Field application of ACC-deaminase biotechnology for improving chickpea productivity in Bahawalpur

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Abstract

The use of biofertilizer in crop production is gaining importance in modern agriculture, especially under stressful environments. In drought-hit areas of the world, improvement in drought tolerance of major food crops is one of the way forwards to ensure food security. Under water limited conditions, the use of ACC-deaminase biofertilizers can mitigate the adverse effects of drought stress on crop production. Present study was conducted for the field evaluation of ACC-deaminase based biofertilizer (*Rhizobium* and *Pseudomonas* strains) for improving chickpea productivity in Bahawalpur. Six field trials were conducted in different villages of Bahawalpur. Biofertilizer alone and in combination with enriched compost was evaluated under field conditions. Different treatments used in the experiment were control, P-Enriched compost, biofertilizer and P-Enriched compost + biofertilizer. The experiments were conducted in Randomized Complete Block Design with three replications. Results showed that enriched compost and biofertilizer, individually and in combination significantly improved the number of nodules plant⁻¹, nodules fresh weight and nodules dry weight of chickpea. The combined application of P-enriched compost and biofertilizer resulted in maximum increase in number of nodules plant⁻¹ and nodules dry weight as compared to control. The integrated use also gave better results in improving plant height and shoot dry weight, yield, number of pods plant⁻¹, number of grains pod⁻¹, and 1000 grain weight as compared to separate use of biofertilizer and P-enriched compost. A significant increase in nitrogen and phosphorus contents of chickpea was also observed with combined application of biofertilizer and compost. It can be concluded that the integrated use of ACC-deaminase containing biofertilizer and P-enriched compost is an effective biotechnology to improve chickpea productivity under field conditions and should be recommended to the farmers in the region, where water shortage is the main hindrance to agricultural sustainability.

Key words: Water shortage, agriculture sustainability, ACC-deaminase biotechnology, food

Introduction

Chickpea is one of the important pulses in human diet and is cultivated in over 50 countries worldwide. Its total global production during 2014 was 14 million tons (FAO, 2015) that is third among the pulse crops during that year. It is a rich source of protein along with minerals (calcium, magnesium, phosphorus, zinc and iron), β -carotene and vitamins (Jukanti *et al.*, 2012). The protein quality of chickpea is better than other available sources and is more economical to produce thus affordable for poor people (Arnoldi *et al.*, 2015). In addition to its importance in human diet, chickpea can also improve soil fertility by fixing atmospheric nitrogen with the help of *Rhizobia* present in root nodules (Duc *et al.*, 2014; Rubiales and Mikic, 2015).

Chickpea can be a good alternative to grain crops in arid and semi-arid climates where water availability is an issue. It is a hardy crop that can easily and successfully be

grown with limited availability of water (Singh and Mukherjee, 2009). However, according to available reports, the yield of chickpea is unstable and unpredictable due to number of biotic and abiotic stresses including precipitation in rain-fed areas (FAO, 2015). These stresses pose a number of adverse effects on crop growth and productivity including decreased photosynthetic rate, less water use efficiency, nutritional imbalance (Ahmad *et al.*, 2013a, 2014) and higher levels of ethylene production (Nadeem *et al.*, 2014). The higher levels of ethylene inhibit seed germination, root elongation and nodulation in legumes (Shaharoona *et al.*, 2006).

The ACC deaminase containing plant growth promoting rhizobacteria (PGPR) have the ability to induce stress tolerance in crop plants (Ahmad *et al.*, 2011) thus enabling them to withstand periods of drought and other stresses. Under limited water availability, these PGPR use different mechanisms for crop improvement including increased

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moisture availability, enhanced nutrient acquisition, phytohormones production and decreased ethylene production due to ACC deaminase activity (Bent *et al.*, 2001). Also, the careful selection and inoculation with efficient rhizobial strains can improve the nodulation in legume crops (Ahmad *et al.*, 2011) as some of them can survive well under drought stress as compared to others (Hussain *et al.*, 2014). These rhizobia facilitate the growth of their host plant by fixing atmospheric nitrogen, antibiotics production, siderophores production, and solubilization of minerals (Patten and Glick, 2002). The combined use of these rhizobia and PGPR can be an efficient and environmental friendly approach to improve the growth and productivity of crop plants under unfavourable environmental conditions (Selvakumar *et al.*, 2012; Ahmad *et al.*, 2013b; Nadeem *et al.*, 2014). Thus, the use of multi-strain biofertilizers containing efficient strains of ACC deaminase producing rhizobacteria and Rhizobia can be a resourceful biotechnology for improving growth and productivity of chickpea under limited availability of water.

