*Soil Environ. 40(2): 183-193, 2021*
*DOI:10.25252/SE/2021/202605*
*Online ISSN: 2075-1141*
*Print ISSN: 2074-9546*

# Rhizobial inoculation in soil improves growth, yield and economic returns of irrigated chickpea

***Hafiz Muhammad Azher Fareed[1], Amar Matloob[1*], Khurram Mubeen[1] and Muhammad Baqir Hussain***
[1]Department of Agronomy, MNS University of Agriculture, Multan
[2]Department of Soil and Environmental Sciences, MNS University of Agriculture, Multan
***[Received:** May 01, 2021 **Accepted:** November 24, 2021 **Published Online:** December 04, 2021**]***

## Abstract

*Effective nodulation and subsequent $N_2$-fixation in legumes depends on the quality of inoculum and application technique. Genotypic variation also exists among crop species regarding response to introduced bacterial strains. A field study was conducted to explore the efficacy of different rhizobial inoculants. Seeds of two chickpea varieties belonging to distinct groups, i.e., Desi (Bhakkar-2011) and Kabuli (Noor-2013) were inoculated with "$N_2$-biofertilizer" and "Rhizogold" while soil application of these inoculants was also tested. Effectiveness of inoculants and their application methods was assessed as improvement in growth and yield attributes of chickpea. Both seed and soil inoculation methods significantly improved the yield attributes of chickpea compared to uninoculated control. It was observed that soil inoculation of both inoculants was better than seed inoculation. Desi chickpea (Bhakkar-2011) seed inoculated with Rhizogold produced maximum dry matter (826.67 g $m^{-2}$). Rhizobial inoculation improved chickpea productivity by enhancing nodulation, dry matter, number of branches per plant and number of pods over uninoculated control. Inoculation with Rhizogold resulted in better utilization of resources that resulted in higher harvest index (48.64%) with soil application as compared to other treatments. Maximum net benefits (216438 PKR $ha^{-1}$ and 213825 PKR $ha^{-1}$) were associated with the soil application of Rhizogold in Kabuli and Desi chickpea, respectively. It is therefore concluded that soil inoculation with effective rhizobia should be carried out for successful chickpea production.*

**Keywords:** Irrigated chickpea, inoculation, net benefits, nodulation, rhizobia, seed yield

## Introduction

Chickpea covers 15% of the world's pulse production, and ranks third as major pulse crop after soybean and peas (FAO, 2016). It is also major winter food legume in Pakistan grown on 0.93 million ha, representing 76% of country's total pulses area with 74% share in total pulses production (Rani *et al*., 2014; PARC, 2018). Pakistan's share in global chickpea production is 10% (Muehlbauer and Sarker, 2017). Over years, area and production of chickpea in Pakistan is fluctuating with a declining trend observed especially for production and average yield (AMIS, 2018). During 2020-21, the production of chickpea witnessed a decrease of 47% compared to 2019-20 (Government of Pakistan, 2021). Since independence following partition of subcontinent in 1947, country's area under chickpea has increased by 10% till date. However, the average yield has declined from 0.54 t $ha^{-1}$ to 0.34 t $ha^{-1}$ during the same period (AMIS, 2018) owing to the fact that chickpea is grown on marginal lands under rainfed conditions with minimal inputs. Average chickpea productivity in Pakistan is lower than Australia, India, Syria, Mexico and Turkey (Muehlbauer and Sarker, 2017). Even under South Asian context, productivity of chickpea in Pakistan was lower by 36, 40 and 62% as compared to Bangladesh, India and Myanmar (FAO, 2015). Over years, the gap between demand and supply of chickpea has widened and Pakistan is the 4th largest importer of pulses (FAO, 2016). During 2016-17 cropping season, Pakistan spent 465 million AU$ on the import of 0.4 million tons of Australian chickpea (Anonymous, 2018). Such import bill can be cut short by increasing domestic production of chickpea and for this geographic range of chickpea needs to be extended beyond its traditional belt where it is grown on marginal lands by resource poor farmers with minimal inputs. Chickpea introduction in irrigated cereal-based cropping systems will help to break disease and weed cycle, improve soil health and system productivity. From 1991 to

***Email:** amar.matloob@mnsuam.edu.pk
**Cite This Paper:** Fareed, H.M.A., A. Matloob, K. Mubeen, M.B. Hussain. 2021. Rhizobial inoculation in soil improves growth, yield and economic returns of irrigated chickpea. *Soil Environ*. 40(2): 183-193, 2021.

2016, numerous high yielding varieties of chickpea viz. Noor-91, CM-98, Punjab-2000, Punjab-2008, Bittal-2016, NIAB CH-2016 have been introduced. Nevertheless, yield potential of these varieties has seldom been realized (Government of Pakistan, 2018). Such a yield gap of chickpea is due to improper agro-technology used by the farmers. It seems that existing yield gap can be reduced by adopting advanced production technology package including the high yielding varieties, weed management, balanced nutrition and use of inoculum.

Nitrogen (N) is a critical input and studies indicate that chickpea is able to meet 60-80% of its N requirement (corresponding to 60-176 kg $ha^{-1}$) via biological $N_2$-fixation (Giller, 2001; Shiferaw *et al*., 2004; SPG, 2016). The lack of compatible rhizobial species in the soil or their low native population often limits nodulation and subsequent $N_2$-fixation in chickpea (Kantar *et al*., 2010; Wolde-meskel *et al*., 2018). Inoculation with effective rhizobial strains is recommended at the time of sowing especially if the native population of bacteria is less than 50 cells $g^{-1}$ soil (Thies *et al*., 1991 a & b). Positive effects of rhizobial inoculation on chickpea productivity are exhibited as increase in biomass, growth and seed yield (Bhuiyan *et al*., 2008; Khaitov *et al*., 2016; Tena *et al*., 2016; Wolde-meskel *et al*., 2018). Recently, Wolde-meskel *et al*. (2018) reported a 21% increase in seed yield of chickpea owing to inoculation in Ethiopia. However, the outcomes of $N_2$-fixation are difficult to generalize since there is immense variation regarding inoculation and nodulation responses owing to prevailing agro-climatic and ecological conditions (Heerwaarden *et al*., 2018). Aspects such as crop genotypes, sources and quality of inoculum, and their application technique in conjunction with contrasting soil physico-chemical properties and management factors could modulate rhizobial inoculation and overall $N_2$-fixation (Kantar *et al*., 2007; Tena *et al*., 2016). Rhizobial strains also differ significantly regarding their stress tolerance and nodulation ability in chickpea (Aynalem *et al*., 2018).

It has been suggested that chickpea yield can be significantly improved by growing high yielding chickpea genotypes in conjunction with effective seed inoculation (Bhuiyan *et al*., 2008). Pakistani soils are generally deficit in N and it is suggested that inoculation is an essential agronomic input to fulfill N demand especially when legumes are either not grown or being grown for first time. The N fixation can be greatly enhanced by appropriate inoculation treatment (Gul *et al*., 2014). The previous work assessing the chickpea performance in terms of growth and yield to rhizobial inoculation is either restricted to greenhouse studies or studies undertaken under rain-fed conditions. Information regarding application of rhizobial inoculum to Desi and Kabuli chickpea grown under irrigated conditions of Pakistan is not readily available. This study was therefore, designed to compare inoculating methods and to evaluate genotypic variations for growth and yield of chickpea in response to rhizobial inoculation.

