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# Effect Of Rhizobial Inoculation On Nodulation And Yield Of Chickpea (Kabuli)

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**Abstract**

Native soil rhizobial populations are insufficient in N2-fixation. Rhizobium seed inoculation may be used to replace expensive N fertilizers and give a valuable method of achieving long-term productivity. In soils with no/weak bacterial presence, seed inoculation of chickpea with an effective and importunate rhizobial strain is essential to supply an adequate rhizobial population in the rhizosphere and has shown a positive effect on nodule number and mass, growth, yield, and other attributes compared to un inoculated ones. The purpose of this research was to see how rhizobium inoculation affected nodule development and chickpea plant growth, and also to find the interaction effect of different genotypes on nodulation and yield of chickpea (kabuli). Three field experiments were carried out during three consecutive rabi seasons of 2011-2012-2013 and 2013-14 at pulses research institute, Faisalabad, Pakistan. Five genotypes of chickpea (Kabuli) such as K-060062, K-60054, K-06006, K-07008, and Noor-2009 were tested with and without rhizobium inoculation. The basal dose of NPK (25-60-0kg) was applied. Peat-based rhizobia inoculum was used for inoculation treatment. The Rhizobium strain was diazotroph inoculate. The experiment was laid out (RCBD). Results show that inoculated plants gave significantly higher nodule numbers, number of branches, number of pods, and grain yield. All varieties performed better in different growth parameters but maximum grain yield was produced in line K60054. It was concluded that with Rhizobium inoculation, chickpea growth was enhanced. The findings of this research suggest the use of rhizobium inoculation in low Nitrogen soil.

**Keywords**: chickpea, rhizobium, nodulation, yield

## Introduction

The growing demand for crops and food to feed such a large population has sparked interest and necessitated the usage of biofertilizers to improve the health of these crops and even the soil (Mitter et al.2021). Biofertilizers can be an excellent complement to chemical fertilizers (Elnahel et al. 2022). The availability of nitrogen is one of the most important limiting variables in crop productivity (Rebi et al.2022; Rebi et al. 2021a; Rebi et al.2021b; Nawaz et al.2021). Because chemical nitrogenous fertilizers are limited in terms of production, availability, and use, biologically fixed nitrogen will play an essential role in enhancing crop output (Mehmood et al.2021). Pakistan is the fourth-largest producer of chickpea. Chickpea (Cicer arietinum L.) is an important leguminous crop because of its nutritional value (17-23 percent protein) in the country's huge vegetarian population (Shurtleff and Aoyagi et al.2022). According to (Wardell et al.2022) chickpea output has decreased over the last decade, and it has been suggested that traditional native soil rhizobial populations are insufficient and ineffectual in biological nitrogen fixing. Seed inoculation of legumes with an effective rhizobial strain is required to ensure an optimal rhizobial population in the rhizosphere. This aids in improving nodulation, N2 fixation, and leguminous crop development and yield (Gough et al.2021). The Rhizobium sp. invades the root hairs of chickpea and forms nodules, where free air nitrogen is fixed. Although these bacteria are found in most soils, their numbers, efficacy in nodulation, and N-fixation differ (Hameed et al.2021). The country is estimated to cultivate chickpea 2.2 million ha. However, until lately, there has been insufficient focus on expanding production, which is either static or declining. Poor soils, insufficient moisture, harsh weather conditions, weeds, and a lack of fertilizer availability are all major obstacles to the growing chickpea output (Irshad et al.2022). Farmers are unable to provide any plant nutrition due to unpredictable rains, inadequate incentives, and poor economic circumstances. Most farmers do not use chemical fertilizers in the production of chickpeas and other grain legumes (Wahab et al.2022). Furthermore, the Rhizobium population is low due to the hard climate, resulting in lower yields. Even in fields where chickpeas have been farmed for many years, inoculation with Rhizobium strains can boost chickpea productivity. Pakistan's main pulse crop is chickpea (Cicer arietinum). It is possible to boost production by improving root and rhizosphere colonization through the administration of efficient nitrogen-fixing bacteria to the seed or to the soil. This will help to reduce the need of nitrogenous fertilizer, which is expensive in this country (Janati et al.2021). Chick-pea cultivars with high yields, as well as efficient rhizobia strains, can boost yields. Some microorganisms have the ability to create intimate bonds with plants, which can be harmful or beneficial to the plants. Rhizobium has been studied extensively in the root nodules of legumes, where it fixes atmospheric nitrogen, but new research suggests that Rhizobium can also have plant growth-promoting (PGP) activity in non-legumes (Wu et al.2022) After isolating these tiny living creatures from the root nodules of local legumes, researchers researched them in combination with some non-legumes (cereals) and reported on their beneficial benefits (Liu et al.2022). Plant growth-promoting rhizobacteria (PGPR) are a group of helpful free-living soil bacteria that stimulate plant development. The purpose of this research was (1) to see how rhizobium inoculation affected nodule development and chickpea plant growth, (2) to find the interaction effect of different genotypes on nodulation and yield of chickpea.

