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RESEARCH ARTICLE

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Evaluation of Endophytes for Enhancing the Growth and Seed Yield in Chickpea (*Cicer arietinum* L.)

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ABSTRACT

Background: Endophytes are microorganisms that reside in the tissues of living plants are relatively unstudied as a potential source of novel natural products for exploitation in medicine, agriculture and industry. In present study, the endophytes were extracted from aerial parts of chickpea (stem and leaves) brought from three different villages of Vijayapur district.

Methods: The isolation of bacteria was done and their morphological and biochemical properties were studied. The screening was done to select efficient endophytes based on compatibility test between *Rhizobium* GV-2 and the isolated endophytic bacteria and indole acetic acid test. Accordingly, three efficient endophytes BL-4, MS-1 and MS-4 were selected and identified as *Bacillus thuringiensis, B. cereus* strain LPDB2 and *B. cereus* strain LPDB5 based on 16S rRNA molecular identification. These three efficient endophytes along with the *Rhizobium* GV-2 were treated with the chickpea seeds in different combinations and sown in pot culture and field condition to check the effect of endophytes on crop performance in chickpea. The observations on plant height and dry matter at 30 DAS, 60 DAS and at harvest, number of branches per plant, number of pods per plant, seed yield per plant, seed yield per net plot, seed yield per hectare were recorded.

Result: The significant difference was observed among the treatments and the results revealed that treatment with *B. thuringiensis*+*B. cereus* strain LPDB2+*B. cereus* strain LPDB5+*Rhizobium* GV-2 noticed significantly higher performance in growth and yield parameters.

Key words: Chickpea, Crop performance, Endophytes.

INTRODUCTION

Legumes are highly important crops in human and animal nutrition and are grown globally under a wide range of agro-climatic conditions as a cash crop and as a source of nitrogen assimilation via nitrogen fixation. Chickpea is cultivated in nearly 50 countries around the world and accounts for more than 20 per cent of the world pulse production and much of the world chickpea supply (70%) comes from India. India ranks first in area and production of chickpea in the world, with an area of 105.61 lakh ha (70.90% in world), production of 112.29 lakh tonnes (67.41% in world) and productivity of 1063 kg/ha (Anonymous, 2019). Madhya Pradesh is leading state in terms of area and production as it contributes around 34 and 40 per cent share to the total area and production of chickpea in the country (Anonymous, 2018). Karnataka is one of the major chickpea producing states in the country and it is grown mainly in rabi season in black soil areas particularly in the northern districts of the state *viz*., Vijayapur, Dharwad, Belagavi, Gadag, Bagalkote and Bidar. Even though India is the largest producer of chickpea, it still imports chickpea from Australia and several other countries. At present, India represents the biggest importer of chickpeas accounting for around one-fifth of the total global import volumes. Keeping in view, the ever-increasing demand for this legume crop, it is essential to improve the production and area under cultivation, at the same time minimizing the stress on this crop plant. Slower growth of developing seedlings under various abiotic factors or biotic factors limits the growth and yield of crops, development of techniques for fast and homogeneous growth of seeds could be a sustainable approach for better agricultural productivity (Osburn and Schroth, 1989). In this aspect, improving the quality and germination and establishment of good plant stand through 'seed priming' is a sustainable approach to enhance yields and performance of plants (McDonald, 2000).

The use of microbes for seed priming is a viable and promising approach in the context of improvement in seed characteristics under changing environmental conditions. Seed priming with the use of endophytic microbial strains appears as more beneficial or stable than rhizospheric microbial strains due to better colonization adaptability and suitability under biotic and abiotic stress conditions (Ajaykumar *et al*., 2019). Inoculation with plant growth promoting bacteria has been devised as a beneficial strategy for improving the fitness of crop plants especially under harsh environmental conditions.

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Hence an attempt has been made to examine the effect of endophytes on crop performance in chickpea under pot culture and field condition.

