Int.J.Curr.Microbiol.App.Sci (2018) *7*(8): 2592-2599

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 7 Number 08 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.708.266

Screening of Multi-trait Mesorhizobium Isolates for Plant Growth Promotion and Nitrogen Fixation in Chickpea (*Cicer arietinum* L.)

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Keywords

Chickpea, *Cicer arietinum* L. Mesorhizobium, Nodulation, Plant dry matter, Nitrogen and Phosphorus content, Nitrogen fixation

Article Info

Accepted:
15 July 2018
Available Online:
10 August 2018

A B S T R A C T

Use of nitrogen fixing and phosphate solubilizing microorganisms (biofertilizers) has become an important aspect of pulse crops production technology. The efficiency of these microorganisms differs widely in accruing the benefits of their application in crops. Ten *Mesorhizobium* isolates having various plant growth promoting (PGP) traits including phosphate solubilization were screened for their symbiotic efficiency in terms of root nodulation, plant dry matter production, nitrogen and phosphorus content and nitrogen fixation by chickpea plant in a field experiment during 2015-16. The isolates showed considerable variations in the symbiotic performance by producing nodule number from 17.3 to 21.8 plant^{-1}, nodule dry weight from 68.0 to 76.1 mg nodule plant^{-1} and plant dry weight from 2.74 to 3.53 g plant^{-1} at 60 DAS registering significant increases over the uninoculated control. The shoot and root length at 60 DAS due to inoculation of different isolates also varied significantly and were higher than the uninoculated control. The different isolates accumulated 92.8 to 127.1 mg more nitrogen plant^{-1} indicating fixation of 34.3 to 68.6 mg nitrogen plant^{-1} over the native population at 60 DAS. *Mesorhizobium* isolate CR-53 was found most promising and followed by the isolates CR-3 and CR-40 in symbiotic performance, nitrogen accumulation and fixation in chickpea. The results suggested that *Mesorhizobium* isolates having different PGP traits could be a better option for obtaining the benefits of inoculation and improving pulse production.

Introduction

Chickpea (*Cicer arietinum* L.) is one of the important grain legumes and cultivated mainly in the Indian subcontinent, Mediterranean region the West Asia and North Africa (WANA) region, and Eastern Africa. India is the largest producer and consumer of chickpea in the world sharing about 71.0 % of the global acreage and production. Chickpea is grown in India on about 8.39 m ha with annual production of 7.05 metric tons grains and productivity of 889 kg ha^{-1}. Chickpea grains contain 29% protein, 59% carbohydrate, 3% fibre, 5% oil and 4% ash. The protein is rich in lysine and arginine but most deficient in sulphur containing amino acids such as methionine and cysteine (Iqbal *et al.*, 2006). It is also a good source of absorbable Ca, P, Mg, Fe and K. Being a leguminous crop, chickpea restores and maintains soil fertility through symbiotic nitrogen fixation in association with *Mesorhizobium* species in addition to its valuable nutritional quality (Nour *et al.*, 1994). The deep root system and leaf shading habits of chickpea also contributes significantly in improving nutritional and physical health of soil.

Rhizobial populations residing in soils are dynamic and continuously evolving communities. Their diversity stems from the size and plasticity of rhizobial genomes (Black *et al*., 2012) which enables them to survive in different habitats. Rhizobia often spread from their initial habitats (Stepkowski *et al.*, 2007); however, the success of their introduction into new environments depends upon their ability to adapt in various biotic and abiotic factors (Trotman and Weaver, 1995). The efficiency of the *Mesorhizobium* sp. strains differ across the locations. Several reports indicated that application of effective *Rhizobium* inoculants in chickpea results increased crop productivity and soil health (Sahai and Chandra, 2011; Dudeja *et al.*, 2011). Chickpea obtain a significant portion of its nitrogen requirement through symbiotic N_2 fixation when grown in association with effective and compatible *Rhizobium* strains. Recently, rhizobia have been shown to promote the plant growth through PGP mechanisms that are different from the atmospheric N_2 fixation. PGP traits such as secretion of phyto-hormones like indole acetic acid that enhances plant growth, synthesis of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which can lower plant ethylene levels, solubilization of minerals such as phosphorus making it more readily available for plant growth, siderophore production that can sequester iron from the soil and provide it to plant cells and by conferring increased resistance against plant pathogens have been reported (Antoun *et al*., 1998; Deshwal *et al.*, 2003). The present study was conducted to compare the performance of different *Mesorhizobium* isolates having PGP traits for plant growth promotion and nitrogen fixation in chickpea under field conditions.