Keeping in view the entire scenario, a multi-strain biofertilizer containing novel strains (*Mesorhizobium cicri*,

Pseudomonas sp. and *Bacillus* sp.) was developed through a series of experiments under laboratory, green house, and field conditions. In the present paper, we report the results of experiments conducted on farmer's fields at Bahawalpur to evaluate the use of biofertilizer for improving chickpea productivity under limited water resources.

Materials and Methods

Preparation of biofertilizer

Biofertilizer was prepared at the using novel strains of *Mesorhizobium cicri*, *Pseudomonas* sp. and *Bacillus* sp. at University of Agriculture Faisalabad. The already installed biofertilizer production unit was used to prepare biofertilizer. The biofertilizer was used to inoculate the seed for field experiments.

Sowing of crop in the field

Field trials were conducted at six locations in different villages of Bahawalpur zone (Figure 1 and 2). The chickpea crop was sown in November, 2014 according to the treatment plan viz. control, P-enriched compost,

Figure 1: Maximum and minimum temperatures during crop season

Figure 2: Rainfall distribution during crop season

Source: Cotton Research Institute, Regional Agricultural Research Institute Bahawalpur

biofertilizer and P-enriched compost + biofertilizer. The experiments were conducted in Randomized Complete Block design with three replications. Recommended dose of P and K @ 60 kg ha⁻¹ while half of the recommended dose of nitrogen (20 kg ha⁻¹) was applied to each plot as basal dose and seeds of chickpea inoculated with multi-strain biofertilizer containing *Mesorhizobium ciceri*, *Pseudomonas* sp. and *Bacillus* sp. were sown. Sowing was done by hand drill and row to row distance was maintained 30 cm while plant to plant distance 5-7 cm. All the agronomic practices were carried out as and when required.

Data recording and analysis

Nodulation data were recorded at flowering by carefully uprooting the plants with enough surrounding soil keeping in view that minimal damage to roots occur. Five plants per experimental unit were uprooted along with rhizospheric soil, washed thoroughly under running water on a sieve of mesh size that don't allow nodules to pass through but the soil. Nodules were counted and fresh as well as oven dry weight was recorded. At harvesting, plant agronomic data were recorded and samples were collected for analyses. Dried ground plant material was digested according to the method of Wolf (1982) and N, P and K concentration was determined as described by Ryan *et al.* (2001).

Physiological parameters i.e. chlorophyll-a, and chlorophyll-b contents in leaves were recorded at physiological maturity by extracting leaf material (0.05 g) in 10 mL dimethylsulfoxide (Hiscox and Israelstam, 1979). The samples were heated at 65 °C for 4 h and then the absorbance of extract was recorded at 663 and 645 nm as described by Arnon (1949). Data were subjected to statistical analysis by using LSD technique at 5% level of significance (Steel *et al.*, 1997).

Results

Results showed that the application of biofertilizer and P-enriched compost improved the root growth and nodulation in chickpea grown under field conditions (Figure 3). The data regarding number of nodules plant⁻¹ (Table 1) showed that the application of biofertilizer and P-enriched compost individually and in combination showed significant increase in number of nodules in chickpea at all sites. With the application of biofertilizer maximum increase in number of nodules plant⁻¹ as compared to control was observed at site 16BC that was 126% higher than control. The combined application of P-enriched compost and biofertilizer resulted in maximum increase (230%) in number of nodules as compared to un-inoculated control at the same site. There was a variation in the effectiveness of treatments at different sites.

The results showed that nodules fresh weight (Table 2) was significantly improved with the application of biofertilizer and P-enriched compost solely and in combination. When P-enriched compost was applied there was 2 fold increase in nodules fresh weight and with the application of biofertilizer 3 fold increase in nodules fresh weight was observed as compared to control at 15BC. The combined application of P-enriched compost and biofertilizer resulted in maximum increase (5 fold) in nodules fresh weight at the same site and it was also significantly better than other treatments at all experimental sites with exception of 13 BC where no significant difference in nodules fresh weight was observed among the combined or sole application of biofertilizer and P-enriched compost. As in case of nodules fresh weight nodules dry weight was also significantly improved with the application of biofertilizer and P-enriched compost solely and in combination (Table 3). Maximum increase was observed in case of combined application of P-enriched compost and biofertilizer at 15 BC that resulted up to 3 fold increase in nodules dry weight as compared to control.