MATERIALS AND METHODS

Effect of endophytes on crop performance in chickpea under pot culture

The pot culture experiment was conducted in the green house of Agricultural College, Vijayapur during 2020-21. The soil having the properties of pH 6.5, EC 0.30 dsm^{-1} available nitrogen 458 Kg/ha, available P_2O_5 40 Kg/ha and K_2O 190 kg/ha was passed through a 4 mm sieve and mixed along with farmyard manure at 2: l proportion and filled in pots @ 10 kg/pot as detailed below:

Experimental details

T_1: Control.
T_2: Chickpea seeds treated with *Bacillus thuringiensis*.
T_3: Chickpea seeds treated with *B. cereus* strain LPDB2.
T_4: Chickpea seeds treated with *B. cereus* strain LPDB5.
T_5: Chickpea seeds treated with *Rhizobium* GV-2.
T_6: Chickpea seeds treated with *B. thuringiensis*+*Rhizobium* GV-2.
T_7: Chickpea seeds treated with *B. cereus* strain LPDB2+ *Rhizobium* GV-2.
T_8: Chickpea seeds treated with *B. cereus* strain LPDB5+ *Rhizobium* GV-2.
T_9: Chickpea seeds treated with *B. thuringiensis*+*B. cereus* strain LPDB2+ *B. cereus* strain LPDB5+*Rhizobium* GV-2.
Replication: 3.
Design: Completely randomized design.

The endophytic bacteria (*Bacillus thuringiensis*, *B. cereus* strain LPDB2, *B. cereus* strain LPDB5) and *Rhizobium* were grown on nutrient broth for 48 h at room temperature. The bacterial cells harvested by centrifugation at 1000 rpm for 15 minutes and biofertilizer was made using talc. Talc was prepared separately containing four different bacteria along with different combinations. Chickpea seeds were treated accordingly @ 1250 g per hectare and sown in pots as well as in field (Sushma, 2015).

Observations recorded

Plant height

Plant height at 30 days, 60 days and at harvest were recorded and expressed in cm. After harvest, the plants were uprooted carefully and were dipped in water for 30 min to loosen the soil.

Number of branches per plant

The branches arising from the main stem was counted at harvest of the crop and the mean was worked out and expressed as number of branches per plant.

Number of pods per plant

Number of filled pods present was counted individually and the average was worked out and expressed as number of pods per plant at harvest.

Seed yield per plant

The seeds harvested from individual plant were dried, threshed, cleaned, weighed and expressed in grams.

Plant dry matter accumulation per plant

The plant samples were oven dried at 60°C for 4-6 days and dry weight of samples were recorded and expressed in gram after harvest depicted in Plate 1.

Effect of endophytes on crop performance in chickpea under field condition

The experiment was conducted in seed farm, Department of Seed Science and Technology Block at College of Agriculture, Vijayapur which is situated in Northern dry zone of Karnataka (Zone 3), It is situated at 160 49’ North latitude, 750 43’ East longitude and at an altitude of 593.8 m above the mean sea level (MSL).

Experimental details

Title of experiment: Effect of endophytes on crop performance in chickpea (*Cicer arietinum* L.), crop: chickpea, variety: JG-11, season: *Rabi,* location: seed farm, College of Agriculture, Vijayapur, design of experiment: RBD, treatments: 9, replications: 3, spacing: 45 × 10 cm, fertilizer: $4N:10P_2O_5$ kg ha^{-1}, plot size (gross plot): 3.15 m × 3 m, net plot size: 2.7 m × 2.4 m, seed rate: 20 kg/acre and seed source: seed unit, RARS, Vijayapur.

Treatment details

The similar treatments were imposed as detailed in pot culture experiment with three replications laid out in RBD concept.

Observations recorded

on growth and yield components.

