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Research Paper

Synergistic interaction of *Piriformospora indica* and microbial inoculants on symbiotic potential, plant nutrition and productivity of chickpea (*Cicer arietinum*)

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ABSTRACT

The present study was carried out during the winter season of 2011–12 and 2012–13 at Indian Agricultural Research Institute, New Delhi, to evaluate the efficacy of microbial bioinoculants in chickpea (*Cicer arietinum* L.) using combinatorial approach. A group of microbial inoculants (*Mesorhizobium ciceri*). Phosphate-solubilizing microorganisms and plant growth-promoting rhizobacteria) in presence and absence of P fertilizer was used to testify its synergistic effect with *Piriformospora indica* on symbiotic traits, growth and yield of chickpea under field conditions. Significant differences were observed with respect to symbiotic potential and other associated parameters with combined inoculation. Inoculation with *Mesorhizobium ciceri* enhanced nitrogenase activity and leghemoglobin content significantly over un-inoculated control. Significant improvement in N and P nutrition with combined inoculation amended with 30 kg P/ha emphasized the beneficial effect of these and similar trend was reflected in productivity enhancement as well.

Key words : Chickpea, Leghemoglobin, *Mesorhizobium ciceri,* Nitrogenase activity, Nutrient availability, *Piriformospora indica*

In India, chickpea is an important cool season leguminous crop, mainly cultivated under rainfed conditions with minimum chemical inputs. Nitrogen requirement for the chickpea growth is gained from symbiotic association with *Mesorhizobium ciceri* (Nour *et al.,* 1994). An adequate supply of phosphorus (P) is important for symbiotic nitrogen fixation (SNF). Since Indian soils contain low to medium soil P fertility, application of P is recommended for optimum plant growth. However, P-use efficiency of applied phosphatic fertilizers ranges from 10 to 25% as the applied phosphorus is quickly fixed in soil. High P-fixation capacity of soil and low P availability can affect the plant growth and nodule function. On the other hand, availability of non-renewable mineral resource of P is also rapidly depleting worldwide (Vance *et al*., 2003). Therefore, to boost the plant productivity under available soil P conditions, efforts were made to integrate microbial inoculants particularly phosphate- solubilizing microorganisms (PSMs) as a sole source or supplements to chemical P fertilizer. Inoculation with PSMs, viz. *Pseudomonas, Enterobacter, Bacillus, Penicillium, Aspergillus* and *Trichoderma,* are reported to reverse the P-fixation particularly when the crop is grown with marginal chemical fertilizers. Seed application of PSMs promoted *Rhizobium*-legume symbiosis (Rosas *et al*., 2006). These PSMs are capable of solubilizing mineral P by organic acid secretion (Sagoe *et al*., 1998). Phosphatase and phytase activity of these organisms also stimulate P mineralization and improve plant P uptake. Besides these, arbuscular mycorrhiza fungi (AMF) can also influence plant P content by absorbing and transferring P from root-depletion zone (Smith and Smith, 1990). The AMF colonize the roots of many plants species and enhance the availability of nutrients including P, Cu^{2+} and Zn^{2+}. P influx in mycorrhizal roots is 3-5 folds higher than non-mycorrhizal roots (Smith and Read, 1999). The plant growth promoting rhizobacteria (PGPR) including *Arthrobacter, Azotobacter, Azospirillium, Bacillus, Flavobacterium,* and *Pseudomonas* can also improve the plant growth by nitrogen fixation, P solubilization, phytohormone production and disease suppression (Bashan and de-Bashan, 2010). The synergistic interaction of *Rhizobium* and PGPR could stimu-

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late the common bean growth under low P conditions (Korir *et al*., 2017).

