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ORIGINAL ARTICLE

Enhanced yield of diverse varieties of chickpea (*Cicer arietinum* L.) by different isolates of *Mesorhizobium ciceri*

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Abstract

In present investigation, six potential candidates of *Mesorhizobium ciceri* were isolated from five different districts of Eastern Uttar Pradesh and were characterized based on biochemical characteristics as well as 16S rDNA sequences. Isolates were analyzed for their multiple plant growth promoting traits, resistance to various environmental stresses such as temperature, pH and salt and were tested individually for growth and yield of three popular varieties of chickpea viz. Avarodi, Uday and PUSA-372, cultivated in the mid-Gangetic region of India. All the isolates exhibited siderophore production and were able to solubilize the inorganic phosphate and zinc. Among total, 50% isolates were found positive to produce ammonia and HCN whereas, IAA production was exhibited in 33.3% isolates. Most of the isolates were found able to tolerate environmental stresses. The growth and yield of Avarodhi and Uday chickpea varieties were found significantly higher when treated with *M. ciceri* strain S3N1 while in variety PUSA-372 it was exhibited when treated with *M. ciceri* strain VAR2.2. Present investigation concluded that a particular *M. ciceri* strain might not be wholly effective for a wide range of chickpea varieties. These strains may be effective bioinoculant for the growth and yield enhancement of chickpea.

Keywords Chickpea · *Mesorhizobium ciceri* · PGPB · Nodulation

Introduction

Chickpea (*Cicer arietinum* L.), is thought to have originated in Anatolia, Turkey (van der Maesen 1984). The earliest record of chickpea in northern India (Uttar Pradesh) is dated at 2000 BC, and much later from south India (Vishnu-Mittre 1974). It is grown in tropical, subtropical and temperate regions and about 90% of the world's chickpea is grown under rain-fed conditions (Kumar and Abbo 2001). It belongs to subfamily Papilionaceae of the family Leguminosae (Fabaceae), with a chromosome number $2n = 16$. Chickpea is an important legume crop, which contributes to about 38% of pulse production in India. India is the largest chickpea producing country with 67.5% of the global chickpea production (Singh et al. 2017a, b). Seeds of chickpea contain 16.4–31.2% protein, 38.1–73.3% carbohydrates, and is a rich source of fiber, vitamins B and C and many important minerals like Ca, P, Mg, Zn, K, Fe (Huda et al. 2003; Ozer et al. 2010).

According to climatic conditions as well as soil texture, different varieties of chickpea are recommended in different geographical locations. Chickpea varieties Avarodhi, Uday (KP-75) and Pusa-372 are more popular and widely cultivated varieties in mid-Gangetic region of Uttar Pradesh (Yadav 2009). The chickpea variety Avarodhi is a high yielding variety (25–30 q/ha) and is resistant to wilt disease [a disease caused by a fungus, *Fusarium oxysporum* f. sp. *ciceris* (Sankar et al. 2018)]. This variety is also recommended and is frequently used in Bihar and northeastern states of India. The Uday variety is suitable for late planting, is moderately resistant to wilt, and specially developed for the soils

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of eastern Uttar Pradesh. The PUSA-372 variety is short durative with low yield potential (18–22 q/h) and is used by the farmers of Uttar Pradesh, Orissa and West Bengal.

In the last decades, crop yield has not increased proportionally with increasing fertilizer inputs, leading to low nutrient efficiency and strong environmental imbalances (Zhang et al. 2009) and chickpea is no exception. To overcome this problem at no expense to soil pollution, there is a need to develop alternative routes of crop production improvement. The plant rhizosphere is the most important habitat where plant–microbe interactions take place. Varieties of microbes, in and around the root, have established a symbiotic, parasitic or neutralistic relationship with plants. Leguminous plants are known to establish symbiotic association with their respective rhizobial strains and form root-nodules.

Many studies have reported increased root and shoot weight, plant vigor, nitrogen fixation and grain yield in various legume crops when inoculated with their respective effective rhizobia (Abdelnaby et al. 2015; Messaoud et al. 2014; Verma et al. 2013; Wolde-meskel et al. 2018). The association between chickpea and *Mesorhizobium* is also known to incur beneficial effects in terms of root nodule formation (Tena et al. 2016), plant growth promotion (Yadav et al. 2018) and such interactions could be exploited for economic gain (Verma et al. 2012, 2013). Chickpea also plays an important role in improving soil fertility by fixation of atmospheric nitrogen in association with *Mesorhizobium*, and fixes up to 141 kg nitrogen per hectare (Rupela 1987).