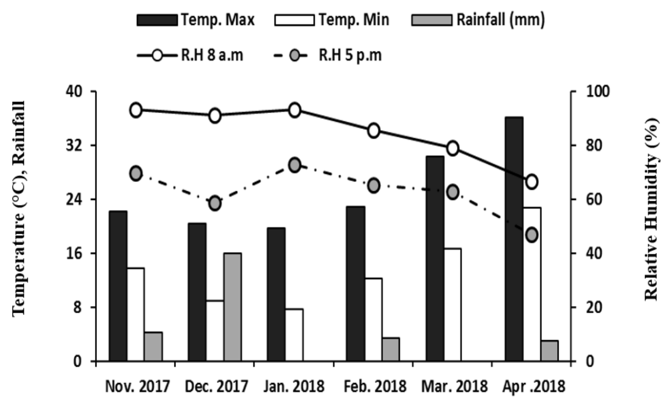


**Figure 1: Agro-metrological data during the course of present study (Source: Automated Weather Station at MNS-University of Agriculture, Multan)**

## Materials and Methods

The proposed study was undertaken at the Research Farm of MNS-University of Agriculture Multan (Latitude 30.16° N, Longitude 71.45° E, altitude 122 m) during Rabi 2017-18. Agro-metrological data comprising mean minimum and maximum air temperature (°C), relative humidity (%) and rainfall (mm) were recorded by Automated Weather Station (AWS) established at MNS-University of Agriculture Multan (Figure 1). Samples of experimental soil were undertaken prior to sowing and immediately after harvest by collecting composite samples from soil depth of 0-15, and 15-30 cm, respectively. The soil samples were analyzed for various parameters (Table 1).

**Table 1: Characteristics of experimental site**

| | | **Pre-Sowing** | | **Post-Harvest** | |
|---|---|---|---|---|---|
| | | **Sampling depth (cm)** | | | |
| **Characteristic** | **Unit** | **0-15** | **15-30** | **0-15** | **15-30** |
| Textural class | - | Loam | Loam | Loam | Loam |
| ECe | dS $m^{-1}$ | 11.3 | 12.8 | 2.34 | 2.48 |
| pH | - | 8.0 | 8.0 | 8.0 | 8.0 |
| OM | % | 0.59 | 0.59 | 0.76 | 0.74 |
| Available Phosphorus | mg $kg^{-1}$ | 7.2 | 7.2 | 8.80 | 8.80 |
| Available Potassium | mg $kg^{-1}$ | 210 | 210 | 230 | 230 |
| Saturation | % | 36 | 36 | 36 | 38 |

Seeds of two chickpea varieties, i.e. Desi (Bhakkar-2011) and Kabuli (Noor-2013) were inoculated with "$N_2$-biofertilizer" from Ayub Agricultural Research Institute, Faisalabad, and "Rhizogold"-a patent biofertilizer of University of Agriculture, Faisalabad. For rhizobial inoculation, two-step method was used and firstly, 10% (w/v) sugar solution was used as a sticking agent and then rhizobial inoculant (30 g per kg seed) was adhered to the chickpea seeds to achieve uniform coating. Inoculation was done under shade and the inoculated seed was air-dried before planting (Wolde-meskel *et al.*, 2018). Soil application of these inoculants was also done by mixing respective inoculum (2.5 kg $ha^{-1}$) with field soil. Seed was sown at the rate of 75 kg $ha^{-1}$. Seeds of chickpea were sown in rows at 40 cm distance using manual drill on November 23, 2017. To avoid risk of cross-contamination, uninoculated control plots (seeds treated with 2 g thiophenate methyl $kg^{-1}$ only) were sown first. The seeds were treated first with fungicide and then inoculation was done (Gaur *et al*., 2010). A basal fertilizer dose of 58 kg $P_2O_5$ and 23 kg N $ha^{-1}$ was used as diammonium phosphate (46% $P_2O_5$ and 18% N). Pre-soaking irrigation (10 cm), and rainfall (26.60 mm) received during the growing season were the only source of water available for crop to grow till maturity. Crop did not show any symptom of water stress, hence, no subsequent irrigation was given. Weeds were removed manually when essential, especially after rainfall to avoid subsequent competitive damage by weeds. Being located in the traditional cotton belt, insect damage especially by pod borer was expected in the experimental plots; therefore, to safeguard crop against pod borer, lufenuron at 375 g $ha^{-1}$ was used at the onset of pod formation. However, diseases like wilt were not observed in the experimental plots since seeds were treated with fungicide. All the other agronomic practices were used as normal and uniform for all the treatments.

The experiment was replicated thrice as per RCBD under factorial arrangements. The net plot size was 1.6 m × 7.6 m. Data about agronomic and yield attributes of chickpea were recorded using standard procedures. Harvest index was calculated as ratio of economic yield to biological yield and was expressed as %. For dry matter and crop growth rate, destructive sampling (1.0 m crop row was harvested leaving appropriate borders) was done at 20 days interval starting from 45 days after sowing (DAS). Crop growth rate (CGR) expressed as increment in dry matter per unit area per unit time over two fixed time intervals was calculated as per Hunt (1979). Data were analyzed through ANOVA technique and differences among the treatments were tested ($p \leq 0.05$) using HSD Tukey's test (Steel *et al*., 1997). Economic returns, and benefit cost ratios were worked out to ascertain the comparative profit of various treatments, marginal analyses were carried out as per CIMMYT (1988).

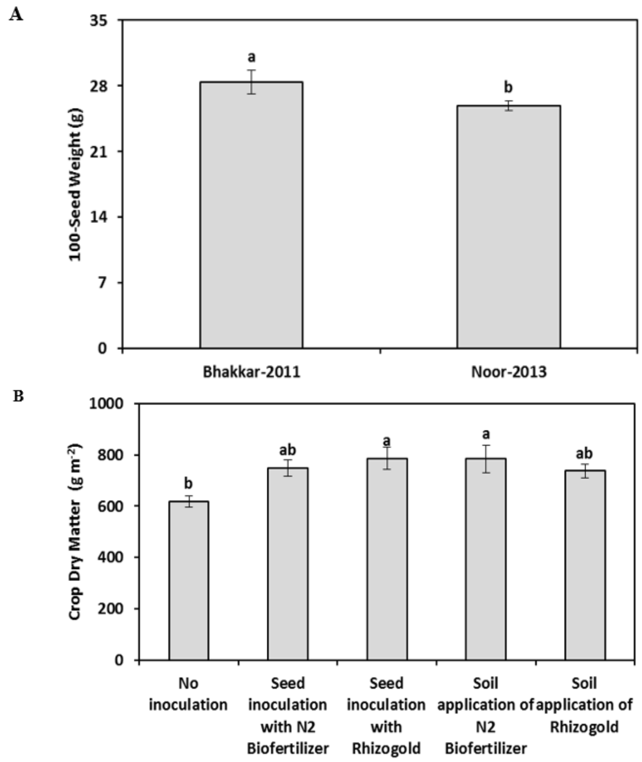


**Figure 2: Influence of chickpea varieties on (A) 100-seed weight (B) Influence of rhizobial inoculation treatments on crop dry matter accumulation. Capped bars show standard errors of three replicates**

