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AGRONOMY

EFFICIENCY OF NITROGEN FIXING, NODULATION FORMATION, PHOSPHATE SOLUBILIZING BACTERIA AND PLANT GROWTH PROMOTING RHIZOBACTERIA ON YIELD OF CHICKPEA

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ABSTRACT

Chickpea is an important nodules producing pulse crop of Thal. To enhance nitrogen fixation for higher yield, the effect of bacterial strains on nodulation was evaluated for yield contributing traits of chickpea at Arid Zone Research Institute, Bhakkar, Pakistan during 2019-20. Six Rhizobium inoculations i.e. *Providencia vermicola, Enterobacter cloacae, Bacillus mojavensis, Mesorhizobium ciceri-11, Mesorhizobium ciceri-1* and *Bacillus subtilis* were tested on two chickpea genotypes i.e. Bhakkar-2011 and Thal-2020. A consortium of six bacterial strains was applied at the time of sowing as a seed coating on a sandy soil. Results showed a significant increase in height/plant, nodulation, 100 grain weight, pods/plant and grain yield (kg/ha) due to bacterial inoculation. Nodules/plant were also increased from 44.08 to 76.16. Maximum number of nodules plant^{-1} were counted as 76.16 with 2.59 g fresh and 0.64 g dry weight per plant in treatment combination viz. Thal-2020 × RP_{08}+RS_{14}+RZ_{11} thus showing the specific symbiotic relationship among the genotypes and Rhizobium inoculum. In comparison to the control, the PSB, nitrogen-fixing bacteria and rhizobium inoculum results were 52% increased in pods/ plant. The maximum number of pods/plant (116.00) and100 grain weight (27.73 g) were recorded in V_1 × T_1 (RP_{08}+RS_{14}+ RZ_{11}). The weight of 100 grains was increased by 40% over the control. T_1 had the highest 100 grain weight of 27.73 g, followed by V1 x T_3 with a 100 grain weight of 26.22 g. PSB, nitrogen fixation bacteria and PSB inoculation substantially improved pulse crop 100 grain weight and yield. Thal-2020 had the highest grain yield of 3732 kg/ha, while V2 (Bhakkar-2011) had the lowest at 3516 kg/ha. Inoculation with rhizobium consortium improved grain yield by 30% (2617 to 3732 kg/ha). Hence, it was concluded that among all the treatments of Rhizobium consortium, N-fixing bacteria and PSB influenced a beneficial effect on chickpea grain yield quality and quantity.

KEYWORDS: *Cicer arietinum*; varieties; inoculation; *rhizobium*; grain yield; Pakistan

