Research Article

Appraisal of Yield and Nitrogen related Traits of Chickpea in Response to Rhizobium Inoculation and Phosphorus Application

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Abstract | Chickpea (*Cicer arietinum* L.) is a major grain legume, cultivated worldwide. This research study was carried-out during the chickpea cropping season (2023–024) at the University of Agriculture Peshawar, Pakistan. Total 20 chickpea genotypes were evaluated under four different environmental conditions, control, rhizobium inoculation, phosphorus treatment, and combined rhizobium-phosphorus treatment. Significant differences were observed among environments, genotypes, and genotype × environment interactions for most of the studied traits. Among genotypes, NKC-10-99, MG1, and FG1 exhibited early maturity (171.96 days). The highest number of reproductive branches and pods per plant were recorded for genotype NKC-10-99. However, maximum seed yield was observed in IG-2 (1636.1 KG ha^{-1}). The highest nitrogen uptake (139.93 kg/ha) was recorded for genotype NKC-10-99, while K-08021 excelled in biological nitrogen fixation. Among treatments, the interaction of rhizobium and phosphorus treatment resulted in early maturity (175.97 days), highest values for seed yield (1548.3 kg/ha) and largest amount of nitrogen uptake (118.12 kg ha^{-1}). Genotypes K-01209, K-01210, NKC-10-99, FG, IG-2 and AG1 performed best in most undertaking growing conditions, and are recommended for future breeding programs. The combined application of rhizobium and phosphorus is suggested for optimal chickpea production.

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Introduction

Chickpea (*Cicer arietinum* L.) is a widely cultivated grain legume, ranking after peas and dry beans, and serving as a crucial source of protein, fiber, vitamins, and essential minerals. Originating from southeastern Turkey, chickpea is a diploid species with 16 chromosomes and primarily self-pollinates due to its cleistogamous flowers. Globally, chickpea contributes 15.9% to total pulse production, with India being the largest producer, accounting for 25-28% of global production. In Pakistan, chickpeas are cultivated on 0.94 million hectares, with a significant portion grown in Khyber Pakhtunkhwa. Chickpea cultivation is influenced by factors such as climate, soil type and agricultural practices, necessitating optimal planting dates and agronomic strategies for maximum yield (Yadav *et al*., 2015). The capability of chickpea to fix atmospheric nitrogen through a symbiotic relationship with rhizobium bacteria improves soil fertility, minimizing the need for synthetic nitrogen fertilizers (Giller *et al*., 2002). The genetic diversity within chickpea genotypes offers opportunities for breeders to develop varieties suited to local agro-ecosystems and climate challenges (Varshney *et al*., 2015)

Two essential practices in chickpea cultivation are the inoculation with rhizobium bacteria and the efficient application of phosphorus. These methods can greatly influence chickpea yield and nutrient absorption, although their effectiveness varies across different chickpea genotypes. Studies examining the combined effects of rhizobium inoculation and phosphorus application on various chickpea genotypes seek to understand how specific genotypes respond to these practices and the mechanisms that influence their growth, development, and nutrient uptake (Hufnagel *et al*., 2014). This knowledge can help provide practical guidelines for optimizing chickpea yield and nutrient content while reducing environmental impact (Giller *et al*., 2002). Furthermore, these insights have the potential to significantly contribute to global food security efforts, as chickpeas play a vital role in sustaining populations, particularly in regions where they are a staple food (Abbo *et al*., 2003). Enhancing cultivation practices for chickpeas could lead to increased food production, reduced malnutrition, and improved livelihoods for communities reliant on this versatile legume.

Keeping in view the given facts this study aimed to evaluate genotype-by-environment interaction across different growing conditions. To investigate the response of chickpea genotypes to rhizobium inoculation and phosphorus fertilization separately and combined in terms of growth, yield and nutrient uptake, estimate the heritability of various traits under different environmental conditions, and identify superior genotypes with enhanced adaptability, yield, and nutrient efficiency. Understanding these factors is essential for improving chickpea productivity and ensuring its role in sustainable agriculture and food security. Furthermore, the study was carried out with the hypothesis, that rhizobium inoculation and phosphorus application will synergistically increase the yield and nitrogen-related traits of chickpea genotypes by promoting better nodulation, nitrogen fixation, and nutrient utilization and there could be difference in genotypes response to given strain of rhizobium and dose of phosphorus fertilizer.