## Results

### Agronomic and yield attributes

Plant height was significantly influenced by inoculation treatments (Table 2). Maximum height of chickpea plants was recorded (57.90 and 57.57 cm) in plots where soil application of both inocula was carried out. Nevertheless, these plants were statistically at par (55.52 and 55.50 cm) with plants growing in plots where seed inoculation was used. However, chickpea height observed under soil application of rhizobial inoculation was 9% higher as compared to uninoculated plots. Maximum branches $plant^{-1}$ (5.27) were recorded in plots where seed inoculation was carried out, nevertheless, these plants were statistically at par (5.08) with plants growing in plots where no inoculation was done (Table 2). However, chickpea branches $plant^{-1}$ observed under soil applications of both rhizobial inoculations were significantly lower (4.42 and 4.85) as compared to the plants growing in non-inoculated plots. Maximum number of pods plant$^{-1}$ of chickpea (16.52 and 16.02) were recorded in plots where soil application of both inocula was carried out, nevertheless, these plants were statistically at par with 14.75 and 14.42 pods plant$^{-1}$ found in plots where seed inoculation was used (Table 2). However, pods per plant produced under soil application of rhizobial inoculation were significantly higher as compared to the plants growing in uninoculated plots (14.12). Seeds per pod were not affected by the main effect of tested factors and their possible interaction. Maximum number of nodules (13.47) of chickpea plants was recorded in plot where soil application of Rhizogold was carried out. Nevertheless, these plants were statistically at par with those growing in other inoculated plots i.e. seed application of $N_2$-Biofertilizer, seed application of Rhizogold and soil application of Rhizogold having values 20.43, 21.37 and 21.07 nodules plant$^{-1}$, respectively (Table 2). However, number of nodules plants$^{-1}$ observed under soil application of rhizobial inoculation was significantly higher as compared to the plants growing in uninoculated plots. Maximum 100-seed weight was recorded in plots of Desi chickpea compared to Kabuli type (Figure 2A).

The interaction between chickpea varieties and rhizobial inoculation treatments was significant for biological yield, while the main effect of chickpea varieties as well as that of rhizobial inoculation treatments were non-significant (Table 2). Under uninoculated conditions, both chickpea varieties recorded similar biological yields. Seed inoculation with $N_2$-biofertilizer enhanced yield over control plots. However, such an increase was similar for both chickpea varieties. Seed inoculation with either of the inoculants improved biological yield to a similar extent in plots of Desi chickpea. However, seed inoculation with Rhizogold was more effective in improving yield of Kabuli type than $N_2$-biofertilizer. Highest biological yield (4.95 t ha$^{-1}$) was recorded for plots of Desi chickpea (Bhakkar-2011) under soil application of Rhizolgold. Although effective in improving the biological yield significantly over uninoculated control, the soil application of both inoculants resulted in similar biological yield regardless of chickpea variety. For seed yield of chickpea, the interactive effect of chickpea varieties with inoculation treatment was significant ($p \leq 0.0.5$). Under uninoculated conditions, both chickpea varieties recorded similar seed yield. However significant yield increments were observed under various inoculation treatments. In plots of Desi chickpea, inoculation resulted in 77-117% yield advantage as compared to the uninoculated plots. Likewise, inoculation resulted in 84-139% yield increase in case of Kabuli chickpea. Seed treatment of both inocula recorded similar yield in plots of both chickpea varieties. Nevertheless, the soil application of Rhizogold was superior as compared to $N_2$-biofertilizer and resulted in 17 and 10% yield increment as compared to the seed inoculated plots of Desi and Kabuli, respectively. For harvest index, the interaction of chickpea varieties and rhizobial inoculation was significant. Maximum harvest index was recorded for plots of Kabuli chickpea under soil application of Rhizogold (50.81%) that was 60% higher compared to control. Seed inoculation of both inoculants resulted in similar harvest index in plots of both chickpea varieties. Soil application of $N_2$-biofertilizer improved harvest index in plots of Kabuli type to a much greater extent than same treatment applied to plots of Desi type. However, lowest harvest index (31.61%) was observed for uninoculated plots of Kabuli chickpea (Table 2).

## Chickpea growth

Inoculation treatments had a significant effect on the dry matter of chickpea (Figure 2B). All the inoculation treatments were at par with each other regarding dry matter accumulation by chickpea. However, dry matter recorded in response to seed inoculation with Rhizogold and soil application of $N_2$-biofertilizer was significantly different from that recorded for uninoculated plots.

Pattern for dry matter accumulation of Desi chickpea (Bhakkar-2011) are shown in Figure 3A. Dry matter exhibited a temporal rise and then curve became stable towards maturity. The highest increment was recorded between 65 and 85 DAS in plots where seed inoculation with Rhizogold was done. At 105 DAS, least dry matter was recorded for control plots. Dry matter of Kabuli chickpea (Noor-2013) manifested a slight increase between 45 to 65 DAS (Figure 3B). Afterwards, a sharp increase was noticed till 105 DAS and dry matter curve leveled off thereafter. The highest dry matter was recorded at 105 and 125 DAS in plots where soil application of Rhizogold was made. Soil application of the $N_2$-biofertilizer was the next effective treatment in this regard. Comparative dry matter accumulation of Desi and Kabuli chickpea is shown in Figure 3C. The dry matter accumulated by two varieties was almost similar at all sampling intervals. At last sampling, numerically higher dry matter was recorded for Kabuli chickpea.

A significant interaction between tested factors was observed for the seasonal CGR ($p \leq 0.0.5$). Under uninoculated conditions, the CGR of Kabuli chickpea (12.11 g m$^{-2}$ d$^{-1}$) was significantly higher (50%) as compared to CGR (8.06 g m$^{-2}$ d$^{-1}$) of the Desi chickpea. Irrespective of chickpea variety, seed application of both the inocula resulted in similar CGR. Soil application of the used inocula resulted in similar CGR in case of Kabuli chickpea. However, the soil application of $N_2$-biofertilizer to plots of Desi chickpea was superior as compared to the soil application of Rhizogold regarding seasonal CGR. The former treatment had 33% more CGR as compared to the later one (Table 2).

