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Symbiotic relationship of chickpea genotypes and pgpr consortium for nodulation, yield productivity and grain protein contents

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Abstract

Four chickpea genotypes *viz.* Bittle-2016 (V_1), TG1218 (V_2), Bhakkar-2011 (V_3), TG1221 (V_4) and five PGPR strains *viz. Enterobacter cloacae* (Z_{11}), *Bacillus subtilis* (Z_{22}), *Providencia vermicola* (P_1), *Bacillus majavensis* (P_8), *Bacillus zhangzouensis* (S_{14}) and *Bacillus majavansis* (S_{19}) in five different consortium C_1 (Z_{11}+P_1+S_{11}), C_2 (Z_{11}+P_8+S_{19}), C_3 (Z_{22}+P_1+S_{14}), C_4 (Z_{22}+P_8+S_{19}) and C_5 (Z_{11}+P_1+S_{19}) along with uninoculated (C_o), were studied in split plot arrangements. Chickpea varieties/lines and PGPR consortiums constituted main plot and subplots, respectively. This study was carried out at Arid Zone Research Institute, Thal area, Punjab, Pakistan in Rabi (winter) season 2018-19 aimed to determine the symbiotic efficiency of chickpea genotypes with *PGPR* consortia to exploit its natural symbiosis to enhance nodulation and thereby increasing yield and protein contents of chickpea grains under arid to semiarid conditions of Thal region, Pakistan. Strong symbiosis was found among chickpea and PGPR consortium which significantly increased the yield and yield components. Maximum number of pods plant^{-1} (113.3) and100 grain weight (28.73 g) was recorded in $V_4 \times C_4$ (Z_{22}+P_8+S_{19}). Inoculation with PGPR consortium increased grain yield by 30 % (1665 to 2196 kg ha^{-1}. Nodules plant^{-1} was also encouraged from 37.33 to 87.83 by seed inoculation. Inoculation with C_4 (Z_{22}+P_8+S_{19}) endorsed higher yield from 1590 to 2491kg ha^{-1}. It was concluded that, rhizibium consortium showed a specific symbiotic relationship with genotypes and inoculation is recommended for better yield and protein contents in chickpea.

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Introduction

The natural success of chickpea is its ability to fix atmospheric N in symbiosis with rhizobia, contributing directly to grain protein and reducing the need for N fertilizer for the crops to follow after it and thereby, has great potential to improve soil N status (Ben Romdhane *et al.*, 2008; Funga *et al.*, 2016; Khaitov *et al.*, 206; Tena *et al.*, 2016) and also plays an important role in human diet (Siddiqi and Mahmood, 2001; Kantar *et al.*, 2007). There are many factors contributing to achieve good crop harvest. Crop nutritional imbalance and poor nodulation appear to be the major issue, which can be managed during crop season. Farmers have false perception that chickpea, being a legume crop, does not need any nutrition and it can grow on marginal soil (Aslam *et al.*, 2010; Chianu *et al.*, 2011). It is proved that poor grain yields in legumes are often the outcome of poor soils (Franke *et al.*, 2016), this might be a major reason for its low grain yield in Pakistan. Inorganic fertilizers are not generally applied to chickpea; however, some progressive farmers apply a little amount of N and P_2O_5 fertilizer through broadcast as a starter dose. Application of phosphorus to legume crops improves grain yield considerably (Hussain, 1983). Grain yield of chickpea was increased significantly with PGPR and phosphorus application (Raut and Kohire, 1991 and Aslam *et al.* 2010). Legume yields and nitrogen fixation depends upon the legume genotype or variety (V), the PGPR strain/consortium (C) and the interactions of these with the bio-physical environment and management practices (Giller *et al.*, 2013).

The phosphorus application improves crop growth with efficient root system alongwith active nodulation. Supplement phosphorus improves pod filling and ultimately enhances the grain yield (Gupta *et al.*, 1998; Reddy *et al.*, 1993). Most of the soils in Pakistan are alkaline and calcareous in nature which binds phosphorus tightly. Therefore, Phosphatic fertilizer is required almost everywhere to support efficient plant growth. Phosphate solublizing bacteria can play a vital role to save the resources; increase the availability of phosphorus and ultimately the grain yield.