INTRODUCTION

Chickpea (*Cicer arrentinum* L.) is important pulse crop of Pakistan thal area which usually got low yield due to various reasons. Seed quality, sowing times, weeds, diseases and pests are some major factors which contributes to low yield (Akhtar amd Siddiqui, 2009). Soil is generally lacking nitrogen, which is very most important element in protein synthesis and metabolism of plants. Yield of chickpea can be significantly improved by using seed of resistant varieties to diseases, balanced fertilizer, and good agronomic practices and efficient bacteria used as bio fertilizer (Chen *et al.,* 2018). Production of crop can be also increased by the application of organic, chemical and biological fertilizer (Oliveira *et al.,* 2017; Tang *et al.,* 2020). During 2019-2020, it was cultivated on an area of 940 thousands hectare with a total production 545 thousand tons. Nutritionally, it is an important source of protein (16-20%) in vegetarian diet. It is also anironic source of vitamins fat, minerals, carbohydrates, total dietary fiber and trace elements. It also plays a vital role in human diet (Kantar *et al.*, 2010). Chickpea has another beneficial attributes to fix atmospheric nitrogen (N) in symbiotic relationship to rhizobia, which contribute directly increases in protein content, nodulation, and decreases the N fertilizer need of subsequent crops. Therefore, chickpea crop has potential to improve soil contents, it quality and nitrogen status (Tena *et al*., 2016; Funga *et al*., 2016). 60-80% of nitrogen requirement were fix by nodules of chickpea. Amounting to 60–176 N kg/ha (Endalkachew *et al.,* 2018). In chickpea, a numbers of researchers have been reported that rhizobia seed inoculation increases root & shoot length, primary branches nodulations, number of pods, and grain yield (Giller *et al*., 2013). Of the total world fertilizer used, about 20-30 % of total applied fertilizer is ued by the plants. Some the varieties have poor nutrient take efficiency. Due to this about 50 % of applied fertilizer is lost in the environment (Fageria, 2014). So a phenomena is must require to minimize these losses. This can only be possible by using different N and P fixing microbes. Among the various microbiome (Cordeiro and Echer, 2020), most of the bacteria known as plant growth-promoting rizobacteria (PGPR) have positive interaction with the plant and promote their growth and survival (Kumar and Dubey, 2020). Rhizobium strains increases crop growth rate at high rates when used in combination with phosphorous solubilizing bacteria (Billah *et al.,* 2019). Due to phosphorous availability the surface area, volume and root length increases due to because phosphorous promotes water absorption from untapped soil surface (Nannipieri *et al.,* 2011). Phosphate solubilizing microbes releases phosphatase enzyme which is responsible for P-solublizition from soil having fixed phosphorous (Kour *et al.,* 2019). Different bacteria genera are important components which are involved in various biotic activities to make soil dynamic for nutrient turn over and sustainable for crop production. They stimulate plant growth through mobilizing nurtients in soil, producing numerous plant growth regulators, protecting from phyto-pathogens by improving soil structure by degrading various soil pollutants and heavy metals (Ahemed *et al.,* 2009; Ahmed, 2012). Therefore the rhizobacteria are the dominant deriving forces in recycling the soil nutrients which are important for the soil fertility (Glick, 2012). In drought conditions P-fixation occurs due to phosphatase enzyme inactivity. Thus PGPR contributes to a massive increase in pulse crop yield (Maliha *et al.,* 2004). Field trials proved that nearly 50% N fertilizer saved through rhizobium inoculation with significant increase in yield and yield traits (Burbano *et al.,* 2015; Lipa and Janczarek, 2020). Rhizobium consortium inoculum mainly increased nitrogen fixation, plant growth and yield traits in gram (Fatima *et al*., 2008). The microorganism efficacy also effected by soil type, source of rhizobia, test crop and as well as quantity of soil nutrients. Rhizobium inoculation not only increases the grain yield but also improves quality of the produce with protein contents, fat, crude fiber, carbohydrates contents and ash in chickpea (Alishahi *et al.,* 2020). Therefore, the present study was carried out to sort out bacterial strains in form of rhizobia consortia on nodulation and yield contributing traits of chickpea.

MATERIALS AND METHODS

The experiment was carried out in 71.02º longitude and 31.37º latitude, with in elevation range of 184 meters from the sea level during 2019-2020 at Arid Zone Research Institute, Bhakkar, Pakistan. Average temperature during the cropping season generally

Table 1. Bacterial strains

Accession No	Strain's Name	Isolation source	Functions
MK880587	*Enteroabacter cloacae* (RP_{08})	Nodules	N-fixing
MK880588	*Bacillus subtilis* (RP_{01})	Nodules	N-fixing
MK880582	*Providenciavermicola* (RS_{15})	Rhizoplane soil	PSB
MK880584	*Bacillus mojavensis* (RS_{14})	Rhizoplane soil	PSB
MN601741	*Mesorhizobium ciceri-11* (RZ_{22})	Rhizospheric soil	Growth promoter
MN601357	*Mesorhizobium ciceri-1* (RZ_{11})	Rhizospheric soil	Growth promoter

Table 2. Treatments

Sr. No	Treatment	N-fixing bacteria, PSB and PGPR consortium
1	T_1 (RP_{08}+ RS_{14}+ RZ_{11})	*Enteroabacter cloacae + Bacillus mojavensis + Mesorhizobium ciceri-1*
2	T_2 (RP_{01}+ RS_{14}+ RZ_{11})	*Bacillus subtilis + Bacillus mojavensis + Mesorhizobium ciceri-1*
3	T_3 (RS_{15}+ RP_{08}+ RZ_{11})	*Providenciavermicola + Enteroabacter cloacae + Mesorhizobium ciceri-1*
4	T_4 (RS_{15}+ RP_{01}+ RZ_{11})	*Providen ciavermicola + Bacillus subtilis + Mesorhizobium ciceri-1*
5	T_5 (RS_{14}+ RP_{08}+ RZ_{22})	*Bacillus mojavensis + Enteroabacter cloacae + Mesorhizobium ciceri-11*
6	T_6 (RS_{14}+ RP_{01}+ RZ_{22})	*Bacillus mojavensis + Bacillus subtilis + Mesorhizobium ciceri-11*
7	T_7 (RP_{08}+ RS_{15}+ RZ_{22})	*Enteroabacter cloacae + Providenciavermicola + Mesorhizobium ciceri-11*
8	T_8 (RS_{15}+ RP_{01}+ RZ_{22})	Providencia vermicola + Bacillus subtilis + Mesorhizobium ciceri-*11*

remained at 12 ºC with 60-70 % relative humidity. The soil was sandy had 0-30 cm layer consisting of 0.014% nitrogen (N), 166 ppm available potassium (K), 3.34 ppm phosphate (PO_4^{-3}) and 0.27% organic matter with soil pH 8.2.

