cultivar× inoculation treatment interaction was non-significant at IARI, whereas, rhizobial inoculation with the cultivar Shubhra was found to be superior for this trait at ICRISAT (Table 4). Similar trend was observed for nutrient uptake (both N and P uptake) at IARI. N uptake (39.54 mg/plant) was found higher with the cultivar JG11 at ICRISAT; whereas, the effect of inoculation on N uptake was insignificant. Rhizobial inoculation and addition of DAP significantly improved P uptake (up to 37%) in all three cultivars at IARI, whereas, it was only for JAKI 9218 (up to 15%) at ICRISAT (Table 5). Hence, it can be concluded that seed inoculation with rhizobia has positive effect on P uptake.

At crop maturity stage, data were collected only at ICRISAT location as it was not possible at IARI due to hostile weather conditions. At ICRISAT location, T_3 (rhizobial inoculation and addition of DAP)significantly enhanced shoot weight (up to 25%), pod weight (up to 37%), pod number (up to 16%), grain yield (up to 25%), stover yield(up to 8%) and P uptake(up to 10%) over $T_4$the absolute control. Among the cultivars, Shubhra recorded higher yield (1772 kg ha^{-1}), followed by JAKI 9218 (1622 kg ha^{-1}) and JG 11 (1599 kg^{-1}), whereas P uptake was found high in Shubhra (2.3 mg plant^{-1}) (Tables 6-7).

P deficiency limits SNF, since it has been shown to have a strong impact on the growth and survival of rhizobia as well as host plant [10]. In the present study, application of P and rhizobial inoculation has been shown to affect SNF positively in chickpea at both IARI and ICRISAT locations. Further, rhizobia in association with P have also shown to enhance plant growth and grain yields in chickpea. Application of PGP bacteria in the rhizosphere is known to enhance root and shoot growth, root hair development, nitrogen fixation, grain yield, stover yield, plant hormone regulation, solubilization of minerals and the suppression of pathogens in crops, including pea, soybean and chickpea [11-17]. However, reports on association of rhizobia with P are very limited. The identification of chickpea cultivars with contrasting utilization of P for SNF can provide excellent models to study the molecular mechanisms underlying N_2 fixation impairment under P deficiency. The results from the current study will certainly make significant advances beyond the current state of the art.

Table 4. Response of chickpea cultivars to rhizobial inoculation and P fertilization on shoot weight (g plant^{-1}) at flower initiation stage

	IARI				ICRISAT			
Treatment	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean
T_1	1.97	1.98	1.74	1.90^{a}	10.45^{gh}	11.61^{efg}	13.70^{c}	11.92^{b}
T_2	1.78	1.81	1.69	1.76^{b}	10.97^{gh}	16.80^{a}	12.54^{de}	13.44^{a}
T_3	1.97	1.97	1.86	1.93^{a}	11.53^{efg}	15.58^{b}	13.03^{cd}	13.38^{a}
T_4	1.62	1.95	1.62	1.73^{b}	10.09^{h}	11.22^{fgh}	12.23^{def}	11.18^{c}
Mean	1.84^{a}	1.93^{a}	1.73^{b}		10.76^{c}	13.80^{a}	12.88^{b}	
	C	0.10			0.54			
CD	T	0.12			0.63			
(p=0.05)								
	CxT	NS			1.08			

T_1 - Positive control (100 % DAP); T_2 - Rhizobia (IC-76+IC-59); T_3 - Rhizobia (IC-76+IC-59) + DAP; T_4 -Absolute control (No rhizobia and DAP). C_1- JAKI 9218; C_2- Shubhra; C_3- PUSA 372 at IARI; JG 11 at ICRISAT

Table 5. Response of chickpea cultivars to rhizobial inoculation and P fertilization on plant nutrition at flower initiation stage

	N Uptake (mg plant^{-1})								P Uptake (mg plant^{-1})							
	IARI				ICRISAT				IARI				ICRISAT			
Treatment	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean
T_1	50.75	43.72	40.92	45.13^a	37.89^b	38.00^b	39.62^a	38.50	1.44	1.43	1.30	1.39^b	2.07^{ab}	2.01^{bc}	2.05^{abc}	2.04^a
T_2	44.53	37.83	36.44	39.60^b	40.08^a	37.09^{bc}	38.28^b	38.48	1.24	1.08	1.02	1.11^c	2.11^a	2.02^{bc}	1.97^c	2.03^a
T_3	50.85	43.30	43.30	45.82^a	40.69^a	36.09^c	40.21^a	39.00	1.62	1.44	1.48	1.51^a	2.11^a	1.81^d	2.09^{ab}	2.00^a
T_4	40.33	39.04	32.73	37.37^b	36.59^c	39.50^a	40.06^a	38.72	1.13	1.16	0.94	1.08^c	1.80^d	1.98^c	2.02^{bc}	1.93^b
Mean	46.62^a	40.97^b	38.35^b		38.81^b	37.67^c	39.54^a		1.36^a	1.28^a	1.19^b		2.02^a	1.96^b	2.03^a	
	C			2.67					0.59				0.08	0.04		
CD	T	3.09		NS					0.09				0.04			
(p=0.05)																
	C x T	NS		1.19					NS				0.08			