Chickpea can fix 60–80% of its nitrogen requirement (Giller, 2001; SPG, 2016), amounting to 60–176 kg N ha^{-1} (Beck *et al.*, 1991; Shiferaw *et al.*, 2004). It was witnessed that inoculation enhanced the nodulation of chickpea, stimulated more nitrogen fixation and increased N uptake (Endalkachew *et al.* 2018). It is selective in its symbiotic requirement, nodulating with only a specific group of rhizobium species (Tena *et al.*, 2016; SPG, 2016). The absence of compatible strains and the small rhizobial population in the soil are major limitations for nodule formation in chickpea (Kantar *et al.*, 2010). Inoculation with effective strains at planting time is recommended if the soil population density of compatible rhizobia is less than 50 cells per gram of soil (Thies *et a*l., 1991). There is an evidence to suggest that inoculation enhances plant growth, grain and biomass yield in chickpea (Ben Romdhane *et al.*, 2008; Funga *et al.*, 2016; Khaitov *et al.*, 2016; Tena *et al.*, 2016).

In addition chickpea requires about 13 to 41 kg Nitrogen ha^{-1} for better growth and development. Nitrogen (N_2) fixing *Rhizobia* could increase yield at a low cost (Kucuk and Kıvanc, 2008). Nitrogenous fertilizer applications are generally considered not necessary as chickpea plant fixes (60-80 percent of its requirements) atmospheric N through symbiosis with nitrogen fixing bacteria (*Rhizobium cicer*) provided that proper conditions of soil moisture, temperature and soil pH. The bacteria, however, must be present in the soil for N fixation (Rhinhart *et al.*, 2003). If full benefit from grain legume crop is to be harvested in terms of maximum yield and soil improvement, the seed should be inoculated with its own specific and suitable *Rhizobium* strain before planting. El-Hadi and Sheikh (1999) and Aslam *et al.*, 2010 reported that *Rhizobium* inoculation application significantly increased total nodule numbers $plant^{-1}$, 100 grain weight, yield (70-72%) and protein contents of chickpea grains. Rhizobium plus phosphate-solubilizing bacteria significantly improved crop yield components (Meena *et al.,* 2001). Grain yield and quality was improved up to 50% in chickpea with seed inoculation (Kyei -Boahen *et al.*, 2002). Karadavut and Ozdemir (2001) reported that Rhizobium significantly increased seed yield (20% higher than control), total above ground dry matter and number of pods plant^{-1} in chickpea. Fatima, *et al.* (2008) narrated that *Rhizobium* inoculum generally increased plant growth, yield and yield components and nitrogen fixation in chickpea.

So that a hypothesis was formulated that chickpea cultivars together with PGPR consortium should be evaluated for their ability to produce nodules and nitrogen fixation potential of produced nodules. The *Rhizobium* strain should be of such a nature to colonize in soil, tolerate environmental stresses, form effective nodules and should have no deleterious effects on ecosystem. Therefore, the following experiment was conducted to sort out varieties/lines of chickpea having better symbiotic relationship with *Rhizobium* inoculation strains for higher yield and improved grain protein content.

Materials and methods

Location

The experimental site located within an elevation range of (31° .37/ N latitude and 71°. 02/ East longitude) above the sea level with annual mean temperature 19.04–33.8 °C, and annual average rainfall of 150–350 mm. A field experiment was conducted at the Arid Zone Research Institute, Bhakkar Punjab, Pakistan during Rabi (winter) season 2018-19 on sandy loam soil with field capacity and permanent wilting point values of 14.67 and 5.40 % on volume basis, respectively.