The data regarding plant height (Table 4) revealed that the application of biofertilizer and P-enriched compost significantly improved the plant height of chickpea, grown at different locations. When P-enriched compost was applied individually, there was 11% increase in plant height in the experiment conducted at Baghdad. With the separate use of biofertilizer, plant height increased up to 16% at different locations. While the combined application of biofertilizer and P-enriched compost gave significantly better results and resulted in 22% increase in plant height at Baghdad. Significant increase in shoot dry weight with the application of P-enriched compost and biofertilizer has also been observed. Separate application of biofertilizer and P-enriched compost significantly increased shoot dry weight in chickpea. The combined application of biofertilizer and P-enriched compost showed significantly higher increase in shoot dry weight as compared to other treatments where 74% increase in shoot dry weight was observed as compared to control at the experimental site 118 DB (Table 5).

The results (Table 6) showed that the use of biofertilizer and P-enriched compost separately as well as in combination caused a significant increase in number of pod plant⁻¹. The sole application of P-enriched compost and biofertilizer resulted in up to 27 and 44% increase in number of pods plant⁻¹, respectively. The combined application of biofertilizer and enriched compost resulted in significantly higher increase in number of pod plant⁻¹ as compared to P-enriched compost and biofertilizer where 61% increase in number of pods plant⁻¹ as compared to control was observed at the experimental site Jageer bhati

dari. The number of grains pod^{-1} also significantly improved with the application of biofertilizer and P-enriched compost, solely and in combination (Table 7). Maximum increase in number of grains pod^{-1} was observed when biofertilizer was applied in combination with P-enriched compost. However, no significant difference was observed between sole application of biofertilizer and in combination with P-enriched compost.

The data presented in Table 8 showed that of 1000 grain weight was significantly improved with the application of biofertilizer and P-enriched compost. When

enriched compost and biofertilizer (Table 9). The separate application of biofertilizer and P-enriched compost increased the grain yield up to 32 and 10%, respectively, while the combined application of biofertilizer and P-enriched compost resulted in significantly higher increase in grain yield as compared to other treatments and the maximum increase was 51% as compared to control at the experimental site 15 BC.

The results regarding chlorophyll contents in leaves revealed that the use of biofertilizer and P-enriched compost resulted in significant increase in chlorophyll

Table 1: Effect of enriched compost and biofertilizer on number of nodules of chickpea

Treatment	No. of nodules (plant^{-1})					
	13 BC	15 BC	16 BC	Baghdad	118 DB	Jageer bhati dari
Control	40 d	27 c	23 d	37 d	38 d	30 d
Enriched compost (EC)	52 c	26 c	38 c	51 c	49 c	39 c
Biofertilizer (B)	62 b	49 b	52 b	59 b	61 b	57 b
EC + B	82 a	60 a	76 a	77 a	78 a	70 a
LSD ($p \leq 0.05$)	3.0518	3.7820	5.6115	3.5082	2.2828	4.8231

Table 2: Effect of enriched compost and biofertilizer on nodules fresh weight of chickpea

Treatment	Nodules fresh weight (g plant^{-1})					
	13 BC	15 BC	16 BC	Baghdad	118 DB	Jageer bhati dari
Control	0.81 b	0.38 d	0.51 d	0.75 d	0.74 d	0.67 d
Enriched compost EC)	1.59 a	1.17 c	0.70 c	0.94 c	0.96 c	1.01 c
Biofertilizer (B)	1.31 ab	1.88 b	1.79 b	1.09 b	1.09 b	1.31 b
EC + B	1.81 a	2.53 a	2.28 a	1.33 a	1.30 a	1.51 a
LSD ($p \leq 0.05$)	0.5423	0.1045	0.2001	0.0443	0.0622	0.0537

Table 3: Effect of enriched compost and biofertilizer on nodules dry weight of chickpea