**Material and methods**

The trial was conducted during three consecutive rabi seasons of 2011-2012, 2012-2013, 2013-2014 at the pulses research institute, Faisalabad. The treatments were designed in a Randomized complete Block having 3 replications for each treatment. The unit plot size was 4m x1.2m. Five genotypes of chickpea namely K-060062, K-60054, K-06006, K-07008, and Noor-2009 were tested with and without rhizobium inoculation. The soil was analyzed to determine the different Physico-chemical properties of soil. The soil was loam in texture. Basal application of NPK was made @ 25-6-0kg /ha. Peat-based rhizobia inoculum 1.5 kg/ha was used for inoculation treatment. The rhizobium strain was named Diazotroph and was used as an inoculant. The seeds were coated with rhizobium inoculum before the sowing was done on 15 November 2011, 15 November 2012, and 15 November 2014. Row to row distance was 30 cm and plant to plant distance of 15 cm was maintained. Thinning, weeding and other intercultural operations were carried out when necessary. Randomly selected plants were uprooted at 50 % flowering stage from each plot to record nodule numbers. Crops were harvested in April 2011-2012-2014. After harvesting, the number of branches, number of pods/plant, and seed yield for each unit plot were recorded. The recorded data were statistically analyzed using statistical package statistical 8.1

**Results and Discussion**

**1. Growth and yield attributes of different genotypes of chickpea 2011-2012**

Different genotypes effects significantly ($P<0.05$) on different growth parameters of chickpea in 2011-2012, which have been presented in Table 1. Maximum branches (12.33plant$^{-1}$) were produced with K06005 and K60062 and the minimum by K-60054 with 9.67plant$^{-1}$. The maximum pod (118.00plant$^{-1}$) was produced with K60054 and the minimum with Noor-2009. Maximum grain yield (2462.5 kgha$^{-1}$ was produced with K60054, and the minimum by K60062 with 1791. Kgha$^{-1.}$ Maximum nodules was produced with K06006 (19.67plant$^{-1}$). Inoculation of chickpea genotypes with Rhizobium strains has led to increasing soil carbon, nitrogen and phosphorous contents (Allito et al. 2020). This study's findings are supporting with those of (Wang et al. 2022; Alemayehu et al. 2022; Kaur et al. 2015)

**Table 1: Growth and yield attributes of different genotypes of chickpea 2011-2012**

| Sr. No | Genotypes | Branches/ plant | Pods /plant | Grain yield kg/ha | Nodules/plant |
|---|---|---|---|---|---|
| **1** | K06005 | 12.333A | 107.00B | 1832.8C | 18.67A |
| **2** | K06006 | 9.333B | 98.50C | 2038.0B | 19.67A |
| **3** | K60054 | 9.67BC | 118.00A | 2462.5A | 12.50C |
| **4** | K60062 | 12.333A | 105.33B | 1791.0C | 16.17C |

| | | | | | |
|---|---|---|---|---|---|
| **5** | Noor-2009 | 8.000C | 57.33D | 2063.7B | 12.17C |
| | SE | 0.5043 | 3.1394 | 65.221 | 0.9629 |
| | CV% | 10.4984 | 6.5955 | 137.02 | 2.101 |