Soil analysis

Composite soil samples were collected at depth of 0–15 and 16-30cm before planting. The samples were weighed, air-dried and ground to pass through a 2 mm sieve before analysis. The soil profile was a sandy loam and the 0–30cm layer had 0.27% organic matter, 0.014% nitrogen (N), 3.34ppm available phosphate (PO_4^{-3}) and 166ppm available potassium (K) with soil pH 8.2. The objectives of the study were to probe the nodulation, yield productivity and grain protein contents of chickpea as affected by symbiosis of chickpea varieties/lines and PGPR consortium. The sandy loam soil (Table 1) was well prepared before sowing of crop. The chickpea varieties/lines [(Bittle-2016 (V_1), TG1218 (V_2), Bhakkar-2011 (V_3), TG1221 (V_4)] were kept in main plot while six bacterial strains (Table 2) were randomly mixed to make five PGPR consortiums [C_1 (Z_{11}+P_1+S_{11}), C_2 (Z_{11}+P_8+S_{19}), C_3 (Z_{22}+P_1+S_{14}), C_4 (Z_{22}+P_8+S_{19}) and C_5 (Z_{11}+P_1+S_{19})] alongwith control were randomized in subplots. The sub plot size was 7 m x 1.2 m. Seeds were inoculated at a rate of 5 g of PGPR consortium per kg of seed using sugar solution as a sticker. Inoculation was done under shade and the inoculated seeds were kept for few minutes until air drying before planting. The graded seed at the rate of 75 kg ha^{-1} was sown with a single row drill.

The recommended dose of fertilizer (22 Kg N and 57 Kg P_2O_5 ha^{-1}) was applied at the time of seed bed preparation.

The crop was inter-cultured twice at 35 and 70 days after sowing. Ten randomly selected plants were uprooted carefully, washed and the number of nodules plant^{-1} were recorded on days 100, 120 and 140 after sowing from each plot and the average number of grains pod^{-1} were recorded from 20 randomly selected pods taken from ten randomly selected plants. Average weights of three samples were recorded for 100 grain randomly taken from grain yield of each treatment where as grain yield (kg ha^{-1}) was recorded on plot basis.

Grain protein value

The seed N content was determined by Kjeldahl's method and the estimated N content was multiplied by a factor of 6.25 to compute the total protein content (Jackson, 1962). Data were subjected to analysis of variance (Steel *et al.*, 1997) to determine the significance of differences between treatments. The Least significant difference (LSD) test was applied for comparison of means of individual treatments (Statistics 8.1).

Results and discussion

Nodules plant^{-1}

Count of nodules, fresh and dry weight of nodules plant^{-1} are presented in Table 3, 4 and 5. It is evident that differences between the means of treatments were significant. Nodules count ranged from 37.33 to 87.83 per plant while dry weight of nodules varied from 0.203 to 0.677 g plant^{-1}. Maximum number of nodules plant^{-1} were counted as 87.83 with 2.445g fresh and 0.677 g dry weight per plant in treatment combination V_4 (TG1221) × C_4 (Z_{22}+P_8+S_{19}) thus showing the specific symbiotic relationship among the genotype and PGPR consortium.

Table 1. Physiochemical characteristic of soil.

Property	Units	Value	
		Before sowing	After harvesting
pH	-	8.2	8.00
ECe	dSm^{-1}	0.44	-
P	Ppm	3.34	4.75
K	Ppm	66	60
Texture	-	Sandy loam	-
Organic matter	%	0.27	0.34
N	%	0.014	0.017
Field capacity	% by volume	14.60	14.67
Bulk density	g^{-3}	1.26	1.26
Permanent wilting point	% by volume	5.4	5.4

Source: Soil and Water Testing Laboratory Ayub Agricultural Research Institute, Faisalabad.

Table 2. Summary of N-fixing bacteria, PSB and PGPR isolated from chickpea nodules, rhizoplane and rhizospheric soil of Thal desert.

Accession #	Strain's Name	Isolation source	Functions
MK880587	*Enteroabacter cloacae*	Nodules	N-fixing
MK880588	*Bacillus subtilis*	Nodules	N-fixing
MK880582	*Providencia vermicola*	Rhizoplane soil	PSB
MK880584	*Bacillus mojavensis*	Rhizoplane soil	PSB
MK880590	*Bacillus zhangzhouensis*	Rhizospheric soil	Growth Promoter
MK880589	*Bacillus mojavensis*	Rhizospheric soil	Growth Promoter

