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Evaluation of *Mesorhizobium ciceri* from saline soil on nodulation and grain yield of Chickpea

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Abstract
A field experiment was conducted during *Rabi* 2017- 18 and 2018-19 at Pulses Research Unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (M.S.) to evaluate the effect of various *Mesorhizobium* isolates of Chickpea on nodulation and grain yield of chickpea variety JAKI-9218. The experiment comprising of thirty-three treatments including uninoculated control of which thirty-two treatments was of *Mesorhizobium* isolates which obtained from chickpea root nodules from different villages of saline track of Purna river of Vidarbha (M.S.). Experiment was laid out in randomize block design with three replications. The carrier-based culture of *Mesorhizobium* isolates were inoculated @ 25 g/kg of seed. Pooled results of the present study revealed that the seed inoculation of *Mesorhizobium* isolate (ACR-10) recorded higher grain yield (1641 kg/ha) and maximum number of nodulation (28.07 nodules /plant), nodules dry weight (100.94mg/pl) and plant dry weight (4.45g/pl) among all the isolates. Among the total studied strains ACR-10, BCR-35 and BCR-36 were reported as most promising strains and were identified as *Mesorhizobium ciceri*.

Keywords: Chickpea, *Mesorhizobium*, nodulation, biofertilizers

Introduction
Chickpea (*Cicer arietinum* L.) is a grain legume crop grown primarily for its nutritional value. Because of high protein contents, it is considered as an economical source of quality vegetable protein. The yield gap of chickpea may be attributed to improper agro-technology used by the farmers. It can be abridged, by adopting the advanced production technology accompanying with the use of inoculums, balanced nutrition, weed management and high yielding varieties (Hakoomat Ali *et al*., 2004) [6].

Chickpea is considered to sustain cropping system productivity due to its ability to fix atmospheric nitrogen. It has highly specific symbiotic association, with a unique group of rhizobia necessary for formation of nodules and nitrogen fixation. Absence of suitable strains, small population size and poor survival of rhizobia cause problems in nodules formation (Kantar *et al*., 2007) [7]. Presence of appropriate nodule forming bacteria in the soil is essential for management and utilization of atmospheric nitrogen. If nodulating crop has not been sown in recent past and grown for the first time then seed inoculation is essential before sowing. Further, to avoid uncertainty about natural inoculation, the seed should be inoculated every time. Nitrogen fixing potential of chickpea genotypes can be increased significantly by rhizobial inoculation. Grain yield of chickpea increased considerably with rhizobial application (Khattak *et al*., 2006) [9]. Chickpea yield can be enhanced by inoculation with competitive rhizobia and is especially economical promising to increase chickpea production (Romdhane *et al*., 2008). Artificial seed inoculation of chickpea in those soils lacking native effective rhizobia is a very useful practice for improving root nodulation and yield of the crop (Khattak *et al*., 2006, Muhammad *et al*., 2010) [9, 12]. Botir Khaitov *et al*. (2020) [4] through his field experimentation on chickpea was concluded that indigenous rhizobial strain have the characteristics of broad host range, effective stimulation, higher nodulation efficiency, greater salt tolerance and can be considered as a biofertilizers for enhancing chickpea productivity in saline soil of Uzbekistan. Evaluation of *Mesorhizobium* isolates in chickpea and other pulses crop have been reported by many earlier workers. Bhuiyan *et al*. (2008) [2] carried out field experiment during two consecutive Rabi seasons in chickpea with a view to assessing the effect of *Mesorhizobium* inoculation on four varieties of chickpea and obtained highest nodule number, nodule dry weight and grain yield.

Keeping this in view, the present investigation was conducted to evaluate the effect of *Mesorhizobium* isolates collected from saline soil tract on nodulation and grain yield in chickpea under field conditions.

Material and Methods

Isolation and molecular Identification of chickpea nodulating bacteria

Healthy plants of chickpea from saline area of Vidarbha region (M.S.), India were collected by random sampling methodology and have been processed for nodule reside rhizobia isolation. The methodology was done according to the Singh *et al*. (2016) [15].