**2. Growth and yield attributes of different genotypes of chickpea 2012-2013**

Different genotypes effects significantly ($P<0.05$) different growth parameters of chickpea in 2012-2013, which have been presented in Table 2. Maximum branches (4.22 plant$^{-1}$) were produced with K06006, and the minimum by K60062 with (3.00 plant$^{-1}$). The number of pods was maximum (65.97) in Noor-2009 and the minimum by K6006 with (34.83). Maximum grain yield was found in K60054 (2591 Kgha$^{-1}$). The number of nodules was the same as in 2011-2012. The study findings are similar to those of (Zetochová et al. 2022; Cheema and Garg et al. 2022; Zaheer et al. 2016).

**Table 2: Growth and yield attributes of different genotypes of chickpea 2012-2013**

| Sr. No | Genotype | Branches/ plant | Pods /plant | Grain yield kg/ha | Nodules/plant |
|---|---|---|---|---|---|
| **1** | K06005 | 3.70B | 45.77B | 2238.5B | 18.67A |
| **2** | K06006 | 4.22A | 34.83C | 1447.3C | 19.67A |
| **3** | K60054 | 3.53BC | 46.23B | 2591.8A | 12.50C |
| **4** | K60062 | 3.00D | 46.20B | 2182.0B | 16.17C |
| **5** | Noor-2009 | 3.40C | 65.07A | 2566.0A | 12.17C |
| | SE | 0.1291 | 1.9217 | 63.320 | 0.9629 |
| | CV% | 0.2713 | 4.0373 | 133.03 | 2.101 |

**3. Growth and yield attributes of different genotypes of chickpea 2013-2014**

Different genotypes performed differently with respect to growth parameters of chickpea in 2013-2014, which have been presented in Table 3. Maximum branches (4.47 plant$^{-1}$) were observed in K06006 and the minimum by K06005 with (3.48 plant$^{-1}$). The number of pods was maximum (68.7 plant$^{-1}$) in K60054 and the minimum by K06005 with (37.00 plant$^{-1}$). Maximum grain yield was found in Noor-2009 with (1899.8 Kg ha$^{-1}$). Rhizobium strains inoculated into chickpea genotypes increased soil carbon, nitrogen, and phosphorus content (Allito et al. 2020). Lower levels of organic matter, nitrogen, and phosphorus in chickpea cultivated without Rhizobium may be due to reduced microbial activity and, as a result, lower C input into the soil (Ramakrishnan et al.2017). The study findings are similar to (Zaheer et al. 2016; Wang et al. 2022; Kaur et al. 2015)

**Table 3: Growth and yield attributes of different genotypes of chickpea 2013-2014**

| Sr. No | Genotypes | Branches/ plant | Pods /plant | Grain yield kg/ha | Nodules/plant |
|---|---|---|---|---|---|
| **1** | K06005 | 3.48D | 37.00C | 1516.7C | 9.10A |
| **2** | K06006 | 4.47A | 41.00C | 1485.5C | 6.74B |

| | | | | | |
|---|---|---|---|---|---|
| **3** | K60054 | 4.01BC | 68.7A | 1849.5A | 6.90B |
| **4** | K60062 | 3.64CD | 41.7C | 1706.7B | 6.18BC |
| **5** | Noor-2009 | 4.25AB | 54.87B | 1899.8A | 5.96C |
| | SE | 0.2002 | 2.8860 | 28.279 | 0.3519 |
| | CV% | 0.4206 | 6.0634 | 59.412 | 0.7394 |

**4. Pooled growth and yield attributes of different genotypes of chickpea 2011-2014**

The pooled performance of different genotypes shows significantly ($P<0.05$) on different growth parameters of chickpea in 2011-2014, which have been presented in Table 4. The number of pods (77.67plant$^{-1}$) and grain yield (4657.0 Kgha$^{-1}$) were higher in the case of the lines K60054 and K06006 respectively. The highest number of nodules (13.90 plant$^{-1}$) was produced with K06005. The findings are similar with those (Khan et al. 2022; Abdiev et al.2019; Wani et al. 2007).