Treatment	Nodules dry weight (g plant^{-1})					
	13 BC	15 BC	16 BC	Baghdad	118 DB	Jageer bhati dari
Control	0.13 d	0.08 d	0.11 d	0.36 d	0.13 d	0.16 d
Enriched compost (EC)	0.19 c	0.11 c	0.21 c	0.41 c	0.15 c	0.21 c
Biofertilizer (B)	0.24 b	0.17 b	0.26 b	0.52 b	0.22 b	0.36 b
EC + B	0.31 a	0.33 a	0.32 a	0.61 a	0.29 a	0.41 a
LSD ($p \leq 0.05$)	0.0204	0.0931	0.0279	0.0296	0.0734	0.0831

Means sharing same letters are statistically show insignificant results at par at 5 % level of probability ($n = 3$)

P-enriched compost was applied, maximum increase was observed at 118 DB where 17% increase in 1000 grain weight as compared to control was observed and with the sole application of biofertilizer 33% increase in 1000 grain weight as compared to control was observed at the same experimental site. Moreover, the combined application of P-enriched compost and biofertilizer resulted in maximum increase (40%) in 1000 grain weight as compared to control at 118 DB. The grain yield was also significantly improved with the application of P-

contents in leaves of chickpea. The combined application of biofertilizer and P-enriched compost resulted in significantly higher increase in chlorophyll 'a' contents in leaves as compared to other treatments (Table 10) where the increase was up to 19%. Data regarding chlorophyll-b contents in leaves (Table 11) showed that in most of trials, there was no significant difference in chlorophyll-b contents in leaves between combined application of biofertilizer with P-enriched compost and sole application of biofertilizer.

Data (Table 12) showed that the sole application of P-enriched compost and biofertilizer significantly enhanced nitrogen concentration of grains in chickpea. The combined application of biofertilizer and P-enriched compost resulted in maximum increase in nitrogen concentration in grains of chickpea. The phosphorus concentration in grains also significantly increased with the application of biofertilizer and P-enriched compost (Table 13). The combined use of P-enriched compost and biofertilizer was more effective than separate use and resulted in maximum increase in phosphorus concentration in grains of chickpea. The application of treatments increased potassium concentration in grains of chickpea but there was increase was not significant as compared to control (Table 14).

Rhizobia make symbiotic associations with legume plants and help plants to grow vigorously through atmospheric nitrogen fixation and several other mechanisms. The ACC deaminase containing rhizobacteria in combination with Rhizobia have been well documented to improve the efficiency of Rhizobial inoculation especially under stressful environments (Ahmad *et al.*, 2012, 2013c) thus they can be excellent supplement to chemical fertilizers for improving crop productivity.

Chickpea is a hardy crop that can grow under limited water conditions and sandy soils thus has the potential to fit well in several cropping sequences such as with maize, fodders and millets (Verma *et al.*, 2013). It is generally grown on marginal lands (sandy soils) under irrigation

Table 4: Effect of enriched compost and biofertilizer on plant height of chickpea

Treatment	Plant height (cm)					
	13 BC	15 BC	16 BC	Baghdad	118 DB	Jageer bhati dari
Control	37.47 c	41.66 c	46.21 c	38.98 c	47.00 b	43.07 c
Enriched compost (EC)	41.25 b	44.61 b	48.96 b	43.15 b	51.00 a	46.48 b
Biofertilizer (B)	44.63 a	47.80 a	53.81 a	44.55 b	54.00 a	49.78 ab
EC + B	45.58 a	48.59 a	54.63 a	47.71 a	54.33 a	51.46 a
LSD ($p \leq 0.05$)	2.4158	1.9489	1.8949	1.7532	3.6476	3.3838

Table 5: Effect of enriched compost and biofertilizer on shoot dry weight of chickpea

Treatment	Shoot dry weight (g plant ⁻¹)					
	13 BC	15 BC	16 BC	Baghdad	118 DB	Jageer bhati dari
Control	10.29 c	12.04 c	10.78 d	15.36 c	6.39 b	7.11 c
Enriched compost (EC)	12.18 b	13.89 bc	12.71 c	16.27 c	7.26 ab	9.04 b
Biofertilizer (B)	15.29 a	15.17 b	15.09 b	17.87 b	7.28 ab	9.78 b
EC + B	15.36 a	17.70 a	16.54 a	19.43 a	11.10 a	11.10 a
LSD ($p \leq 0.05$)	1.7079	1.9631	1.1834	1.4724	1.0641	1.2259

Table 6: Effect of enriched compost and biofertilizer on number of pods plant⁻¹ in chickpea