In recent years, a great attention has been given to the cultivable endophytic fungus *Piriformospora indica* for improvement in P nutrition. In contrast to AM fungi which are obligate biotrop and uncultivable in nature, *P. indica* can be axenically grown as pure culture. This fungus also improves plant growth and health by P mobilization, phytohormone production and N_2 fixation. This also confers tolerance against biotic and abiotic stresses and can suppress soil borne pathogens (Franken, 2012). Hence the deployment of microbial inoculants for improved chickpea production has been a focus of research for a number of years. However, microbe-mediated plant growth promotion approach generally involves the use of single or dual microbial inoculants. In our study, the crop-specific *Mesorhizobium* supplemented with different microbial inoculants belonging to functionally diverse groups was used for improving PGPR efficiency as the application of a mixture of microbial inoculants closely resembles the natural situation and can promote plant growth through combination of different PGP mechanisms**.** The study was undertaken to evaluate the effect of combined inoculation of *P. indica* with triple inoculants (*Mesorhizobium,* PSM and PGPR) on symbiotic traits, nutrient uptake and yield of chickpea under field conditions.

MATERIALS AND METHODS

Field Experiment

A field experiment was conducted during the winter (*rabi*) seasons of 2011–12 and 2012–13 at Indian Agricultural Research Institute Field, New Delhi to evaluate the synergistic effect of *Piriformospora indica* and three microbial inoculants, namely *Mesorhizobium ciceri* – (CH 1233), *Trichoderma* sp. (PSM), *Pseudomonas* sp. (PGPR), on growth, nodulation and nutrient (nitrogen and phosphorus) uptake in chickpea variety 'Pusa 1103'. The experiment was laid in randomized block design with 3 replications. The soil samples (0–15 cm depth) were taken at the beginning of the experiment. The soil was sandy-loam, with *p*H 8.75, organic carbon 0.6% and electrical conductivity (EC) 0.75-dS/m. The alkaline $KMnO_4$-oxidizable-N was 65.8 kg/ha and 0.5 M $NaHCO_3$-extractable-P was 20 kg/ha. The previous crop in the experimental site was summer mungbean followed by fallow soil during the *kharif* season. The experiment comprised 7 treatments with the plot size of 10 m^2. The plots with application of 100% recommended dose of fertilizer (RDF, 100 kg DAP/ha-contains 18 : 46 kg N and P_2O_5 without inoculants served as a positive control (T_1). Inoculation with *Mesorhizobium ciceri* (T_2) alone was added to compare the treatment effect on symbiotic potential. Treatment (T_3) involving triple inoculants consisting of *M. ciceri* + PSM + PGPR were used to compare the interactive effect of mixed inoculants. Synergistic effect of *Piriformospora indica* with triple inoculants was compared under P-deficient (without rock phosphate - T_4, T_5) and P-sufficient (30 kg P_2O_5/ha applied in the form of rock phosphate – T_6, T_7) conditions. Details of treatments are given below and the seed inoculation was performed as per the method described earlier (Swarnalakshmi *et al*., 2011): T_1, 100% RDF (positive control); T_2, *Mesorhizobium ciceri;* T_3, Triple inoculants (*M. ciceri* + *Trichoderma* sp. + *Pseudomonas* sp.); T_4, *Piriformospora indica;* T_5, *P. indica* + triple inoculants; T_6, *P. indica* + 30 kg P_2O_5; T_7, *P. indica* + triple inoculants + 30 kg P_2O_5.

Chickpea plant samples were collected at initiation of flowering stage (75 days after sowing) and before harvesting. Symbiotic potential was studied in terms of nitrogenase activity and leghemoglobin content in nodules at 75 days after sowing (DAS). Chlorophyll, N and P content of plant samples were also simultaneously estimated. Biomass and seed yield were recorded at harvesting stage of the crop.

Nitrogenase activity

Nodule nitrogenase activity was estimated by acetylene reduction assay (ARA, Hardy *et al.,* 1973). The excised root-systems bearing nodules were placed in 50 ml glass vials and capped with a suba seal. Acetylene (10%) was injected after ejecting equal amount of air and samples were incubated for 30 min. One ml of the sample was withdrawn and injected into pre-conditioned Nucon-Model Gas Chromatograph (5500) housing Porapak N stainless steel column and Flame Ionization Detector. The column temperature was maintained at 75°C and injector and detector temperatures were at 110°C. Nitrogen gas with a flow rate of 30 ml/min was used as carrier gas. The rate of ethylene formed was measured and expressed as n moles ethylene/mg nodule fresh weight/hr.