**Table 4: Pooled performance of different genotypes of chickpea (Kabuli)**

| Sr. No | Genotypes | Branches/ plant | Pods /plant | Grain yield kg/ha | Nodules/plant |
|---|---|---|---|---|---|
| **1** | K06005 | 6.53A | 63.25B | 1862.7C | 13.90A |
| **2** | K06006 | 6.00B | 58.10C | 1657D | 13.22A |
| **3** | K60054 | 5.40C | 77.67A | 2301.3A | 9.72C |
| **4** | K60062 | 6.33AB | 64.43B | 1893.2C | 11.18B |
| **5** | Noor-2009 | 5.22C | 59.08C | 2176.5B | 9.07C |
| | SE | 0.1888 | 1.2530 | 40.02 | 0.5146 |
| | CV% | 0.3966 | 2.6325 | 84.08 | 1.5289 |

**5. Effect of rhizobial inoculation on growth and yield attribution of chickpea (Kabuli)**

Effect of rhizobial inoculation on growth parameters of chickpea was significant and presented in (table 5).In this table shows the comparison of both inoculated and uninoculated seed effects on growth of chickpea. Maximum branches (6.37/plant) were produced in inoculated followed by un inoculated with (5.43/plant). The maximum number of pods (69.76/plant) was produced by inoculated seed. While, (59.25 /plant) was produced by un inoculated seeds. In the same way, grain yield and nodules per plant were higher in inoculated seed rather than un inoculated. The result of this study supports with (Uzma et al.2022; Kumawat et al.2022).

**Table 5: Effect of rhizobial inoculation on growth and yield attribution of chickpea (Kabuli)**

| | Branches/ plant | Pods /plant | Grain yield kg/ha | Nodules/plant |
|---|---|---|---|---|
| **Inoculated** | 6.37A | 69.76A | 2052A | 12.11A |
| **Un inoculated** | 5.43B | 59.25B | 1940B | 10.13A |
| **SE** | 0.119 | 0.793 | 25.312 | 0.326 |

| **CV%** | 0.251 | 1.665 | 53.18 | 0.684 |
|---|---|---|---|---|

**6. Interaction effects of rhizobial inoculant with different chickpea genotypes in 2011-2012**

The interaction effect of rhizobia inoculant with different genotype of chickpea was significant and presented in table 6 in 2011-2012. Maximum branches (14.33plant$^{-1}$ and 10.33plant$^{-1}$ were produced in K06005 and K60062, minimum with Noor-2009. The maximum number of pods and grain yield were produced in K60054 (136 plant $^{-1}$), (2837kgha$^{-1}$) respectively. K06006 produced a maximum number of nodules per plant (21.333). Uptake of Nitrogen gives the highest results and other which shows the lowest results may be due to the lowest nitrogen and other environmental factors such as drought, high temperature, and less availability of water. The findings are similar to the results of (Xu et al.2022; Li et al. 2022; Khan et al. 2022)

**Table 6: Interaction effects of rhizobial inoculant with different genotype of chickpea in 2011-2012**

| Sr No | Genotypes | Branches/ plant | Pods /plant | Grain yield kg/ha | Nodules/plant |
|---|---|---|---|---|---|
| **1** | K06005* | 14.33A | 117.67B | 1802.0E | 18.333B |
| | K06005 | 10.33B | 96.33C | 1863.7E | 19.00AB |
| **2** | K06006* | 11.00B | 119.967B | 1958.0D | 21.333A |
| | K06006 | 7.67D | 77.33D | 2118.0CD | 18.00B |
| **3** | K60054* | 9.07BC | 136.0A | 2837.7A | 17.00BC |
| | K60054 | 7.67D | 100.0C | 2087.3CD | 8.00F |
| **4** | K60062* | 14.33A | 111.33B | 1414.7F | 17.67B |
| | K60062 | 10.33B | 99.33C | 2167.3BC | 14.67CD |
| **5** | Noor-2009* | 7.33D | 56.67E | 2312.0B | 13.333DE |
| | Noor-2009 | 8.67CD | 58.00E | 1815.3E | 11.000E |
| | SE | 0.7132 | 4.4397 | 92.238 | 1.3617 |
| | CV% | 10.4 | 10.5 | 4.0 | 9.2 |