The chickpea varieties/lines i.e. Thal-2020 (V_1), Bhakkar-2011 (V_2) were kept in main plot while six bacterial strains (*Providencia vermicola, Enterobacter cloacae, Bacillus mojavensis, Mesorhizobium ciceri-11, Mesorhizobium ciceri-1* and *Bacillus subtilis*) isolated from chickpea nodules and soil of Thal Desert (Table 1) were randomly mixed to make eight treatments [T_1 (RP_{08}+ RS_{14}+ RZ_{11}), T_2 (RP_{01}+ RS_{14}+ RZ_{11}), T_3 (RS_{15}+ RP_{08}+ RZ_{11}), T_4 (RS_{15}+ RP_{01}+ RZ_{11}), T_5 (RS_{14}+ RP_{08}+ RZ_{22}), T_6 (RS_{14}+ RP_{01}+ RZ_{22}), T_7 (RP_{08}+ RS_{15}+ RZ_{22}) and T_8 (RS_{15}+ RP_{01}+ RZ_{22})] along with control (T_0). The experiment was designed in split plot design with three replication. The sub plot size was 7× 1.2 m each having 4 rows with 15/30 cm intra/inter row spacing. Field were prepared by pre sowing irrigation and one bag of DAP (18% N, 46% P_2O_5) fertilizer @ 125 kg/ha was applied at the time of seed bed preparation. Seeds were inoculated @ 60 g of rhizobium treatment per kg of seed. The graded seed was sown @ 75 kg/ha with a single row drill. After sowing crop was inter-cultured thrice at 35, 45 and 70 days. The sugar solution used as sticker. Inoculation was carried out under shade and before planting seed inoculated with consortia kept for few minutes for air drying. Two irrigations were applied i.e. at seedling and flowering. Three randomly selected plants were uprooted carefully, washed and the number of nodules/plant were recorded on days 45, 90 and 120 DAS (days after sowing) from each plot. Similarly, grain/pod were counted from randomly 20 selected plants. 100 grain were collected randomly from every treatment and grain yield was recorded on plot basis.

STATISTICAL ANALYSIS

To assess the importance of variations between procedures, data were subjected to analysis of variance (Steel *et al.,* 1997). For comparing the means of individual procedures, the Least Significant Difference (LSD) test was used by using computer based statistical Microsoft software (Katz, 2010; Minitab, 2013)

RESULTS AND DISCUSSION

Nodules/plant

Data pertaining number of nodules, fresh and dry weight of nodules plant^{-1} is presented in Table 3, 4 and 5, respectively. It was observed difference between treatments of mean were significant. Nodules amount ranged from 41.5 to 76.16 per plant while dry weight of nodules varied from 0.22 to 0.64 g/plant.Maximum number of nodules/plant were counted as 76.16 with

Table 3. Effect of bacterial inoculation on number of nodules/plant

PSB & Rhizobium inoculum	Chickpea genotypes		Mean
	Thal-2020 (V_1)	Bhakkar-2011 (V_2)	
T_0 (Control)	44.08 c	41.5 c	57.05
T_1 (RP_{08}+ RS_{14}+ RZ_{11})	76.16 a	72.98 a	74.57
T_2 (RP_{01}+ RS_{14}+ RZ_{11})	55.99 b	57.00 b	56.50
T_3 (RS_{15}+ RP_{08}+ RZ_{11})	63.95 ab	48.19 bc	56.07
T_4 (RS_{15}+ RP_{01}+ RZ_{11})	56.35 b	68.91 a	62.63
T_5 (RS_{14}+ RP_{08}+ RZ_{22})	57.96 b	60.50 b	59.23
T_6 (RS_{14}+ RP_{01}+ RZ_{22})	58.25 b	66.33 ab	62.29
T_7 (RP_{08}+ RS_{15}+ RZ_{22})	69.35 ab	42.84 c	56.10
T_8 (RS_{15}+ RP_{01}+ RZ_{22})	57.50 b	54.43 b	55.97