RESULTS AND DISCUSSION

Effect of endophytic bacteria and *Rhizobium* on growth parameters under pot culture and field condition in chickpea

Significant differences were observed in the growth parameters like plant height at 30 DAS, 60 DAS and at harvest, number of branches per plant and plant dry matter accumulation at 30 DAS, 60 DAS and at harvest between the treatments (Table 1 and 2). The highest plant height in pot culture and field condition was recorded in treatment T_9 (15.63 and 17.97 cm) *Bacillus thuringiensis*+*B. cereus* strain LPDB2+*B. cereus* strain LPDB5+*Rhizobium* GV-2 followed by T_6 (14.93 and 17.27cm) *B. thuringiensis*+*Rhizobium* GV-2 and treatment T_7 (14.49 and 16.83 cm) *B. cereus* strain LPDB2+*Rhizobium* GV-2 while, least plant height was recorded in Control (12.74 and 15.07 cm) at 30 DAS. At 60 days after sowing, highest plant height in pot culture and field condition was recorded in T_9 (22.53 and 24.07 cm) *B. thuringiensis*+*B. cereus* strain LPDB2+*B. cereus* strain LPDB5+*Rhizobium* GV-2 followed by T_6 (21.07 and 23.40 cm) *B. thuringiensis*+*Rhizobium* GV-2 and treatment T_7

Plate 1: General view of the post culture experiment.

Table 1: Effect of endophytic bacteria and *Rhizobium* on growth and yield parameters under pot culture in chickpea.

Treatments	Plant height (cm)			Number of branches/ plant	Dry matter accumulation/ plant (gm)	Number of pods/ plant	Seed yield/ plant (gm)
	30 DAS	60 DAS	At harvest				
T_1: Control	12.74	18.60	36.27	5.97	22.13	52.67	18.53
T_2: *Bacillus thuringiensis*	13.60	20.47	37.80	6.97	23.21	55.67	19.57
T_3: *B. cereus* strain LPDB2	13.13	19.13	36.93	6.47	22.58	53.33	18.74
T_4: *B. cereus* strain LPDB5	12.96	19.23	36.63	6.20	22.22	53.00	18.63
T_5: *Rhizobium* GV-2	12.94	19.70	37.07	6.77	23.00	54.00	19.11
T_6: *B. thuringiensis*+*Rhizobium* GV-2	14.93	21.07	39.40	7.50	24.13	58.67	20.44
T_7: *B. cereus* strain LPDB2+*Rhizobium* GV-2	14.49	20.93	38.53	7.27	23.93	57.00	19.97
T_8: *B. cereus* strain LPDB5+*Rhizobium* GV-2	14.16	20.40	38.67	7.10	23.52	56.67	19.86
T_9: *B. thuringiensis*+*B. cereus* strain LPDB2+*B cereus* strain LPDB5+*Rhizobium* GV-2	15.63	22.53	40.60	7.87	24.66	59.33	20.65
Mean	13.84	20.23	37.99	6.90	23.27	55.59	18.81
S.Em (±)	0.17	0.51	0.62	0.37	0.10	1.09	0.17
CD @ 5%	0.50	1.53	1.85	1.11	0.31	3.28	0.24

Table 2: Effect of endophytic bacteria and *Rhizobium* on growth and yield parameters under field conditions in chickpea.