Thus PGPR consortium increased nodules plant^{-1} by 135 % over control. The different PGPR consortium behaved differently regarding nodules count, fresh and dry weight of nodules plant^{-1}. The nodules plant^{-1} were increased by 103, 106, 107, 114 and 135 % over the control by treatment interaction V_4 × C_2, V_3 × C_4, V_1 × C_4, V_2 × C_4 and V_4 × C_4, respectively. PGPR consortium, a combination of nitrogen fixing bacteria, phosphate solublizing and growth promoting bacteria showed a good symbiotic relationship with genotype TG1221 and gave maximum increase of 135 % in number of nodules plant^{-1}. Similarly increments in fresh and dry weight of nodules plant^{-1} were observed in treated plots with PGPR consortium against untreated plots. The yield components were also increased in similar fashion and ultimately the grain yield was increased from 1496 to 2491 kg ha^{-1}. With the increase in fresh and dry weight of nodules plant^{-1}, nodules count was also increased which showed the enhanced microbial activities increased N uptake by the crop plants and thus the yield as well. Tippannavar and Desai (1992), Shah *et al.* (1994), Biswas *et al*. (2003) and Aslam *et al*. (2010) reported that seed inoculation increased the number of nodules plant^{-1}, fresh and dry weight of nodules plant^{-1}. These results are also in conformity with the findings of El-Hadi and Sheikh (1999) as they reported Rhizobium inoculation significantly increased total number of nodules plant^{-1}. Yadav *et al.* (1994) observed an increase in the number of nodules and grain yield due to grain inoculation. Roy et al. (1995) also reported that grains inoculation increased the nodules number plant^{-1} and gave the highest harvest index and 100 grain weight. Chickpea is well acknowledged for its specificity towards rhizobial supplies (SPG, 2016; Tena *et al.*, 2016). A positive response of chickpea to inoculation was reported by Ben Romdhane *et al.* (2008), Funga *et al.* (2016), and Khaitov *et al.* (2016). Moreover, legumes are seriously affected by drought for attachment of bacteria to root hair, nodulation and N fixation (Zahran, 1999).

Table 3. Nodules plant^{-1} affected by symbiotic relationship of chickpea genotypes and PGPR Consortium.

PGPR Consortium	Chickpea genotypes				Mean
	Bittle- 2016 (V_1)	TG1218 (V_2)	Bhakkar 2011 (V_3)	TG1221 (V_4)	
Control (C_0)	37.33	40.00	42.83	40.50	40.17
C_1 ($Z_{11}+P_1+S_{11}$)	48.06	55.50	47.00	74.50	56.26
C_2 ($Z_{11}+P_8+S_{19}$)	64.49	71.37	60.56	75.70	68.03
C_3 ($Z_{22}+P_1+S_{14}$)	57.44	69.97	58.22	68.87	63.63
C_4 ($Z_{22}+P_8+S_{19}$)	77.44	80.00	76.83	87.83	80.53
C_5 ($Z_{11}+P_1+S_{19}$)	62.22	62.89	51.44	64.33	60.22
Mean	57.83	63.29	56.15	68.62	

$LSD_{0.05}$ (Varieties/Lines) = 3.866, $LSD_{0.05}$ (PGPR Consortium) = 3.414

$LSD_{0.05}$ (Varieties/Lines × PGPR Consortium) = 6.827

Table 4. Fresh weight of nodules plant^{-1} affected by symbiotic relationship of chickpea genotypes and PGPR Consortium.

PGPR Consortium	Chickpea genotypes				Mean
	Bittle- 2016 (V_1)	TG1218 (V_2)	Bhakkar 2011 (V_3)	TG1221 (V_4)	
Control (C_0)	0.927	1.060	0.823	0.953	0.941
C_1 ($Z_{11}+P_1+S_{11}$)	1.660	1.363	0.960	1.483	1.367
C_2 ($Z_{11}+P_8+S_{19}$)	1.493	1.520	1.540	1.857	1.603
C_3 ($Z_{22}+P_1+S_{14}$)	1.253	1.893	1.560	1.227	1.483
C_4 ($Z_{22}+P_8+S_{19}$)	1.337	1.417	1.380	2.443	1.644
C_5 ($Z_{11}+P_1+S_{19}$)	1.567	1.543	1.300	1.293	1.426
Mean	1.373	1.467	1.261	1.543	

$LSD_{0.05}$ (Varieties/Lines) = 0.161, $LSD_{0.05}$ (PGPR Consortium) = 0.188

$LSD_{0.05}$ (Varieties/Lines × PGPR Consortium) = 0.376.