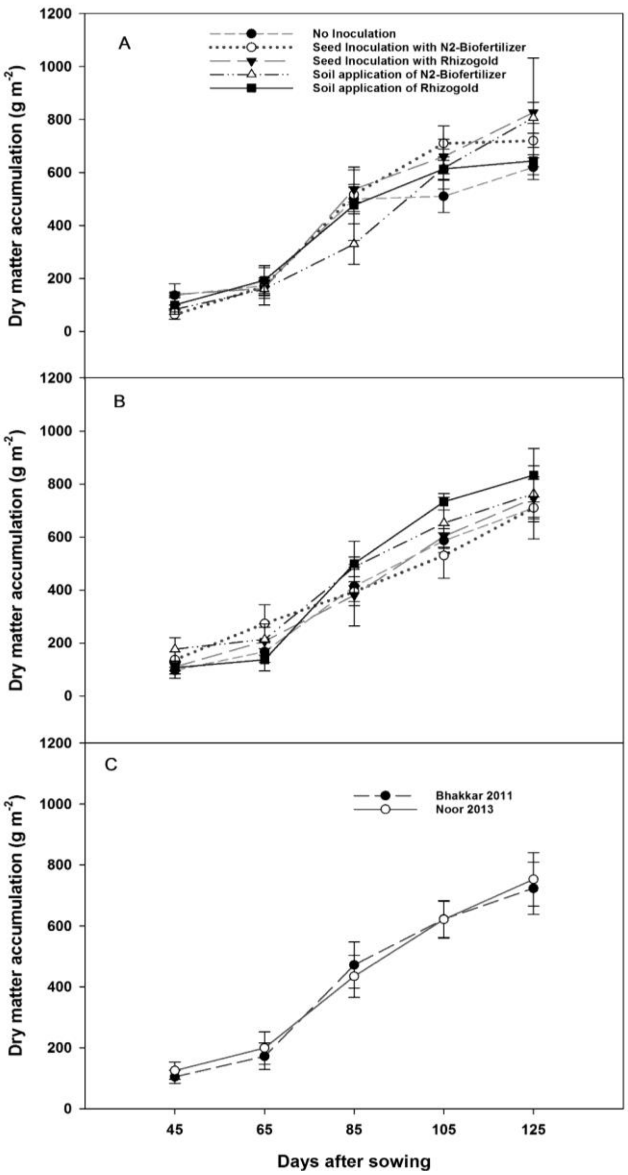


**Figure 3: Influence of rhizobial inoculation treatments on patterns of dry matter accumulation by (A) Bhakkar-2011, (B) Noor-2013, (C) Comparative dry matter accumulation by Bhakkar-2011 and Noor-2013. Capped bars show standard errors of three replicates**

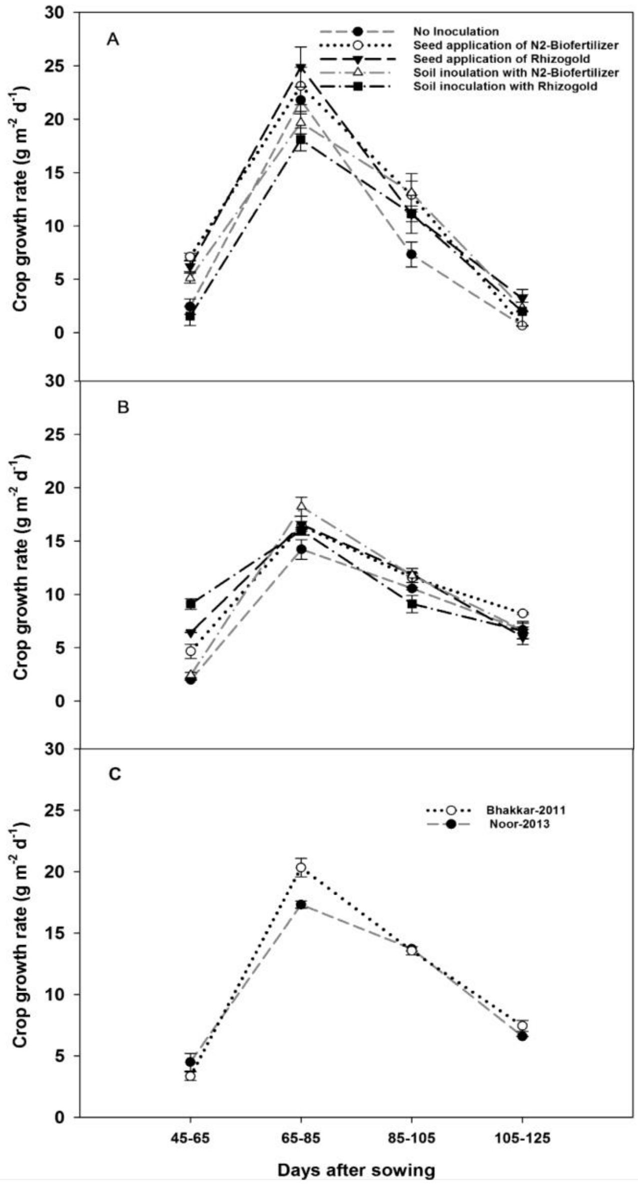


**Figure 4: Influence of rhizobial inoculation treatments on patterns of crop growth rate of (A) Bhakkar-2011, (B) Noor-2013, (C) Comparative crop growth rate of Bhakkar-2011 and Noor-2013.Capped bars show standard errors of three replicates**

Regarding pattern for CGR of Desi chickpea (Bhakkar-2011; Figure 4A), the highest CGR was recorded between 65-85 DAS in plots where seed application of Rhizogold was used. This treatment was followed by seed inoculation with $N_2$-biofertilizer. Between 85-105 DAS, unincoulated plots had the lowest CGR. For CGR of Kabuli chickpea (Noor-2013) (Figure 4B), the highest CGR was recorded

**Table 2: Influence of rhizobial inoculation treatments on agronomic and yield parameters of chickpea**

| Rhizobial Inoculation | Plant height (cm) | Number of branches | Number of pods | Number of nodules | Biological yield (t ha$^{-1}$) | | Seed yield (t ha$^{-1}$) | | Harvest index (%) | | Seasonal crop growth rate (g m$^{-2}$ d$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Desi | Kabuli | Desi | Kabuli | Desi | Kabuli | Desi | Kabuli |
| No inoculation | 53.05 B | 4.98 B | 14.12 B | 5.38 B | 2.80e | 2.99e | 1.05 e | 0.94 e | 36.97 de | 31.61 e | 8.06 c | 12.11 a |
| Seed inoculation with $N_2$Biofertilizer | 55.50 AB | 5.68 A | 14.75 B | 20.43 A | 3.75d | 3.76d | 1.86 bcd | 1.73 d | 49.79 ab | 46.01 abc | 10.94 ab | 10.22 abc |
| Seed inoculation with Rhizogold | 55.52 AB | 5.55 AB | 14.40 B | 21.37 A | 3.95cd | 4.44abc | 1.96 bc | 1.83 cd | 49.89 ab | 41.34 bcd | 11.44 ab | 10.61 abc |
| Soil application of $N_2$Biofertilizer | 57.90 A | 5.82 A | 16.02 A | 21.07 A | 4.85ab | 4.21bcd | 1.95 bc | 2.04 b | 39.93 cde | 48.95 ab | 12.06 a | 9.78 abc |
| Soil application of Rhizogold | 57.57 A | 5.80 A | 16.52 A | 23.47 A | 4.95a | 4.43abc | 2.28 a | 2.24 a | 46.47 abc | 50.81 a | 9.06 bc | 9.56 abc |
| **HSD *$p \leq 0.05$*** | 4.43 | 0.63 | 1.04 | 4.55 | 0.64 | | 0.186 | | 8.71 | | | |

