



Effectiveness of bacterial inoculation for improving grain yield and quality of chickpea

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Abstract

Field experiments were carried out for three consecutive Rabi seasons (2011-12 to 2013-14) at Pulses Research Institute, Faisalabad to study the effect of bacterial inoculation on grain yield and quality of chickpea (*desi*). One variety (Pb-2008) and four advanced lines viz. D-03009, D-06002, D-06003 and D-06052 were tested with and without bacterial inoculation (*Mesorhizobium ciceri*, *Pseudomonas* spp. and *Bacillus* spp.). On a loamy soil ($EC = 1.8 \text{ dS m}^{-1}$, $pH = 7.6$), a consortium of three bacteria spp. was applied as seed coating at the time of sowing. The experiments were laid out in split plot design with three replications. Results showed a significant increase in nodulation (17.5%), plant height (4.0%), number of pods per plant (7.8%) and grain yield (7.5%) due to bacterial inoculation. The increase/decrease in the number of branches per plant (3.8%), 100 grain weight (1.9%), protein (-4.5%), carbohydrate (0.0%), crude fiber (2.0%) and mineral matter (2.5%), remained statistically non-significant. The interaction among local cultivar / advanced lines and microbial inoculation was significant for improving grain yield. The advanced line D-06052 yielded with highest grains (2890 kg ha^{-1}) due to bacterial inoculation. Hence, it can be argued that the bacterial inoculation positively influenced the grain yield and its components without fading the quality of produce. Bacterial inoculation is recommended for better chickpea yield.

Keywords: Bacterial inoculation, chickpea, yield, protein

Introduction

Chickpea (*Cicer arietinum* L.) is an important pulse crop in Pakistan. During the year 2013-14, it was cultivated on an area of 949.6 thousands hectare with a total production 399.0 thousand tons and an average of 420 kg ha^{-1} (Government of Pakistan, 2014). It is a cheap source of high quality protein in the diets of millions of people in developing countries, who cannot afford animal protein for balanced nutrition. In addition to proteins, it is a good source of carbohydrates, minerals and trace elements (Huisman and Poel, 1994). Average yield of chickpea in Pakistan is usually low. Soils of Pakistan are generally deficient in nitrogen; the most important element in the metabolism of plants and protein synthesis. Its deficiency in soil usually results in low crop yield (Romdhane *et al.*, 2008). Yield of chickpea can be improved by using seed of good varieties, balanced fertilizers, good agronomic practices and the use of efficient bacteria as biofertilizer. Crop productivity can be increased by the application of chemical, organic and biological fertilizers (Elliott and Abbott, 2003). Inoculation increases soil nitrogen along with the increase in root and shoot nitrogen (Ahmed *et al.*, 2008). It has been reported by a number of researchers that rhizobial inoculation increases the root/ shoot length, number of pods, nodulation and yield of chickpea (Gupta and Namdeo, 1997, Sarma *et al.*, 2009, Akhtar and Siddiqui, 2009 and Zahir *et al.*, 2010). Field trials showed

that nearly 50% N fertilizer can be saved through rhizobial inoculations with considerable increase in yield (Rewari and Nkvb, 1988, Tilak, 1993, Ekhlas *et al.*, 2011 and Tahir *et al.*, 2011). The efficacy of microorganism is also affected by soil type, source and quantity of soil nutrients as well as the test crop (Bhuiyan *et al.*, 2008, Mehboob *et al.*, 2008, Javed and Bajwa, 2011). *Rhizobium* inoculation not only increases the yield but also improves the quality of produce with increases in protein, crude fiber, fat, ash and carbohydrate contents in chickpea (Aslam *et al.*, 2010, Abdalla *et al.*, 2013, and Singh *et al.*, 2014).

The present study was therefore, carried out to compare the effect of *microbial* inoculation on the yield, nodulation and grain quality of various lines/variety of chickpea (*desi* gram).