Mesorhizobium ciceri are specific for chickpea and their performance in terms of plant growth promotion, however, yield improvement also depends on chickpea variety and geographical location. The *M. ciceri* strains exhibit several PGP traits like symbiotic nitrogen fixation (Boddey and Dobereiner 1995), production of phytohormones like indole acetic acid (IAA; Arshad and Frankenberger 1993), solubilization of mineral phosphates and other nutrients (De Freitas et al. 1997; Gaur 1990), production of Fe chelating agent siderophores (Bhagat et al. 2014) and hydrogen cyanide (HCN) production (Singh et al. 2015). Hence, in the present investigation, attempts have been made to isolate and characterize the potential *M. ciceri* isolates and to evaluate the contribution of these strains in terms of plant growth, nodulation and yield of three different chickpea (*Cicer arietinum* L.) varieties viz. Avarodhi, Uday and PUSA-372 in the alluvium of the Mid-Gangatic plains of Uttar Pradesh, India.

Materials and methods

Sample collection

Chickpea crop fields from five districts of Eastern Utter Pardesh viz. Allahabad (25.45°N and 81.84°E), Varanasi (25.28°N and 82.96°E), Deoria (26.67°N and 83.53°E), Mau (25.56°N and 83.33°E) and Kushinagar (26.74°N and 83.89°E), were selected for sampling of root nodules and rhizospheric soil. These sites lie within semiarid or subhumid regions and are characterized by saline and sodic soils. At the early crop stage, chickpea plants were uprooted to collect healthy root nodules and adhered rhizospheric soil for bacterial isolation.

Isolation of bacterial isolates

Root nodules were surface sterilized by treating with $HgCl_2$ (0.1%) for 30 s followed by ethanol washing (95%) for 10 s and finally washing with sterile water. These nodules were crushed with sterile water using a mortar and pestle to obtain nodular exudates. Rhizospheric soil associated bacterial isolates were isolated using a serial dilution method [10^{-6}, (Ben-David and Davidson 2014)]. These bacterial exudates were spread-plated on to Congo-red Yeast Extract Mannitol agar (CRYEMA) medium and subsequently incubated at 28 ± 2 °C for 5 days (Vincent 1970). Repeated sub-culturing using single colonies was done to obtain pure cultures.

Culture, media and growth conditions

Mesorhizobium ciceri isolates were grown on yeast extract mannitol agar (YEMA) medium, maintained by periodic transfer on YEMA media at 28 °C for 48–72 h and stored at 4 °C for further studies. All the media ingredients used in this study were sourced from Hi-Media Lab, Pvt. Ltd., Mumbai, India.

Identification of bacterial isolates

Finally, the bacterial isolates were identified based on colony morphology (Jida and Assefa 2012), growth characterization (Rai et al. 2012) and biochemical tests such as catalase, citrate utilization, Gram's staining, gelatin liquefaction, H_2S production, indole production, oxidase, MR-VP test, starch hydrolysis and urease (Cappuccino and Sherman 1992).

The isolates were also identified using 16S rRNA gene sequence analysis. Pelleted bacteria from Yeast Extract Mannitol (YEM) medium culture were resuspended in distilled water and DNA was isolated with Wizard®DNA Purification Kit (Promega). The universal primer pairs 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-GTT

ACCTTGTTACGACTT-3′) were used for 16S rRNA gene amplification. Amplified PCR products were purified using Nucleopore Quick Gel Recovery Kit (Genetix) and sent for sequencing on an automated sequencer (model ABI 3130xl). The resulting sequences were compared by $BLAST_N$ search against NCBI database to determine the percent similarity. Gene sequence alignment was completed using the ClustalW program (MEGA version 6.0; Tamura et al. 2013). Phylogenetic analyses and the tree topology robustness were completed by bootstrap analysis using 1000 replications of the sequences for the neighbor-joining method. The 16S rRNA gene sequences were deposited in the NCBI database and their accession numbers were obtained.

Physiological and PGP characterization

Isolates were characterized for potent PGP traits like production of ammonia (NH_3; Cappuccino and Sherman 1992), HCN (Bakker and Schipper 1987), IAA (Bano and Musarrat 2003), siderophore (Schwyn and Neilands 1987) and solubilization of minerals; phosphate and zinc (Gaur 1990). Abiotic stress tolerance viz. salt, pH and temperature stress tolerance of the isolates were estimated by growing them on YEMA medium in triplicate with different NaCl concentrations (0.0–5.0% w/v), pH (4.0–10.0) and temperatures (10–40 °C) respectively.

Chickpea varieties

The seeds of three frequently used chickpea varieties in Uttar Pradesh viz. PUSA-372, Avarodhi and Uday were obtained from ICAR-Indian Institute of Seed Science, Mau, Uttar Pradesh, India.

Seed bacterization

Healthy chickpea seeds were surface sterilized with 0.1% mercuric chloride for 2 min and rinsed five times with sterile double distilled water. Sterilized chickpea seeds were inoculated with 5 ml of 4–5 day old broth cultures grown in specific media of inoculants along with 1 ml of 1% (w/v) sticker solution of gum acacia to ensure a bacterial population in the range of 10^3 to 10^4 CFU $seed^{-1}$ per treatment.