Leghemoglobin (Lb) determination

Fresh nodule tissue (500 mg) was homogenized in 5 ml solution of 0.1 m phosphate buffer (*p*H 7.4) followed by addition of alkaline pyridine reagent as described by Appleby and Bergersen (1980). Tissue lysate was centrifuged at 12,000 *g* for 15 min to sediment the bacteroids and 5 ml of alkaline pyridine reagent was added to the supernatant. This solution was divided into 2 equal portions. To one portion, few crystals of sodium dithionate were added to reduce the formation of ferric haemachrome and the absorbance was read at 559 nm. To the other portion, few crystals of potassium hexacyanoferrate were added to oxidize the hemochrome and absorbance was

read at 539 nm. The difference between the values at 2 absorbance (A_{559}-A_{539}) was calculated and leghaemoglobin content was expressed as µmoles/g nodule fresh weight.

Chlorophyll content

Chlorophyll content was measured by using DMSO method. A known biomass (100 mg) of fresh chickpea leaves was placed in a vial containing 5 ml of DMSO and the pigment was extracted after incubating at 65ºC for an hour. Vials were shaken intermittently to allow the complete extraction of pigment and its uniform distribution. The absorbance was read at 645 nm and 663 nm using a UV-visible spectrophotometer (Hiscox *et al.,* 1979).

Plant nutrient analysis

The plants samples were dried in an oven at 60ºC and powdered for nitrogen and phosphorus analysis. The total N content was analyzed using Kjeldahl digestion unit, while total P was determined using a sulfuric–nitric–perchloric acid digest as per procedures described by Prasad *et al.* (2013).

Statistical analysis

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 and the mean values were separated according to LSD test at P <0.05.

RESULTS AND DISCUSSION

In India, chickpea is normally grown under rainfed conditions with minimum chemical inputs. Symbiotic nitrogen fixation by root nodulating *Mesorhizobium* is a major N_2-fixing system in chickpea and the estimated amount of nitrogen fixed was in the range of 3–121 kg/ha. Phosphorus nutrition of the crop is improved by presence of PSMs and mycorrhizal colonization. Co-inoculation of PGPRs such as *Azotobacter, Bacillus* and *Pseudomonas* stimulates plant growth and nodulation in legumes (Korir *et al*., 2017). The beneficial impact of PGPRs is considered to be a direct plant growth promotion by production of plant growth regulators, nitrogen fixation, enhanced access to soil nutrients and disease control (Bashan and de-Bashan, 2010). In recent times, emphasis was given to use an endophytic fungus *P. indica*, which is known to improve P nutrition and influence crop tolerance to biotic/ abiotic stresses (Franken, 2012). The cumulative effect of *P indica*, *Rhizobium,* PSB and PGPR is greater than single or dual inoculation as the different beneficial microorganisms posses varying metabolic potentials such as N fixation, P solubilization, P mobilization and phytohormone production. In the present study, an attempt was made to testify the synergistic effect of *Piriformospora indica* with microbial inoculants (*Mesorhizobium ciceri* + PSB + PGPR) on symbiotic potential, growth, plant nutrition particularly N and P uptake, and yield of chickpea under field conditions.