Treatment	Number of pods plant ⁻¹					
	13 BC	15 BC	16 BC	Baghdad	118 DB	Jageer bhati dari
Control	28.30 d	30.74 d	30.65 d	29.63 b	24.52 d	25.52 c
Enriched compost (EC)	30.19 c	35.02 c	34.02 c	30.86 b	31.22 c	32.06 b
Biofertilizer (B)	36.04 b	40.54 b	37.92 b	33.70 b	35.49 b	40.81 a
EC + B	38.17 a	47.25 a	40.37 a	37.84 a	38.15 a	41.02 a
LSD ($p \leq 0.05$)	1.1986	1.8372	1.7865	4.1106	1.9656	2.1224

Means sharing same letters are statistically show insignificant results at par at 5 % level of probability (n = 3)

Discussion

The rhizosphere is the most active region of soil where diverse plant-soil microbe interactions take place. Endophytic and rhizospheric microbes have been reported to have beneficial effects on plant growth and productivity through a number of direct and indirect mechanisms (Nadeem *et al.* 2014). Among endophytic bacteria,

conditions and in rainfed areas where water scarcity and increasingly variable and extreme weather conditions are the main problems. The ACC deaminase containing rhizobacterial based biofertilizers have been well documented to help crop plants to withstand extreme environmental conditions (Arshad *et al.*, 2008; Ahmad *et al.*, 2013b; Nadeem *et al.*, 2014). The present study thus explores the effectiveness of multi-strain ACC deaminase

containing biofertilizer to improve the productivity of chickpea under field conditions in Bahawalpur region where water availability is the major problem.

The results from our study showed that the inoculation with multi-strain biofertilizer containing novel strains of *Mesorhizobium cicri*, and ACC deaminase containing

Table 7: Effect of enriched compost and biofertilizer on number of grains pod⁻¹ in chickpea

Treatment	Number of grains pod ⁻¹					
	13 BC	15 BC	16 BC	Baghdad	118 DB	Jageer bhati dari
Control	1.31 c	1.32 c	1.25 c	1.16 c	1.09 c	1.29 b
Enriched compost (EC)	1.36 b	1.39 b	1.31 b	1.27 ab	1.22 b	1.33 b
Biofertilizer (B)	1.43 a	1.47 a	1.41 a	1.24 b	1.28 a	1.40 a
EC + B	1.40 a	1.49 a	1.44 a	1.29 a	1.30 a	1.37 a
LSD ($p \leq 0.05$)	0.0354	0.0593	0.0499	0.0435	0.0620	0.0421

Table 8: Effect of enriched compost and biofertilizer on 1000 grain weight in chickpea

Treatment	1000 grain weight (g)					
	13 BC	15 BC	16 BC	Baghdad	118 DB	Jageer bhati dari
Control	199.00 c	259.33 d	245.00 d	274.67 b	191.33 d	202.00 d
Enriched compost (EC)	205.33 b	273.33 c	279.0 c	274.00 b	224.67 c	225.00 c
Biofertilizer (B)	217.33 a	281.67 b	301.67 b	282.67 a	253.67 b	244.67 b
EC + B	220.67 a	294.00 a	339.67 a	288.67 a	267.00 a	255.33 a
LSD ($p \leq 0.05$)	5.2120	6.5166	9.6220	7.5859	10.738	7.3332

Table 9: Effect of enriched compost and biofertilizer on grain yield of chickpea

Treatment	Grain yield (kg acre ⁻¹)					
	13 BC	15 BC	16 BC	Baghdad	118 DB	Jageer bhati dari
Control	648.33b	781.70c	749.30c	948.30b	848.30d	948.30b
Enriched compost (EC)	661.00b	861.00bc	827.7b	967.70b	927.70c	967.70b
Biofertilizer (B)	730.33b	1030.33ab	897.0b	997.00ab	997.00b	997.00ab
EC + B	985.00a	1180.30a	1047.0a	1087.34a	1113.70a	1072.31a
LSD ($p \leq 0.05$)	199.78	210.12	73.162	68.646	42.876	68.646

Table 10: Effect of enriched compost and biofertilizer on chlorophyll-a contents in leaves of chickpea