Materials and Methods

The study evaluated 20 chickpea genotypes (Table 1) at the Research Farm of the University of Agriculture, Peshawar, during the Rabi Season of 2023-2024. The experiment consisted of four environments: a control group, rhizobium-inoculated, phosphorus-treated, and a combination of rhizobium and phosphorus treatment. The field trial followed a randomized complete block design with three replications, each containing 20 plots with three rows plot^{-1}, with a row-to-row distance of 30 cm and a plant-to-plant spacing of 10 cm, while row length was maintained at 4 m. Single Super Phosphate (SSP) was used as the phosphorus source, providing 18% phosphorus content, with 7.2 kg SSP applied to the phosphorus-treated fields. Standard agronomic practices were followed uniformly across all genotypes.

Inoculant preparation and application

Rhizobium inoculant Bizote-MAX was obtained from the National Agriculture Research Center (NARC), Islamabad. A slurry was prepared by mixing the inoculant with a 5% sugar solution to enhance seed adhesion. The chickpea seeds were coated with the inoculum in a clean jar under shaded conditions to protect bacterial viability.

Data collection

Various growth and yield parameters were recorded on ten randomly selected plants per plot and then averaged. Number of reproductive branches, Pods plant^{-1} and number of nodules plant^{1} were counted on randomly selected plants before harvest and averaged. The data to maturity was taken by counting the number of days from the date of sowing to the time when 90% of the pods turned brown and were ready for harvest Seeds yield obtained after threshing each plot individually was weighed in kilograms separately and was converted to kg ha^{-1} using the following relationship.

$$\text{Yield (kg ha}^{-1}) = \frac{\text{Yield (kg)}}{3\text{m}\times 0.3\text{m}2\times 3} \times 10{,}000\text{m}^2$$

Where: 10,000 = m^2 in one hectare, 3 = number of rows, 3 = row length, $0.3m^2$ = row to row distance

Biological nitrogen fixation was estimated using the nitrogen difference method as described by Peoples *et al*. (2008). In this method total N uptake was determined in plant samples taken at maximum plant biomass stage. For this purpose, a known area of wheat grown under control environment (no N application) adjacent to chickpea crop was harvested and analyzed for total N uptake. The amount of N_2 fixed was calculated as difference in n uptake of N of wheat and chickpea crops as follows.

N_2 fixed (kg $ha^{-1)}$ = Total N in chickpea (kg $ha^{-1)}$ – Total N in wheat (kg $ha^{-1)}$

The total N uptake by chickpea plants was determined from multiplication of its total N concentration with total biomass according to the following formula.

$$Total\ N\ uptake\ (kg\ ha^{-1}) = \frac{Total\ N\ Conc.(\%)x\ Total\ biomass\ (kg\ ha^{-1})}{100}$$

Statistical analysis was conducted on the gathered data using ANOVA techniques based on the methods outlined by Araújo (2003).

Table 1: *List of chickpea genotypes evaluated at The Uni. of Agric. Peshawar during 2023-24*

Genotypes	Source	Genotypes	Source
DG3	PBG, UAP	K-6066	AARI
MG1	PBG, UAP	K-08021	AARI
AG1	PBG, UAP	K-01209	AARI
DG4	PBG, UAP	K-01210	AARI
BG5	PBG, UAP	K-01201	AARI
BG4	PBG, UAP	K-60075	AARI
MG3	PBG, UAP	K-60069	AARI
IG2	PBG, UAP	K-01153	AARI
FG1	PBG, UAP	K-01151	AARI
Karak-1	ARSAK	NKC-10-99	NIFA

NIFA: Nuclear institute of food and Agriculture (Peshawar). ARSAK: Agriculture Research Station Ahmad wala Karak and AARI: Ayub Agriculture Research Institute, PBG, UAP: Plant Breeding and Genetics, The University of Agriculture Peshawar