Net-house experiment

The pot experiments were conducted at ICAR-NBAIM (National Bureau of Agriculturally Important Microorganism, Mau, UP, India) net-house during 2014–2015. Soil collected from the NBAIM field was slightly alkaline (pH 8.36) containing high amount of organic carbon (6.7), calcium (7.9) and sulphur (8.5). Fresh plastic pots of 30 cm diameter were filled with 5 kg of sterilized soil and sand in 3:1 ratio. Sand was mixed to increase the aeration of the mix which positively affects the growth and yield of crop (Hogg 1976). The pot experiments were conducted with six treatments in three replications. Ten inoculated seeds were sown in each pot. After 1 week of germination, thinning was done resulting in 5 seedlings per pot. Control pots were sown with un-inoculated seeds treated with YEM broth. Pots were arranged in a randomized block design. Pots were irrigated with sterile tap water as needed and the experiment was terminated 120 days after inoculation.

Effect of *M. ciceri* on growth and yield of chickpea

Measurements of plant height, shoot dry weight (SDW), root dry weight (RDW), number of nodules (NN) and nodule dry weight (NDW) were taken from randomly uprooted plants from each pot at 70 days after sowing (DAS). Adhered soil particles at the root surface were washed in running water very gently without the breakage of root or root-nodules. Nodules were detached from plants and kept at 70 °C for 72 h in a hot air oven. The dried plant materials were used for weight measurement. Pod $plant^{-1}$, seed pod^{-1} and seed $plant^{-1}$ were recorded at the time of harvesting. Seeds were weighed after complete drying. Total chlorophyll content was determined in the fresh and fully matured leaves at 30 DAS as per the method described by Arnon (1949).

Statistical analysis

All the necessary parameters were recorded and analyzed statistically. All data was statistically analyzed following the students "T" test technique.

Results and discussion

Agriculture could benefit from symbiotic relationships between microbes and plants by improving production by manipulating the composition of soil microbial communities (Asei et al. 2015; Pérez-Fernández and Alexander 2017). Many plant growth promoting (PGP) bacteria have the capability to induce growth of diverse crops (Mathu et al. 2017; Nieto-Jacobo et al. 2017; Sammauria and Kumawat 2018) either through induction of nitrogen fixation or by the production of different plant hormones (Pérez-Fernández and Alexander 2017; Ulzen et al. 2016). Biological nitrogen fixation is a property of a specific group of prokaryotes known as diazotrophic bacteria with a nitrogenase enzyme complex that converts atmospheric inert nitrogen to plant utilizable ammonia (Araujo et al. 2015). Various plant growth promoting substances are also produced by such diazotrophic bacteria (Malik and Sindhu 2011) which give them potential

to be used as biofertilizers in different crops for sustainable agriculture.

In the present investigation, a total of six *M. ciceri* strains (PHD1 (NCBI accession no KP992877), PHD2 (KP992878), PHD8 (KP992884), PHD11 (KP992887), S3N1 (KM926557) and VAR2.2 (KY515332)) were isolated from five different districts of Uttar Pradesh, India and were analyzed for their PGP traits, resistance against various environmental stresses such as temperature, pH and salt stresses and their yield enhancement abilities against three frequently used chickpea varieties (Avarodhi, Uday and PUSA-372) in Eastern Uttar Pradesh, India.

Soil characteristics

Physicochemical analysis of soil samples (Table 1) revealed that soils were slightly alkaline to alkaline (pH 7.18 ± 0.22–8.22 ± 0.31) with a high concentration of organic carbon (5.08 ± 0.93–6.60 ± 1.00 g/kg of soil), calcium (6.73 ± 0.26–8.36 ± 0.060) and sulfur (8.74 ± 0.71–9.96 ± 1.77) with moisture levels at 8.96 ± 0.89–10.68 ± 1.81%. The soil color varied from grey to pale brown.

Identification of bacterial isolates

None of the isolates changed the color of CRYEMA medium supplemented with CR indicating distinctive rhizobial characteristics. The colonies formed had a circular shape with a continuous margin, whereas, the color varied from watery-to-milky translucent to creamy-to-white opaque. The isolates formed medium to large colonies (1.8–4.2 mm) with copious mucus production under optimum growth conditions. Based on generation time (GT) in YEM broth culture, 84.3% of the isolates were identified as moderately slow growers (GT-4.1–5.4 h) whereas, 15.7% were marked as fast growers (GT-2.8–3.8 h). The rhizobia were rod shaped, Gram negative and exhibited motility.