A field experiment was conducted during *Rabi* 2017-18 and 2018-19 at Pulses Research Unit, Dr. PDKV, Akola (M.S). The experiment was laid out in randomized block design with thirty-three treatments. Besides uninoculated control there were thirty-two different treatments of seed inoculation of *Mesorhizobium* which obtained from chickpea root nodules from different villages of saline tract of Purna river of Vidarbha region (M.S.) of India. Seeds were inoculated with respective carrier base culture of *Mesorhizobium* inoculants prior to sowing using 25 g kg^{-1} of seed. Each treatment has gross plot size 3.00 X 2.40 m^2 and net plot 2.80 X 1.80 m^2 and replicated thrice with chickpea variety JAKI-9218. The data on nodulation was recorded at 35 DAS from five randomly selected plants from each plot. The roots of uprooted plants were gently washed with water and counted the active nodules. Seed yields were also recorded after crop harvest. Data obtained through experimentation were subjected to analysis of variance (ANOVA) using Panse and Sukhatme (1967) [13].

Molecular Identification of Potential isolates

Based on the pooled data of 2017-18 & 2018-19, the first three potent isolates were taken for the molecular identification and was done according to the Maheshwari *et al*. (2021) [9]. Genomic DNA of studied isolates was isolated through Genomic DNA Purification Kit (Promega) and partial 16S rRNA gene was amplified in an ABI PCR System from universal primer pairs (Singh *et al*. 2019). Further, amplified PCR product was examined by horizontal electrophoresis in 1.2% agarose for quality check. Further, the amplified PCR amplicon were purified with Nucleo-Pore gel elution kit. Purified 16S rRNA gene was directly sequenced using the ABI sequencer (Model 3130xl). Annotation of sequenced gene was done by BLASTN search analysis and curated sequences were deposited in Gene Bank.

Results and Discussion

Table 1: Effect of prominent *Mesorhizobium* isolates on nodulation and grain yield of Chickpea (Pooled data of two years 2017-18 & 2018-19)

Isolates	No. of Nodules/ plant	Nodule dry wt/ plant (mg)	Plant dry weight/pl (g)	Grain Yield Kg/ha
ACR-10	28.07	100.94	4.45	1641
ACR-20	26.67	97.20	4.38	1582
BCR-34	26.04	95.54	4.35	1553
BCR-35	27.67	99.71	4.42	1630
BCR-36	27.60	98.67	4.40	1606
BCR-40	26.77	98.03	4.39	1593
BCR-46	25.40	93.19	4.35	1545
Control	12.44	32.01	3.23	965
S.E.(m)+	1.03	1.08	0.13	59
C.D. P=0.05	3.02	3.31	0.36	174

Effect on Nodulation: Seed inoculation with *Mesorhizobium* isolates increased the nodule numbers as compared to uninoculated control (12.44plant^{-1}). The data from the table and Fig 1 graphically indicated that the treatment of *Mesorhizobium* ACR-10 isolate recorded the highest nodules number and was followed by *Mesorhizobium* BCR-35 and BCR-36 (27.67 & 27.60 nodules plant^{-1}). The variation in nodule number was due to better compatibility and efficiency of inoculated *Mesorhizobium* compared to the native rhizobia in forming effective nodules in the rhizosphere of chickpea. The inoculation with *Mesorhizobium* isolates significantly increased nodulation in all the isolates tested. ACR-10 *Mesorhizobium ciceri* increased (55.68%) nodules per pant over uninoculated control in the present studies. The results of nodulation are in agreement with Bhuiyan *et al*. (1998) [3] who reported the *Rhizobium* inoculation increased nodulation and seed yield upto 35% in chickpea. The significance of *Mesorhizobium* bacteria in improving nodulation in salt tolerance and salt stress soil in legumes has also been extensively reported by Afrasayab *et al*. 2010 [1] and Mhadhbi *et al*. 2004 [10].

Effect on Nodules and Plant Dry Weight

The pooled interaction effect of *Mesohizobium* isolates on nodules and plant dry weight was significant showed by graphically (Fig 3). The maximum nodules dry weight (100.94mg/plant) 68.28% and plant dry weight (4.45 g/plant) 27.41% was recorded in *Mesohizobium ciceri* ACR-10 followed by BCR-35 (99.71mg/plant and 4.42g/plant) 67.90% and 26.92% over uninoculated control. The increased dry weight of nodules, shoot and root of chickpea due to *Mesorhizobium* isolates over control in saline soil and *Mesorhizobium* inoculation increased the biomass of tested nine chickpea genotypes by 3.3 to 33.9% relative to the control was reported earlier (Botir Khaitov *et al*. 2020) [4].