Pods plant^{-1}

Number of pods plant^{-1} is a primitive determinant in pulse crops. Data regarding number of pods plant^{-1} are presented in Table 6. The analysis of variance showed that the differences among the means of treatments were significant. Pods plant^{-1} ranged from 74.7 to 113.3 in control and treatment interaction $V_4 \times C_4$. Thus PGPR consortium yielded an increase of 52% in number of pods plant^{-1} over control. This may be due to more availability of nutrients in the form of nitrogen and phosphorus due to the enhanced activities of nitrogen fixing, phosphate solublizing bacteria along with growth promoting bacteria included in PGPR consortium. PGPR consortium significantly increased the number of pods plant^{-1}. Maximum number of pods plant^{-1} (109.42) were recorded in C_4 ($Z_{22}+P_8+S_{19}$) followed by C_2 ($Z_{11}+P_8+S_{19}$) with an average of 104.83 pods plant^{-1}. Minimum number of pods plant^{-1} (76.58) was recorded in uninoculated plots. On an average, the number of pods plant^{-1} were increased by 27, 29, 30, 37, and 43% over control by inoculating the seeds with PGPR consortium treatments viz. C_5, C_3, C_1, C_3 and C_4, respectively.

Table 5. Dry weight of nodules plant^{-1} affected by symbiotic relationship of chickpea genotypes and PGPR Consortium.

PGPR Consortium	Chickpea genotypes				Mean
	Bittle- 2016 (V_1)	TG1218 (V_2)	Bhakkar 2011 (V_3)	TG1221 (V_4)	
Control (C_0)	0.280	0.227	0.203	0.393	0.276
C_1 ($Z_{11}+P_1+S_{11}$)	0.373	0.250	0.257	0.550	0.358
C_2 ($Z_{11}+P_8+S_{19}$)	0.297	0.433	0.320	0.553	0.401
C_3 ($Z_{22}+P_1+S_{14}$)	0.253	0.370	0.450	0.273	0.337
C_4 ($Z_{22}+P_8+S_{19}$)	0.400	0.373	0.263	0.677	0.428
C_5 ($Z_{11}+P_1+S_{19}$)	0.363	0.313	0.277	0.537	0.373
Mean	0.328	0.328	0.295	0.497	

LSD $_{0.05}$ (Varieties/Lines) = 0.041, LSD $_{0.05}$ (PGPR Consortium) = 0.052

LSD $_{0.05}$ (Varieties/Lines × PGPR Consortium) = 0.104.

Table 6. Pods Plant^{-1} affected by symbiotic relationship of chickpea varieties/lines and PGPR consortium.

PGPR Consortium	Chickpea genotypes				Mean
	Bittle- 2016 (V_1)	TG1218 (V_2)	Bhakkar 2011 (V_3)	TG1221 (V_4)	
Control (C_0)	74.7	80.0	76.0	75.7	76.58
C_1 ($Z_{11}+P_1+S_{11}$)	97.7	94.3	101.0	104.3	99.33
C_2 ($Z_{11}+P_8+S_{19}$)	109.7	110.7	96.7	102.3	104.83
C_3 ($Z_{22}+P_1+S_{14}$)	86.7	103.3	104.7	101.7	99.08
C_4 ($Z_{22}+P_8+S_{19}$)	110.7	102.7	111.0	113.3	109.42
C_5 ($Z_{11}+P_1+S_{19}$)	93.3	98.7	98.7	94.7	96.33
Mean	95.44	98.28	98.00	98.67	

LSD $_{0.05}$ (Varieties/Lines) = 1.59, LSD $_{0.05}$ (PGPR Consortium) = 1.64

LSD $_{0.05}$ (Varieties/Lines × PGPR Consortium) = 3.28.

These results are in accordance with that of Karadavut and Ozdemir (2001), Fatima et *al.* (2008) and Aslam *et al.* (2010) who reported that inoculation significantly increased grain yield over control, total above ground dry matter and number of pods plant^{-1} in chickpea.

Seed inoculation with PGPR consortium might have increased nitrogen and phosphorus supply to crop plants, which ultimately resulted in more number of pods plant^{-1}. These results are in line with those of Ali *et al.* (2003) and Tena *et al.* (2016) who achieved increased number of pods plant^{-1} by seed inoculation.