Materials and Methods

Field experiments are conducted to study the effect of bacterial inoculation (*Mesorhizobium ciceri*, *Pseudomonas* spp. and *Bacillus* spp.) on a variety and the advanced lines of chickpea for improving the grain yield and grain quality for three consecutive years (2011-12 to 2013-14) at Pulses Research Institute, Faisalabad, Pakistan. The experimental design used was factorial (Two factors, inoculation and varieties) laid out in split plot with three replications. The plot size, plant to plant and row to row spacing was $1.2 \text{ m} \times 4 \text{ m}$, 15 cm and 30 cm, respectively. A variety (Pb- 2008)

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Table 1 Effect of bacterial inoculation on the grain yield and 1000 grain weight of some advanced lines of Chickpea.

S. No.	Variety/ Lines	Grain yield (kg ha ⁻¹)			1000 grain weight (g)		
		Un-inoculated	Inoculated	Mean	Un-inoculated	Inoculated	Mean
1	Pb-2008	2152 ef	2345 c	2248 B	223.4	229.0	226.2 A
2	D-03009	1944 h	2111 fg	2028 D	202.2	197.2	199.7 B
3	D-06002	2012 gh	2269 cde	2140 C	188.5	196.8	192.7 B
4	D-06003	2268 cd	2181 def	2224 B	191.7	218.0	205.3 B
5	D-06052	2596 b	2890 a	2743 A	218.0	228.0	223.0 A
	Mean	2194 B	2359 A		204.8	214.8 NS	
	% increase over control		7.5			1.9	
LSD for variety = 78, inoculation = 49.5, for interaction = 110.7					LSD for variety = 30		

Table 2 Effect of bacterial inoculations on the nodules and plant height of some advanced lines of Chickpea.

S. No.	Variety/ Lines	No. of nodules/plant			Plant height (cm)		
		Un-inoculated	Inoculated	Mean	Un-inoculated	Inoculated	Mean
1	Pb-2008	12.6 b	16.1 a	14.4 A	49.1	49.6	49.4 BC
2	D-03009	8.5 h	10.8 de	9.6 D	47.0	49.1	48.1 C
3	D-06002	11.9 bc	12.4 b	12.1 B	47.0	48.4	47.7 C
4	D-06003	9.0 gh	10.1 de	9.6 D	50.1	53.0	51.5 B
5	D-06052	9.5 fg	11.2 cd	10.3 C	55.8	59.1	57.4 A
	Mean	10.3 B	12.1 A		49.8 B	51.8 A	
	% increase over control		17.5			4.0	
LSD for variety = 0.68, inoculation = 0.43, for interaction = 0.959					LSD for variety = 2.63, inoculation = 1.66		

Table 3 Effect of bacterial inoculation on number of pods and branches of some advanced lines of Chickpea.

S. No.	Variety/ Lines	Pods/plant			Branches/plant		
		Un-inoculated	Inoculated	Mean	Un-inoculated	Inoculated	Mean
1	Pb-2008	54	64	59 D	6.0 ab	5.4 cd	5.7 A
2	D-03009	81	86	83 B	4.3 f	6.3 a	5.3 A
3	D-06002	77	86	82 B	4.7 ef	5.0 de	4.8 B
4	D-06003	61	68	64 C	5.6 bc	5.1 cde	5.3 A
5	D-06052	101	108	105 A	5.4 cd	5.5 bcd	5.4 A
	Mean	75 B	82 A		5.2	5.5 NS	
	% increase over control		9.3			5.8	
LSD for variety = 4.82, inoculation = 3.05					LSD for variety = 0.8124, for interaction = 1.15		

and four advanced lines (D-03009, D-06002, D-06003 and D-06052) of chickpea (*Cicer arietinum*) were used. Prepared seed inoculum (consisting of *Mesorhizobium*, *Bacillus* and *Pseudomonas* spp. each @ 10^5 - 10^6 cfu/g) were collected from Soil Bacteriology Section, Ayub Agricultural Research Institute, Faisalabad. Seeds were inoculated by hand mixing of inoculum with seed @ one packet of inoculum per acre. The experimental area was divided into two sub plots (one for inoculated and other for un-inoculated seed). One set of seeds was sown after inoculation while other set of seeds was sown without

inoculation as control. Nodules were collected/ counted at flowering and at maturity, five plants from each replication of each treatment were selected randomly and observations regarding the plant height, number of branches, number of pods, and 1000 grain weight were recorded. Grain yield was recorded after harvesting whole plot. Grain samples were collected for protein, fat, fiber, ash and carbohydrate analysis. Standard methods given in AOAC (2000) were followed to analyze quality parameters mentioned above. Data for three years were pooled and results are given and discussed as an average of three years data. Data were



subjected to statistical analysis and means were compared using LSD test at 0.05 level of probability Steel *et al.* (1997).