Effect on grain yield: In the present investigation, seed inoculation with *Mesorhizobium ciceri* ACR-10 isolate significantly increased the grain yield over uninoculated control (965 kg ha^{-1}). Graphical representation of grain yield is showed in graph (Fig 2), it is revealed that the seed inoculation with *Mesorhizobium ciceri* ACR-10 recorded the highest grain yield 41.19% and was closely followed by BCR-35 i.e. 40.80% over uninoculated control. Yield advantages over uninoculated control occurred due to seed inoculation with ACR-10 and BCR-35 respectively. Increase in grain yield could be attributed due to better crop growth, better nodulation and seed inoculation with efficient and better

Mesorhizobium isolate. Inoculation with *Mesorhizobium* isolates obviously enhanced the yield of chickpea and was found statistically different over control. Bhuiyan *et al*. (1998) [3] found that *Rhizobium* inoculation increased seed yield upto 35%. Gupta and Namdeo (1996b) [5] found that seed inoculation with *Rhizobium* increased chickpea seed yield by 9.6 to 27.9%. *Many* researchers declared that inoculation with appropriate *Mesorhizobium* strains is effective measurers to increase N_2 fixation, promote N nutrition and enhance yield in legumes (Mirza *et al*. 2007 Thomashow & Bakker 2015) [11, 17].

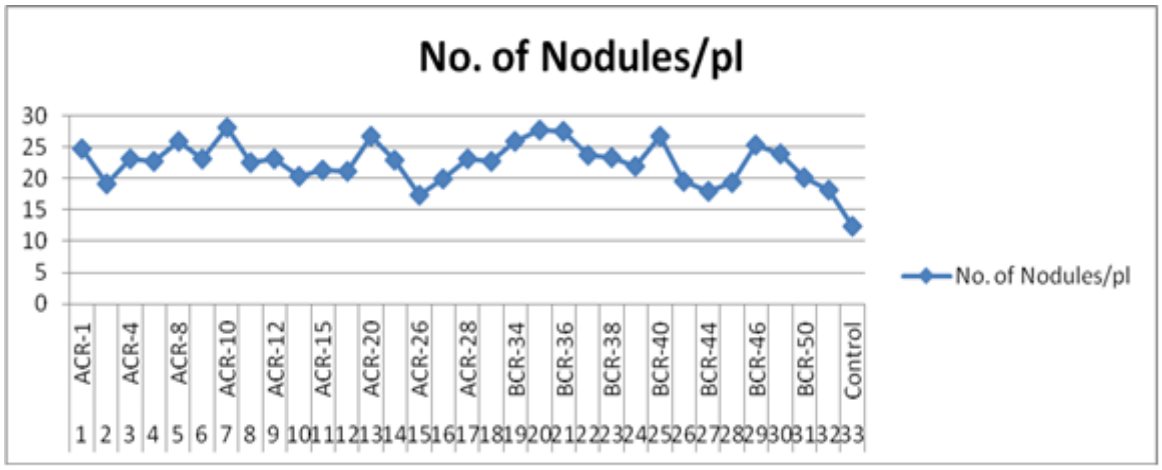

Fig 1: Effect of seed inoculation with *Mesorhizobium* isolate on nodulation of chickpea