T_1 - Positive control (100 % DAP); T_2 - Rhizobia (IC-76+IC-59); T_3 - Rhizobia (IC-76+IC-59) + DAP; T_4 -Absolute control (No rhizobia and DAP). C_1- JAKI 9218; C_2- Shubhra; C_3- PUSA 372 at IARI; JG 11 at ICRISAT

Table 6. Response of chickpea cultivars to rhizobial inoculation and P fertilization at harvest stage at ICRISAT, Patancheru

	Shoot weight (g plant^{-1})				Pod weight (g plant^{-1})				Pod number (plant^{-1})				100 seed weight (g)			
Treatment	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean
T_1	5.94	7.86	4.92	6.42	14.27	15.10	14.37	14.58	49.0	49.4	50.4	49.6	22.7	27.4	22.4	24.2
T_2	5.67	6.78	5.28	5.91	13.21	15.67	12.85	13.91	49.4	48.8	47.3	48.5	21.6	27.4	22.0	23.7
T_3	5.66	9.13	5.44	6.57	16.77	18.30	16.44	17.17	52.1	49.4	53.1	51.6	22.7	27.5	22.5	24.2
T_4	4.72	6.89	5.11	5.57	10.58	15.26	15.04	13.63	43.9	47.5	50.6	47.3	21.5	27.7	22.3	23.8
Mean	5.50	7.66	5.19		13.71	16.08	14.68		48.6	48.8	50.3		22.1	27.5	22.3	
	C	0.44			1.06				1.43				0.19			
CD	T	0.75			1.50				1.78				0.41			
(p=0.05)																
	C xT	0.96			2.12				2.73				0.47			

T_1 - Positive control (100 % DAP); T_2 - Rhizobia (IC-76+IC-59); T_3 - Rhizobia (IC-76+IC-59) + DAP; T_4 -Absolute control (No rhizobia and DAP). C_1- JAKI 9218; C_2- Shubhra; C_3- PUSA 372 at IARI; JG 11 at ICRISAT

Table 7. Response of chickpea cultivars to rhizobial inoculation and P fertilization on yield traits and plant nutrition at harvest stage at ICRISAT, Patancheru

Treatment	Grain yield (kg ha^{-1})				Stover yield (kg ha^{-1})				N Uptake (mg plant^{-1})				P Uptake (mg plant^{-1})			
	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean
T_1	1331^d	1837^a	1536^c	1568^a	1670	1772	1488	1643		52.11^a	48.69^{cd}	51.93^a	50.90^a	2.22^{cd}	2.30^{bc}	2.23^{cd}
2.30^a																
T_2	1224^d	1754^a	1345^d	1440^b	1674		1687		1441		1601	47.18^e	51.71^a	50.43^b	49.80^b	2.14^{ef}
2.39^a	2.23^{cd}	2.30^a														
T_3	1622^{bc}	1772^{ab}	1599^{bc}	1664^a	1805	1779	1505	1696	45.23^f	49.07^c	48.11^d	47.50^c	2.18d^e	2.31^b	2.12^{ef}	2.20^b
T_4	1212^d	1740^{ab}	1325^d	1426^b	1670	1685	1427	1594	46.99^e	46.38^e	49.35^c	47.60^c	2.04^g	2.08^{fg}	2.10^{fg}	2.10^c
Mean	1347^c	1776^a	1451^b		1705	1731	1465		47.90^c	49.00^b	50.00^a		2.20^b	2.30^a	2.20^b	
	C	90.57			25.12				0.44				0.04			
CD	T	104.58			47.08				0.50				0.04			
(p=0.05)																
	C xT	181.15			57.43				0.87				0.08			

T_1 - Positive control (100 % DAP); T_2 - Rhizobia (IC-76+IC-59); T_3 - Rhizobia (IC-76+IC-59) + DAP; T_4 -Absolute control (No rhizobia and DAP). C_1- JAKI 9218; C_2- Shubhra; C_3- PUSA 372 at IARI; JG 11 at ICRISAT

ACKNOWLEDGEMENTS

ICRISAT is a member of CGIAR Consortium and this work has been undertaken as part of the CGIAR Research Program on Grain Legumes. We also thank Mr. PVS Prasad for his significant contribution in the laboratory and field studies.