Grains pod^{-1}

Grains pod^{-1} were affected significantly by interaction of PGPR consortium and genotypes (Table 7). The maximum number of grains pod^{-1} were recorded as 2.33 in V_4 (TG1221) x PGPR consortium C_4 ($Z_{22}+P_8+S_{19}$) closely followed by V_3 (Bhakkar 2011) x C_4 ($Z_{22}+P_8+S_{19}$) while minimum grains pod^{-1} were recorded as 1.60 in untreated plot. Inoculation with

PGPR consortium increased grains pod^{-1} from 1.68 to 2.13 while genotypes also significantly affected the grain pod^{-1} which ranged from 1.7 to 1.97. The maximum number of grains pod^{-1} (2.33) was recorded in treatment combination V_4 x C_4 closely followed by V_4 x C_2 (2.20). The minimum number of grains pod^{-1} was recorded in uninoculated treatment (1.60). Inoculation with PGPR consortium C_3, C_5, C_1, C_2, and C_4 increased the number of grains pod^{-1} by 7, 9, 9, 15, and 27%, respectively over the control.

Table 7. Grains pod^{-1} affected by symbiotic relationship of chickpea varieties/lines and PGPR inoculation strains.

PGPR Consortium	Chickpea genotypes				Mean
	Bittle- 2016 (V_1)	TG1218 (V_2)	Bhakkar 2011 (V_3)	TG1221 (V_4)	
Control (C_0)	1.73	1.73	1.60	1.67	1.68
C_1 (Z_{11}+P_1+S_{11})	2.00	1.87	1.60	1.87	1.83
C_2 (Z_{11}+P_8+S_{19})	1.87	1.87	1.80	2.20	1.93
C_3 (Z_{22}+P_1+S_{14})	1.80	1.93	1.53	1.93	1.80
C_4 (Z_{22}+P_8+S_{19})	2.07	2.07	2.07	2.33	2.13
C_5 (Z_{11}+P_1+S_{19})	1.73	2.07	1.73	1.80	1.83
Mean	1.87	1.92	1.72	1.97	

$LSD_{0.05}$ (Varieties/Lines) = 0.206, $LSD_{0.05}$ (PGPR Consortium) = 0.172

$LSD_{0.05}$ (Varieties/Lines × PGPR Consortium) = 0.344.

The interaction between genotypes and inoculation consortium were also significant and the maximum number of grains pod^{-1} (2.13) was recorded in V_2 (TG1218) with PGPR consortium C_4 (Z_{22}+P_8+S_{19}), while the minimum number of grains pod^{-1} (1.60) was recorded in V_3 (Bhakkar 2011) x C_0 (control). The treatment V_4 x C_4 yielded 45 % increase over control treatment (V_3 x C_0).

Increase in the number of grains pod^{-1} was probably due to more availability of nitrogen and phosphorus due to microbial activities in treated plots.

Table 8. 100-Grain weight affected by symbiotic relationship of chickpea varieties/lines and PGPR inoculation strains.

PGPR Consortium	Chickpea genotypes				Mean
	Bittle- 2016 (V_1)	TG1218 (V_2)	Bhakkar 2011 (V_3)	TG1221 (V_4)	
Control (C_0)	21.60	20.45	21.67	22.32	21.51
C_1 (Z_{11}+P_1+S_{11})	24.47	23.35	22.75	25.42	24.00
C_2 (Z_{11}+P_8+S_{19})	22.09	23.99	23.35	25.63	23.76
C_3 (Z_{22}+P_1+S_{14})	23.28	24.05	24.23	24.47	24.01
C_4 (Z_{22}+P_8+S_{19})	26.51	27.48	26.25	28.73	27.724
C_5 (Z_{11}+P_1+S_{19})	24.04	24.27	23.46	23.72	23.87
Mean	23.67	23.93	23.62	25.04	

$LSD_{0.05}$ (Varieties/Lines) = 0.88, $LSD_{0.05}$ (PGPR Consortium) = 0.55

$LSD_{0.05}$ (Varieties/Lines × PGPR Consortium) = 1.11.