Table 1. Synergistic effect of Piriformospora indica and microbial inoculants on chickpea symbiosis and chlorophyll content

Treatment		Winter season (*rabi*) 2011–12			Winter season (*rabi*) 2012–13		
		Nitrogenase activity (nmole/mg nodule fresh weight/hr)	Leghemoglobin content (µmol/g fresh weight nodule)	Chlorophyll content (mg/g fresh weight of leaves)	Nitrogenase activity (nmole/mg nodule fresh weight/hr)	Leghemoglobin content (µmol/g fresh weight nodule)	Chlorophyll content (mg/g fresh weight of leaves)
T_1	100% RDF	1,408.9^{e}	9.66^{d}	2.6^{c}	250.57^{e}	6.40^{b}	1.77^{bc}
T_2	*Mesorhizobium ciceri*	3,247.7^{a} (130.46)	29.59^{a} (206.31)	2.30^{f} (–11.54)	668.59^{a} (166.83)	14.78^{a} (130.94)	1.68^{cd} (–5.08)
T_3	Triple inoculants *(M. ciceri* + *Trichoderma* sp. + *Pseudomonas* sp.)	2,116.5^{b} (50.22)	15.60^{bc} (64.49)	2.44^{d} (–6.15)	540.43^{c} (115.68)	7.11^{b} (11.09)	1.84^{ab} (3.95)
T_4	*Piriformospora indica*	1,689.5^{d} (19.92)	14.32^{c} (48.24)	2.44^{d} (–6.15)	619.62^{ab} (147.28)	7.34^{b} (14.69)	1.48^{d} (–16.38)
T_5	*P. indica* + Triple inoculants	1,775.4^{c} (26.01)	17.54^{b} (81.57)	2.39^{e} (–8.08)	671.37^{a} (167.94)	6.90^{b} (7.81)	1.97^{ab} (11.30)
T_6	*P. indica* + 30 kg P_2O_5	1,591.7^{d} (12.97)	13.39^{c} (38.61)	2.88^{a} (10.77)	382.41^{d} (52.62)	7.15^{b} (11.72)	2.04^{ab} (15.25)
T_7	*P. indica* + Triple inoculants + 30 kg P_2O_5	1,837.9^{c} (30.45)	15.15^{bc} (56.83)	2.67^{b} (2.69)	583.38^{bc} (132.82)	7.41 ^{b}(15.78)	2.16^{a} (22.03)
	SEm±	67.73	1.32	0.03	23.51	0.61	0.14
	CD (P=0.05)	145.27	2.83	0.06	50.42	1.32	0.30

Letters in superscripts indicate mean separation by Duncan's multiple range test (P≤0.05).
Values in parentheses indicate percentage increase over control (T_1)

Symbiotic nitrogen fixation and associated traits

Greater differences in nodule nitrogenase activity were observed among various treatments. The nitrogenase activity was significantly better in *Mesorhizobium*-inoculated nodules, followed by the treatments involving triple inoculants (Table 1). The increase in the nitrogenase activity under treatment with *Mesorhizobium ciceri* (T_2) over the control (100% RDF) was from 130.5% to 166.8% during the second year of experiment and the activity under *P. indica* (T_4 and T_5) inoculation was higher or at par with additional application of P fertilizers (T_6 and T_7). The maximum activity was recorded (3,247 nmole/mg nodule fresh weight/hr) in *Mesorhizobium ciceri* (T_2) treatment, followed by triple inoculants (T_3) and *P. indica* + triple inoculants (T_5 and T_7) during *rabi* season of 2011–12. Similarly, during 2012–13, *Mesorhizobium ciceri* (T_2) treatment recorded higher nodule nitrogenase activity (668.59 nmol/mg nodule fresh weight/hr) which was at par with the treatment involving *P. indica* + microbial inoculants (T_3), emphasizing the positive effect of inoculation in the soil with low available nitrogen (<120 kg/ ha) content. Further, the treatment with triple inoculants (T_3) increased the nodule activity significantly over un-inoculated control, indicating the possible role of PSMs in symbiotic nitrogen fixation. Increased nitrogenase activity in treatment augmented with quadruple inoculants over fertilizer control in both the years emphasized a stimulatory role of *P. indica* and PSMs with N_2-fixing *Mesorhizobium ciceri*. The response of symbiotic traits to additional P was supported by the earlier findings of ATP requirement for nitrogenase activity (Leidi *et al.,* 2000), whereas P deprivation can negatively affect the nodule formation and nodule function by enhanced proton release (Tang *et al.,* 2001). On the other hand, un-inoculated chemical treatment with 100% RDF (T_1) exhibited significant reduction in nitrogenase activity indicating that the application of chemical nitrogen had an inhibitory effect on symbiotic nitrogen fixation as reported earlier (Voisin *et al*., 2002). Nitrogen nutrition can also improve N assimilation which in turn reduces nitrogenase activity or N fixation potential (Neo and Layzell, 1997).