Results and Discussion

Grain yield

Bacterial inoculation significantly enhanced (7.5%) the grain yield over non-inoculation (Table 1). The interactions between inoculation and Advanced lines were significant. Difference among the variety/Advanced lines was also significant. Advance line D-06052 gave the highest grain yield (2743 kg ha⁻¹) while D-03009 gave the lowest grain yield (2028 kg ha⁻¹). The highest grain yield (2890 kg ha⁻¹) was recorded in inoculated Advanced line D-06052 while the lowest (1944 kg ha⁻¹) in the un-inoculated chickpea Advanced line D-03009. Akhtar and Siddiqui (2009), Bhuiyan *et al.* (2008), Giri *et al.* (2010), Sarma *et al.* (2009) and Zahir *et al.* (2010) reported an increase in yield with microbial inoculation. Similar results were recorded by Gupta and Namdeo (1997).

Bacterial inoculation increased the nodulation and hence yield increased due to the enhanced availability of nitrogen. Similar results were obtained by Wani *et al.* (2007). Tena *et al.* (2016) also reported that inoculation had a pronounced effect on grain yield of chick pea.

1000 grain weight

Results in table 1 showed that bacterial inoculation had no significant effect on 1000 grain weight. The check variety Pb-2008 was statistically at par with advanced line D-06052 and differed significantly from the other advanced lines which had non-significant differences among themselves. Interaction among the inoculation and variety/advanced lines was also non-significant. The results are more or less similar to the findings of Bhuiyan *et al.* (2009) who quoted non-significant differences in 100 grain weight among chickpea genotypes and bacterial inoculation during the first year and reported a significant difference during the 2nd year. The highest 1000 grain weight (229.0 g) was observed in inoculated check variety Pb- 2008 while the lowest 1000 grain weight (188.5 g) was found in un-inoculated advanced line D-06002.

Number of nodules

A significant effect of bacterial inoculation on nodulation was observed (Table-2). Variety/advanced lines differed significantly from one another. The highest number of nodules per plant (14.4) was

observed in the check variety Pb-2008. The interaction among variety /advanced lines and inoculation were also found significant. Maximum number of nodules per plant (16.1) was found in the inoculated variety Pb-2008. These findings are supported by the results of Akhtar and Siddiqui (2009), who found an increase in number of nodules in chickpea by bacterial inoculation.

Plant height

Results in table -2 showed that bacterial inoculation significantly increased the plant height. Maximum plant height (59.1 cm) was found with bacterial inoculation in advanced line D-06052 which was 16.9% higher than un-inoculated check variety Pb-2008. The results are in line with the findings of Akhtar and Siddiqui (2009) who reported that inoculation of chickpea with *Rhizobium* spp. caused a significant increase in growth, yield and number of nodules per root system as compared to no inoculation under field conditions. The increase in plant height may be attributed to the symbiotic nitrogen fixation, release of plant nutrients and growth hormones due to microbial activity.

Number of pods per plant

The data in table 3 showed that bacterial inoculation significantly improved the number of pods per plant (9.33%) but interaction of advanced lines with bacterial inoculation were non-significant. The highest number of pods per plant (108) was observed in the inoculated advanced line D-06052 and the lowest number of pods per plant (54) was found in variety Pb-2008 without inoculation. Sarma *et al.* (2009) and Namvar and Shariff (2011) also reported a significant increase in number of pods per plant with microbial inoculation to chickpea.

Number of branches per plant

The response of different variety/advanced lines to bacterial inoculation was found inconsistent (Table 3). Overall effect of inoculation on branches per plant was non-significant. Bacterial inoculation significantly increased the number of branches only in the advanced line D-03009. Previous studies of Javad (2006) and Khan *et al.* (2006) confirm this statement which state that the response of legumes to microbial application is highly variable ranging from significantly negative to significantly positive. Climate variations also have large impact on the effectiveness of applied bacterial strains (Zaidi *et al.*, 2009).