LSD+P Value$_{0.05}$(Varieties) = 5.28+0.001, LSD$_{0.05}$ (Rhizobium) = 3.51+0.000, LSD$_{0.05}$(Varieties × Rhizobium) = 4.97+0.002

Table 4. Effect of bacterial inoculation on number of fresh weight (g) nodules/plant

PSB & Rhizobium inoculum	Chickpea genotypes		Mean
	Thal-2020(V_1)	Bhakkar-2011 (V_2)	
T_0 (Control)	0.88 c	0.60 c	0.74
T_1 (RP_{08}+ RS_{14}+ RZ_{11})	2.59 a	2.57 a	2.58
T_2 (RP_{01}+ RS_{14}+ RZ_{11})	1.30 b	1.38 b	1.34
T_3 (RS_{15}+ RP_{08}+ RZ_{11})	0.96 c	1.45 b	1.21
T_4 (RS_{15}+ RP_{01}+ RZ_{11})	1.23 bc	1.14 c	1.19
T_5 (RS_{14}+ RP_{08}+ RZ_{22})	1.48 b	1.06 c	1.27
T_6 (RS_{14}+ RP_{01}+ RZ_{22})	1.27 b	1.21 bc	1.24
T_7 (RP_{08}+ RS_{15}+ RZ_{22})	0.96 c	1.48 b	1.22
T_8 (RS_{15}+ RP_{01}+ RZ_{22})	1.05 bc	1.78 ab	1.42

LSD+P Value$_{0.05}$(Cultivars) = 0.22+0.021, LSD$_{0.05}$ (Rhizobium) = 0.34+0.001, LSD$_{0.05}$ (Cultivars × Rhizobium) = 0.49+0.032

Table. 5. Effect of bacterial inoculation on number of dry weight (g) nodules/plant

PSB & Rhizobium inoculum	Chickpea genotypes		Mean
	Thal-2020(V_1)	Bhakkar-2011 (V_2)	
T_0 (Control)	0.24 c	0.22 c	0.23
T_1 (RP_{08}+ RS_{14}+ RZ_{11})	0.64 a	0.55 ab	0.60
T_2 (RP_{01}+ RS_{14}+ RZ_{11})	0.35 b	0.38 b	0.37
T_3 (RS_{15}+ RP_{08}+ RZ_{11})	0.28 b	0.42 b	0.35
T_4 (RS_{15}+ RP_{01}+ RZ_{11})	0.38 b	0.27 c	0.33
T_5 (RS_{14}+ RP_{08}+ RZ_{22})	0.37 b	0.43 b	0.40
T_6 (RS_{14}+ RP_{01}+ RZ_{22})	0.34 b	0.47 b	0.41
T_7 (RP_{08}+ RS_{15}+ RZ_{22})	0.39 b	0.40 b	0.40
T_8 (RS_{15}+ RP_{01}+ RZ_{22})	0.42 b	0.35 b	0.39

LSD+P Value$_{0.05}$(Cultivars) = 0.067+0.001, (Rhizobium) = 0.067+0.061,(Cultivars×Rhizobium)=0.99+0.000

Table 6. Effect of rhizobium inoculum on pods/plant

PSB & Rhizobium inoculum	Chickpea genotypes		Mean
	Thal-2020(V_1)	Bhakkar-2011 (V_2)	
T_0 (Control)	83.67 c	82.33 c	83.00
T_1 (RP_{08}+ RS_{14}+ RZ_{11})	116.00 a	110.33 a	113.17
T_2 (RP_{01}+ RS_{14}+ RZ_{11})	96.00 ab	92.67 b	94.34
T_3 (RS_{15}+ RP_{08}+ RZ_{11})	105.33 ab	103.00 ab	104.17
T_4 (RS_{15}+ RP_{01}+ RZ_{11})	98.67 ab	100.67 ab	99.67
T_5 (RS_{14}+ RP_{08}+ RZ_{22})	100.67 ab	103.00 ab	101.84
T_6 (RS_{14}+ RP_{01}+ RZ_{22})	98.33 ab	99.00 ab	98.67
T_7 (RP_{08}+ RS_{15}+ RZ_{22})	96.33 ab	100.33 ab	98.33
T_8 (RS_{15}+ RP_{01}+ RZ_{22})	104.67 ab	100.22 ab	102.45

