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In Vitro Studies on Effect of Endophytic Bacteria and Rhizobium on Seed Quality Parameters in Chickpea

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ABSTRACT: 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. 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. The investigation has aimed in seed quality parameters under *in vitro* condition. Seed quality parameters include seed germination percentage, seedling length, seedling vigour index-I and test weight. 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 seed quality parameters.

Keywords: Chickpea, Seed quality, Endophytes

Chickpea (*Cicer arietinum* L.) is an ancient crop of modern times and 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. 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 (3). 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 other countries. 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 (10). In this aspect, improving the seed quality and establishment of good plant stand through 'seed priming' is a sustainable approach to enhance seed quality of plants (9).

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 (2). 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.

Therefore, an attempt has been made to examine the invitro studies on effect of endophytic bacteria and rhizobium on seed quality parameters in chickpea.

MATERIALS AND METHODS

The plant samples were collected from Basavanabagewadi (16°33′36.814′′ N and 75°51′8.955′′ E), Huvinahipparagi (16°33′8.105′′ N and 76°5′0.985′′

E) and Muddebihal (16°25′33.033′′ N and 76°7′11.476′′ E) villages of Vijayapur district. The endophytic bacteria were isolated for their morphological and biochemical properties were studied. Morphological properties encompass cell and colony morphology whereas, biochemical properties include Indole test, MRVP test, Citrate utilization test, Starch hydrolysis test, Urea hydrolysis test, Catalase test, Oxidase test (5). The screening was done to select efficient endophytes based on compatibility test between rhizobium GV-2 and the isolated endophytic bacteria and IAA test. Three efficient endophytes were selected to study *invitro* effect of endophytic bacteria and rhizobium on seed quality parameters in chickpea.

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. This biofertilizer containing four different bacteria along with different combinations were treated accordingly (Sushma, 2015). Using the between paper method, *in vitro* experiment was carries out.

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 and T_9: Chickpea seeds treated with *B. thuringiensis* + *B. cereus* strain LPDB2+ *B. cereus* strain LPDB5 + *Rhizobium* GV-2.

The observations recorded on seed quality parameters

Germination test was conducted as per the International Seed Testing Association procedure by adopting between paper method in a germinator maintained at 25-30°C and 95±3 per cent relative humidity in three replications each of 100 seeds. The first count on fourth day and second count on tenth day of germination for normal seedling was made and expressed as percentage (8).

The seedlings shoot and root length were measured after the final count in standard germination test. Ten normal seedlings were selected randomly from each replicate. The shoot length was measured from point of the attachment of the cotyledon to the tip of the seedling. Similarly, the root length was measured from the point of attachment to the tip of the root. Average shoot or root length (cm) was computed by dividing the total shoot or root length by total number of normal seedlings measured (6).

Seedling vigour index-I was calculated by using the below formula as suggested by (1), expressed in whole number.
Vigour Index-I (VI) = Germination (%) x Seedling length (cm)

Hundred seeds were counted manually from a sample drawn randomly from each treatment in eight replications and weighed. The mean weight of the samples was recorded as hundred seed weight and expressed in grams.

RESULTS AND DISCUSSION

The observations were recorded on standard germination test, seedling length, seedling vigour index-I and test weight of chickpea seeds under *in vitro* condition.

The maximum germination percentage of chickpea seeds under *in vitro* was observed in treatment T_9 (95.67 %) *Bacillus thuringiensis* + *B. cereus* strain LPDB2 + *B. cereus* strain LPDB5 + *Rhizobium* GV-2 followed by T_6 (95.50 %) and T_7 (94.83 %) compared to T_1 (92.83 %) Control and their superiority was to an extent of 2.96, 2.79, 2.10 per cent compared to control respectively. The maximum seedling length of chickpea seeds under *in vitro* was recorded in treatment T_9 (25.69 cm) *B. thuringiensis* + *B. cereus* strain LPDB2 + *B. cereus* strain LPDB5 + *Rhizobium* GV-2 while least seedling length was recorded in T_1 (21.03 cm) Control. The maximum seedling vigour index-I of chickpea seeds under *in vitro was* observed in treatment T_9 (2458) *B. thuringiensis* + *B. cereus* strain LPDB2 + *B. cereus* strain LPDB5 + *Rhizobium* GV-2, least seedling vigour index-I was recorded in T_1 (1951) Control. The maximum test weight of chickpea seeds under *in vitro (after* seed treatment) was observed in treatment T_9 (32.28 gm) *B. thuringiensis* + *B. cereus* strain LPDB2 + *B. cereus* strain LPDB5 + *Rhizobium* GV-2, least test weight was recorded in T_1 [23.37 (without seed treatment) gm] Control.

Increase in germination percentage in treated seeds is due to minimum electrical conductivity *i.e.,* very low seed leachates than untreated and hence subsequent increase in seedling length and seedling vigour index was

Table 1. *Invitro* studies on effect of endophytic bacteria and rhizobium on seed quality parameters in chickpea

Sl. No.	Treatments	Test weight (after seed treatment) (gm)	Seed germination (%)	Seedling length (cm)	Seedling vigour index-I
1	T_1: Control	23.37	92.83	21.03	1951
2	T_2: *Bacillus thuringiensis*	31.60	94.17	23.73	2235
3	T_3: *B. cereus* strain LPDB2.	31.59	93.50	23.33	2182
4	T_4: *B. cereus* strain LPDB5.	31.58	93.17	21.63	2017
5	T_5: *Rhizobium* GV-2	31.60	93.83	23.47	2202
6	T_6: *B. thuringiensis* + *Rhizobium* GV-2	32.20	95.50	24.90	2378
7	T_7: *B. cereus* strain LPDB2+ *Rhizobium* GV-2	32.20	94.83	24.73	2346
8	T_8: *B. cereus* strain LPDB5 + *Rhizobium* GV-2	32.18	94.50	24.27	2293
9	T_9:*B. thuringiensis* + *B. cereus* strain LPDB2 + *B. cereus* strain LPDB5 + *Rhizobium* GV-2	32.28	95.67	25.69	2458
10	Mean	30.96	94.22	23.64	2229.0
11	S.Em (±)	0.11	0.84	0.44	46.95
12	CD @ 1 %	0.45	3.48	1.80	193.93

observed. The increased germination percentage might be due to combined effect of increased production of hydrolytic enzymes, protease and amylase and production of growth hormones like IAA, gibberellins or by inducing the host hormone production (11) which regulates the endogenous hormone level and *metabolism* due to endophytic seed treatments. Increase in root and shoot length due to endophytic seed treatment might be due to enhanced production of cytokinin and auxin which induces cell division and elongation. It is reported that increase in root and shoot weight was due to production of cytokinin in *B. subtilis* treated seeds as it increases the cell division and also due to production of other endogenous hormones like IAA and gibberellins(4). The increase may be also due to the reduction in endogenous ACC by ACC deaminase bacteria (*Bacillus* sp.,) (7). Indistinguishable hike was noticed in other parameters also *viz.,* seedling length and vigour index over control seeds due to increased root and shoot length.

CONCLUSION

Significantly higher test weight, seed germination, seedling length and seedling vigour index under *invitro condition* was observed in treatment with *Bacillus thuringiensis* + *Bacillus cereus* strain LPDB2 + *Bacillus cereus* strain LPDB5 + *Rhizobium* GV-2.

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