Results and Discussion

Days to 90% maturity

The combined analysis of variance for days to 90% maturity showed highly significant differences among genotypes (p≤0.05) and genotype × environment interactions, while the environment alone had no significant effect (Table 2). Previous studies by Gul *et al* (2011) reported similar findings, though environmental differences may have influenced results. The mean data for days to 90% maturity ranged from 171.67 (DG4, K-08021, K-01201, and NKC-10-99) to 185 days (K-01210 and K-01209) under a control environment, 171 (NKC-10-99) to 185 days (DG4) for rhizobium inoculated, 171.67 (NKC-10-99, DG3, MG1, and FG1) to 185 days (DG4) for the phosphorus-treated environment, and 171 (IG2 and NKC-10-99) to 188.33 days (K-01209) for rhizobium and phosphorus-treated growing condition. Genotypes mean across environments ranged from 171.33 to 182.33 days. The genotype NKC-10-99 matured earlier followed by genotype FG1 i.e.171.33 and 171.96 days respectively. Genotype K-01151 took the maximum number of days for 90% maturity followed by MG3 i.e. 182.33 and 181.42 days respectively. The genotype × environment interaction means of chickpea genotypes for the said trait varied from 171 to 188.33 days, fewer days to 90% maturity was noted for genotype NKC-10-99, whereas genotype K-01209 took a maximum number of days for 90% maturity. Environment means across genotypes ranged from 175.97 to 177.42 days. Minimum days for 90% maturity were observed in rhizobium-inoculated plants i.e., 175.97, while rhizobium and phosphorus-treated environments were late to mature by taking 177.42 days (Figure 1).

Number of productive branches plant^{-1}

Pooled Analysis of Variance showed significant variations in reproductive branches across genotypes, environments (p≤0.05), and genotype × environment interactions. Similar findings were reported by Yousaf *et al* (2022) and Wazir Rehan *et al* (2018) (Table 2). The mean values for number of productive branches varied from 7.13(MG3) to 10.40 (MG1) under control environment, 9.13 (KARAK-1) to 16.40 (NKC-10-99) under rhizobium inoculated, 8.33 (K-01209) to 15.40 (NKC-10-99) for phosphorus-treated environment, and 8.07 (K-01209) to 16.40 (NKC-10-99) for rhizobium and phosphorus-treated condition. (Figure 2). Genotype mean across environments ranged from 8.47 to 14.47. The genotype NKC-10-99 showed the maximum number of branches followed by genotype FG1 i.e. 14.47 and 10.4 respectively. Genotype K-01209 showed the least number of branches followed by K-01210 i.e. 8.47

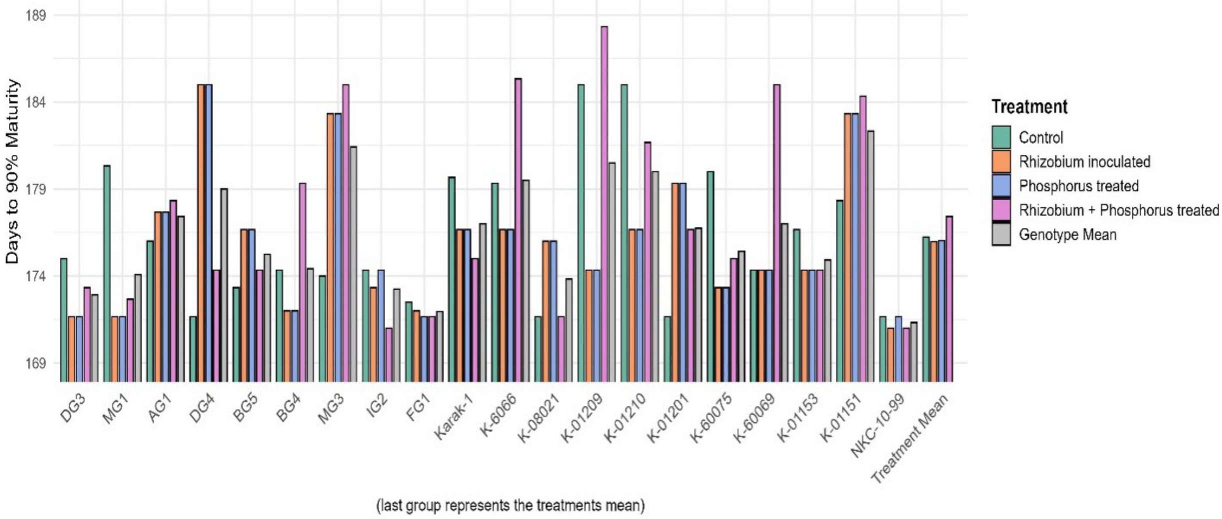

Figure 1: *Mean values of chickpea genotypes for days to 90% Maturity under four different growing conditions at The University of Agriculture Peshawar during 2023-24*