Protein contents and protein yield

Results revealed that bacterial inoculation did not affect the protein contents as well as total protein yield



which were observed statistically at par with un-inoculated plants (Table 4). Among the advanced lines, line D-06052

was recorded in the advanced line D-03009 with inoculation and 303 kg ha⁻¹ protein by the variety Pb- 2008 without

Table 4 Effect of bacterial inoculation on protein contents and protein yield of some advanced lines of Chickpea.

S. No.	Variety/ Lines	Crude protein (%)			Protein yield (kg ha ⁻¹)		
		Un-inoculated	Inoculated	Mean	Un-inoculated	Inoculated	Mean
1	Pb-2008	19.0 b	17.8 c	18.4 C	303 b	350 ab	327 B
2	D-03009	19.8 ab	17.2 c	18.5 C	352 ab	345 ab	348 AB
3	D-06002	19.2 b	19.6 b	19.4 B	383 ab	375 ab	379 AB
4	D-06003	19.4 b	17.7 c	18.5 C	444 ab	451 a	448 A
5	D-06052	19.9 ab	20.7 ab	20.3 A	373 ab	428 ab	401 AB
	Mean	19.6 NS	18.60		371 NS	390	
	% increase over control		-8.2			5.1	
LSD for variety= 0.76, for interaction = 1.07				LSD for variety=92.5 , for interaction = 130.8			

Table 5 Effect of bacterial inoculation on carbohydrates and crude fat of some advanced lines of Chickpea.

S. No.	Variety/ Lines	Carbohydrates (%)			Fat (%)		
		Un-inoculated	Inoculated	Mean	Un-inoculated	Inoculated	Mean
1	Pb-2008	71.64 ab	69.96 cde	70.80 AB	3.62 g	3.97 efg	3.80 D
2	D-03009	69.97 cde	71.90 a	70.93 AB	4.13 cdef	4.39 abcd	4.26 AB
3	D-06002	70.41 bcd	69.38 de	69.90 B	4.04 def	4.74 a	4.39 AB
4	D-06003	69.09 ef	71.23 abc	70.16 B	4.70 ab	4.48 abc	4.59 A
5	D-06052	69.51 de	67.88 f	68.70 C	3.79 fg	4.33 bcde	4.06 CD
	Mean	70.12	70.10 NS		4.06 B	4.38 A	
	% increase over control		0.0			7.9	
LSD for variety= 1.00, for interaction = 1.421				LSD for variety= 0.273, inoculation= 0.173, for interaction = 0.386			

responded to bacterial inoculation prominently with 20.7% protein contents against 19.9% protein contents in non-inoculated plants of same advanced line. The highest protein contents (20.7%) was produced by the same advanced line (D-06052) as compared to others 3 advanced lines and a variety Pb-2008 while minimum protein contents (17. 2%) was observed in the advanced line D-3009. The results of present study are contradictory to the results of Aslam *et al.* (2010), Tahir *et al.* (2011), Ekhlas *et al.* (2011), Bejandi *et al.* (2012), Rugheim and Abdelgani (2012) and Abdalla *et al.* (2013) who reported higher protein contents due to the rhizobium inoculation to chickpea.

In case of total protein yield, the advanced line D-06003 proved better as compared to check variety Pb-2008 and other advanced lines. This advanced line yielded 451 kg ha⁻¹ protein with inoculation and 444 kg ha⁻¹ protein without inoculation while the lowest protein yield (345 kg ha⁻¹)

inoculation. Grain yield increased with the application of microbial inoculation but total protein yield did not increase accordingly due to lower protein contents in inoculated plant.