The phylogenetic assignment was undertaken by Blast analysis of 16S rRNA gene sequences of total six isolates which clearly revealed that all these isolates fell within the genus *Mesorhizobium* sp. *ciceri* with 99–100% sequence similarity (Fig. 1). The phylogenetic reconstruction of 16S

Table 1 Physiochemical properties of soil

Region	pH	Organic carbon (gm/kg of soil)	Calcium (ppm)	Sulfur (%)	Moisture (%)
Allahabad	7.42 ± 0.43	5.66 ± 0.18	8.32 ± 0.89	9.96 ± 1.77	8.96 ± 0.89
Varanasi	7.86 ± 0.33	5.35 ± 1.04	8.36 ± .060	9.11 ± 1.00	10.68 ± 1.81
Kushinagar	7.57 ± 0.45	6.27 ± 0.97	6.73 ± 0.26	8.83 ± 0.93	9.75 ± 0.37
Mau	8.22 ± 0.31	6.60 ± 1.00	8.12 ± 1.33	8.74 ± 0.71	9.38 ± 0.65
Deoria	7.18 ± 0.22	5.08 ± 0.93	8.13 ± 0.97	8.90 ± 0.88	9.25 ± 1.01

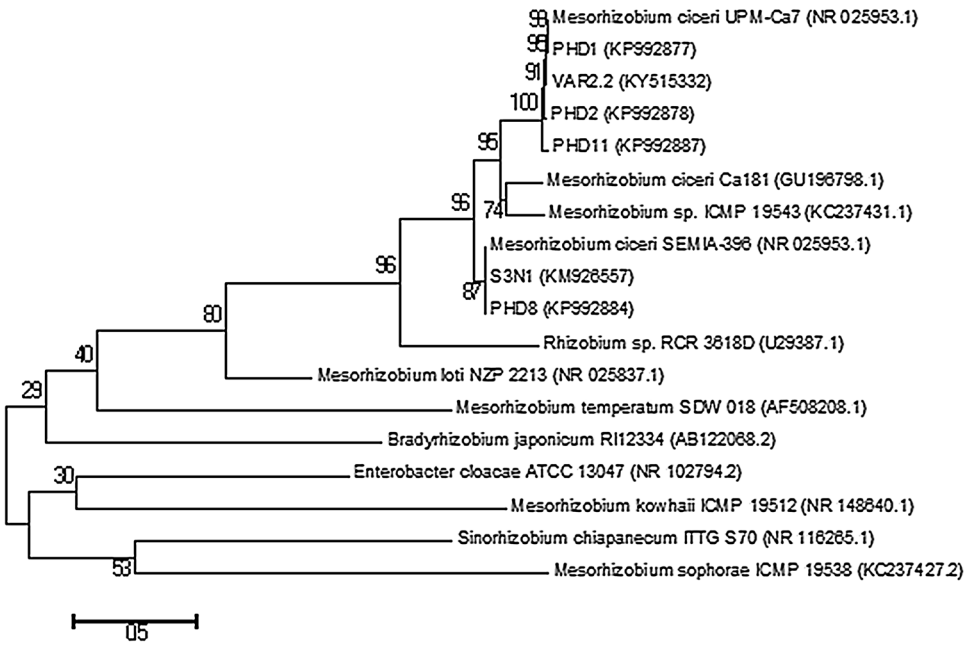

Fig. 1 Phylogenetic tree showing the relationship between *M. ciceri* isolates and related rhizobia based aligned 16S rDNA sequences for structure analysis; bootstrap values derived from 1000 replicates

rRNA gene sequences showed that these isolates were clustered in a common sub-group along with reference strains of *Mesorhizobium* sp. *ciceri* (Fig. 1).

Physiological and PGP characterization

The selected isolates were positive when tested for oxidase, citrate utilization and catalase production, and were negative when tested for the production of indole and H_2S, MR-VP test, urease, starch hydrolysis and gelatin liquefaction. Different isolates showed varied efficiency in siderophore production (among all six isolates, five rhizobia significantly produced siderophores and PHD 8 recorded the maximum solubilization zone (28 mm)), in phosphate solubilization (all isolates significantly produced a solubilization zone and VAR 2.2 recorded the maximum solubilization zone (19 mm)) and in zinc solubilization (all isolates significantly produced a solubilization zone and S3N1 recorded the maximum solubilization zone (23 mm; Table 2). Furthermore, isolates produced different amounts of fungicide agents like HCN and ammonia (NH_3) in in vitro studies. For NH_3 production, among all isolates 3 were positive and S3N1 produced high (+++) amounts whereas PHD8 produced moderate (++) amounts while for HCN production, among all isolates, 3 were positive and S3N1 produced high (+++) amounts, VAR2.2 produced moderate (++) amounts. For IAA production, among all tested isolates, two isolates viz. PHD2 and PHD11 were positive and produced 178.3 and 163.5 ml/mg of IAA, respectively.