LSD+P Value$_{0.05}$(Cultivars) = 3.10+0.000, (Rhizobium) = 1.70+0.005, (Cultivar × rhizobium) = 2.41+0.001

2.59 g fresh and 0.64 g dry weight per plant in treatment combination V_1 (Thal-2020) × T_1 (RP_{08}+ RS_{14}+ RZ_{11}) thus showing the specific symbiotic relationship among the genotype and rhizobium inoculum. PGPR, phosphate solubilizing and growth promoting bacteria showed a good effect with genotype TG1410and gave maximum increase of 23% in number of nodules/plant. In comparison to untreated plots, treated plots with rhizobium inoculum showed similar increases in fresh and dry weight of nodules/plant, with genotype Thal-2020 showing the highest increase of 331 percent in fresh weight of nodules/plant. Nodules count increased as fresh and dry weight of nodules/plant increased, suggesting that increased microbial activities increased N uptake by crop plants and yield. Crop inoculation increased the amount of nodules/plant, fresh and dry weight of nodules/plant according to Martins-da-Costa *et al.* (2020). Grain inoculation increased the number of nodules and grain yield, according to Cordeiro and Echer (2019). Moreover, legumes are seriously affected by drought for attachment of bacteria to root hair, nodulation and N fixation. Further studies (Akhtar and Siddiqui, 2009) showed involvement of *Glomus*, *Pseudomonas putida*, *P. alcaligenes*, *P. aeruginosa*, *A. awamori* and *Rhizobium* sp. were noted for growth, nodule production and in the case of chickpea disease rot under field conditions. Installation of Rhizobium sp. resulted in a greater increase in growth and yield than *P. putida*, *P. aeruginosa* or *G. intraradices*. The number of nodules in the root system was very high in plants treated with Rhizobium sp. compared to plants without *Rhizobium* sp. The installation of P. caused a similar decrease in concentration as caused by *P. putida*. Our results also confirmed by (Korir *et al.,* 2017) in a comparative analysis of difference between the combination of rhizobia species and PGPR compared with a single rhizobia implant in dry root weights. These results suggest that co-inoculation of PGPR and Rhizobia has an effect on bean growth. The use of PGPR may enhance the effectiveness of Rhizobium bio fertilizers in the production of standard beans.

Pods/plant

In pulse crops, the number of pods per plant is a basic determinant of yield. Table 6 showed data on the number of pods produced per plant. Plant/pods ranged from 82.33 to 116.00. T_1 (RP_{08}+ RS_{14}+ RZ_{11}) had the most pods/plant (116.00), followed by T_3 (RS_{15}+ RP_{08}+ RZ_{11}) with an average of 105.33 pods/plant. A total of 83.67 pods/plant were found in the un-inoculated plot. Over the control, the PSB, nitrogen-fixing bacteria, and rhizobium inoculum increase the number of pods/plant by 52 percent. The number of pods plant^{-1} was substantially increased by PSB and rhizobium

Table 7. 100-Grain weight affected by PSB and rhizobium inoculum

PSB & Rhizobium inoculum	Chickpea genotypes		Mean
	Thal-2020(V_1)	Bhakkar-2011 (V_2)	
Control (T_0)	22.89 b	23.28 a	23.09
T_1 (RP_{08}+ RS_{14}+ RZ_{11})	27.73 a	25.51 a	26.62
T_2 (RP_{01}+ RS_{14}+ RZ_{11})	25.01 ab	24.30 ab	24.70
T_3 (RS_{15}+ RP_{08}+ RZ_{11})	26.22 ab	26.95 a	26.59
T_4 (RS_{15}+ RP_{01}+ RZ_{11})	24.57 a	24.01 ab	24.29
T_5 (RS_{14}+ RP_{08}+ RZ_{22})	25.45 a	24.99 ab	25.22
T_6 (RS_{14}+ RP_{01}+ RZ_{22})	24.53 a	25.38 a	26.95
T_7 (RP_{08}+ RS_{15}+ RZ_{22})	24.92 a	24.48 a	24.70
T_8 (RS_{15}+ RP_{01}+ RZ_{22})	25.81 ab	26.09 a	25.95

Table 8. Beneficial effect Rhizobium inoculum and PSB on grain yield (kg/ha)