Means not sharing a letter in common differ significantly at 5% probability by Tukey's HSD test

**Table 3: Economic analyses of seed and soil applications of inoculation for Desi (Bhakkar-2011) chickpea genotype**

| Parameter | $I_1$ | $I_2$ | $I_3$ | $I_4$ | $I_5$ | Remark |
|---|---|---|---|---|---|---|
| Chickpea seed yield | 1.05<br>1046.7 | 1.86<br>1858.3 | 1.96<br>1961.1 | 1.95<br>1950 | 2.28<br>2275 | t ha$^{-1}$<br>kg ha$^{-1}$ |
| 10% loss | 104.67 | 185.83 | 196.11 | 195 | 227.5 | To bring at farmer level |
| Adjusted seed yield | 942 | 1672.5 | 1765 | 1755 | 2047.5 | 10% discount |
| Income from seed yield | 94200 | 167250 | 176500 | 175500 | 204750 | PKR ha$^{-1}$<br>(PKR 100 per kg) |
| Straw yield | 1.75<br>1753.33 | 1.89<br>1891.67 | 1.99<br>1988.89 | 2.90<br>2900.00 | 2.68<br>2675.00 | t ha$^{-1}$<br>kg ha$^{-1}$ |
| 10% straw loss | 175.33 | 189.17 | 198.89 | 290.00 | 267.50 | To bring at farmer level |
| Adjusted straw yield | 1578.00 | 1702.50 | 1790.00 | 2610.00 | 2407.50 | 10% discount |
| Income from straw yield | 7890 | 8512.5 | 8950 | 13050 | 12038 | PKR ha$^{-1}$<br>PKR 5 per kg |
| Gross income | 102090 | 175763 | 185450 | 188550 | 216788 | PKR ha$^{-1}$ |
| Inoculum | 0 | 150 | 150 | 350 | 350 | 75 PKR per pack |
| Cost varied | 0 | 150 | 150 | 350 | 350 | PKR ha$^{-1}$ |
| Net benefits | 102090 | 175613 | 185300 | 188200 | 216438 | PKR ha$^{-1}$ |

$I_1$ = No inoculation; $I_2$ = Seed application of $N_2$-Biofertilizer; $I_3$ = Soil application of $N_2$-Biofertilizer; $I_4$ = Seed application of Rhizogold; $I_5$ = Soil application of Rhizogold

during 65-85 DAS in plots where soil application of $N_2$-biofertilizer was done. Control plots had the lowest CGR at these time intervals. At last sampling, plots where $N_2$-biofertilizer was used for seed inoculation had the highest CGR. Comparative periodic CGR of Desi (Bhakkar-2011) and Kabuli (Noor-2013) chickpea varieties are shown in the Figure 4C. The growth rate of two varieties was alike between 45-60 DAS and 75-90 DAS. However, highest CGR was recorded between 65-85 DAS in plots of Kabuli chickpea (Noor-2013). Rest of the time, the CGR did not vary between the two chickpea varieties. However, the initial vegetative crop growth (45-60 DAS) was accompanied with minimum CGR than rest of the sampling intervals.

## Economic analyses

In case of economic analysis, our study exhibited that inoculated treatments brought up a net benefit over uninoculated plots. Economic analyses showed that highest net benefit of 216438 PKR ha$^{-1}$ was obtained from plots of Desi chickpea (Bhakkar-2011) where soil application of Rhizogold was done (Table 3). Analysis also depicted that second highest net benefit of 211825 PKR ha$^{-1}$ was achieved from plots of Kabuli chickpea (Noor-2013) under same inoculation treatment (Table 4).

Higher benefit to cost ratio was recorded for inoculated plots in comparison to non-inoculated plots. For Desi chickpea (Bhakkar-2011), maximum benefit cost ratio (2.846) was recorded in plot where Rhizogold was soil applied (Table 5). On the other hand, reduction in benefit-cost ratio (1.347) was recorded in uninoculated plots. In case of Kabuli chickpea (Noor-2013), maximum benefit cost ratio (2.812) was obtained where seed application of Rhizogold was used. The lowest benefit cost ratio (1.242) was observed in uninoculated plots. Highest marginal rate of return (MRR) was associated with seed inoculation with $N_2$-biofertilizer in both chickpea varieties (Table 6). The next higher MRR was recorded for soil application of same inoculum in plots of both chickpea varieties.

**Table 4: Economic analyses of seed and soil applications of inoculation for Kabuli (Noor-2013) chickpea genotype**

| Parameter | $I_1$ | $I_2$ | $I_3$ | $I_4$ | $I_5$ | Remark |
|---|---|---|---|---|---|---|
| Chickpea seed yield | 0.94 | 1.73 | 1.83 | 2.04 | 2.24 | t ha$^{-1}$ |
| | 944.44 | 1725 | 1830.6 | 2044.44 | 2244.4 | kg ha$^{-1}$ |
| 10% loss | 94.444 | 172.5 | 183.06 | 204.444 | 224.44 | To bring at farmer level |
| Adjusted seed yield | 850 | 1552.5 | 1647.5 | 1840 | 2020 | 10% discount |
| Income from seed yield | 85000 | 155250 | 164750 | 184000 | 202000 | PKR 100 per kg |
| Straw yield | 2.04 | 2.03 | 2.61 | 2.16 | 2.71 | t ha$^{-1}$ |
| | 2042.22 | 2031.00 | 2609.44 | 2163.56 | 2705.56 | kg ha$^{-1}$ |
| 10% straw loss | 204.22 | 203.10 | 260.94 | 216.36 | 270.56 | To bring at farmer level |
| Adjusted straw yield | 1838.00 | 1827.90 | 2348.50 | 1947.20 | 2435.00 | 10% discount |
| Income from straw yield | 9190 | 9139.5 | 11743 | 9736 | 12175 | PKR 5 per kg |
| Gross income | 94190 | 164390 | 176493 | 193736 | 214175 | PKR ha$^{-1}$ |
| Inoculum | 0 | 150 | 150 | 350 | 350 | 75 PKR per pack |
| Cost varied | 0 | 150 | 150 | 350 | 350 | PKR ha$^{-1}$ |
| Net benefit | 94190 | 164240 | 176343 | 193386 | 213825 | PKR ha$^{-1}$ |

$I_1$ = No inoculation; $I_2$ = Seed application of $N_2$-Biofertilizer; $I_3$ = Soil application of $N_2$-Biofertilizer; $I_4$ = Seed application of Rhizogold; $I_5$ = Soil application of Rhizogold