The rhizospheric microbiota is a complex biosystem that have several benefits to plant systems including plant health and growth. PGP microbial candidates including those that reside in root nodules are helpful to plants without having any harmful effects on the host, the nearby ecology and the environment. In plants, zinc is one of the key constituents as it performs varied functions in plant metabolism, regulation and developmental processes including floral development, gametogenesis, fertilization and seed development (Kamran et al. 2017; Khande et al. 2017; Mishra et al. 2017). In present investigation, it is clear that chickpea root nodule and rhizosphere dwelling *M. ciceri* are promising for the solubilization of zinc from insoluble zinc compounds. These promising isolates may be useful to make zinc available for plants in the soil system. Phosphorus (P) is a vital component of the nucleic acid structure and plays a major role in the regulation of protein synthesis, growth of new tissue, division of cells, photosynthesis, storage and transfer of energy, respiration and proper development of the roots and hastening of maturity in plants (Muneer and Jeong 2015; Yan et al. 2015). Phosphorus is considered a primary nutrient for plant growth (Hinsinger 2001) and is needed to sustain optimum plant production and quality (Zapata and Zaharah 2002). However, this significant nutrient is largely present in immobilized form in the soil where it is unavailable for plants and, therefore, phosphate solubilizing bacteria play a key role in plant growth through making this available to the plants (Baliah et al. 2016; Bhattacharyya and Jha 2012; Hameeda et al. 2008; Sharma et al. 2013a, b). IAA is reported as one of the most important plant hormones and plays a vital role in signaling and also in the regulation of plant development, cell elongation, cell division and differentiation in plants (Singh et al. 2013).

There are many microbial candidates that cause host plant infection. Therefore, another important role of PGPB is to provide protection to plants against phytopathogens. *M. ciceri* are well documented for both their multiple plant growth promoting activities, attributed to their capacity of stress tolerance and for reduced disease symptoms caused by plant pathogens (Ahmad et al. 2006). Another important trait of PGPB is the production of ammonia. Being a volatile compound, NH_3 is toxic to many pathogenic fungi (Ahmad et al. 2006) and inhibits the germination of spores and mycelium growth of various fungi (Adams et al. 2009), and thus, is indirectly involved in plant growth promotion. NH_3 is also involved in environmental alkalinization and increases the pH of soil (Vylkova 2017); a strategy to conquer pathogens. HCN producer rhizobacteria has been postulated to play a vital role in the biological control of pathogens (Bhattacharyya and Jha 2012; Pandey et al. 2018).

Table 2 Plant growth promoting traits of *M. ciceri* isolates from chickpea root nodule and rhizosphere

Strains	Siderophore zone clearance (mm)	P, solubilization zone clearance (mm)	Zn, solubilization zone clearance (mm)	NH3 production	HCN production	IAA production (mg/ml)
PHD1	15	16	10	Nd	Nd	Nd
PHD11	11	11	17	Nd	Nd	163.5
PHD2	22	16	17	Nd	+	178.3
PHD8	28	6	12	++	Nd	Nd
S3N1	17	16	23	+++	+++	Nd
VAR2.2	≤ 2	19	19	+	++	Nd

Nd not detected

All the *M. ciceri* exhibited growth in response to temperature, salinity and pH stress. Growth under 28 °C was considered as the control. All isolates revealed a broad range of temperature tolerance (10–35 °C). Similarly, growth evaluation of isolates under salt stress was also examined and growth without NaCl was considered as the control. Five isolates viz. PHD1, PHD2, PHD8, S3N1 and VAR2.2 exhibited satisfactory growth in a range of 0.5–2.0% salt concentration whereas PHD11 showed growth up to a concentration of 3.0% NaCl. Likewise, the isolates also exhibited tolerance against slightly acidic and alkaline pH. Growth under pH 7.0 was considered as the control. PHD11, S3N1 and VAR2.2 showed growth across a broad range of pH (4.0–10.0). Whereas other three isolates grew well in pH ranges between 6.0 and 9.0.

Climate change promises to increase the average global temperature. Crop production is largely affected by temperature swings caused by climate change because weather is an essential input into agricultural production. Tolerance to high temperature is a desirable trait for PGPB to improve plant growth in the high temperature environment. Generally, the optimum growth temperature for chickpea rhizobia is 25–30 °C. However, in our study, several *M. ciceri* strains exhibited growth in a wide range of temperatures between 10 and 35 °C. Several researchers reported that chickpea rhizobia are endowed with thermotolerant properties and could be able to grow up to 40 °C (Jida and Assefa 2012; Küçük and Kıvanç 2008; Maâtallah et al. 2002). Increased temperature optima of *M. ciceri* in this study may suggest they have potential as bioinoculant in temperature stressed conditions.