Table 2: *Mean squares of days to 90% maturity, number of reproductive branches plant^{-1}, number of nodules per plant, number of pods per plant, nitrogen uptake, relative nitrogen fixation, biological nitrogen fixation, nitrogen derived from atmosphere, seed yield and biological yield*

Source	Environment	Genotype	Reps w/n environment	G X E	Error	CV (%)
Degrees of freedom	3	19	12	57	152	-
Day to 90% maturity	27.79	127.54**	44.32	41.06**	16.07	2.27
Number of Productive branches plant^{-1}	30.31**	19.38**	2.98	2.98**	0.61	7.92
Number of nodules plant^{-1}	5017**	100.42	364.90	80.51	67.76	21.43
Number of pods plant^{-1}	3662.96**	315.94**	203.98	203.98**	101.30	19.27
Nitrogen uptake	26990**	6913**	78.1	384.2**	174.6	13.43
Biological nitrogen fixation	27187**	6945**	76.0	384.2**	175.0	7.56
Seed yield	5935430**	1632269**	379149.5	348237	294963	48.83

*** *= Significance at 1% and 5% probability level, Df= Degree of freedom, CV =coefficient of variance*

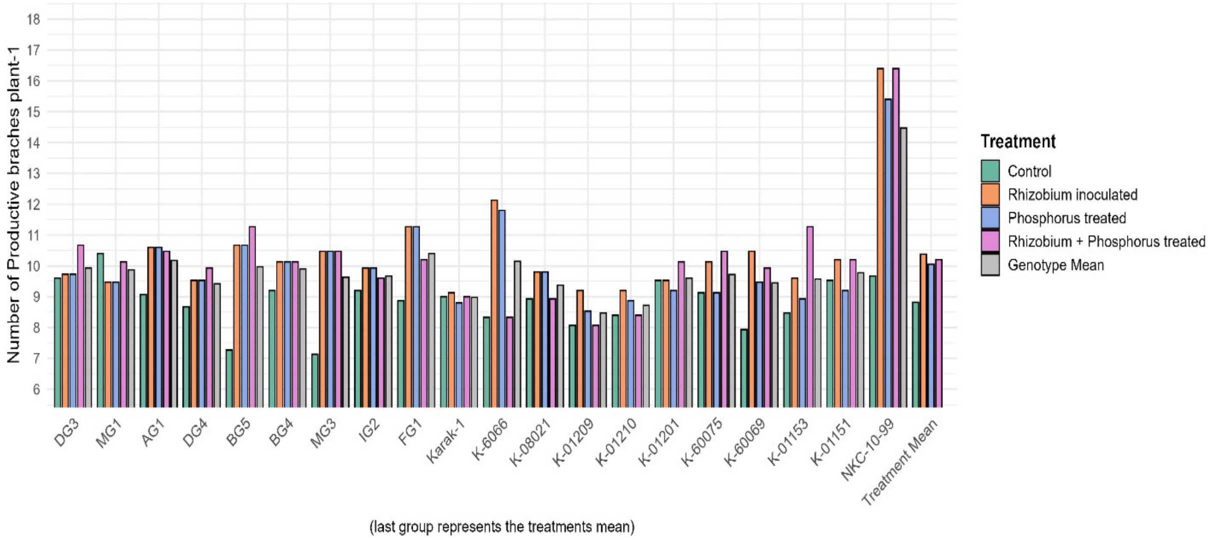

Figure 2: *Mean values of chickpea genotypes for number of Productive branches plant^{-1} under four different growing conditions at The University of Agriculture Peshawar during 2023-24*

and 8.72 respectively. The genotype × environment interaction means of chickpea genotypes for the number of productive branches varied from 7.13 to 16.4, the lowest number of productive branches was noted for genotype MG3, whereas genotype NKC-10-99 showed the maximum number of productive branches per plant. Environment means across genotypes ranged from 8.82 to 10.38. The maximum number of branches was observed in only the rhizobium inoculated environment i.e., 10.38, while the control environment showed the least number of productive branches i.e., 8.83 (Figure 2).

