Endophytic bacteria improve mesorhizobial nodulation, plant growth and yield in chickpea (Cicer arietinum)

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ABSTRACT

The aim of our study was to determine the symbiotic effectiveness of Mesorhizobium ciceri and plant growth promotion of endophytic bacteria in field-grown chickpea during 2016–17 and 2017–18. Co-inoculation of M. ciceri along with various endophytes has significantly improved soil dehydrogenase activity in chickpea rhizosphere. The combined inoculation of M. ciceri with nodule endophytes, viz. Bacillus cereus (25.7–51.9%) and Bacillus aerophilus (18.6–27.8%) showed higher nodule weight than uninoculated control which is at par with inoculation of M. ciceri with root endophyte (Pseudomonas flourescens). Co-inoculation of M. ciceri + B. aerophilus increased chickpea growth (13.7–21.5%) at 50% flowering stage and grain yield (15.6–18.2%) at harvest stage which is at par with the treatment with P. flourescens (17.5–20.1%). A positive correlation was observed between nodule dry weight with growth and yield of chickpea in the second year. Co-inoculation of endophytic bacteria has improved the symbiotic efficiency, growth and productivity of chickpea through synergistic interaction with Mesorhizobium.

Keywords: Chickpea, Endophytic bacteria, Mesorhizobium, Nodulation, Productivity

In India, chickpea cultivation is generally practised under rainfed conditions. Chickpea forms symbiotic association with Mesorhizobium species which can convert atmospheric nitrogen into ammonia through symbiotic nitrogen fixation (Nour et al. 1994). The amount of nitrogen fixed in Chickpea–Mesorhizobium symbiosis varies from 3-121 N/kg/ha (Peoples et al. 1995). Growing chickpea in crop rotation benefits the succeeding crop by enhanced N supply from the soil. Plant growth promoting rhizobacteria (PGPR) and endophytic bacteria influenced rhizobial symbiosis positively (Swarnalakshmi et al. 2019, Swarnalakshmi et al. 2020). The combined inoculation of rhizobia and rhizobacteria is more effective as the latter can modify nodule formation and nitrogen fixation in grain legumes (Verma et al. 2013). The endophytic microorganisms induced nodulation and growth in chickpea and exhibit more pronounced plant growth-promoting effects than rhizobacteria (Ahmad et al. 2019). A large number of non-rhizobial endophytic bacterial genera, viz. Agrobacterium, Pseudomonas, Enterobacter, Pantoea, Bacillus and Paenibacillus are more frequently isolated from root, shoot and nodule tissues of different legumes (Kan et al. 2007, Li et al. 2008, Ahmad et al. 2019). The presence of nodule associated endophytes, viz. Agrobacterium, Bacillus, Curtobacterium, Enterobacter, Erwinia, Herbaspirillum, Mycobacterium, Paenibacillus, Pseudomonas, Phyllobacterium, Ochrobactrum, Sphingomonas, Rhizobium, Ensifer, Mesorhizobium, Burkholderia, Phyllobacterium and Devosia are reported in leguminous plants (Dudeja et al. 2012). They can enter into the plant tissues along with the nodule bacteria and capable of co-habiting with Rhizobium in root nodules. These endophytic bacteria are reported to enhance plant growth promotion through phosphate solubilisation, nitrogen assimilation, iron chelation and pathogen suppression (Rosenblueth and Martínez-Romero 2006, Liu et al. 2009).

As chickpea is grown under marginal lands, emphasis has been given on seed inoculation with Mesorhizobium and other beneficial microorganisms to provide the plant nutrition. Interactive effects of Mesorhizobium and rhizobacteria have been demonstrated. However, reports on the synergistic effect of endophytic bacteria with Mesorhizobium on growth and productivity of chickpea under field conditions are scanty. The present investigation was carried out to evaluate the comparative performance of various endophytic bacterial strains along with Mesorhizobium on nodulation, plant growth and productivity of chickpea under field conditions.

MATERIALS AND METHODS

Field experiment was conducted at ICAR-Indian Agricultural Research Institute, New Delhi, India (28°40'
N latitude and 77°12' E longitude, 229 m amsl) during rabi (November–April) season of two consecutive years (2016–17 and 2017–18). The climate of an experimental unit is semi-arid with an average annual rainfall of approximately 650 mm, 80% of which is received through south-west monsoon during July to September and the rest is received during the 'Western Disturbances' in the months of December to February. The chickpea crop was sown with the residual moisture during rabi. The mean daily maximum temperature during the crop growth period varied from 25–34°C, whereas December–January are the coldest months with the mean daily minimum temperature ranging from 5–8°C.