The trend in leghemoglobin content was similar to that of nitrogenase activity (Table 1). It was the highest with *Mesorhizobium ciceri* (T_2) treatment during *rabi* 2011–12 and 2012–13, followed by the treatment involving triple inoculants (T_3) and *P. indica* + triple inoculants (T_7) in the presence of additional P application. The chlorophyll content was more pronounced in treatment, viz. T_6 and T_7. It is interesting to notice that the increase in nitrogenase activity was apparently reflected in terms of leghemoglobin content which is an indicator of nodule effectiveness and a positive correlation was recorded between Lb content and nitrogenase activity. The leghemoglobin which is an oxygen-diffusing protein exclusively localized in the nitrogen-fixing root nodules appears to play a regulatory role in symbiotic nitrogen fixation. It has been proposed that the presence of Lb protects the O_2-labile nitrogenase enzyme by reducing the free oxygen concentration by 7–11 nmol in nodule cytoplasm while maintaining high oxygen flux for respiration to synthesize ATP (Downie, 2005). The decrease in leghemoglobin content is associated with either reduced N fixation or onset of nodule senescence (Dupont *et al.,* 2012). Besides this, auto oxidation of Lb can generate H_2O_2 in nitrogen-fixing root nodules, which, in turn, can impart innate immune response in plants (Neill *et al.,* 2002). Our results corroborated with previous studies indicating that the application of microbial inoculants such as *Rhizobium, Bacillus* and PGPR can enhance chlo-

Table 2. Synergistic effect of *Piriformospora indica* and microbial inoculants on plant nutrition of chickpea

S. No.	Treatment	*Winter season (rabi)* 2011–12		*Winter season (rabi)* 2012–13	
		N uptake (mg/plant)	P uptake (mg/plant)	N uptake (mg/plant)	P uptake (mg/plant)
T_1	100% RDF	42.77^{d}	3.67^{b}	47.97^{d}	4.96^{e}
T_2	*Mesorhizobium ciceri*	48.15^{cd}	3.89^{b}	53.65^{cd}	5.22^{e}
T_3	Triple inoculants *(M. ciceri* + *Trichoderma* sp. + *Pseudomonas* sp.)	75.94^{a}	6.59^{a}	62.63^{b}	6.27^{d}
T_4	*Piriformospora indica*	39.07^{d}	4.17^{b}	56.00^{c}	7.18^{c}
T_5	*P. indica* + Triple inoculants	62.18^{bc}	4.06^{b}	65.93^{b}	7.74^{c}
T_6	*P. indica* + 30 kg P_2O_5	40.58^{d}	7.46^{a}	76.67^{a}	9.45^{b}
T_7	*P. indica* + Triple inoculants + 30 kg P_2O_5	75.12^{a}	6.31^{a}	80.99^{a}	10.25^{a}
	SEm±	6.91	0.63	3.04	0.34
	CD (P=0.05)	14.83	1.36	6.52	0.72

Letters in superscripts indicate mean separation by Duncan's multiple range test (P≤0.05).
Values in parentheses indicate percentage increase over control (T_1)

rophyll content. The increased chlorophyll content may improve light harvesting capacity of plants which in turn will enhance the release of photosynthates through root exudation. The root-derived products can fuction as substrates for establishment of introduced inoculants in the rhizosphere, which, in turn might stimulate plant growth through N fixation, P solubilization and mobilization as well as phytohormone production.