The growth of *M. ciceri* and process of biological nitrogen fixation is largely affected by soil acidity and alkalinity (Laranjo and Oliveira 2011). Similarly, salinity also has numerous detrimental effects on the activity of rhizobia, including declining the cell number due to inhibition of cell division, restricting root infection and inhibiting nodulation (Brígido et al. 2012; Laranjo and Oliveira 2011; Moussaid et al. 2017). Not only the rhizobia but the host legume plants are also sensitive to NaCl exposure (Bertrand et al. 2015; Qu et al. 2016). Likewise, all *M. ciceri* isolates in this study showed tolerance to salt stress and grew well in salt concentrations up to 2.0%. However, isolate PHD11 showed growth in salt concentrations up to 3.0% NaCl. Elizabeth et al. (2000) observed the effectiveness of acid tolerant *Rhizobium leguminosarum* strains for the growth and development of clover plants. pH tolerant PGPB are also involved in the scaling up of the photosynthesizing area of the leaf (Bertamini et al. 2006). Singh et al. (2015) also reported an increase in nodulation and yield of chickpea crop after inoculation with high pH tolerant strains of *M. ciceri*. Several researchers observed an increase in leguminous plant growth, nodulation and nitrogen fixation after inoculation of salt tolerant rhizobia under saline conditions (Bertrand et al. 2015; Qu et al. 2016; Talbi et al. 2013; Wang et al. 2016). Dong et al. (2017) also reported the increased nodulation in leguminous plant after inoculation with salt tolerant rhizobial strains. Studying such characteristics *i.e.* salt and pH tolerance in *M. ciceri* may provide useful information for future improvement of chickpea crops.

Effect of *M. ciceri* on growth and yield of chickpea

Plant height was measured at 70 DAS. A significant increase was clearly noted in the plants treated with *M. ciceri* strains (Table 3). In the Avarodhi, variety, the recorded plant height ranged from 39.3 to 48.3 cm. Maximum plant height (48.3 cm) was observed in the plants treated with strain S3N1 followed by treatments with PHD11 (47.6 cm), which were significantly higher than control plants (33.6 cm). Similarly, in the Uday variety, plant height of inoculated plants was higher compared to the control plants. Maximum plant height was recorded in the plants treated with strain PHD11 (40.6 cm) whereas, minimum plant height was seen in the uninoculated control plants. In the PUSA-372 variety, greater plant growth could be seen in all the treated plants in comparison to control plants where plant heights ranged from 26.3 (control) to 39.9 cm (VAR2.2). Singh et al. (2017a, b), also observed the positive effect of inoculation of *M. ciceri* on chickpea height.

The effect of inoculation of *M. ciceri* on root and shoot dry weight of chickpea cultivars is provided in Table 4. At 70 DAS, maximum shoot biomass in the Avarodhi variety was recorded in plants treated with strain S3N1 (1.584 g per plant). In the Uday variety of chickpea, maximum shoot dry weights were recorded in the plants inoculated by strain S3N1 (1.390 g/plant) which was significantly higher in comparison to the un-inoculated control (0.597 g/plant). Plants of the PUSA-372 variety of chickpea exhibited maximum shoot biomass when treated with the same strain (S3N1;

Table 3 Inoculation effect of *M. ciceri* over the height of chickpea plants

S. N	Bacterial strain	Average plant height (cm)		
		Avarodhi	Uday	PUSA-372
1	C	33.6	26.6	26.3
2	PHD1	39.3	36.6	33.6
3	PHD2	42.3	38.0	36.0
4	PHD8	39.0	33.3	31.0
5	PHD11	47.6*	40.6*	37.9
6	S3N1	48.3*	40.0*	37.6
7	VAR2.2	40.0	35.9	39.9*

*$p < 0.05$

Table 4 Inoculation effect of *M. ciceri* on shoot biomass of chickpea

S. N	Bacterial strain	Shoot dry weight/root dry weight (g)		
		Avarodhi	Uday	PUSA-372
1	C	0.8/0.2	0.6/0.1	0.6/0.1
2	PHD1	1.1/0.3	1.1/0.2	1.0/0.2
3	PHD2	1.4/0.3	1.2/0.3*	1.1/0.2
4	PHD8	1.3/0.2	1.1/0.2	1.1/0.2
5	PHD11	1.5*/0.3	1.3*/0.3*	1.2/0.3*
6	S3N1	1.8*/0.4*	1.4*/0.4**	1.2/0.3*
7	VAR2.2	1.4/0.3	1.1/0.3*	1.3*/0.4*

*$p < 0.05$; **$p < 0.005$

1.528 g/plant). Subsequently, the highest root dry weight increase (87.09%) over the control was observed when seeds were inoculated with S3N1 followed by an enhancement of 69.67% in PHD11 and 58.70% in PHD1. Minimum increase in root dry matter accumulation (29.67%) was observed in plants treated with strain PHD2. Similarly, in the Uday variety, the maximum root dry weight was exhibited by strain S3N1 where dry root weight had increased by 148.67% over the control. In the remaining strains, root dry matter weight was recorded and ranged from 117.69 to 69.11% over the un-inoculated control. In the PUSA-372 chickpea variety, the maximum root dry weight was seen in seed treated with strain S3N1 (124.62%) followed by VAR2.2 (76.11%) over the control. Minimum root dry mass enhancement was seen in plants treated with strain PHD1 where an increase of 45.52% was noted. Endophytic and rhizospheric bacteria are known to play an important role in plant yield and growth promotion, plant health, and protection (Kaur et al. 2015; Sharma et al. 2013a, b). Koli and Swarnalakshmi (2017) also reported the increased value of these secondary characteristics in chickpea cultivars when inoculated with *M. ciceri*.