The experiment was laid out in randomized block design and replicated three times with the plot size of 10 m². The soil samples (0-15 cm depth) were taken at the beginning of the experiment. The soil was sandy–loam in texture, with a pH of 7.34, organic carbon, 0.6% (Walkley and Black 1934) and electrical conductivity (EC) was 0.23 dS/m. The alkaline KMnO₄ oxidizable–N was 213.3 kg/ha (Subbiah and Asija 1956) and 0.5 M NaHCO₃ extractable P was 18.9 kg/ha (Olsen et al. 1954). The experiment comprised 10 treatments. The treatment without inoculants served as a control (T₁). A single inoculation of Mesorhizobium ciceri- CH 1233 (T₂) was added to compare the co-inoculation effect. The co-inoculation of M. ciceri with different endophytic bacteria strains (T₃ to T₁₀) were compared with reference check (T₃; M. ciceri + CNE 1) and combined inoculation of M. ciceri + PGPR (T₄; M. ciceri + Pseudomonas aeruginosa). All endophytes were isolated from chickpea nodules (NE) except the Pseudomonas flourescens (T₅) which was isolated from chickpea roots (RE).

The details of treatments are; T₁: Uninoculated control; T₂: Mesorhizobium ciceri CH 1233; T₃: M. ciceri + CNE 1 (ref. check); T₄: M. ciceri + Pseudomonas aeruginosa (PGPR); T₅: M. ciceri + Pseudomonas flourescens (RE); T₆: M. ciceri + Pseudomonas aegroginosa (NE); T₇: M. ciceri + Bacillus cereus (NE); T₈: M. ciceri + Bacillus aerophilus (NE); T₉: M. ciceri + Pseudomonas flourescens (NE); T₁₀: M. ciceri + Enterobacter sp. (NE).

Chickpea seeds (cv. BG 372) were inoculated using carrier (vermiculite) based endophytic microbial inoculants containing 10⁹ CFU (colony forming unit)/g using carboxy methyl cellulose (1%) as a sticker. The inoculated seeds were sown in the field during the first week of November 2017 and 2018. Each plot had 10 rows with 30 cm × 10 cm spacing. Fertilizer was applied at 100 kg DAP as a basal dose. No irrigation was given during the crop growth period. Plant and rhizosphere soil samples were collected at 50% flowering stage and analysed for nodulation potential, plant growth and dehydrogenase activity. The biomass and grain yield were recorded at the harvesting stage.

Dehydrogenase activity: Dehydrogenase activity of the soil was determined using 2,3,5-Triphenyl tetrazolium chloride (TTC) test given by Casida (1977). About 5g soil was taken in test tubes, and 1 ml of 3% TTC was added. The tubes were sealed airtight and incubated at room temperature for 24 hr in the dark. After the incubation period, 10 ml methanol was added, and the content was filtered using Whatman No.1 filter paper. The red colour developed due to TPF (Triphenyl formazan) formation was measured at 485nm.

Nodulation potential: The chickpea plants were uprooted with an intact root system and washed with running tap water to remove the adhering soil particles. The root nodules were detached carefully and the nodule number per plant was recorded. These nodules were then dried at 70°C for two days in a hot air oven and nodule dry weight (mg/plant) was weighed.

Plant growth and yield: The plant growth was determined by separating both the root and shoot of the plant samples. The samples were dried in the hot air oven at 70°C till the constant weight is achieved. At the harvest stage, the biomass and grain yield per plot was recorded.

Statistical analysis: The data recorded on various parameters were subjected to analysis of variance (ANOVA) and post hoc mean separation was performed by the Duncan’s Multiple Range Test (DMRT). The mean values were separated according to LSD test at P<0.05. All statistical analysis was performed using SPSS (version 18.0) software.