Treatments	Plant height (cm)			Number of branches/ plant	Dry matter accumulation/plant (gm)			Number of pods/ plant	Seed yield (kg/net plot)	Seed yield (kg/ha)
	30 DAS	60 DAS	At harvest		30 DAS	60 DAS	At harvest			
T_1: Control	15.07	20.93	38.60	7.00	1.54	9.97	24.17	55.33	1.21	1281
T_2: *Bacillus thuringiensis*	15.93	22.73	40.13	7.80	1.59	10.17	25.25	59.00	1.36	1440
T_3: *B. cereus* strain LPDB2.	15.30	21.57	39.27	7.50	1.56	10.09	24.62	57.00	1.28	1354
T_4: *B. cereus* strain LPDB5.	15.27	21.47	38.97	7.23	1.55	10.07	24.26	55.67	1.25	1323
T_5: *Rhizobium* GV-2	15.50	22.03	39.40	7.67	1.58	10.11	25.02	58.00	1.32	1397
T_6: *B. thuringiensis+Rhizobium* GV-2	17.27	23.40	41.73	8.53	1.63	10.61	26.17	61.33	1.43	1515
T_7: *B. cereus* strain LPDB2+ *Rhizobium* GV-2	16.83	23.27	41.00	8.30	1.62	10.29	25.96	60.00	1.40	1485
T_8: *B. cereus* strain LPDB5+*Rhizobium* GV-2	16.50	22.80	40.87	8.13	1.61	10.19	25.56	59.33	1.38	1459
T_9: *B. thuringiensis+B. cereus* strain LPDB2+*B. cereus* strain LPDB5+*Rhizobium* GV-2.	17.97	24.07	42.93	8.90	1.64	10.76	26.70	61.67	1.46	1545
Mean	16.18	22.47	40.32	7.90	1.59	10.25	25.30	58.59	1.34	1422
S.Em (±)	0.17	0.61	0.62	0.37	0.01	0.11	0.17	1.34	0.01	5.9
CD @ 5%	0.51	1.84	1.85	1.12	0.02	0.32	0.51	4.03	0.02	17.7

(20.93 and 23.27 cm) *B. cereus* strain LPDB2+*Rhizobium* GV-2 while, least plant height was recorded in T_1 (18.60 and 20.93 cm) Control and at harvest, the maximum plant height in pot culture and field condition was seen in T_9 (40.60 and 42.93 cm) *B. thuringiensis+B. cereus* strain LPDB2+*B. cereus* strain LPDB5+*Rhizobium* GV-2 followed by T_6 (39.40 and 41.73 cm) *B. thuringiensis+Rhizobium* GV-2 and treatment T_7 (38.53 and 41.00 cm) *B. cereus* strain LPDB2+*Rhizobium* GV-2 while, least plant height was recorded in T_1 (36.27 and 38.60 cm) Control. Similar work was also carried out by Verma *et al.,* (2013) who revealed that mutual inoculation improves the plant height as compared to uninoculated control. It may be because of the growth promoting hormones and nutrient uptake ability. Increase in the vegetative and reproductive growth in all the combination of isolates could have been lead to the better solubilization of phosphorous, development of disease resistance and production of plant growth hormones (Sturz *et al.,* 1997). The increased plant height could be attributed to increased cell elongation and multiplication because of enhanced nutrient content in plants following inoculation of *Rhizobium* sp. and endophytic bacteria. Similar results were also reported earlier by Tomar *et al.* (1996).