The accelerated effects of seed inoculation on number of seeds pod^{-1} were also observed by Malhur *et al.* (2003) and Aslam *et al.* (2010), they reported increased number of seeds pod^{-1} by seed inoculation. These results are also confirm the findings of Zai *et al.* (1999) who concluded that Rhizobium inoculation had a significant effect on growth, N contents and uptake in shoots, yield and yield attributes, and grain protein content in chickpea.

100-grain weight

The data regarding 100 grain weight of chickpea as affected by genotypes and PGPR consortium inoculation are presented in Table 8. It is obvious that different treatments significantly affected the 100 grain weight. Maximum 100 grain weight of 28.73g was recorded in V_4 x C_4 compared to 20.45g in V_2 x C_o (uninoculated). 100 grain weight was increased by 40% over uninoculated plots. The interaction between inoculation and genotypes showed significant differences and interaction of genotype V_4 with C_4 gave 100 grains weight of 27.24 g closely followed by V2 x C4 as 27.48g while minimum 100 grain weight of 20.45 g was recorded in check. On average, V_4 x C_4 treatment gave the maximum 100 seed weight of 27.73 g depicting 29% increase over control (V_4 x C_o). This increase in 100 grains weight may be due to the more availability of nutrients like nitrogen and phosphorus in the rhizosphere due to microbial activities.

Table 9. Grain protein contents affected by symbiotic relationship of chickpea varieties/lines and PGPR inoculation strains.

PGPR Consortium	Chickpea genotypes				Mean
	Bittle- 2016 (V_1)	TG1218 (V_2)	Bhakkar 2011 (V_3)	TG1221 (V_4)	
Control (C_o)	20.37	19.73	19.77	20.20	20.02
C_1 ($Z_{11}+P_1+S_{11}$)	23.43	22.57	22.40	22.50	22.73
C_2 ($Z_{11}+P_8+S_{19}$)	21.57	22.47	22.73	23.27	22.51
C_3 ($Z_{22}+P_1+S_{14}$)	23.63	23.73	23.80	22.47	23.41
C_4 ($Z_{22}+P_8+S_{19}$)	22.57	23.90	23.77	23.13	23.34
C_5 ($Z_{11}+P_1+S_{19}$)	21.47	21.80	21.80	22.57	21.91
Mean	22.17	22.37	22.38	22.36	

LSD $_{0.05}$ (Varieties/Lines) = 151, LSD $_{0.05}$ (PGPR Consortium) = 99.81

LSD $_{0.05}$ (Varieties/Lines × PGPR Consortium) = 199.62.

Inoculation with PGPR consortium also increased the 100 grain weight in chickpea. Maximum 100 grains weight of 27.24g was recorded in C_4 followed by C_3 with 100 grain weight of 24.01g whereas the minimum 100 grain weight of 21.51g was recorded in the control treatment. On average, 100 grains weight was increased by 10, 11, 11, 12, and 27 % by inoculating seeds with C_2, C_3, C_5, C_1 and C_4, respectively.

Better growth and development of crop plants due to seed inoculation might have affected the nutrient supply (N&P) to plants resulting in producing more assimilates which might have partitioned more efficiently from source to sink and ultimately gained more seed weight. Similar results were reported by Alam *et al.* (1999), El-Hadi and Sheikh (1999), Meena *et al.* (2001), Kyei-Boahen *et al.* (2002), Aslam *et al.* (2010) and Tena *et al.* (2016) who stated that *PGPR* inoculation significantly increased 100 seed weight and yield. The results suggest that consortium contributed much to increase the efficiency of major nutrient and thus led to the higher grains weight and yield.

Grain protein content

Grain protein content is considered to be an important character in chickpea. Data regarding grain protein content are presented in Table 9 wherein, it is apparent that different treatments affected the grain protein content of chickpea significantly. It can be assumed that availability of more nitrogen and phosphorous in the root zone might have increased grain protein content. Seed inoculation with PGPR Consortium significantly influenced the protein content of chickpea.