Materials and Methods

The screening of the 10 different multi-trait mesorhizobial isolates having PGP traits (P solubilization, IAA, GA, Siderophore and HCN production) was done in a field experiment at Pantnagar at Norman E. Borlaug Crop Research Centre of the University (29° N latitude, 79.3° E longitude and at an altitude of 243.8 m above the mean sea level) during Rabi season of 2016 to 2017. The experimental soil was Sandy loam of pH 6.90 and EC 0.24dSm^{-1} having 0.67 % Organic C and 180.2, 20.1 and 261.4 kg ha^{-1} available N, P and K, respectively. Treatments consisting seed inoculation with 10 mesorhiobial isolates (CR-3, CR-11, CR-21, CR-25, CR-40, CR-41, CR-46, CR-47, CR-53 and CR-62) with an uninoculated control and RDF (20 kg N ha^{-1} + 40 kg P_2O_5 ha^{-1}) were laid out in plots of 3.0 m x 4.0 m size following randomized block design with 3 replications.

The Chickpea (cv. PG-186) was sown on 23rd November 2016 with a seed rate of 100 kg ha^{-1}. Seeds were surface sterilized by soaking in 0.1% $HgCl_2$ for 2 minutes and then thoroughly washed with sterilized distilled water. The inoculums of each *Mesorhizobium* isolate were prepared by multiplying in YEM broth for 4 days. Seeds were treated with the inoculums of different *Mesorhizobium* isolates separately by mixing with 4 ml inoculum kg^{-1} seed at the time of sowing. Then treated seeds were sown immediately in tractor opened furrows at 3-4 cm depth. Nitrogen (20 kg ha^{-1}) and phosphorous (40 kg P_2O_5 ha^{-1}) were applied as per treatment through urea and single super phosphate, respectively.

Recommended dose of Potassium (20 kg ha^{-1}) was given uniformly to all plots through muriate of potash. All the fertilizers were applied in planting furrows prior to seed showing. The crop was raised and protected from insects, pests and diseases as per the standard recommended practices.

Five plants from each plot were randomly uprooted from the side rows along with a soil block of about 25 cm diameter at 60 days after sowing (DAS). Adhered soil with roots was washed off with tap water carefully by keeping the roots of plants in sieve. Nodules were removed from the roots; counted and their dry weight was recorded after drying in hot air oven to constant weight at 65˚C. The shoot and root lengths of the uprooted plants from each plot was also recorded. The plants were then dried at 65˚C to constant weight and their dry weights were recorded. The N in finely grinded (40 mesh) plant sample was estimated using rapid N-cube analyzer (Elementar) and nitrogen accumulation in plants was computed by multiplying with the plant dry weight. The concentration of phosphorous in chickpea plants at 60 DAS was estimated by molybdovandate phosphoric acid yellow colour method after wet digestion in di-acid (HNO_3: $HClO_4$, 9:4) (Page, 1982). The obtained experimental data were subjected to statistical analysis appropriate to the design by STPR3 software of G.B.P.U.A. & T Pantnagar. The treatments difference was tested by using F-test of significance at 5% level of probability (Panse and Suchatme, 1978).

Results and Discussion

Nodule number

Inoculation of different Mesorhizobial isolates influenced the root nodule number significantly at 60 DAS (Table 1). Nodule number $plant^{-1}$ due to different treatments ranged from 14.9 to 21.8. All the *Mesorhizobium* isolates, except CR-11 and CR-21, gave significantly higher nodule number, by 22.1 to 46.3 %, over the uninoculated control and was followed by CR-3 and CR-40 in nodule number. *Mesorhizobium* isolate CR-53 recorded significantly more nodule number than CR-11, CR-21 and CR-46 and was at par with remaining isolates. Isolates CR-53, CR-3 and CR-40 also produced significantly more nodule number than application of 20kg N+40kg P_2O_5 ha^{-1} of 48.2, 43.4 and 41.0 %, respectively. These results are in agreement with the earlier report of Gupta (2006) and Bhatt and Chandra (2014) who similarly reported significant increases in nodule number in chickpea due to *Mesorhizobium* inoculation in field conditions. The significant and variable effects of inoculated *Mesorhizobium* isolates may be attributed to better survival of the inoculated *Mesorhizobium* isolates in soil to cause infection for nodule development.