PSB & Rhizobium inoculum	Chickpea genotypes		Mean
	Thal-2020(V_1)	Bhakkar-2011 (V_2)	
Control (T_0)	2617 c	2610 c	2614
T_1 (RP_{08}+ RS_{14}+ RZ_{11})	3732 a	3516 a	3624
T_2 (RP_{01}+ RS_{14}+ RZ_{11})	3167 b	3088 b	3127
T_3 (RS_{15}+ RP_{08}+ RZ_{11})	3607 a	3123 b	3365
T_4 (RS_{15}+ RP_{01}+ RZ_{11})	3292 ab	3306 ab	3298
T_5 (RS_{14}+ RP_{08}+ RZ_{22})	3360 ab	3116 b	3233
T_6 (RS_{14}+ RP_{01}+ RZ_{22})	3188 b	3215 ab	3101
T_7 (RP_{08}+ RS_{15}+ RZ_{22})	3095 b	3125 b	3110
T_8 (RS_{15}+ RP_{01}+ RZ_{22})	2935 b	2986 b	2959

$LSD_{0.05}$(Cultivars) = 101.05, $LSD_{0.05}$ (Rhizobium) = 121.38, $LSD_{0.05}$ (Cultivars × rhizobium) = 171.66

inoculum. A total of 83.67 pods/plant were found in the un-inoculated plot. In comparison to the control, the PSB, nitrogen-fixing bacteria, and rhizobium inoculum result in a 52 percent increase in pods/plant. PSB and rhizobium inoculum increased the number of pods/plant significantly. According to Fatima *et al.* (2008), inoculation substantially increased number of pods/plant and grain yield in chickpea. Seed inoculation with PSB and growth-promoting bacteria may have increased the supply of nitrogen and phosphorus to crop plants, resulting in more pods/plant. According to Tena *et al.* (2016), seed inoculation resulted in a rise in the amount of pods/plant.

100-grain weight

Data in Table 5 showed that 100 grain weight of chickpea as a feature of genotypes and rhizobium inoculation increased by treatments application. Different treatments had a considerable impact on the 100 grain weight. V1 × T_1 had a maximum 100 grain weight of 27.73g, compared to 22.89g in V1 x T_0. The weight of 100 grains was increased by 40% over the control. T_1 had the highest 100 grain weight of 27.73g, followed by V1 * T_3 with a 100 grain weight of 26.22 g, and control had the lowest 100 grain weight of 22.89g. PSB, nitrogen fixation bacteria, and PSB inoculation substantially improved pulse crop 100 grain weight and yield, according to Tena *et al.* (2016) which are in agreement with our findings. Other study (Aamir *et al.,* 2013) showed similar findings that a single and integrated inoculation improved 1000 grain weight and grain yields by 14 and 30%, respectively, compared to the unvaccinated controls. In addition, name-cutting, water-related content (RWC) and total dry (TDM) are improved in the event of a water intake/implant plant. Similarly, improved protein content (48%) and K × N ratio (95%) grains were detected in single and combined inoculation. Therefore, rhizobium and PGPR vaccination can be a sustainable way to improve plant growth. Better crop plant growth and development due to seed inoculation may have influenced nutrient supply, resulting in the production of more assimilates that partitioned more efficiently from source to sink and, as a result, increased seed weight. The findings indicate that using different rhizobium strains improves the efficiency of major nutrients, resulting in increased grain weight and yield.

Grain yield (kg/ha)

It was revealed that various treatments had a big impact on chickpea grain yield (Table 8). T_1 (V1) had the highest grain yield of 3732 kg/ha, while V2 (Bhakkar-2011) had the lowest at 3516 kg/ha. In terms of chickpea yield, V1 out performed V2 by 24% on average. It may be due to genotype yield potential differences. This increase in yield could be attributed to successful nodulation, which improved the exploitation of phosphorus and atmospheric nitrogen supply, resulting in higher yield. These results are consistent with those of Cordeiro and Echer (2019), who found that inoculation increased seed yield by 13%. Our findings are consistent with those of Fatima *et al.* (2008), who found that Rhizobium inoculum application increased growth, yield components, and nitrogen fixation in general. Endalkachew *et al.* (2018) found that using inoculum alone or in combination with fertilizer increased chickpea grain yield. The current study suggests that the use of rhizobium inoculum should be promoted in order to increase chickpea yield.

CONCLUSION

The application of N-fixing bacteria, PSB and a particular PGPR consortium improved chickpea grain yield and quality by having a positive impact on agronomic parameters such as plant height, nodules/plant, number of pods/plant, 100 grains weight, grain yield, and grain contents, as shown in the preceding accounts. For better soil improvement and chickpea crop production, the use of N-fixing bacteria, PSB, and the PGPR consortium is recommended. It is possible that grain consistency, which is a significant characteristic, can be improved by chickpea inoculation.