Carbohydrates and fat

There was no significant effect observed on carbohydrates and fat contents due to the application of bacterial inoculum to chickpea (Table 5). The highest carbohydrate contents (71.90%) were observed in advanced line D-03009 while the lowest carbohydrate contents (69.09%) were found in advanced line D-06003. When means for variety/lines were compared, advanced lines D-03009, D- 06002, D-06003 and variety Pb-2008 were found statistically at par among themselves while the advanced line D-06052 ranked at the lowest position. Ekhlas *et al* (2011) found that *Bradyrhizobium* inoculation decreased the carbohydrate contents in cowpeas while Rugheim and Abdelgani (2012) found a decrease of carbohydrate in faba bean with *Rhizobium* inoculation. On the other hand,



Senthikumar and Sivagurunathan (2012) found that *Rhizobium* inoculation increased the carbohydrates in green gram.

Bacterial inoculation significantly increased fat contents in chickpea (Table 5). The highest fat contents (4.74%) was observed in advanced line D- 06002 with inoculation and 4.04% fat contents without inoculation while the lowest fat contents (3.97%) was found in variety Pb-2008 with inoculation and 3.6% fat contents without inoculation. On comparing the means for advanced

fiber contents. In contrast to the results of this study, many researchers found an increase in crude fiber and ash contents of chickpea by rhizobium inoculation (Ekhlas *et al.* (2011), Rugheim and Abdelgani (2012), Abdalla *et al.* (2013) and Singh *et al.* (2014).

Conclusion

Responses of different genotypes of chickpea with respect to yield and its components and quality parameters varied to bacterial inoculation. Bacterial inoculation

Table 6 Effect of bacterial inoculation on crude fiber and ash contents of some advanced lines of Chickpea.

S. No.	Variety/Lines	Crude fiber (%)			Ash (%)		
		Un-inoculated	Inoculated	Mean	Un-inoculated	Inoculated	Mean
1	Pb-2008	3.84 ab	3.91 a	3.88 A	3.10 ab	3.16 ab	3.13 AB
2	D-03009	3.22 cde	3.35 bcde	3.28 CD	3.33 a	3.38 a	3.35 A
3	D-06002	3.00 de	2.90 e	2.95 D	3.00 ab	3.21 ab	3.10 AB
4	D-06003	3.51 abcd	3.38 bcde	3.44 BC	3.16 ab	3.09 ab	3.12 AB
5	D-06052	3.62 abc	4.00 a	3.81 AB	3.24 ab	3.16 ab	3.20 B
	Mean	3.43	3.51 NS		3.17	3.20 NS	
	% increase over control		2.3			0.95	
LSD for variety= 0.37, for interaction = 0.5252				LSD for variety = 0.363, for interaction = 0.513			

lines/variety, it was found that the advanced lines D-06002, D-06003 and D- 03009 were statistically at par but better than the advanced line D- 06052 and variety Pb-2008. Fat contents in advanced lines D-06052 and D-06002 were significantly improved with inoculation. Results of present study are supported by Rugheim and Abdelgani (2012) who reported an increase in fat contents of faba bean with *Rhizobium* inoculation.

Crude fiber and ash

Bacterial inoculation did not affect the crude fiber and ash contents in chick pea (Table 6). The highest fiber content (4.0%) was found in advanced line D-06052 with inoculation. On the other hand, the lowest value of crude fiber (2.90%) was recorded in advanced line D-06002 with inoculation. When means for lines/variety were compared, it was observed that performance of variety Pb- 2008 was the best but it was statistically at par with advanced line D-06052. The advanced line D- 06002 ranked at the lowest position (Table 6). Ash contents found in all the four advanced lines and a variety were statistically at par among themselves. When means for advanced lines/variety were compared, it was found that all the advanced lines were statistically at par however; advanced line D-03009 produced the highest ash contents (3.38 and 3.33% with and without inoculation respectively). In agreement with our results, Elsheikh *et al.* (2009) reported that inoculation with *Bradyrhizobium* to soybean had no significant effect on

significantly increased the grain yield, plant height, nodulation, pods per plant and fat contents while 1000 grain weight, protein, carbohydrate, crude fat, crude fiber and ash contents, remained unaffected. On the basis of findings of present study it is recommended to inoculate chickpea seed with bacterial inoculation (*Mesorhizobium ciceri*, *Pseudomonas* spp. and *Bacillus* spp.) for cropping a good yield.

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