**Table 5: Influence of rhizobial inoculation treatments on economic benefits and benefit cost ratio of chickpea genotypes**

| Parameter | Variable cost | Fixed cost | Total cost | Gross income | Net benefit | Net return | BCR |
|---|---|---|---|---|---|---|---|
| | | | | (PKR ha$^{-1}$) | | | |
| **Treatment** | | | | **Bhakkar-2011** | | | |
| No inoculation | 0 | 75815 | 75815 | 102090 | 102090 | 26275 | 1.347 |
| Seed inoculation with $N_2$-Biofertilizer | 150 | 75815 | 75965 | 175762.5 | 175612.5 | 99797.5 | 2.314 |
| Seed inoculation with Rhizogold | 150 | 75815 | 75965 | 185450 | 185300 | 109485 | 2.441 |
| Soil application of $N_2$-Biofertilizer | 350 | 75815 | 76165 | 188550 | 188200 | 112385 | 2.476 |
| Soil application of Rhizogold | 350 | 75815 | 76165 | 216787.5 | 216437.5 | 140622.5 | 2.846 |
| | | | | **Noor-2013** | | | |
| No inoculation | 0 | 75815 | 75815 | 94190 | 94190 | 18375 | 1.242 |
| Seed inoculation with $N_2$-Biofertilizer | 150 | 75815 | 75965 | 164389.5 | 164239.5 | 88424.5 | 2.164 |
| Seed inoculation with Rhizogold | 150 | 75815 | 75965 | 176492.5 | 176342.5 | 100527.5 | 2.323 |
| Soil application of $N_2$-Biofertilizer | 350 | 75815 | 76165 | 193736 | 193386 | 117571 | 2.544 |
| Soil application of Rhizogold | 350 | 75815 | 76165 | 214175 | 213825 | 138010 | 2.812 |

BCR = Benefit Cost Ratio

## Discussion

The plant height was maximum under inoculation of soil applied $N_2$-biofertilizer that was significantly higher than uninoculated plots. This increase was probably due to greater CGR and better nodulation (Meena *et al.*, 2013; Kaur *et al.*, 2015). Soil application of $N_2$-biofertilizer was superior to uninoculated plots regarding plant height (Sharar *et al.*, 2000; Namwar *et al.*, 2011). The improvements recorded for the number of branches plant$^{-1}$ may be due to microbial mediated production of phytohormones which can induce morpho-physiological changes in roots, thus can increase nutrient uptake from soil (Sharma *et al.,* 2013). Effective nodulation and hence concurrent increase in $N_2$-fixation might explain the observed difference among the treatments.

2015). Rhizobial strains differ significantly regarding their stress tolerance and nodulation ability in chickpea (Aynalem *et al*., 2018). The nodule number varied from 9 to 22 nodules per plant; while, plant shoot dry biomass ranged from 1.18 to 1.84 g plant$^{-1}$. Contrarily, in present study, the interactive effect between chickpea varieties and rhizobial inoculation was non-significant. The differences in 100-seed weight observed between tested chickpea varieties could be explained in part due to morpho-physiological divergence present between the tested varieties and their differential dry matter accumulation and its partitioning and variable CGR. El Hadi and Elsheikh (1999) found genotypic variation regarding response to the applied inoculum, and cultivar Gabel Marra recorded the highest percentage of protein due to N fixation compared to the other cultivars. The improvement in biological yield with rhizobial inoculation was presumably because of improved plant height, number of branches, dry matter accumulation, nodulation and yield attributes. Greater CGR in inoculated plots due to better nodulation and hence $N_2$-fixation was conducive to increased dry matter which was ultimately reflected as higher biological yield. The positive effects of N include increase in vegetative growth, canopy size and interception of light and hence photosynthetic gains (Namvar *et al*., 2011, 2013). These findings are in close proximity with those of Dutta and Bandyopadhyay (2009) in chickpea. These reports are in agreement with those of Namwar *et al.* (2011) and Singh *et al.* (2015) who stated that *Rhizobium* inoculation has a positive effect on the pods per plant, 100-seeds weight and hence the seed yield of chickpea. The increment in seed yield due to rhizobial inoculation was due to

**Table 6: Dominance and marginal analyses for Desi (Bhakkar-2011) and Kabuli (Noor-2013) chickpea genotype subjected to different inoculation materials and application methods**

| Parameter | Variable cost | Net benefit | Marginal cost | Marginal net benefit | MRR (%) |
|---|---|---|---|---|---|
| | | | **PKR ha$^{-1}$** | | |
| **Treatment** | | | Bhakkar-2011 | | |
| No inoculation | 0 | 100090 | - | - | - |
| Seed inoculation with $N_2$-Biofertilizer | 150 | 173612.5 | 150 | 73522.5 | 49015.0 |
| Seed inoculation with Rhizogold | 150 | 183300 | 0 | 9687.5 | D |
| Soil application of $N_2$-Biofertilizer | 350 | 186200 | 200 | 2900 | 1450.0 |
| Soil application of Rhizogold | 350 | 214437.5 | 0 | 28237.5 | D |
| | | | **Noor-2013** | | |
| No inoculation | 0 | 92190 | - | - | - |
| Seed inoculation with $N_2$-Biofertilizer | 150 | 162239.5 | 150 | 70049.5 | 46699.7 |
| Seed inoculation with Rhizogold | 150 | 174342.5 | 0 | 12103 | D |
| Soil application of $N_2$-Biofertilizer | 350 | 191386 | 200 | 17043.5 | 8521.8 |
| Soil application of Rhizogold | 350 | 211825 | 0 | 20439 | D |

In our study, number of nodules were significantly higher under various rhizobial inoculation treatments. These results are in corroboration with those of Das *et al.* (2013) who reported overall improvement in the crop growth under the influence of microbial inoculation and attributed the same to impact on nutritional environment and involvement in various physiological processes in the plant system. The less number of branches realized under soil application could be due to less $N_2$-fixation owing to poor survival or suboptimal rhizobial population under such treatments. The enhancement in pods plant$^{-1}$ with rhizobial inoculations may be attributed to better root development, nodulation and greater nutrient acquisition resulting in vigorous plant growth which in turn resulted in better flowering and partitioning of photo-assimilates during seed formation (Dutta and Bandyopadhyay, 2009; Singh *et al.,* 2010; Singh *et al*., 2015). More nodules plant$^{-1}$ can be ascribed to production of phytohormones, suppression of pathogens or better nutrient acquisition that may contribute to promote nodulation (Kaur *et al.,* 2015). The corroborated results have also shown an increase in number of nodules due to rhizobial application (Erman *et al*., 2011. Such increments in nodules number with biofertilizers has also been reported elsewhere (Malik and Sindhu, 2011; Chandra and Pareek, promotion of number of branches and pods plant$^{-1}$ due to better plant growth. Moreover, such an increase could also be explained in terms of better crop dry matter accumulation and growth rates. Bhattacharjya and Chandra (2013) explained the positive effect of seed inoculation on growth and yield attributes over that of uninoculated control which was attributed to dissolution and mobilization of fixed P in soil to the crop plants by *Pseudomonas diminuta* and its synergistic effect with increased nodulation. Inoculation related improvement in growth, biomass and yield of chickpea have been reported (Bhuiyan *et al*., 2008; Khaitov *et al*., 2016). Bhuiyan *et al*. (2008) recorded a seed yield of 1.29 t ha$^{-1}$ in plots sown with chickpea seed inoculated with rhizobial strain RCa-220 that was 15% higher over untreated control plots.