REFERENCES

1. ENGELSTAD OP AND GL TERMAN (1980). Agronomic effectiveness of phosphate fertilizers. In: Khasawneh FE, Sample E and Kamprath, E. (Eds.). The role of phosphorus in agriculture. Madison, WI. 311-322, American Society of Agronomy.

2. DIVITO GA AND V SADRAS (2014). How do phosphorus, potassium and sulphur affect plant growth and biological nitrogen fixation in crop and pasture legumes? A meta-analysis. *Field Crops Research*, **156**: 161-171.

3. AE N, J ARIHARA AND K OKADA (1991). Phosphorus response of chickpea and evaluation of phosphorus availability in Indian Alfisols and Vertisols. In: Johansen C *et al* (Eds.), Phosphorus nutrition of grain legumes in the semi-arid tropics, ICRISAT, Patancheru, India, 33-44.

4. FAOSTAT (2017). Statistical Database 2017. Available at)#http://faostat.fao.org*#.

5. GOPALAKRISHNAN S, V SRINIVAS AND S SAMINENI (2017). Nitrogen fixation, plant growth and yield enhancements by diazotrophic growth-promoting bacteria in two cultivars of chickpea (*Cicer arietinum* L.). *Biocatalysis and Agricultural Biotechnology*, **11**: 116-123.

6. SWARNALAKSHMI K, S SINGH AND AK SAXENA (2014). Root exudate profiling of chickpea cultivars exhibiting differential nodulation potential. Proceedings of national Conference on "Empowering mankind with Microbial Technologies" Annual Conference of AMI, TNAU, Coimbatore. AM S4: 1-3.

7. NOVOZAMSKY I, VJG HOUBA, ECKR VAN AND W VANVARK (1983).A novel digestion technique for multiple element analysis. *Communications in Soil Science and Plant Analysis,* **14**: 239-249.

8. OLSEN SR AND LE SOMMERS (1982). Phosphorus. In: Methods of soil analysis, Agron No 9, Part 2, 'chemical and microbial properties', 2nd edition, Am. Soc. Agron. Page AL. (ed.), Madison WI, USA, 403-430.

9. SANZ-SAEZ A, F MORALES, C ARRESE-IGOR AND I ARANJUELO (2017). P deficiency: A major

limiting factor for rhizobial symbiosis. In: Legume nitrogen fixation in soils with low phosphorous availability, Sulieman S and SP Tran (eds.), 21-39, Springer International Publishing AG.

10. MARTINS D, A MACOVEI, P LEONETTI, A BALESTRAZZI AND S ARAUJO (2017). The influence of phosphate deficiency on legume symbiotic N_2 fixation. In: Legume nitrogen fixation in soils with low phosphorous availability, Sulieman S and SP Tran (Eds.), 41-75, Springer International Publishing AG.

11. TOKALA RK, JL STRAP, CM JUNG, DL CRAWFORD, MH SALOVE, LA DEOBALD, JF BAILEY AND MJ MORRA (2002). Novel plant-microbe rhizosphere interaction involving *Streptomyces lydicus* WYEC108 and the pea plant (*Pisum sativum*). *Applied and Environmental Microbiology*, **68**: 2161-2171.

12. LUCAS JA, BR SOLANO, F MONTES, J OJEDA, M MEGIAS AND FJG MANERO (2009). Use of two PGPR strains in the integrated management of blast disease in rice (*Oryza sativa*) in Southern Spain. *Field Crops Research,* **114**: 404-410.

13. MINORSKY PV (2008). On the inside. *Plant Physiology*, **146**: 323-324.

14. RICHARDSON AE, JM BAREA, AM MCNEILL AND CP COMBARET (2009). Acquisition of P and N_2 in the rhizosphere and plant growth-promotion by microorganisms. *Plant Soil*, **321**: 305-339.

15. SOE KM, A BHROMSIRI AND D KARLADEE (2010). Effects of selected endophytic actinomycetes (*Streptomyces* sp.) and Bradyrhizobia from Myanmar on growth, nodulation, nitrogen fixation and yield of different soybean varieties. *Chiang Mai University Journal of Natural Sciences,* **9**: 95-109.

16. GOPALAKRISHNAN S, V SRINIVAS, G ALEKHYA, B PRAKASH B, H KUDAPA AND RK VARSHNEY (2015). Evaluation of *Streptomyces* sp. obtained from herbal vermicompost for broad spectrum of plant growth-promoting activities in chickpea. *Organic Agriculture,* **5**: 123-133.

17. GOPALAKRISHNAN S, V SRINIVAS, S SRINIVASAN AND CV SAMEER KUMAR (2016). Plant growth-promotion and biofortification of chickpea and pigeonpea through inoculation of biocontrol potential bacteria, isolated from organic soils. *SpringerPlus,* **5**: 1882.