The maximum number of branches per plant in pot culture and field condition at harvest was observed in T_9 (7.87 and 8.90) *Bacillus thuringiensis+B. cereus* strain LPDB2+*B. cereus* strain LPDB5+*Rhizobium* GV-2 followed by T_6 (7.50 and 8.53) *B. thuringiensis+Rhizobium* GV-2 and T_7 (7.27 and 8.30) *B. cereus* strain LPDB2+*Rhizobium* GV-2 while, least number of branches per plant was recorded in T_1 (5.97 and 7.00) Control. Similarly, maximum dry matter accumulation of chickpea seeds from field condition at 30 DAS was observed in T_9 (1.64 gm) *B. thuringiensis+B. cereus* strain LPDB2+*B. cereus* strain LPDB5+*Rhizobium* GV-2 followed by treatment T_6 (1.63 gm) *B. thuringiensis+Rhizobium* GV-2 and treatment T_7 (1.62 gm) *B. cereus* strain LPDB2+ *Rhizobium* GV-2 while, least dry matter accumulation was recorded in T_1 (1.54 gm) Control. At 60 DAS, the maximum dry matter accumulation of chickpea seeds from field condition was observed in treatment T_9 (10.76 gm) *B. thuringiensis+B. cereus* strain LPDB2+*B. cereus* strain LPDB5+*Rhizobium* GV-2 followed by treatment T_6 (10.61 gm) *B. thuringiensis+Rhizobium* GV-2 and treatment T_7 (10.29 gm) *B. cereus* strain LPDB2+ *Rhizobium* GV-2 while, least dry matter accumulation was recorded in T_1 (9.97 gm) Control. At harvest, the maximum dry matter accumulation of chickpea seeds from pot culture and field condition was observed in treatment T_9 (24.66 and 26.70 gm) *B. thuringiensis+B. cereus* strain LPDB2+*B. cereus* strain LPDB5+*Rhizobium* GV-2 followed by treatment T_6 (24.13 and 26.17 gm) *B. thuringiensis+Rhizobium* GV-2 and treatment T_7 (23.93 and 25.96 gm) *B. cereus* strain LPDB2+ *Rhizobium* GV-2 while, least dry matter accumulation was recorded in T_1 (22.13 and 24.17 gm) Control. Coinoculation of these rhizobial and nonrhizobial endophytic isolates resulted in increase in plant biomass, plant height and number of branches per plant in chickpea crop under pot culture and field condition when compared with single inoculation and uninoculated control. Zhao *et al*. (2011) reported that the legume *Sophora alopecuroides* on co-inoculation with Zong 1 *P. chlororaphi* (endophyte)+SQ1 *Mesorhizobium* sp. showed high growth under greenhouse conditions than those of single inoculation and showed a significant difference ($p < 0.05$) when compared to a negative control, suggesting that strains of Zong 1 and *Mesorhizobium* sp. SQ1 have better synergistic or addictive effect. Increase in plant growth parameters might be due to the production of phytohormones like IAA which was stated by Liao *et al*. (2014) which invigour the cellular division fostering root growth or it may be due to the siderophores production, which can amend the bioavailability of plant nutrients and thereby bracing growth (Joseph *et al*., 2012).

Effect of endophytic bacteria and *Rhizobium* on yield parameters under pot culture and field condition in chickpea

Significant differences were observed in the yield parameters like number of pods per plant, seed yield per plant, seed yield per net plot and seed yield per hectare between the treatments and depicted in in figure. At harvest, the maximum number of pods per plant in pot culture and field condition was observed in T_9 (59.33 and 61.67) *Bacillus thuringiensis*+*B. cereus* strain LPDB2+*B. cereus* strain LPDB5+*Rhizobium* GV-2 followed by T_6 (58.67 and 61.33) and T_7 (57.00 and 60.00) compared to T_1 (52.67 and 55.33) Control and their superiority was to an extent of 11.22 and 10.28, 10.22 and 9.78, 7.59 and 7.78 per cent respectively. The maximum seed yield per plant in pot condition was observed in T_9 (20.65 gm) *B. thuringiensis*+*B. cereus* strain LPDB2+*B. cereus* strain LPDB5+*Rhizobium* GV-2 followed by T_6 (20.44 gm) and T_7 (19.97 gm) compared to T_1 (18.53 gm) Control and their superiority was to an extent of 10.26, 9.34 and 7.21 per cent respectively. The maximum seed yield per net plot in field condition was recorded in T_9 (1.46 kg/plot) *B. thuringiensis*+*B. cereus* strain LPDB2+*B. cereus* strain LPDB5+*Rhizobium* GV-2 followed by T_6 (1.43 kg/plot) and T_7 (1.40 kg/plot) compared to T_1 (1.21 kg/plot) Control and their superiority was to an extent of 17.12, 15.38 and 13.57 per cent respectively. The maximum seed yield per hectare in field condition was observed in T_9 (1545 kg/ha) *B. thuringiensis*+*B. cereus* strain LPDB2+*B. cereus* strain LPDB5+*Rhizobium* GV-2 followed by T_6 (1515 kg/ha) and T_7 (1485 kg/ha) compared to T_1 (1281 kg/ha) Control and their superiority was to an extent of 17.08, 15.44 and 13.73 per cent respectively Fig 1.