REFERENCES

Aamir, M., Aslam, A., Khan, M.Y., Jamshaid, M., Ahmad, M., Asghar, H., Zahir, Z. 2013. Co-inoculation with Rhizobium and plant growth promoting rhizobacteria (PGPR) for inducing salinity tolerance in mung bean under field condition of semi-arid climate. J. Asian J Agri Biol, 1(1):7-12.

Ahemed M., M.S. Khan, A. Zaidi and P.A. Wani. 2009. Remediation of herbicides contaminated soil using microbes M.S., Khan, A. Zaidi and J. Musarrat (Eds.), Microbes in Sustainable Agriculture, Nova Science Publishers, New York, USA.

Ahmed, 2012. Implications of bacterial resistance against heavy metals in bioremediation: a review. IIOABJ. 3:39-46.

Akhtar, M. and Z. Siddiqui. 2009. Effects of phosphate solubilizing microorganisms and Rhizobium sp. on the growth, nodulation, yield and root-rot disease complex of chickpea under field condition. J. African Journal of Biotechnology, 8(15):36-41.

Alishahi, F., H.A. Alikhani, N.A. Khoshkholgh-Sima, Etesami, H. 2020. Mining the roots of various species of the halophyte Suaeda for halotolerant nitrogen-fixing endophytic bacteria with the potential for promoting plant growth. Int Microbiol, 23(3):415-427.

Burbano, C.S., J.L. Gronemeyer, T. Hurek, B. Reinhold-Hurek. 2015. Microbial community structure and functional diversity of nitrogen-fixing bacteria associated with Colophospermum mopane. FEMS Microbiol Ecol, 91(4):177-185.

Billah, M., M. Khan, A. Bano, T.U. Hassan, A. Munir and A.R. Gurmani. 2019. Phosphorus and phosphate solubilizing bacteria: Keys for sustainable agriculture. Geomicrobiol. J. 36:904-916.

Chen, J., P.F. Wang, C. Wang, X. Wang and H. Gao. 2018. Effects of decabromodiphenyl ether and planting on the abundance and community composition of nitrogen-fixing bacteria and ammonia oxidizers in mangrove sediments: A laboratory microcosm study. Sci Total Environ, 616:1045-1055.

Cordeiro, C. and F.R. Echer. 2019. Interactive Effects of Nitrogen-Fixing Bacteria Inoculation and Nitrogen Fertilization on Soybean Yield in Unfavorable Edaphoclimatic Environments. Sci Rep.9(1): 15606-15613.

Cordeiro, C., F.R. Echer. 2020. Publisher Correction: Interactive Effects of Nitrogen-Fixing Bacteria Inoculation and Nitrogen Fertilization on Soybean Yield in Unfavorable Edaphoclimatic Environments. Sci Rep, 10(1):12115-12121.

Endalkachew, W., J.V. Heerwaardenb, B. Abdulkadira, S. Kassac, I. Aliyid, T. Degefue, K. Wakweyaf, F. Kanampiug and K. E. Giller. 2018. Additive yield response of chickpea (*Cicer arietinum* L.) to *rhizobium* inoculation and phosphorus fertilizer across smallholder farms in Ethiopia. Agri. Eco. Environ. 261:144-152.

Fageria, N. 2014. Yield and yield components and phosphorus use efficiency of lowland rice genotypes. J. Plant Nutr. 37: 979-989.

Fatima, Z., A. Bano, R. Sial, M.Aslam. 2008. Response of chickpea to plant growth regulators on nitrogen fixation and yield. Pak. J. Botany. 40:13-17.

Funga, A., O. Chris, O.C. Ojiewo, L. Turoop, S.G. Githiri. 2016. Symbiotic effectiveness of elite rhizobia strains nodulatingDesi type chickpea (*Cicer arietinum* L.) varieties. J. Plant Sci. 4 (4):88-94.

Giller, K.E., A.C. Franke, R. Abaidoo, F. Baijukya, A. Bala, S. Boahen, K. Dashiell, S. Kantengwa, J.M. Sanginga, N. Sanginga, A.J. Simmons, A. Turner, J. Wolf, P. Woomer and B.Vanlauwe. 2013. N2 Africa: Putting nitrogen fixation to work for smallholder farmers in Africa. In Agro-ecological Intensification of Agricultural Systems in the African Highlands, London: Routledge. p. 156-174.