Nutrient contribution and yield

The results exhibited a synergistic effect of 4 inoculants under treatment T_7 – (*P. indica* + *M. ciceri* + PSM + PGPR + 30 kg P_2O_5) on nitrogen and phosphorus status of plants. Nitrogen uptake was significantly higher with application of triple inoculants (T_3) which was at par under combined inoculation with P supplement (T_7) in the first year (Table 2). In the second year, there was an enhanced uptake under T_6 and T_7 followed by treatments having triple/ quadruple inoculants over fertilizer control emphasizing the additional requirement of P for chickpea growth. Reduced N uptake observed under the control treatment (T_1) revealed low fertilizer-use efficiency or insufficient nutrients for optimum crop growth. The alkaline *p*H and low organic matter make Indian soils more susceptible to ammonia volatilization which in turn may reduce N uptake (Prasad *et al.,* 2014), whereas microbial inoculation may result in the continuous supply of available nitrogen in the soil till flowering stage of plants by conserving inorganic soil N (Lupwayi *et al.,* 2006). The results also revealed a positive relationship between nodule dry weight and nutrient uptake during both the years (Fig. 1).

Similarly, higher P uptake in chickpea was observed with combined inoculation along with P input and P uptake was significantly higher in presence of microbial inoculants amended with rockphosphate (T_6 and T_7) followed by the use of triple inoculants (T_5) which was also statistically at par under *P. indica* treatment (T_4) (Table 2). This inoculation effect may be attributed to P solubilization or P mobilization potential of PSM and *P. indica* respectively. Similar findings were reported by Mansotra *et al.* (2015) who observed improved P content with co-inoculation of *Mesorhizobium, P. indica* and PGPR. Application of rockphosphate along with P solubilizer is shown to rapidly increase the available P (Barea *et al.,* 2002). The production of organic acids, viz. citric acids, oxalic acid, gluconic acid by PSM, can decrease the soil *p*H and increase dissolution of insoluble P (Sagoe *et al*., 1998). Our findings are in accordance with the results of Wani *et al.* (2007), who reported about two-fold increase in P uptake and enhanced N uptake with P-solubilizing *Pseudomonas* and *Bacillus* sp. as well as higher grain yield in chickpea. Similarly, combined inoculation of *Mesorhizobium* along with *Pseudomonas* and *P. indica* improved the nutrient uptake and yield of chickpea (Mansotra *et al.,* 2015) in which *P. indica* enhanced the ability of *Mesorhizbium* and PGPR. A large fraction of P in tropical climate is adsorbed on soil minerals and PSM inoculation can increase the solubility of P and its availability in the soil solution which subsequently can enhance the effectiveness of P mobilizers (Smith and Smith, 1990). Similar to mycorrhizal mediated P uptake in plant, application of *P. indica* absorbs the soluble P in soil.

Microbial inoculants improved plant biomass and grain yield significantly at harvest stage (Fig. 2) (Table 3) and no significant differences were observed in terms of 100-seed weight. Biomass and grain yield of chickpea increased significantly under treatments T_6 and T_7 (*P. indica* + 30 kg P_2O_5 and *P. indica* + *M. ciceri* + PSM + PGPR + 30 kg P_2O_5) followed by the treatment involving triple inoculants (T_3). The treatment consisting of P. *indica* + *M. ciceri* + PSM + PGPR + 30 kg P_2O_5 recorded more biomass and grain yield (1,900 and 1,331 kg/ha) over uninoculated control (1,483 and 983 kg/ha). Our results confirm the findings of Valverde *et al.* (2006) where the co-inoculation of *Mesorhizobium* with *Pseudomonas* in-

Table 3. Synergistic effect of *Piriformospora indica* with microbial inoculants on biomass and grain yield of chickpea