The increase in seed yield is due to the higher number of pods, branches and seed size in treated seeds than control. Several other reasons which contribute to high yield are better seedling emergence and vigour, increased nutrient supply, increased photosynthetic pigments and phytohormone production with reduced disease and pest incidence. Liao *et al*. (2014) stated that the impact of yield might be due to early vegetative growth that help the plants

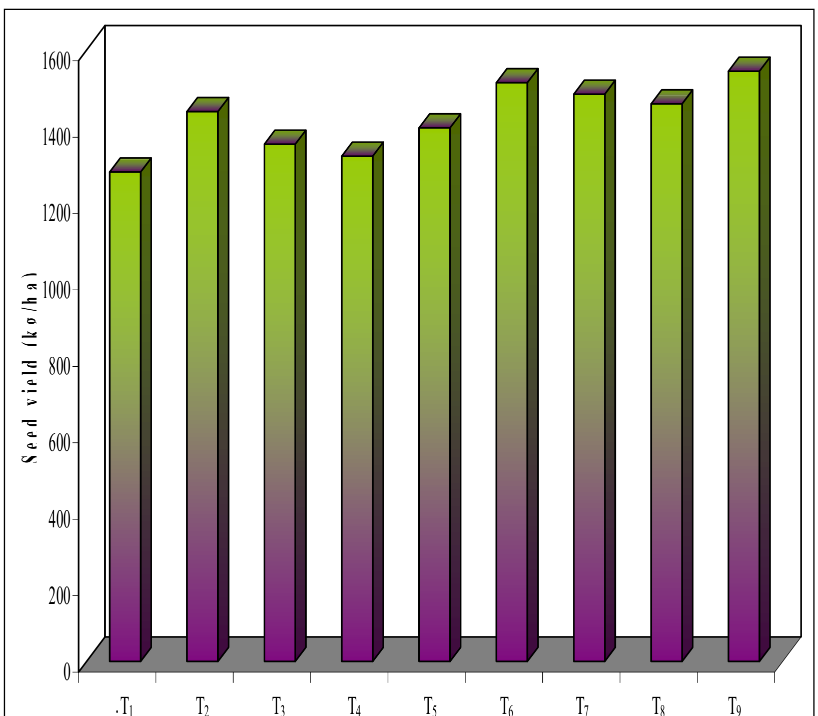

Fig 1: Effect of endophytic bacteria and *rhizobium* on seed yield per hectare under field condition.

T_1: Control; T_2: *Bacillus thuringiensis*; T_3: *B. cereus* strain LPDB2; T_4: *B. cereus* strain LPDB5; T_5: *Rhizobium* GV-2; T_6: *B. thuringiensis*+*Rhizobium* GV-2; T_7: *B. cereus* strain LPDB2+*Rhizobium* GV-2; T_8: *B. cereus* strain LPDB5+*Rhizobium* GV-2; T_9: *B. thuringiensis*+*B. cereus* strain LPDB2+*B. cereus* strain LPDB5+*Rhizobium* GV-2.

to outpass the biotic and abiotic stress condition. This outcome is in accordance with Kabaluk and Ericsson (2007) who reported that stand density and yield of corn increased by controlling the wireworm. Oyetunji *et al*. (2019) reported that increase in grain number was observed in rice treated with *M. anisopliae* and *B. bassiana* along with reduction in adults of African gall midge. As seed yield depends on various environmental variables like plant density (Diepenbrock, 2000), increase in seed yield might be due to better field emergence and plant density.

CONCLUSION

Seed treatment with *B. thuringiensis*+*B. cereus* strain LPDB2+*B. cereus* strain LPDB5+*Rhizobium* GV-2 were treated @ 1250 g per hectare and sown in pots as well as in field has recorded higher growth and seed yield attributing parameters followed by *B. thuringiensis*+*Rhizobium* GV-2 as compared to untreated under pot and field conditions.

Conflict of interest: None.

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