Togay *et al*. (2008) reported that inoculation with *Rhizobium* significantly increased the CGR and dry matter in chickpea. Malik *et al*. (2006) found that seed inoculation with *Rhizobium* significantly increased the plant total dry matter, seed yield and harvest index. These results corroborate the observations of Kaur *et al.* (2014) and Zaidi *et al.* (2003) who also reported that the inoculation with *Rhizobium* spp. resulted in significantly higher dry matter as compared to uninoculated plots. The rapid increase in total dry matter at the later stages of growth was due to the development of a considerable amount of leaf area compared to early stages (Yasari and Patwardhan, 2006).

Application of granular inoculant to the soil just below the seed improved the yield of Kabuli chickpea by 36 and 14% as compared to seed inoculation with liquid or peat based powder, respectively (Boahen *et al*., 2002). They also reported that relative benefits of seed inoculation depend on placement depth of the inoculum which is related to method of application. Inoculum applied to the seed or granular inoculum applied at seeding depth caused nodulation in the crown region. Greater profitability as evident from higher net returns, benefit cost ratios and MRR of applied rhizobial treatments relative to non-inoculated plots provided insights in to enhanced yields and associated monetary benefits suggesting the cost-effectiveness. These reports are parallel with those of Jain *et al.* (2006) who also obtained higher gross returns with inoculation of *Rhizobium* as compared to non-inoculated plots.

## Conclusion

Application of rhizobial inoculum to chickpea varieties significantly improved the growth and yield of chickpea. The best yield performance of both chickpea varieties was observed in plots where soil application of both inoculums was used. It is therefore, concluded that soil inoculation with effective rhizobia must be carried out for profitable chickpea production in irrigated conditions. Future research studies should be undertaken to explore the rhizobial survival and $N_2$-fixation under abiotic stresses. Moreover, comparative performance of these inocula under varying water regimes (rain-fed vs irrigated) also needs to be explored considering diverse chickpea genotypes.

## References

AMIS. 2018. Province vise production of Gram from 1947-48 to 2016-17. http://www.amis.pk/Agristatistics/Data/HTML%20Final/Gram/Production.html. [Accessed, August 30, 2018].

Anonymous. 2018. Pulses import set to increase on bad crop. The News. February 16, 2018. https://www.thenews.com.pk/print/281517-pulses-import-set-to-increase-on-bad-crop.

Aynalem, B., T. Temesgen, Y. Bezie and S.W. Yohannis. 2018. Phenotypic characterization and symbiotic effectiveness test of chickpea (*Cicer arietinum* L.) rhizobia isolated from Dejen and Aneded Districts, East Gojjam Zone, Amahara Region, Ethiopia. *African Journal of Biotechnology* 17: 730-738.

Bhattacharjya, S. and R. Chandra. 2013. Effect of inoculation methods of *Mesorhizobium ciceri* and PGPR in chickpea (C*icer arietinum* L.) on symbiotic traits, yield, nutrient uptake and soil properties. *Legume Research* 36: 331-37.

Bhuiyan, M.A.H., D. Khanam, M.F. Hossain and M.S. Ahmed. 2008. Effect of *rhizobium* inoculation on nodulation and yield of chickpea in calcareous soil. *Bangladesh Journal of Agricultural Research* 33: 549-554.

Boahen, S., A.E. Slinkard and F.L. Walley. 2002. Evaluation of rhizobial inoculation methods for chickpea. *Agronomy Journal* 94: 851-859.

Chandra, R. and N. Pareek. 2015. Comparative performance of plant growth promoting rhizobacteria with rhizobia on symbiosis and yields in urdbean and chickpea. *Journal of Food Legumes* 28: 86-89.

CIMMYT. 1988. From Agronomic Data To Farmer Recommendations: An Economic Training Manual. Completely revised Edition, Mexico.

Das, S., B.L. Pareek, A. Kumawat and S.R. Dhikwal. 2013. Effect of phosphorus and biofertilizers on productivity of chickpea (*Cicer arietinum* L.) in north western Rajasthan, India. *Legume Research* 36: 511-514.

Dutta, D. and P. Bandyopadhyay. 2009. Performance of chickpea (*Cicer arientinum* L.) to application of phosphorus and bio-fertilizer in laterite soil. *Archives of Agronomy and Soil Science* 55: 147-55.

El Hadi, E.A. and E.A.E. Elsheikh. 1999. Effect of *rhizobium* inoculation and nitrogen fertilization on yield and protein content of six chickpea (*Cicer arietinum* L.)

cultivars in marginal soils under irrigation. *Nutrient Cycling and Agroecosystem* 54: 57-63.

Erman, M., S. Demir, E. Ocak, S. Tufenkci, F. Oguz and A. Akkopru. 2011. Effects of *Rhizobium*, arbuscular mycorrhiza and whey applications on some properties in chickpea (*Cicer arietinum* L.) under irrigated and rainfed conditions 1-Yield, yield components, nodulation and AMF colonization. *Field Crops Research* 122: 14-24.

FAO. 2015. (Food and Agriculture Organization) FAOSTAT Statistical Database of The United Nation Food And Agriculture Organization (FAO) Statistical Division. Rome. http://faostat.fao.org/site/339/default.aspx. [Assessed, October 7, 2015].

FAO. 2016. (Food and Agriculture Organization) International year of pulses 2016: Nutritious seeds for a sustainable future. http://www.fao.org/pulses-2016/faq/en. [Assessed, June 3, 2016].

Gaur, P.M., S. Tripathi, C.L.L. Gowda, G.V.R. Ranga, H.C. Sharma, S. Pande and M. Sharma. 2010. Chickpea Seed Production Manual. International Crops Research Institute for the Semi-Arid Tropics. Patancheru 502 324, Andhra Pradesh, India. 28p.

Giller, K.E. 2001. Nitrogen Fixation in Tropical Cropping Systems. CAB International, Wallingford. pp. 274-282.

Government of Pakistan. 2021. Ministry of Finance, Pakistan economic survey 2020-2021. http:// https://www.finance.gov.pk/survey/chapters_21/02-Agriculture.pdf. [Accessed on, 30, 2021].

Government of Pakistan. 2018. Directorate of Agricultural Information, Crop production Technologies, Gram, https://dai.agripunjab.gov.pk/croptechnologies. [Assessed, August 31, 2018].

Gul, R., H. Khan, N.U. Khan and F.U. Khan. 2014. Characterization of chickpea germplasm for nodulation and effect of *rhizobium* inoculation on nodules number and seed yield. *The Journal of Animal and Plant Sciences* 24: 1421-1429.

Heerwaarden, J., F. Baijukya, S. Kyei-Boahen, S. Adjei-Nsiah, P. Ebanyat, N. Kamai, E. Wolde-meskel, F. Kanampiu, B. Vanlauwe and K. Giller. 2018. Soyabean response to *rhizobium* inoculation across sub-Saharan Africa: Patterns of variation and the role of promiscuity. *Agriculture, Ecosystems and Environment* 261: 211-218.

Hunt. 1979. Plant Growth Analysis. The rationale behind the use of the fitted mathematical function. *Annals of Botany* 43: 245-249.