Pods plant^{-1}

The difference in total number of pods plant^{-1} is significantly high across different environments and genotypes ($p \leq 0.05$). The combined effect of environment and genotype also significantly pretentious the number of pods plant^{-1}($p \leq 0.01$) (Table 2). Yousaf *et al.* (2022) also reported highly significant differences among the genotypes for the number of pods when studying genetic divergence and inter-relationship in 20 chickpea genotypes. When analyzing the impact of phosphorus and rhizobium inoculation on chickpea productivity, Wazir *et al.* (2018) reported similar results for the effect of environment and genotype x environment interaction on the number of pods.

The mean data for number of pods ranged from 23.47 (DG4) to 55.4 (IG2) under control environment, 28 (K-6066) to 73.87 (BG5) under rhizobium inoculated, 26.67 (K-6066) to 74.13 (BG5) for phosphorus-treated environment, and 46.87 (IG2) to 65.73 (NKC-10-99) for rhizobium and phosphorus-treated. Genotype mean across environments ranged from 34.67 to 58.37. The genotype BG5 showed the maximum number of pods followed by genotype FG1 i.e. 58.37 and 57.48 respectively. Genotype K-6066 showed the least number of pods followed by MG1 i.e. 34.67 and 46.55 respectively. The genotype × environment interaction means of chickpea genotypes for the number of pods varied from 23.47 to 74.13, the lowest number of pods was noted for genotype DG4, whereas genotype BG5 showed the maximum number of pods. Environment means across genotypes ranged from 40.58 to 57.3. The maximum number of pods was observed in the rhizobium and phosphorus-treated environment i.e. 45.32, while the control environment showed the least number of pods i.e. 24.99 (Figure 3).

Number of nodules plant^{-1}

The environments ($p \leq 0.05$) significantly affected the number of nodules in a plant. The interaction between genotype and environment and genotypes did not significantly affect the number of nodules per plant (Table 2). Tagore *et al.* (2013) observed a similar difference in the number of nodules across chickpea genotypes while studying the effects of rhizobium and phosphate-solubilizing bacterial inoculants in chickpea genotypes. ARM Solaiman (2012) reported significant effects of environments and genotype × environment interaction on the number of nodules when grown in phosphorus-treated and rhizobium environments. The differences in the results were due to different genotypes being studied.

The mean data for number of nodules ranged from 17.4 (K-01151) to 35.53 (FG1) under control environment, 27.53 (MG1) to 51.6 (K-60075) under rhizobium inoculated, 36.53 (K-01210) to 58.4