S. No.	Treatment /year	2011–12		2012–13	
		Biomass (kg/ha)	Grain yield (kg/ha)	Biomass (kg/ha)	Yield (kg/ha)
T_1	100% RDF	4,650	1,483	3,194	983
T_2	*Mesorhizobium ciceri*	4,800	1,650	3,564	1,006
T_3	Triple inoculants(*M. ciceri* +*Trichoderma* sp. +*Pseudomonas* sp.)	4,817	1,683	3,703	1,018
T_4	*Piriformospora indica*	4,783	1,650	3,703	1,006
T_5	*P. indica* + Triple inoculants	4,883	1,717	3,472	1,018
T_6	*P. indica* + 30 kg P_2O_5	5,117	1,783	4,120	1,307
T_7	*P. indica* + Triple inoculants + 30 kg P_2O_5	5,133	1,900	4,120	1,331
	SEm±	136.9	1,22.8	185.2	48.7
	CD (P=0.05)	292.9	263.3	396.3	104.2

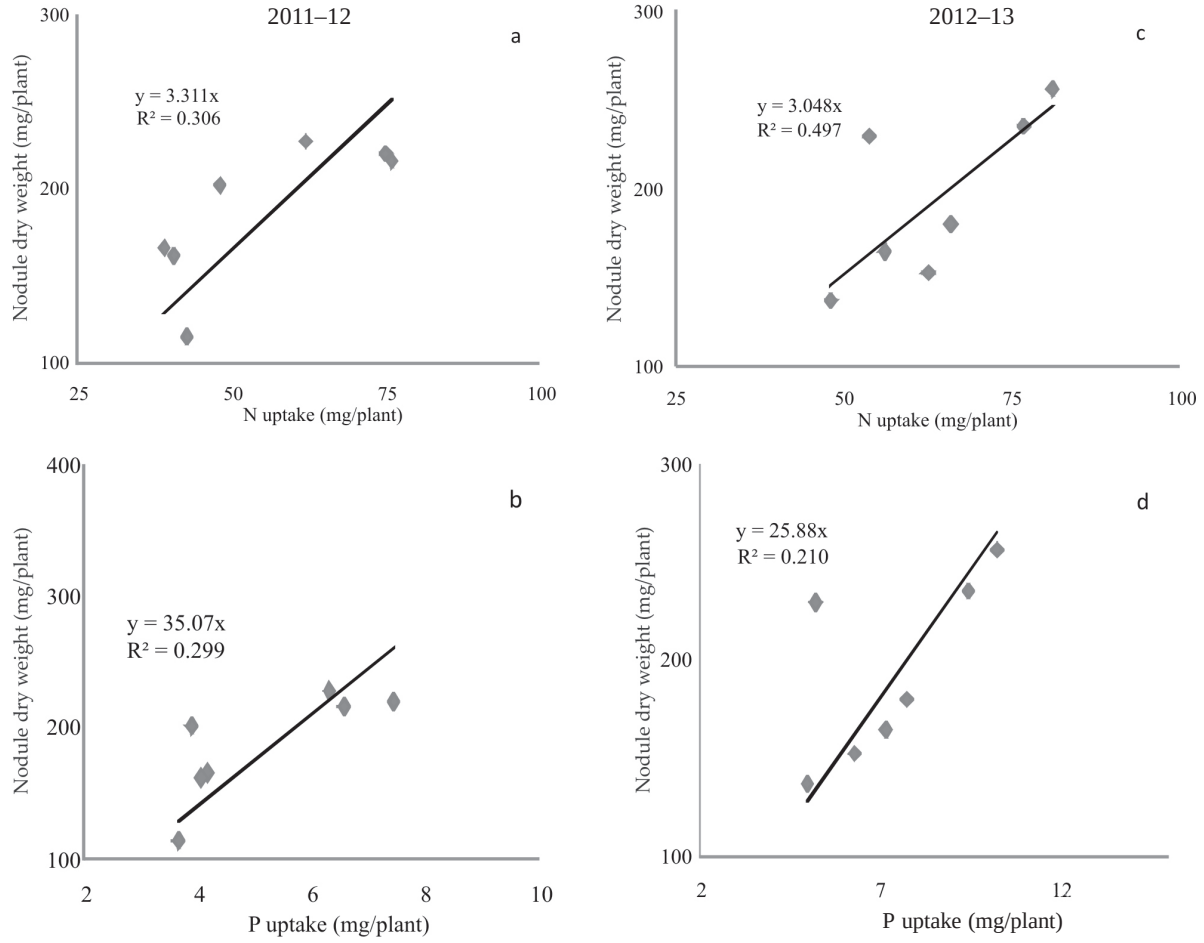

Fig. 1. Relationships between nodule dry weight and nutrient uptake (N and P) in chickpea during two consecutive years (2011–12, 2012–13)