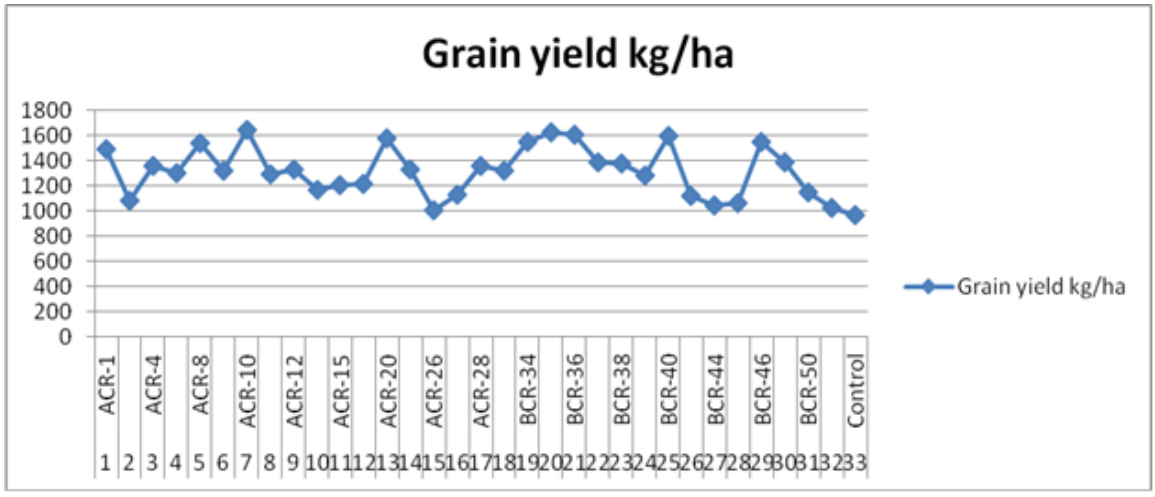

Fig 2: Effect of seed inoculation with *Mesorhizobium* isolate on grain yield of chickpea.

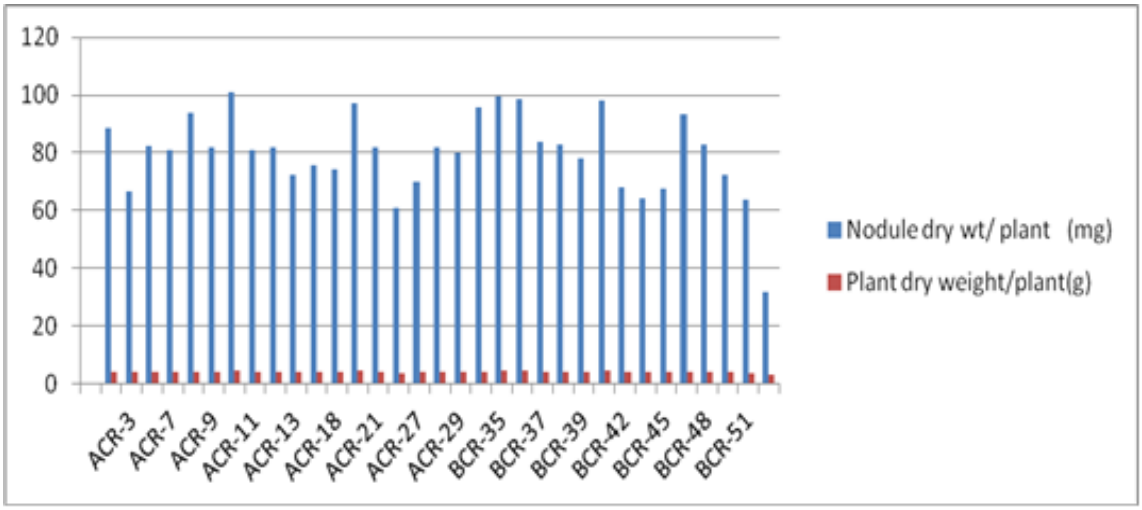

Fig 3: Effect of various *Mesorhizobium* seed inoculation on yield attributing traits of Chickpea.

Molecular identification of Isolates

Molecular identification of the top three symbiotically efficient isolates ACR-10, BCR-35 and BCR-36 were done from partial 16S rRNA gene sequence of1281 bp, 1293bp and

1223bp, respectively. A Blast report with these sequences revealed that strains ACR-10, BCR-35 and BCR-36 fallen within the genus *Mesorhizobium* sp. *ciceri* with 99% to 100% sequence similarity. Further, the annotated sequences ACR-10, BCR-35 and BCR-36 were deposited at NCBI database (*https://www.ncbi.nlm.nih.gov*) under the accession number ON754969, ON754972 and ON754973 respectively. Zhang *et al*. (2019) [18] have also revealed the abundance of *Mesorhizobium* genus in chickpea root nodule. Similarly, Singh *et al*. (2016) [15] have revealed the 55 isolates and all were reported as *Mesorhizobium ciceri*.

Overall study concluded that the *Mesorhizobium* sp. is the core responsible microbiota for the efficient nodulation in chickpea. Seed inoculation with *Mesorhizobium ciceri* (ACR-10) was found most effective isolate for enhancing the yield and yield attributes of Chickpea.

Fig 4: Nodulation due to seed inoculation of *Mesorhizobium ciceri* (ACR-10)

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