Treatment	Chlorophyll-a contents ($\mu\text{g g}^{-1}$)					
	13 BC	15 BC	16 BC	Baghdad	118 DB	Jageer bhati dari
Control	1.310 b	1.330 b	1.243 b	1.306 c	1.130 c	1.223 b
Enriched compost (EC)	1.343 b	1.366 b	1.306 ab	1.320 bc	1.253 b	1.260 ab
Biofertilizer (B)	1.403 a	1.410 a	1.320 ab	1.343 b	1.330 ab	1.306 ab
EC + B	1.406 a	1.420 a	1.383 a	1.390 a	1.353 a	1.360 a
LSD ($p \leq 0.05$)	0.0390	0.0395	0.0847	0.0344	0.0881	0.1208

Table 11: Effect of enriched compost and biofertilizer on chlorophyll-b contents in leaves of chickpea

Treatment	Chlorophyll-b contents ($\mu\text{g g}^{-1}$)					
	13 BC	15 BC	16 BC	Baghdad	118 DB	Jageer bhati dari
Control	0.530 c	0.646 b	0.710 b	0.676 c	0.560 a	0.506 b
Enriched compost (EC)	0.546 bc	0.683 ab	0.750 a	0.696 bc	0.566 a	0.533 ab
Biofertilizer (B)	0.573 b	0.720 a	0.770 a	0.713 b	0.581 a	0.556 a
EC + B	0.610 a	0.720 a	0.776 a	0.753 a	0.610 a	0.570 a
LSD ($p \leq 0.05$)	0.0303	0.0415	0.321	0.0245	0.1194	0.0372

Means sharing same letters are statistically show insignificant results at par at 5 % level of probability (n = 3)

PGPR *Pseudomonas* sp. and *Bacillus* sp. significantly improved the nodulation in chickpea. Improvement in bioavailability of nutrients especially nitrogen is an

important trait of Rhizobium based inoculants.

The improvement in nodulation in these studies might be due to more efficiency of inoculated Rhizobial strain that

Table 12: Effect of enriched compost and biofertilizer on nitrogen concentration in grains of chickpea

Treatment	Nitrogen concentration in grains (%)					
	13 BC	15 BC	16 BC	Baghdad	118 DB	Jageer bhati dari
Control	1.38 c	1.60 c	1.61 c	1.41 b	1.20 c	1.32 b
Enriched compost (EC)	1.48 bc	1.60 c	1.84 b	1.53 b	1.32 b	1.40 ab
Biofertilizer (B)	1.57 ab	1.85 b	1.92 b	1.79 a	1.42 b	1.57 ab
EC + B	1.70 a	2.04 a	2.12 a	2.02 a	1.56 a	1.66 a
LSD ($p \leq 0.05$)	0.1866	0.1005	0.1169	0.2336	0.1010	0.2641

Table 13: Effect of enriched compost and biofertilizer on phosphorus concentration in grains of chickpea

Treatment	Phosphorus concentration in grains (%)					
	13 BC	15 BC	16 BC	Baghdad	118 DB	Jageer bhati dari
Control	0.33 d	0.35 c	0.29 c	0.36 c	0.33 c	0.44 a
Enriched compost (EC)	0.38 c	0.39 b	0.31 b	0.38 b	0.43 a	0.47 a
Biofertilizer (B)	0.41 b	0.38 b	0.31 b	0.39 b	0.36 b	0.45 a
EC + B	0.44 a	0.42 a	0.44 a	0.41 a	0.44 a	0.49 a
LSD ($p \leq 0.05$)	0.0287	0.0301	0.0166	0.0122	0.0161	0.1932

Table 14: Effect of enriched compost and biofertilizer on potassium concentration in grains of chickpea

Treatment	Potassium concentration in grains (%)					
	13 BC	15 BC	16 BC	Baghdad	118 DB	Jageer bhati dari
Control	1.25 a	1.54 a	1.68 a	1.31 a	1.34 a	1.48 a
Enriched compost (EC)	1.26 a	1.56 a	1.70 a	1.40 ab	1.38 a	1.51 a
Biofertilizer (B)	1.29 a	1.57 a	1.69 a	1.35 b	1.37 a	1.50 a
EC + B	1.30 a	1.63 a	1.73 a	1.60 b	1.44 a	1.59 a
LSD ($p \leq 0.05$)	0.1190	0.1018	0.0696	0.2223	0.1443	0.1245

Means sharing same letters are statistically show insignificant results at par at 5 % level of probability ($n = 3$)