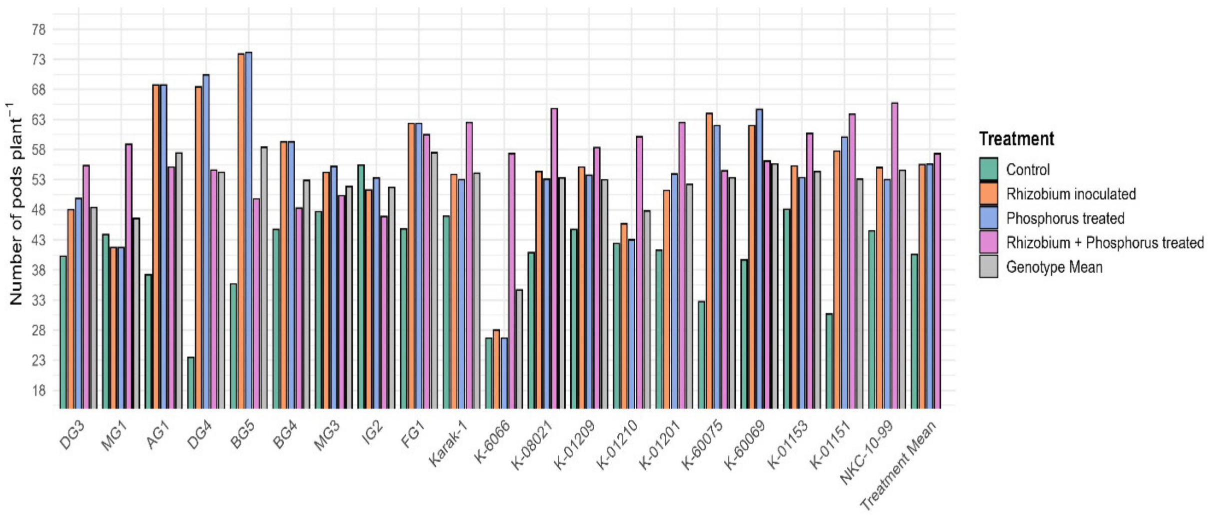

Figure 3: *Mean values of chickpea genotypes for Number of pods plant^{-1} under four different growing conditions at The University of Agriculture Peshawar during 2023-24*

(K-08021) for phosphorus-treated environment, and 33.67 (MG3) to 51.2 (NKC-10-99) for rhizobium and phosphorus-treated, respectively (Figure 4). The mean number of nodules of 20 genotypes across four different sowing conditions ranged from 33.58 to 44.42. The genotype K-08021 showed the maximum number of nodules followed by genotype FG1 i.e. 44.42 and 42.85 respectively. While genotype K-60069 showed the least number of nodules followed by MG1 i.e., 33.58 and 33.72 respectively. The genotype × environment interaction means of chickpea genotypes for the number of nodules varied from 17.4 to 58.4, the lowest number of nodules was noted for genotype K-01151, whereas genotype K-08021 showed the maximum number of nodules. Environment means across genotypes ranged from 24.99 to 45.32. The maximum number of nodules was observed in only the phosphorus environment i.e. 45.32, while the control environment showed the least number of nodules i.e. 24.99 (Figure 4).

Nitrogen uptake (kg ha^{-1})

A highly significant effect of genotype, environment, and their interaction ($p \leq 0.05$) was observed for nitrogen uptake, consistent with findings by Das *et al*. (2013) on phosphorus and biofertilizer treatments. (Table 2). The mean data for nitrogen uptake ranged from 25.05 (K-08021) to 114.7 kgha^{-1} (NKC-10-99) under the control environment, 46.30 (K-08021) to 151.2 kgha^{-1} (NKC-10-99) under the rhizobium inoculated, 67.19 (K-08021) to 156.57 kgha^{-1} (IG2) for phosphorus-treated environment, and 73.71 (K-08021) to 148.42 kgha^{-1} (K-01153) for rhizobium and phosphorus-treated environment (Figure 5).

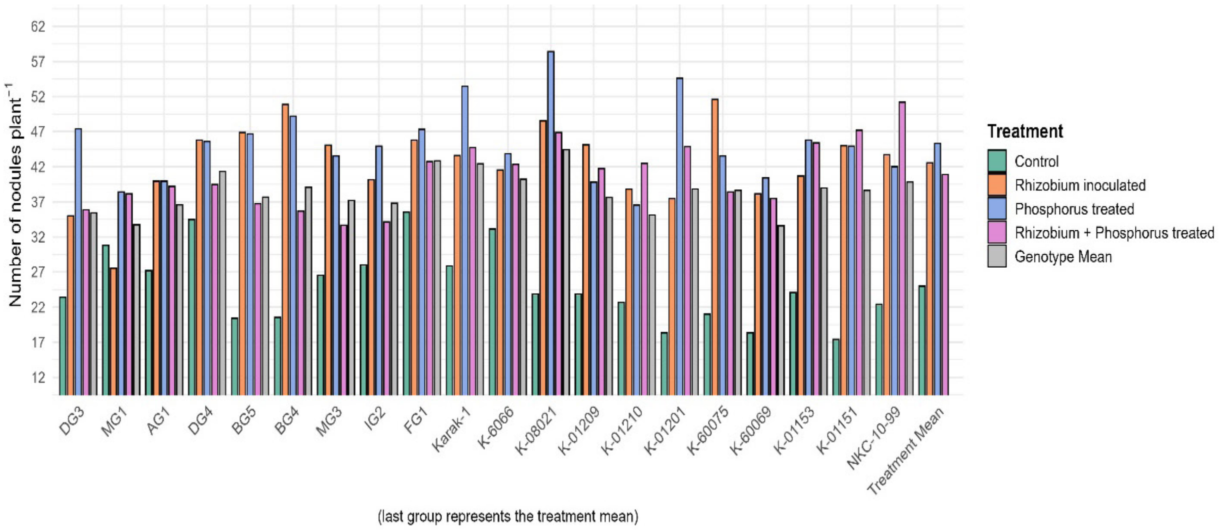

Figure 4: *Mean values of chickpea genotypes for number of nodules plant^{-1} under four different growing conditions at the university of agriculture peshawar during 2023-24*