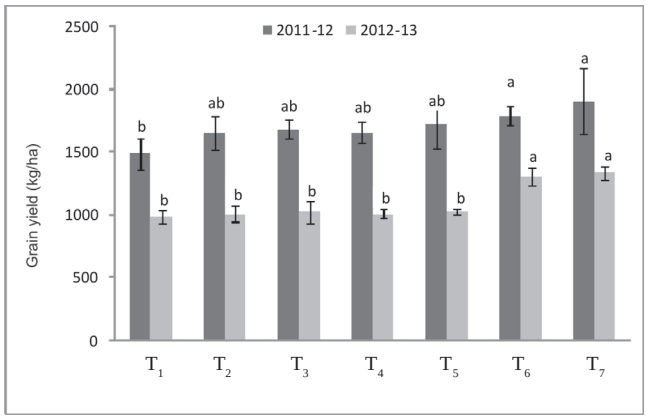

Fig. 2. Synergistic effect of *Piriformospora indica* with microbial inoculants on grain yield of chickpea. [T_1, 100% RDF; T_2, *Mesorhizobium ciceri;* T_3, Triple inoculants *(M. ciceri* + *Trichoderma* sp. + *Pseudomonas* sp.); T_4, *Piriformospora indica;* T_5, *P. indica* + Triple inoculants; T_6, *P. indica* + 30 kg P_2O_5; T_7, *P. indica* + Triple inoculants + 30 kg P_2O_5] *Bars indicate standard error and letters indicate mean separation by Duncan's multiple range test* ($P \leq 0.05$).

fluenced the chickpea growth positively. The crop response to the inoculation has been reported to vary between 14% and 60.3% in soybean (Graham, 1985), which also confirmed our yield data where the mean yield enhancement of 28% and 35% (T_7) over uninoculated control was observed during 2011–12 and 2012–13 respectively. Decrease in yield with 100% RDF (T_1) may be due to unavailability of P due to fixation, whereas under P-sufficient conditions application of P solubilizer/ P mobilizers assured the continuous supply of P to plant growth till harvest stage resulting in improved yield. In the present study, the inoculation with different microbial inoculants with various metabolic potential showed an additive effect on plant growth and nutrient status. The application of microbial inoculants accelerates plant growth and development at early stage which in turn reflects in yield increase at harvest stage.

Overall, synergistic effect of multiple microbial inoculants (*Mesorhizobium,* PSM, PGPR and *P. indica*) on improved symbiotic potential, soil fertility, plant nutrition

and productivity of chickpea was apparently evident and *Mesorhizobium* is known to improve symbiotic nitrogen fixation in chickpea. The PSM and *P. indica* inoculation contributes to the P nutrition, whereas PGPR improves the plant growth by several mechanisms such as N_2 fixation, P solubilization, phytohormone production and antibiosis. These beneficial plant–microbe interactions manifest the nutrient cycle process in the host rhizosphere and improve plant productivity. Enhancement of plant nutrition with combined inoculation indicated a cumulative effect of microbial functional traits in chickpea rhizosphere. The inoculation effect was more pronounced with addition of P fertilizer which signifies the additional requirement of P for chickpea growth under low soil P content. However, the measurement of low critical P requirement of host plant and P- use efficiency can address the role of additional P in plant-improvement activities of chickpea. Further, the survival of microbial inoculants in the rhizosphere during the crop-growth cycle needs to be analysed to understand the functional potential of introduced strains during the crop growth period.

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