Jain, L.K., P. Singh and J.K. Balyan. 2006. Productivity and profitability of chickpea (*Cicer arietinum* L.) cultivation as influenced by biofertilizers and phosphorus fertilization. *Indian Journal of Dryland Agricultural Research and Development* 21: 82-84.

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

Kantar, F., F.Y. Hafeez, B.G. Shivakumar, S.P. Sundaram, N.A. Tejera, A. Aslam, A. Bano and P. Raja. 2007. p. 179-192. In: Chickpea Breeding and Management. S.S. Yadav, R. Redden, W. Chen and B. Sharma (eds.). CAB Int'l. Wallingford, CT, USA.

Kaur, N. 2014. Studies on endophytic rhizobacteria and *Rhizobium* sp. *vigna consortium* on symbiotic efficiency, nodule occupancy on at molecular level and yied in mungbean (*Vigna radiate* L. Wilczek). Ph.D Dissertation. Punjab Agricultural University, Ludhiana, India.

Kaur, N., P. Sharma and S. Sharma. 2015. Co-inoculation of Mesorhizobium sp. and plant growth promoting rhizobacteria Pseudomonas sp. as bio-enhancer and bio-fertilizer in chickpea (*Cicer arietinum* L.). *Legume Research* 38: 367-74.

Khaitov, B., A. Kurbonov, A. Abdiev and M. Adilov. 2016. Effect of chickpea in association with *rhizobium* to crop productivity and soil fertility. *Eurasian Journal of Soil Science* 5: 105-112.

Malik, D.K. and S.S. Sindhu. 2011. Production of indole acetic acid by *Pseudomonas* sp.: Effect of coinoculation with *Mesorhizobium* sp. *Cicer* on nodulation and plant growth of chickpea (*Cicer arietinum* L.). *Physiology and Molecular Biology of Plants* 17: 25-32.

Malik, M.A., M.A. Cheema and H.Z. Khan. 2006. Growth and yield response of soybean (*Glycine max* L.) to seed inoculation and varying phosphorus levels. *Journal of Agricultural Research* 44: 47-53.

Meena, M.R., J. Dawson and M. Prasad. 2013. Effect of biofertilizers and phosphorus on growth and yield of chickpea (*Cicer arietinum* L.). *Bioinfolet* 10: 235-237.

Muehlbauer, F.J. and A. Sarker. 2017. Economic Importance of Chickpea: Production, Value, and World Trade. p. 5-12. In: The Chickpea Genome. R.K. Varshney, M. Thudi and F.J. Muehlbauer (eds.). Springer, Cham, Switzerland.

Namvar, A., R.S. Sharifi, M. Sedghi, R.A. Zakaria, T. Khandan and B. Eskandarpour. 2011. Study on the effects of organic and inorganic nitrogen fertilizer on yield, yield components and nodulation state of chickpea (*Cicer arietinum* L.). *Communications in Soil Science and Plant Analysis* 42: 1097-1109.

Namvar, A., R.S. Sharifi, T. Khandan and M.J. Moghadam. 2013. Seed inoculation and inorganic nitrogen fertilization effects on some physiological and agronomical traits of chickpea (*Cicer arietinum* L.) in irrigated condition. *Journal of Central European Agriculture* 14: 28-40.

PARC (Pakistan Agricultural Research Council). 2018. Pulses Program, Pakistan Agriculture Research Council. http://www.parc.gov.pk/index.php/en/csi/137-narc/crop-sciences-institue/712-national-coordinated-pulses-programme. [Accessed, August 30, 2018].

Rani, S., H. Shah, U. Farooq and B. Rehman. 2014. Supply, demand, and policy environment for pulses in Pakistan. *Pakistan Journal of Agricultural Research* 27: 149-159.

Sharar, M.S., M. Ayub, A. Choudluy and M. Nadeem. 2000. Effect of NP application and inoculation on the growth and yield of gram (*Cicer arietinum* L.). *Pakistan Journal of Agricultural Science*s 37: 155-57.

Sharma, P., V. Khanna, N. Kaur, G. Dhillon, G. Singh, S. Sharma, H. Kaur and A.K. Saxena. 2013. Effect of dual inoculation of *Pseudomonas argentinensis* LPGPR1 and *Mesorhizobium* on growth of chickpea *(Cicer arietinum* L.). *Journal of Research Punjab Agriculture University* 50: 1-4.

Shiferaw, B., M.C.S. Bantilan and R. Serraj. 2004. Harnessing the potentials of BNF for poor farmers: technological, policy and institutional constraints and research needs. pp. 3-27. In: Symbiotic Nitrogen Fixation: Prospects for Enhanced Application in Tropical Agriculture. R. Serraj. (eds.). Oxford & IBH Publishing, New Delhi.

Singh, G., N. Aggarwal and V. Khanna. 2010. Integrated nutrient management in lentil with organic manures, chemical fertilizers and biofertilizers. *Journal of Food Legumes* 23: 149-151.

Singh, S., S.S. Bawa, S. Singh, S.C. Sharma and P. Sheoran. 2015. Productivity, profitability and sustainability of rainfed chickpea under inorganic and biofertilization in foothills of north-west Himalayas. *Archives Agronomy and Soil Science* 61: 1151-1163.

SPG (Saskatchewan Pulse Growers). 2016. http://saskpulse.com/growing/chickpeas-beans/inoculation-and-fertility. [Accessed, November 10, 2016].

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

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

Thies, J.E., P.W. Singleton, and B.B. Bohlool. 1991. Influence of the size of indigenous rhizobial populations on establishment and symbiotic performance of introduced rhizobia on field-grown legumes. *Applied and Environmental Microbiology* 57: 19-28.

Thies, J.E., P.W. Singleton, and B.B. Bohlool. 1991. Modelling symbiotic performance of introduced rhizobia in the field by use of indices of indigenous population size and nitrogen status of the soil. *Applied and Environmental Microbiology* 57: 29-37.

Togay, N., Y. Togay, K.M. Cimrin, M. Turan. 2008. Effect of *Rhizobium* inoculation, sulfur and phosphorus application on yield, yield components and nutrient uptake in chickpea (*Cicer aretinum* L.). *African Journal of Biotechnology* 7: 776-782.

Wolde-meskel, E., J. van Heerwaarden, B. Abdulkadir, S. Kassa, I. Aliyi, T. Degefu, K. Wakweya, F. Kanampiu, K.E. Giller. 2018. Additive yield response of chickpea (*Cicer arietinum* L.) to *rhizobium* inoculation and phosphorus fertilizer across smallholder farms in Ethiopia. *Agriculture, Ecosystems and Environment* 261: 144-152.

Yasari, E., and A.M. Patwardhan. 2006. Physiological analysis of the growth and development of canola (*Brassica napus* L.) under different chemical fertilizer application. Asian Journal of Plant Sciences 5: 745-752.

Zaidi, A., M.S. Khan and M. Amil. 2003. Interactive effect of rhizotrophic microorganisms on yield and nutrient uptake of chickpea (*Cicer arietinum* L.). *European Journal of Agronomy* 19: 15-21.