Nodule dry weight

Root nodule dry weight at 60 DAS due to different *Mesorhizobium* sp. isolates, except CR-21 and CR-11, was significantly more than the uninoculated control. The highest nodule dry weight was recorded with isolates CR-53 followed by CR-40 and CR-3 registering 26.4, 25.6 and 24.8 % significant increases over the uninoculated control. Although, all the isolates were at par, but isolates CR-53, CR-3, CR-25 and CR-40 were observed more effective than others by registering significant increases in nodule dry weight over the application of 20kg N+40kg P_2O_5 ha^{-1}. Such beneficial effects of *Mesorhizobium* inoculation in chickpea have also been reported by Kaur *et al* (2015) due to enhancement in nodule number and synthesis of more nodule tissue because of presence of other PGP traits such as P solubilization, siderophores production, IAA production etc.

Shoot length

Shoot length of chickpea plants at 60 DAS ranged between 24.4 to 31.6 cm. Application of RDF (20kg N+40kg P_2O_5 ha^{-1}) was statistically comparable the uninoculated control, while inoculation of different *Mesorhizobium* isolates significantly increased the shoot length ranging from 12.3 to 29.3% (Table 1). The highest shoot length was recorded with isolate CR-53 followed by isolates CR-3, CR-40, CR-25 and CR-47 registering significant increments of 29.3, 28.0, 28.0, 24.7 and 18.4 % over the uninoculated control, respectively. Isolates CR-53, CR-3, CR-40 and CR-25 were also found significantly superior to 20kg N+40kg P_2O_5 ha^{-1} treatment by recording 21.8, 20.5, 20.5 and 17.4% more shoot length at 60 DAS. This could be viewed in the light of superiority of these isolates than others in root nodulation, which resulted in higher nitrogen fixation and plant growth. Mabrouk and Belhadj (2014) found that inoculation of chickpea by different *Rhizobium* strains showed significant differences in dry matter at 45 DAS and shoot growth was significantly increased by two fold when the chickpea plants were inoculated with *Rhizobium* isolates Pch. Azm and Pch. Bj1 as compared to the uninoculated control.

Root length

Alike shoot length, chickpea root length due to inoculation of different *Mesorhizobium* isolates was higher, by 14.9 to 28.4%, over the uninoculated control (Table 1). All the isolates, except CR-11 and CR-21 were statistically comparable in root length. The highest root length was recorded with isolate CR-53 followed by CR-3, CR-40 and CR-41 registering significant increases of 44.8, 43.0, 41.4 and 34.4% over the uninoculated control, respectively. These isolates also showed significant increases in root length of 30.8, 29.2, 27.4 and 21.4% over the application of 20kg N+40kg P_2O_5 ha^{-1}, respectively suggesting variable influence of the isolates on root length and could be attributed to occurrence of PGP traits in the isolates. Similarly finding was also reported by Lal and Khan, (2014).

Plant dry weight

Application of 20kg N+40kg P_2O_5 ha^{-1} gave numerical increase in plant dry weight of 20.8% over the uninoculated control (Table 1). Inoculation of different *Mesorhizobium* sp. isolates showed significant increases in plant dry weight ranging from 49.7 to 92.9 % over uninoculated control. The highest plant dry weight was recorded with isolate CR-53 registering 92.9 % higher plant dry weight than the uninocualted control. It was followed by CR-3, CR-40 and CR-25 registering significant increases of 84.7, 83.6 and 81.4 % over uninoculated control, respectively.

Isolate CR-53 was at par with CR-3, CR-40 and CR-41 and significantly superior to all other isolates in plant dry weight. All isolates were also found to be significantly better than application of 20kg N+40kg P_2O_5 ha^{-1}. Malik and Sindhu (2011) also reported that inoculation of chickpea seed with *Mesorhizobium* sp. alone increased the plant dry weight that varied from 1.14 to 1.80 times in comparison to uninoculated control. Biologically fixed nitrogen is the most dominating factor influencing the dry weight of shoot (Fatima *et al.*, 2008). Increase in dry weight of shoot compared to uninoculated control was also reported by Akthar and Siddiqui (2009).

Nitrogen and Phosphorus content in plant

Nitrogen and Phosphorus content in plants due to application of 20kg N+40kg P_2O_5 ha^{-1} was comparable to the uninoculated control (Table 2).

Table.1 Inoculation effect of Mesorhizobium isolates on nodulation, plant growth and dry matter production at 60 DAS

Treatment	Nodule number plant^{-1}	Nodule dry weight plant^{-1}	Shoot length (cm)	Root length (cm)	Plant dry weight (g plant^{-1})
Uninoculated control	14.9	60.2	24.43	9.60	1.83
20kg N+40kg P_2O_5 ha^{-1}	16.2	64.5	25.90	10.63	2.21
CR-3	21.0	75.1	31.27	13.73	3.38
CR-11	17.3	68.0	27.43	11.03	2.74
CR-21	17.3	67.9	27.93	11.77	2.77
CR-25	19.3	73.8	30.47	12.80	3.32
CR-40	20.0	75.6	31.27	13.57	3.36
CR-41	19.2	73.9	30.10	12.90	3.22
CR-46	18.2	72.5	28.67	12.13	2.87
CR-47	18.8	72.9	28.93	12.33	2.94
CR-53	21.8	76.1	31.60	13.90	3.53
CR-62	18.8	72.1	29.17	12.37	3.03
CD at 5%	**3.1**	**9.1**	**4.27**	**1.82**	**0.41**