Determination of chlorophyll content in the leaves of plants is an indirect method of estimating crop productivity. Total chlorophyll content was measured at 30 DAS. The chlorophyll content was marginally higher in all treated plants when compared to the control plants (Table 5). In the Avarodhi variety, maximum chlorophyll content was recorded in the leaves of plants treated with strain S3N1 (1.5 mg/g). The results indicate that inoculation of *M. ciceri* strains with chickpea seeds has a synergistic effect on chlorophyll content of leaves. Increased nitrogen supply by the action of *M. ciceri* in treated plants as compared to un-inoculated plants may be the reason for the enhanced leaf chlorophyll content in chickpea plants. Nitrogen is the most important mineral element in the process of chlorophyll biosynthesis (Cecchin and Terezinha 2004). Verma et al. (2012), (2013) also reported that chickpea plants grown in the presence of *M. ciceri* had higher total chlorophyll content than the control.

Nodule formation was observed in the treated plants only and nodules were not exhibited in the un-inoculated control plants in the pot experiment (Table 6). Seed treatment of the Avarodhi variety of chickpea with strain S3N1 exhibited the maximum number of root-nodules (41.66; dry weight 0.101 gm) followed by PHD11 (38; dry weight 0.078 gm). In the Uday variety, strain S3N1 was found to be the most effective with the highest increase in nodules (44.66; dry weight 0.082 gm) followed by PHD11 (35.66; dry weight 0.086 gm). The maximum number of nodules were counted in the PUSA-372 variety of chickpea plants treated with strain VAR2.2 (39; dry weight 0.081 gm) followed by treatment with PHD11 (35.33; dry weight 0.075 gm). Significant enhancement of nodulation and nodule dry matter was reported by many researchers in chickpea plants following inoculation of *M. ciceri* (Verma et al. 2010, 2012, 2013; Yadav et al. 2018). These results are in close agreement with the findings of Chaudhary and Sindhu (2015) who reported the effective nodulation in chickpea by application of *M. ciceri*.

As shown in Table 7, total pods per plant demonstrated a significant response with inoculation of *M. ciceri*. Overall, plants treated with *M. ciceri* S3N1, PHD11 and VAR2.2

Table 5 Inoculation effect of *M. ciceri* on chlorophyll content of the leaves of chickpea

S. N	Bacterial strain	Chlorophyll content (mg/g)		
		Avarodhi	Uday	PUSA-372
1	C	0.9	0. 8	0.8
2	PHD1	1.1	1.0	0.9
3	PHD2	1.3	1.4*	1.3
4	PHD8	1.2	1.3	1.2
5	PHD11	1.3	1.2	1.3
6	S3N1	1.5*	1.3	1.4*
7	VAR2.2	1.2	1.3	1.4*

*$p < 0.05$

Table 6 Inoculation effect of *M. ciceri* on nodulation of chickpea

S. N	Bacterial strain	Nodule/plant (dry weight g/plant)		
		Avarodhi	Uday	PUSA-372
1	C	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
2	PHD1	20.7 (0.05)*	14.7 (0.03) *	20.0 (0.05) *
3	PHD2	29.3 (0.08) **	24.0 (0.07) **	25.7 (0.06) *
4	PHD8	21.3 (0.05) *	19.7 (0.06) *	23.0 (0.06) *
5	PHD11	38.0 (0.08) ***	35.7 (0.09) ***	35.3 (0.08) **
6	S3N1	41.7 (0.10) ***	42.0 (0.08) ***	34.0 (0.07) **
7	VAR2.2	29.0 (0.07) **	26.3 (0.06) **	39.0 (0.08) ***

*$p < 0.05$; **$p < 0.005$; ***$p < 0.0005$

Table 7 Inoculation effect of *M.ciceri* on number of pods of chickpea

S. N	Bacterial strain	Average number of pods plant^{-}		
		Avarodhi	Uday	PUSA-372
1	C	17.7	16.3	16.0
2	PHD1	28.3	25.7	29.0
3	PHD2	30.3	31.3	25.0
4	PHD8	32.7	29.0	33.0*
5	PHD11	34.3*	35.3*	34.3*
6	S3N1	43.3*	43.3*	34.7*
7	VAR2.2	35.3*	36.3*	39.3*

*$p < 0.05$

showed significantly higher numbers of pods/plant as compared to plants inoculated with other strains.