RESULTS AND DISCUSSION

Rhizosphere soil dehydrogenase activity: There was a significant effect of microbial inoculation on rhizosphere soil dehydrogenase activity (Table 1). The uninoculated control showed the lowest dehydrogenase activity whereas the greatest change in the dehydrogenase activity was observed with combined inoculation of M. ciceri with endophytic bacterial strain during both seasons. In 2016-17, comparison between uninoculated control (T₁) with T₈ (M. ciceri + B. aerophilus) showed an increase of 147% followed by T₃ (M. ciceri + P. flourescens) and T₈ (M. ciceri + P. flourescens) which also showed a concurrent increase in nodule dry weight. In the second year, the reference check (T₉) showed an increase of 24% over uninoculated control followed by T₈ (M. ciceri + P. aeruginosa). Gopalakrishnan et al. (2015) found that inoculation of Streptomyces sp. in the soil is found to increase dehydrogenase activity by 21%. Measurement of dehydrogenase activity reflects the changes in the microbial activity in the rhizosphere soil following the inoculation. This enzyme plays a key role in soil organic matter due to transfer of hydrogen from organic substrates to inorganic acceptors which are involved in a reductive process of biosynthesis and thus are an important part of enzyme systems of living microorganisms (Wolinska and Stepienewska 2012). A positive correlation between dehydrogenase activity and nodule dry weight (0.36 in 2016-17 and 0.66 in 2017-18) was observed during both the seasons indicates that inoculation can significantly influence the rhizosphere microbial activity in chickpea, which in turn can improve the nodulation synergistically.

Nodulation potential: Root nodules are the most significant part of legumes–rhizobial symbiosis. Inoculation of legume crop with root nodulating rhizobia has been
Table 1  Synergistic effect of Mesorhizobium ciceri and endophytic bacteria on nodulation and rhizosphere soil dehydrogenase activity at 50% flowering

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nodule formation (50% flowering)</th>
<th>Nodule dry weight (mg/plant)</th>
<th>Dehydrogenase activity (mg TPF/day/100 g soil)</th>
<th>Nodule formation (50% flowering)</th>
<th>Nodule dry weight (mg/plant)</th>
<th>Dehydrogenase activity (mg TPF/day/100 g soil)</th>
</tr>
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<tr>
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<td>121.56&lt;sup&gt;f&lt;/sup&gt;</td>
<td>9.00</td>
<td>90.00&lt;sup&gt;e&lt;/sup&gt;</td>
<td>100.54&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>T₁</td>
<td>13.33</td>
<td>109.33&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>124.54&lt;sup&gt;f&lt;/sup&gt;</td>
<td>11.67</td>
<td>110.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>109.76&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>T₂</td>
<td>11.00</td>
<td>102.00&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>159.80&lt;sup&gt;g&lt;/sup&gt;</td>
<td>9.33</td>
<td>113.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>124.71&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>9.00</td>
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<td>107.16&lt;sup&gt;de&lt;/sup&gt;</td>
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<td>202.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.00</td>
<td>140.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>116.29&lt;sup&gt;abc&lt;/sup&gt;</td>
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<tr>
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<td>142.84&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>115.75&lt;sup&gt;abc&lt;/sup&gt;</td>
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</table>

Letters in superscripts indicate mean separation by Duncan’s multiple range test (P≤0.05).

endophytic bacteria inoculated along with various endophytic bacteria has significantly improved chickpea plant growth at 50% flowering (Table 2). In our study, uninoculated control showed the lowest shoot and root weight in both the seasons. A comparative analysis of the T₁ (uninoculated control) and T₈ (M. ciceri + B. aerophilus) showed an increase of 15.6% shoot growth in 2016-17, which is at par with T₃ and T₉ which comprise endophytic strains. A similar trend was observed with respect to root growth where T₅ (M. ciceri + B. aerophilus) showed 19% increased root growth over T₁ (uninoculated control) during 2016-17. In the second year, T₃ (M. ciceri + Pseudomonas fluorescens) showed 29.9% increased shoot growth over uninoculated control followed by T₉, whereas with respect to root growth T₃ (M. ciceri + P. argentinensis) accounted 43% increase over uninoculated control followed by T₅, T₉, T₆, T₇, T₈, and T₁₀ in the first year, nodule number/plant was positively correlated with shoot growth (0.67) however in the second year a positive correlation between nodule dry weight with shoot (0.7) and root (0.67) growth was observed. Our results were in conformity with Saini et al. (2015) who reported that co-inoculation of endophytic bacteria along with Mesorhizobium sp. could increase root and shoot dry weight of chickpea. The inoculation of Rhizobium along with Azotobacter sp., Bacillus sp., Pseudomonas sp., Piriformospora indica and Trichoderma sp. stimulate plant growth and nodulation in field-grown legumes (Korir et al. 2017, Swarnalakshmi et al. 2017). The increased chickpea growth by combined inoculation of Mesorhizobium sp. and Pseudomonas sp. may be due to improved N and P uptake (Verma et al. 2013). These bacteria also promote plant growth by production of phytohormones and vitamins in the rhizosphere (Swarnalakshmi et al. 2020). Besides that, the endophytic bacteria can induce systemic resistance in plants by inducing phenol production and protect from multiple pathogens, which directly helps in improving the overall plant growth (Rangeshwaran et al. 2008).
and endophytic bacteria can also improve the plant growth at different stages (Ali et al. 2017, Swarnalakshmi et al. 2017). In the present study, it is evident that among the treatment involving various endophytes, *M. ciceri* + *P. flourescens* and *M. ciceri* + *B. cereus* are significantly increased nodule biomass, growth and yield of chickpea. This may be due to the enhanced supply of N from environmental stresses and microbial competitions. The plant growth promoting substances such as indole-3-acetic acid, gibberellins and cytokinins produced by the endophytic bacteria can also improve the plant growth at different stages (Ali et al. 2017, Swarnalakshmi et al. 2019).