It increased from 20.02 to 23.41%. Similarly mild increase in protein contents from 22.17 to 22.38 % in different chickpea genotypes which showed similarity in genotypes while interaction of PGRP consortium and genotypes increased protein contents from19.73 to 23.90% which advocates that PGPR consortium significantly increased the protein contents in chickpea. As nitrogen is a constituent of protein, seed inoculation might have enhanced the nitrogen supply to seed which resulted in higher protein content. Our findings are supported by reports of Ibrahim and Mahmood (1989), who stated that seed inoculation with different strains of bacteria significantly improved pod yield, N content of the plant and average nitrogen recovery. Alam *et al.* (1999), Aslam *et al.* (2010) and Giller *et al.* (2013) also reported that inoculums significantly increased grains protein content. Kyei-Boahen *et al.* (2002) reported that grain yield and quality were improved up to 50 % in chickpea with inoculation.

Table 10. Grain Yield (kg ha^{-1}) affected by symbiotic relationship of chickpea varieties/lines and PGPR inoculation strains.

PGPR Consortium	Chickpea genotypes				Mean
	Bittle- 2016 (V_1)	TG1218 (V_2)	Bhakkar 2011 (V_3)	TG1221 (V_4)	
Control (C_o)	1880	1496	1692	1590	1665
C_1 (Z_{11}+P_1+S_{11})	1949	1620	1945	2180	1923
C_2 (Z_{11}+P_8+S_{19})	2145	2128	1957	2444	2169
C_3 (Z_{22}+P_1+S_{14})	1970	1868	1876	2299	2003
C_4 (Z_{22}+P_8+S_{19})	1927	1855	1970	2491	2061
C_5 (Z_{11}+P_1+S_{19})	2333	1838	1701	2320	2048
Mean	2034	1801	1857	2221	

$LSD_{0.05}$ (Varieties/Lines) = 151, $LSD_{0.05}$ (PGPR Consortium) = 99.81

$LSD_{0.05}$ (Varieties/Lines × PGPR Consortium) = 199.62.

*Grain yield (*kg ha^{-1}*)*

It was observed that different treatments significantly affected the grain yield in chickpea (Table 10). Maximum grain yield of 2221 kg ha^{-1} was recorded in V_4 (TG1221) as compared to V_2 (TG1218) kg ha^{-1}. On average, V_4 gave 24 % edge in yield of chickpea over V_2.

It might be due to different yield potential of genotypes. Seed inoculation also significantly affected the grain yield and, on average, increased 15, 20, 23, 24 and 30 % over control with inoculating the seed with PGPR consortiums C_1 (Z_{11}+P_1+S_{11}), C_3 (Z_{22}+P_1+S_{14}), C_5 (Z_{11}+P_1+S_{19}), C_4 (Z_{22}+P_8+S_{19}) and C_2 (Z_{11}+P_8+S_{19}), respectively. This increase in yield may be due to effective nodulation which in turn enhanced the utilization of atmospheric nitrogen and availability of phosphorus towards higher yield.

These findings are in line with those of Sharma *et al.* (2001) who reported 13 % increase in seed yield by inoculation. Our findings are in conformity with that of Fatima et *al.* (2008) and Aslam *et al.* (2010), who reported that application of *Rhizobium* inoculum, generally increased growth, yield components and nitrogen fixation.

The interaction of genotypes plus PGPR consortium (C_4 (Z_{22}+P_8+S_{19}) gave the maximum average yield of 2491 kg/ha against 1590 kg/ha in uninoculated plot. These results support the idea that inoculum could be more effective and beneficial in enhancing grain yield and agreed with reports published by Meena *et al.* (2001) and Endalkachew *et al.* (2018) who concluded that inoculum singly or in combination with fertilizer increased grain yield in chickpea (Karadavut and Ozdemir, 2001, Khaitov *et al.* 2016). Resultantly, the microbial activities were optimum which increased the nodules N-supply to crop plants and hence increased crop growth, number of pods $plant^{-1}$, grains pod^{-1} and grain yield. It may be concluded from the present study that the use of PGPR consortiums should be encouraged in order to get profitable yield of chickpea under prevailing agro-climatic conditions.

Conclusion

From the foregoing accounts, it may be concluded that the application of specific PGPR consortium improved grain yield and quality of chickpea through a positive effect on agronomic parameters like nodules plant-1, number of pods plant-1, number of seeds pod-1, 100 grains weight and grain protein contents. Therefore, use of PGPR consortium is recommended for better chickpea crop production and soil improvement. It could be recommended that grain quality, being an important character, can be improved by inoculation in chickpea.

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