Figure 3: Effect of enriched compost and biofertilizer on nodulation in chickpea

effectively colonized the plant roots under field conditions (Verma *et al.*, 2012). It might also be due to the effectiveness of ACC deaminase containing rhizobacterial strains which increased root growth and proliferation under unfavorable conditions (Ahmad *et al.*, 2011), increased surface area of roots, provided more infection sites for Rhizobial attachment to make nodules thus improving nodulation in chickpea. Similarly, Ahmad *et al.* (2013c) also reported the increased nodulation in mungbean due to combined use of Rhizobium and PGPR under salt-affected field conditions. Lowest nodulation in un-inoculated control elucidates decreased activity of native rhizobia but application of P-enriched compost and Rhizobium based multi-strain biofertilizer increased nodulation. It is advocated that the application of P-enriched compost resulted in increased Rhizobium bioactivity in rhizosphere and increased the root colonization of bacteria by improving physical properties of soil along with carbon and nutrient availability that promoted the nodulation (Zahran, 1999; Bashan *et al.*, 2013).

In our study, the use of multi-strain biofertilizer and P-enriched compost significantly increased yield attributes of chickpea. The findings are in line with the results from studies of Gharib *et al.* (2008) who reported that the use of biofertilizer and P-enriched compost enhanced crop yield significantly by improving the grain weight and number of grains. In case of combined application, in addition to the positive effects of P-enriched compost on soil physico-chemical properties and crop growth, the bacterial strains present in the multi-strain biofertilizer enhanced the nitrogen and phosphorus availability, suppressed the possible negative effects of water shortage and improved root growth leading to improvement in yield and quality of chickpea. It has been well documented that promotion of root growth under stressed environment occurs due to decrease in ethylene levels by the ACC-deaminase activity of rhizobacterial strains (Shaharoon *et al.*, 2006; Ahmad *et al.*, 2012). Similarly, in previous study, it has been reported that PGPR inoculation increased the grain yield by suppressing the diseases (Vinale *et al.*, 2008).

The combined application of multistrain biofertilizer and P-enriched compost can be more effective than separate use of biofertilizer. In these studies, the increase in growth and yield of chickpea could also be due to role of inoculated bacterial strains in nutrient acquisition by solubilization of phosphorus and other macro as well as micronutrients. Moreover, the bacterial strains might have effectively colonized the plant roots due to positive effect of P-enriched compost on soil properties which might have enhanced their efficiencies like N fixation, P-solubilization and effective pathogen suppression. Similarly, increasing nitrogen fixation due to phosphorus supplementation has been reported by Manjunath and

Bagyaraj, (1984). In another study, Rudresh *et al.* (2005) reported that nutrients availability plays an important role in increasing grains number per pod and grain weight under field conditions.

This study demonstrated the effectiveness of multi-strain biofertilizer in combination with P-enriched compost for improving growth, yield and nutritional quality (N, P and K) of chickpea under field conditions at different locations. The increase in grain quality could be attributed to the enhanced nutrient use efficiency in the presence of organic fertilizers and bio-inoculants. Studies have shown that the composted organic materials release nutrients slowly and may reduce the leaching losses, particularly of N (Muneshwar *et al.*, 2001) and thus enhance nutrient use efficiency (Rudresh *et al.*, 2005; Jutur and Reddy, 2007). This premise is further supported by the fact that total N and P uptakes in chickpea were significantly increased in response to combined application of biofertilizer and P-enriched compost. All these factors result in increased nutrient uptake leading to better quality of grains (Neuens and Reheul, 2003; Otieno *et al.*, 2007). The increase in nutrient uptake may be due to increase in available N and P contents in the soil, and improved soil structure due to higher organic matter that increased nutrients availability and hence enhanced growth and quality of grains (Manna *et al.*, 2001).

Conclusion

In the present study, the application of ACC deaminase containing multi-strain biofertilizer and P-enriched compost significantly enhanced the nodulation, growth, yield, grain quality and physiology of chickpea under field conditions at different locations in Bahawalpur. The integrated use of biofertilizer and P-enriched compost was effective at all experimental locations but their efficacy varied with locations / sites. It can be concluded that the use of multi-strain biofertilizer in combination with P-enriched compost is significantly effective in improving the productivity and profitability of chickpea. So, the farmers should be recommended to adopt the ACC deaminase biofertilizer technology in combination with P-enriched compost for higher productivity of chickpea in the region.

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