Glick. 2012. Plant Growth-Promoting Bacteria: Mechanisms and Applications Hindawi Publishing

Corporation, Scientifica. India.

Kantar, F., B.G. Shivakumar, C. Arrese-Igor, F. Hafeez, E.M. Gonzalez, A. Imran and E. Larrainzar. 2010. Efficient biological nitrogen fixation under warming climates. In: Yadav, S. McNeil, L. David, R. Robert and A. Sharanagouda. (Eds.), Climate Change and Management of Cool Season Grain Legume Crops. Springer, New York. p. 283-306.

Kour, D., K.L. Rana, A.N. Yadav, N. Yadav, V. Kumar, A. Kumar and R. Z Sayyed. 2019. Drought tolerance phosphorus solubilizing microbes. In: Plant Growth Promoting Rhizobacteria for Sustainable Stress Management; Microorganisms for Sustainability; Springer: Berlin, Germany. p. 32-36.

Kumar, A. and A. Dubey, 2020. Rhizosphere microbiome: Engineering bacterial competitiveness for enhancing crop production. J. Adv. Res. 24: 337-352.

Maliha, R., K. Samina, A. Najma, A. Sadia and L. Farooq. 2004. Organic acids production and phosphate solubilization by phosphate solubilizing microorganisms under in vitro conditions. Pak. J. Biol. Sci. 7:187–196.

Nannipieri, P., L. Giagnoni, L. Landi and G. Renella, 2011. Role of phosphatase enzymes in soil. Springer: Heidelberg, Germany. p. 215-243.

Steel, R.G.D., J.H. Torrie and D. Dickey, 1997. Principles and Procedures of Statistics: A Biometrical Approach. 3rd Edition, McGraw Hill Book Co. Inc., New York.

Katz, A. 2010. Microsoft Excel. 2010. J. Style. 2:21-39.

Korir, H., N.W. Mungai, M.Thuita, Y. Hamba, C. Masso. 2017. Co-inoculation effect of rhizobia and plant growth promoting rhizobacteria on common bean growth in a low phosphorus soil. J Frontiers in Plant Science, 8:141-148.

Lipa, P. and M. Janczarek. 2020. Phosphorylation systems in symbiotic nitrogen-fixing bacteria and their role in bacterial adaptation to various environmental stresses. Peer J. 8:8466-8474.

Martins-da-Costa, E., Almeida Ribeiro, P.R., Soares de Carvalho, T., P. Vicentin, R. Balsanelli, F.M. Souza Moreira. 2020. Efficient Nitrogen-Fixing Bacteria Isolated from Soybean Nodules in the Semi-arid Region of Northeast Brazil are Classified as *Bradyrhizobium brasilense*. *Curr Microbiol*. 77(8):1746-1755.

Minitab, L. 2013. Minitab, State College, Centre County, Pennsylvania, United States. PA 16801. Data Universal Number System (DUNS) number. 107326043.

Oliveira, R.S., P. Carvalho, G. Marques, L. Ferreira, M. Nunes, I. Rocha, Y. Ma, M.F. Carvalho, M. Vosatka and H. Freitas. 2017. Increased protein content of chickpea (*Cicer arietinum* L.) inoculated with arbuscular mycorrhizal fungi and nitrogen-fixing bacteria under water deficit conditions. J Sci Food Agric, 97(13):4379-4385.

Tang, A., A.O. Haruna, N.M.A. Majid and M.B. Jalloh. 2020. Potential PGPR Properties of Cellulolytic, Nitrogen - Fixing, Phosphate - Solubilizing Bacteria in Rehabilitated Tropical Forest Soil. Microorganisms, 8(3):277-286.

Tena, W., E. Wolde and F. Walley. 2016. Response of chickpea (*Cicer arietinum* L.) to inoculation with native and exotic Mesorhizobium strains in Southern Ethiopia. Afric. J. Biotech. 15 (35):1920-1929.

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Sr. No.	Author's name	Contribution	Signature
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2.	Muhammad Anayat Ullah	Collected and analysed the data	
3.	Mudassar Khaliq	Helped in research work and write-up the manuscript	
4.	Muneer Abbas	Helped in data analysis	
5.	Zubeda Parveen	Helped in data tabulation	
6.	Muhammad Irshad	Proof read the manuscript