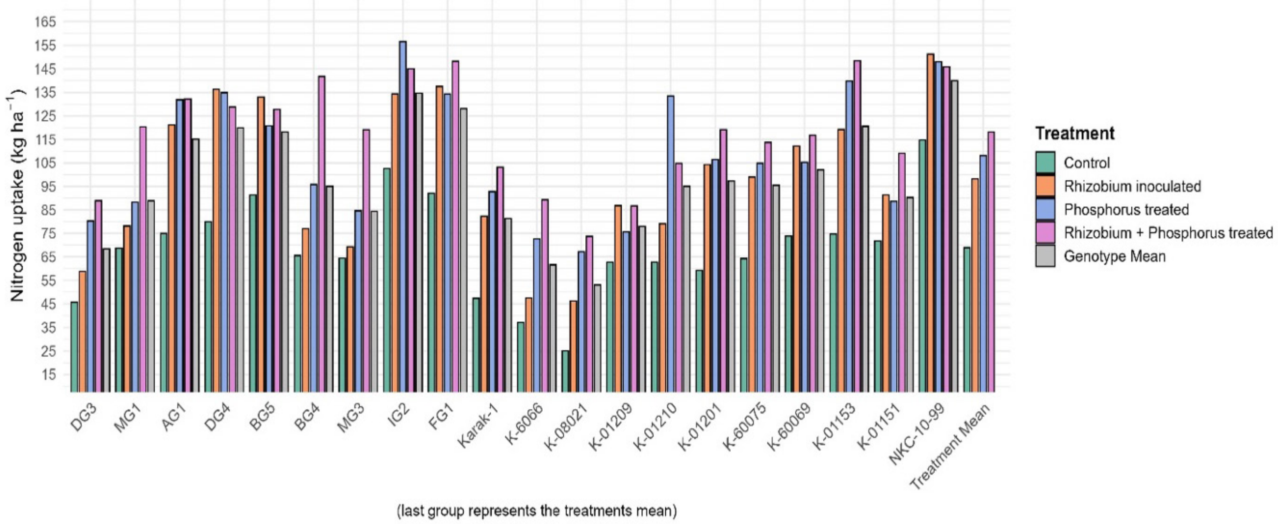

Figure 5: *Mean values of chickpea genotypes for Nitrogen uptake (KG ha^{-1}) under four different growing conditions at The University of Agriculture Peshawar during 2023-24*

Genotype mean across environments ranged from 53.06 to 139.93 kgha^{-1}. The genotype NKC-10-99 showed maximum nitrogen uptake followed by genotype IG2 i.e. 139.93 and 134.62 kgha^{-1} respectively. Whereas, genotype K-08021 showed the least nitrogen uptake followed by K-6066 i.e. 53.06 and 61.65 kgh^{-1} respectively. The genotype × environment interaction means of chickpea genotypes for nitrogen uptake varied from 25.05 to 156.6 kgha^{-1}, the lowest nitrogen uptake was noted for genotype K-08021, while genotype NKC-10-99 showed maximum nitrogen uptake. Environment means across genotypes ranged from 68.96 to 118.12 kgha^{-1}. Maximum nitrogen uptake was observed for the combined rhizobium and phosphorus-treated environment i.e. 118.12, while the control environment showed the least nitrogen uptake i.e. 68.96 (Figure 5).