Table.2 Inoculation effect of Mesorhizobium isolates on nitrogen and phosphorus concentrations and nitrogen fixation by chickpea plant at 60 DAS

Treatment	Nitrogen content (%)	Phosphorus content (%)	Nitrogen accumulation (mg plant^{-1})	Nitrogen fixed over control (mg plant^{-1})	Nitrogen fixed overFertilizer treatment (mg plant^{-1})
Uninoculated control	3.202	0.198	58.5	-	-
20kg N+40kg P_2O_5 ha^{-1}	3.308	0.207	72.7	-	-
CR-3	3.595	0.217	121.5	63	48.8
CR-11	3.376	0.208	92.8	34.3	20.1
CR-21	3.440	0.211	95.3	36.8	22.6
CR-25	3.545	0.213	117.9	59.4	45.2
CR-40	3.571	0.216	119.8	61.3	47.1
CR-41	3.527	0.212	113.4	54.9	40.7
CR-46	3.450	0.211	99.2	40.7	26.5
CR-47	3.470	0.212	102.0	43.5	29.3
CR-53	3.597	0.219	127.1	68.6	54.4
CR-62	3.481	0.212	105.3	46.8	32.6
CD at 5%	**0.361**	**NS**	**20.0**	**-**	**-**

However, inoculation of different *Mesorhizobium* isolates increased the plant nitrogen content ranging from 5.4 to 12.3% and phosphorous content from 5.1 to 10.6 % over the uninoculated control. Isolate CR-53 showed highest nitrogen and phosphorus content and was followed by isolates CR-3 and CR-40 registering significant increases of 12.3, 12.3 and 11.5 % in plant nitrogen content and 10.6, 9.6, 9.1 in plant phosphorous content over the uninoculated control, respectively. These isolates gave statistically comparable nitrogen and phosphorus contents in plant. These results are in agreement with the earlier reports of Sarna *et al* (2008) and Qureshi *et al* (2009) and may be due to increased nitrogen fixation and phosphorus solubilization in soil leading to increased availability for plants. The variations in the ability of nitrogen fixation and phosophorus solubilization by rhizobia have also been reported elsewhere (Dudeja *et al.*, 2011).

Nitrogen fixation

Nitrogen fixation by different *Mesorhizobium* isolates was computed over the indigenous population by difference method. The different isolates accumulated 92.8 to 127.1 mg plant^{-1} more nitrogen indicating fixation of 34.3 to 68.6 mg plant^{-1} over the native population under uninocualted control treatment (Table 2). The highest nitrogen accumulation was obtained with isolate CR-53 followed by CR-3 and CR-40. These isolates fixed 68.6, 63.0 and 61.3 mg plant^{-1} more nitrogen over the uninocualted control and 54.4., 48.8 and 47.1 mg plant $^{-1}$ more nitrogen over application of 20kg N+40kg P_2O_5 ha^{-1}, respectively and were superior to remaining isolates. The fertilizer treatment recorded slightly more nitrogen over the uninoculated control. Nitrogen fixation efficiency is dependent on genetic makeup of the rhizobia and also influenced by soil and environmental factors. Similar variations in the nitrogen fixation efficiency of the rhizobia have been reported by Sarr *et al.* (2016). The *Mesorhizobium* isolates were obtained from diverse locations and it is expected that they differ in their efficiency in establishing the symbiosis and subsequently nitrogen fixation.

In conclusion, results of the study clearly demonstrated the variations in the symbiotic efficiency of the *Mesorhizobium* isolates. Isolate CR-53, CR-3 and CR-40 were found promising in enhancing the rootnodulation, dry weight matter production and nitrogen fixation in chickpea. The further studies are needed to confirm the findings under different agro-ecological situations.

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How to cite this article:

Pushpa Yadav, Ramesh Chandra, Navneet Pareek and Raverkar, K.P. 2018. Screening of Multi-trait Mesorhizobium Isolates for Plant Growth Promotion and Nitrogen Fixation in Chickpea (*Cicer arietinum* L.). *Int.J.Curr.Microbiol.App.Sci.* 7(08): 2592-2599.
doi: https://doi.org/10.20546/ijcmas.2018.708.266