Inoculated*

**7. Interaction effects of rhizobial inoculant with different genotypes of chickpea in 2012-2013**

The interaction effect of rhizobial inoculation on growth parameters of chickpea was significantly presented in table 7. The maximum number of branches (14.33plant$^{-1}$ -10.33plant$^{-1}$ were produced in K06005 and K60062 respectively, and the minimum by Noor-2009. The maximum number of pods and grain yield were produced in K60054 @(136 kgha$^{-1}$), (2837kgha$^{-1}$) respectively. K06006 produced a maximum number of nodules per plant (21.333). Inoculation of chickpea genotypes with rhizobium strains has led to increasing soil carbon, nitrogen, and phosphorous contents (Allito et al. 2020). Lower concentration of organic matter, N and P content under chickpea grown without Rhizobium can depend on the reduced microbial activity and consequently the reduced C input to soil (Ramakrishnan et al.2017). The result is similar with the findings of (Li et al. 2022; Wani et al. 2007; Abdiev et al.2019)

**Table 7: Interaction effects of rhizobial inoculant with different genotypes of chickpea in 2012-2013**

| Sr. No | Genotypes | Branches/ plant | Grains/pod | Grain yield /PLANT | Nodules/plant |
|---|---|---|---|---|---|
| **1** | K06005* | 3.4BC | 1.12FG | 2543.7A | 18.333B |
| | K06005 | 4.00A | 1.08G | 1933.3D | 19.00AB |
| **2** | K06006* | 4.10A | 1.17DEF | 1463.3E | 21.333A |
| | K06006 | 4.33A | 1.15EFG | 1431.3E | 18.00B |
| **3** | K60054* | 3.60B | 1.31BC | 2700.7A | 17.00BC |
| | K60054 | 3.47BC | 1.17DEF | 2483.0BC | 8.00F |
| **4** | K60062* | 3.33BC | 1.22DE | 2330.3BC | 17.67B |
| | K60062 | 2.67D | 1.24CD | 2033.7D | 14.67CD |
| **5** | Noor-2009* | 3.60B | 1.55A | 2588.3A | 13.333DE |
| | Noor-2009 | 3.20C | 1.37B | 2543.7AB | 11.000E |
| | SE | 0.1826 | 0.0376 | 89.548 | 1.3617 |
| | CV% | 0.3837 | 0.0790 | 188.13 | 9.2 |

Inaculated*

**8. Interaction effects of rhizobial inoculant with different genotypes of chickpea in 2013-2014**

The interaction effect of rhizobial inoculant on growth parameters of chickpea was significantly presented in table 8. The maximum number of branches (4.78plant$^{-1}$) in Noor-2009 was produced. The maximum number of pods and grain yield were produced in K60054 Noor-2009 respectively. Line K06005 shows the highest result in the number of nodules per plant. Rhizobium strains inoculated into chickpea genotypes increased soil carbon, nitrogen, and phosphorus content. Lower levels of organic matter, nitrogen, and phosphorus in chickpea cultivated without Rhizobium may be due to reduced microbial activity and, as a result, lower C input into the soil (Ramakrishnan et al.2017).The findings are similar to the findings of (Khan et al. 2022; Zaheer et al. 2016; Zetochová et al. 2022)