The highest number of total pods/plant was recorded in plants treated with strain S3N1 in chickpea varieties Avarodhi and Uday. In chickpea variety PUSA-372, plants treated with strain VAR2.2 exhibited a maximum number of pods per plant (39.3) which is a significant increase when compared to control plants (16.0). Pérez-Fernández and Alexander (2017) also observed a statistically significant increase in the number of pods per chickpea plant with *Mesorhizobium* inoculation.

The grain yield recorded at harvesting stage revealed that inoculation of *M. ciceri* significantly increased the grain yield as compared to un-inoculated control (Table 8). Strain S3N1 exhibited 1.59 and 1.74 seed/pod in Avarodhi and Uday varieties, respectively, over the control (1.26 and 1.19 seeds/pod). Other strains also exhibited a significant increase from 1.38 to 1.51 for this particular trait. Strain VAR2.2 was found to be the most effective strain in terms of seeds/pod with 1.62 seeds/pod over the control (1.30 seeds/pod) in the PUSA-372 chickpea variety. Variance analysis of data indicated that *M. ciceri* inoculation had statistically significant positive effects on the number of grains per plant and the highest magnitude of this trait was recorded in PUSA-362 chickpea plants inoculated with strain S3N1 (234%) followed by inoculation with PHD11 (151%) over the un-inoculated control plants. Other strains also exhibited a significant increase in this trait. The same increasing pattern was exhibited in the Uday chickpea variety where the maximum increase was seen in plants inoculated with S3N1 (238%) as compared to control plants. Furthermore, the greatest effect in terms of the number of seeds per plant in the PUSA-372 chickpea variety was exhibited in plants treated with VAR2.2 (175%) followed by treatment with S3N1 (120%) as compared to the control, in pot experiments. The lowest percent increase over control in terms of total yield was observed in plants treated with PHD2 (54%; Table 8). Our findings are in close agreement with several researchers who have reported the synergistic effects of rhizobia on nodulation and yield of legume crops (Atieno et al. 2012; Kaur and Sharma 2013; Korir et al. 2017; Mishra et al. 2009). It is highly likely that microorganisms with plant growth promoting traits like siderophore production, IAA production and phosphate solubilization activity might have a role in root proliferation when *Mesorhizobium* is introduced in the rhizosphere of chickpea crops resulting in better nodulation and yield as reported by Yadav et al. (2018).

There was a remarkable difference in the weight of 100 seeds amongst these genotypes and within the same genotype treated with different strains (Table 9). Maximum seed weight was observed in the Avarodhi variety plants treated with S3N1 (29.2 g) followed by treatment with PHD11 (27.5 g/100 seeds). In Uday cultivars, maximum seed weight was observed in plants treated with S3N1 (28.2 g) which was statistically similar to PHD11 treatment (27.9 g). In these two varieties, treatment with S3N1 also resulted in higher pods/plant and a greater number of root nodules. The lowest seed yield, for both varieties, was recorded in the un-inoculated control plants. In the PUSA-372 variety of chickpea, the highest seed weight was recorded in plants treated with VAR2.2 (24.8 g/100 seeds) while the lowest seed weight was seen in the control.

Table 8 Inoculation effect of *M.ciceri* on chickpea yield

S. N.	Bacterial strain	Average number of seeds plant^{-}		
		Avarodhi	Uday	PUSA-372
1	C	22	21	24
2	PHD1	46*	39*	39
3	PHD2	44*	46*	38
4	PHD8	43*	51*	44
5	PHD11	55*	52*	48*
6	S3N1	76**	69**	54*
7	VAR2.2	51*	49*	67**

*$p < 0.05$; **$p < 0.005$

Table 9 Inoculation effect of *M. ciceri* on 100 grains weight

S. N	Bacterial strain	100 grain weight (g)		
		Avarodhi	Uday	PUSA-372
1	C	22.2	20.8	19.3
2	PHD1	26.4	23.4	22.0
3	PHD2	24.4	24.3	23.1
4	PHD8	25.3	22.9	19.9
5	PHD11	27.5	27.9	24.7*
6	S3N1	29.2*	28.2*	24.3
7	VAR2.2	24.9	23.9	24.8*

*$p < 0.05$

In the foregoing investigation, inoculation of *M. ciceri* strains as plant growth promoting bacteria resulted in highly significant enhancement of plant growth, chlorophyll content, increased nodules and yield in three frequently used chickpea varieties; Avarodhi, Uday and PUSA-372, in Eastern Uttar Pradesh. It is also concluded that one particular strain may not benefit different varieties, as in our investigation, strain S3N1 was found to be most effective for chickpea varieties Avarodhi and Uday while strain VAR2.2 was the most effective for variety PUSA-372, in terms of plant height, dry mass, chlorophyll content, nodules and yield. To the best of our knowledge, this is the first study noting the effect of different *M. ciceri* strains on three varieties of chickpea crops from five districts of eastern Uttar Pradesh in a single experiment. The study also suggests the requirement of careful selection of inoculants for different chickpea varieties for commercial production and sustainable agriculture production.

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