The highest grain yield was recorded from *T*₅ (*M. ciceri* + *P. flourescens*) with an increase of 17.5% as compared to the uninoculated control followed by *T*₈ (15%) and *T*₉ (15.62%) in 2016-17. The similar trend was observed during second year with *T*₅ leading to 20.1% increase over control (*T*₁) which is at par with *T*₄ (17.9%), *T*₆ (18.2%), *T₇ (14.1%), *T₈ (18.2%), *T₉ (15.7%) and *T*₁₀ (15.7%). Overall, *T*₅ and *T*₈ showed consistent performance over the years in improving nodulation, growth and productivity of chickpea. In 2016-17, the improved shoot growth with microbial inoculation was significantly correlated with biomass and grain yield. In second year (2017-18), nodule dry weight was positively correlated with shoot growth (0.7) and yield (0.76) (P<0.05). The synergistic effect of *Mesorhizobium* with *Bacillus* or *Pseudomonas* on chickpea growth, yield and grain protein was reported by Wani et al. (2007). In accordance with our findings, an increase of 12.5% in yield was recorded due to microbial co-inoculation of *Bacillus megaterium* and *Rhizobium* sp. in chickpea was observed by Aparna et al. (2014). Our previous field trials highlight the positive effects of application phosphatic fertilizer along with inoculation of *Mesorhizobium, Pseudomonas* sp., *Piriformospora indica* and *Trichoderma* sp. which together accounted 35% increase in grain yield over uninoculated control due to improved nitrogen and phosphorus nutrition (Swarnalakshmi et al. 2017). In the present study, it is evident that among the treatment involving various endophytes, *M. ciceri* + *P. flourescens* and *M. ciceri* + *B. cereus* are significantly increased nodule biomass, growth and yield of chickpea. This may be due to the enhanced supply of N through symbiotic nitrogen fixation by *Mesorhizobium* and plant growth-promoting potential of *Pseudomonas* strain. Our results indicate that inoculation of *Mesorhizobium* and endophytic bacteria can augment chickpea production over and above the application of the recommended dose of chemical fertilizer.

Our findings have showed clear evidence that inoculation of endophytic bacteria and *Mesorhizobium ciceri* can significantly improve the nodulation potential, plant growth and rhizosphere dehydrogenase activity. The combined inoculation of *M. ciceri* with *Bacillus* sp. and *Pseudomonas flourescens* enhanced the nodule biomass. The co-inoculation effect of *Mesorhizobium* with endophytic bacteria was more pronounced on plant growth at flowering stage, which was also reflected in improved seed yield at harvest stage. The nodule biomass, plant growth and yield attributes were positively correlated. Our study signifies that dual-inoculation of *M. ciceri* with endophytic bacteria has significant potential to improve nodulation and overall growth and productivity of chickpea crop in a synergistic manner.

### Table 2: Synergistic effect of *Mesorhizobium ciceri* endophytic bacteria on plant growth and yield of chickpea

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoot weight (g/plant)</th>
<th>Root weight (mg/plant)</th>
<th>Biomass (kg/ha)</th>
<th>Yield (kg/ha)</th>
<th>Shoot weight (g/plant)</th>
<th>Root weight (mg/plant)</th>
<th>Biomass (kg/ha)</th>
<th>Yield (kg/ha)</th>
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<tr>
<td><em>T</em>₁</td>
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<td>0.47d</td>
<td>7733.3bc</td>
<td>2666.7c</td>
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<td>0.29ab</td>
<td>6400.0a</td>
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Letters in superscripts indicate mean separation by Duncan’s multiple range test (P≤0.05).
ACKNOWLEDGEMENTS

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