**Table 8: Interaction effects of rhizobial inoculant with different genotypes of chickpea in 2013-2014**

| Sr No | Genotypes | Branches/ plant | Pods /plant | Grain yield kg/ha | Nodules/plant |
|---|---|---|---|---|---|
| **1** | K06005* | 4.00BCD | 38.800C | 1513.3DE | 10.90A |
| | K06005 | 2.95E | 35.200C | 1520.0DE | 7.3BC |
| **2** | K06006* | 4.72A | 38.67C | 1491.3E | 7.83B |
| | K06006 | 4.22ABC | 43.33C | 1479.3E | 5.64DE |
| **3** | K60054* | 3.64CD | 81.87A | 1987.3A | 7.39BC |
| | K60054 | 4.39AB | 55.60B | 1711.7BC | 6.4CD |
| **4** | K60062* | 3.61D | 41.40C | 1788.0B | 6.50CD |
| | K60062 | 3.67CD | 42.07C | 1625.3CD | 5.84DE |
| **5** | Noor-2009* | 4.78A | 54.07B | 2053.0A | 6.67CD |
| | Noor-2009 | 3.72CD | 55.67B | 1746.7BC | 5.25E |
| | SE | 0.2831 | 4.0815 | 63.234 | 0.4977 |
| | CV% | 0.5949 | 8.5749 | 132.85 | 1.0457 |

Inoculated*

**9. Pooled interaction effect of rhizobial inoculant with different genotypes of chickpea (2011-2014)**

The pooled interaction effect of rhizobial inoculant on growth parameters of chickpea was significant, presented in (Table 9). The maximum number of branches (7.27plant$^{-1}$) in K06005 was produced. The maximum number of pods (88.5plant$^{-1}$) and grain yield (2508.6plant$^{-1}$) were produced in K60054. Genotype K06005 shows the highest (14.63plant$^{-1}$) result in the number of nodules per plant. Inoculating chickpea genotypes with Rhizobium strains enhanced soil carbon, nitrogen, and phosphorus levels. Reduced microbial activity and, as a result, lower C input into the soil may cause lower levels of organic matter, nitrogen, and phosphorus in chickpea cultivated without Rhizobium. (Ramakrishnan et al.2017). The findings are similar to the findings of (de Almeida Leite et al. 2022; Khan et al. 2022; Zetochová et al. 2022)

**Table 9: Pooled interaction effect of rhizobial inoculant with different genotypes of chickpea (2011-2014)**

| Sr No | Genotypes | Branches/ plant | Pods /plant | Grain yield kg/ha | Nodules/plant |
|---|---|---|---|---|---|
| **1** | K06005* | 7.27A | 69.70B | 1953.0DE | 14.63A |
| | K06005 | 5.80C | 56.80F | 1772.3FG | 13.17AB |
| **2** | K06006* | 6.60B | 66.33BC | 1637.7H | 14.60A |
| | K06006 | 5.40CD | 49.87G | 1676.2GH | 11.83B |
| **3** | K60054* | 5.63CD | 88.50A | 2508.6A | 12.20B |
| | K60054 | 5.17D | 66.83BC | 2094.0C | 7.23D |
| **4** | K60062* | 7.1AB | 63.67CD | 1844.4EF | 12.10B |
| | K60062 | 5.57CD | 65.20C | 1942.1DE | 10.27C |
| **5** | Noor-2009* | 5.23D | 60.60DE | 2317.8B | 10.00C |
| | Noor-2009 | 5.20D | 57.57EF | 2035.2CD | 8.13D |
| | SE | 0.2831 | 1.7721 | 56.600 | 0.7277 |
| | CV% | 0.5949 | 3.7229 | 118.91 | 1.5289 |

Inoculated*

**Conclusion**

After beans and peas, chickpea (Cicer arietinum L.) is the world's third most important food legume. However, in the last decade, its productivity has decreased, and it has been suggested that native soil rhizobial populations may be insufficient or less efficient in N2-fixation. Seed inoculation with rhizobium may be a viable alternative to expensive nitrogen fertilizers and a means of achieving long-term output. In soils with no/weak bacterial presence, seed inoculation of chickpea with an effective and importunate rhizobial strain is essential to supply an adequate rhizobial population in the rhizosphere, and has shown a positive effect on nodule number and mass, growth, yield, and other attributes when compared to uninoculated ones. Hence, the objectives of this research were to find how rhizobium inoculation affected nodule development and chickpea plant growth, and also to find the interaction effect of different varieties on nodulation and yield of chickpea. From above results shows that rhizobial inoculation with the seed of chickpea gives better results rather than un inoculation. Among all genotypes, K60054 gives best results in most of the parameters. So it was concluded that rhizobial inoculation is the best choice in low nitrogen /low rhizobial population soil.

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