Biological nitrogen fixation (BNF %)

BNF in chickpea was significantly influenced by genotype, environment, and their interaction ($p \leq 0.05$) (Table 2). Similar effects were reported by Das *et al.* (2013) in chickpea genotypes under phosphorus treatment and biofertilizers. The BNF values ranged from 12.10% to 131.6% across different treatments. Under the control environment, genotype K-6066 exhibited the lowest BNF (12.10%), while NKC-10-99 showed the highest (89.70%). Rhizobium inoculation improved BNF, with NKC-10-99 achieving 126.2%. Under phosphorus treatment, IG2 recorded the highest BNF (131.6%), while K-08021 had the lowest (42.20%). The combined rhizobium and phosphorus treatment further enhanced BNF, with K-01153 showing the highest value (123.4 %). Genotype means across environments varied from 28.10% (K-08021) to 114.9% (K-08021), and environmental means ranged from 43.80% in control to 93.1% in the combined treatment (Figure 6).

Seed yield (kg ha^{-1})

Seed yield is a key trait as it directly determines the productivity and economic value of a crop. Analysis of variance showed that the seed yield of chickpea plants was significantly affected by the plant genotypes ($p \leq 0.05$) and the environments. The results of ANOVA did not show any significant effect of interaction between genotype and environment on the seed yield (Table 2). The genotypes of chickpea exhibited a significant difference in seed yield. Wazir Rehan *et al.* (2018) also reported significant effects of genotypes and environment for seed yield while studying the effects of phosphorus and rhizobium inoculation on chickpea genotypes. However, they also observed significant difference in genotype by environment interaction which is contradictory to our results, that might be due to different growing conditions and different genotypes being studied.

The mean data for the seed yield ranged from 186.6 (K-60069) to 1492.3 kgha^{-1} (NKC-10-99) under the control environment, 302.41 (K-60069) to 1611.6 kgh^{-1} (IG2) under rhizobium inoculated, 412.3 (K-6069) to 1611.6 kgha^{-1} (IG2) for phosphorus-treated environment, and 651.7 (K-60069) to 1900.7 kgha^{-1} (IG2) for rhizobium and phosphorus-treated. (Figure 7). Genotypes mean across environments ranged

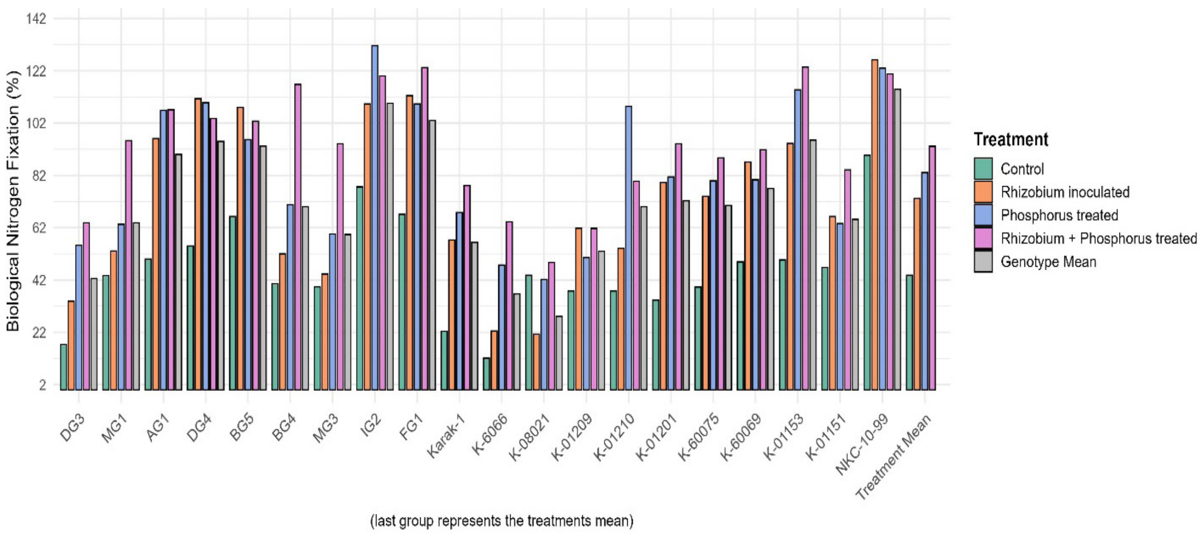

Figure 6: *Mean values of chickpea genotypes for biological nitrogen fixation (%) under four different growing conditions at The University of Agriculture Peshawar during 2023-24*

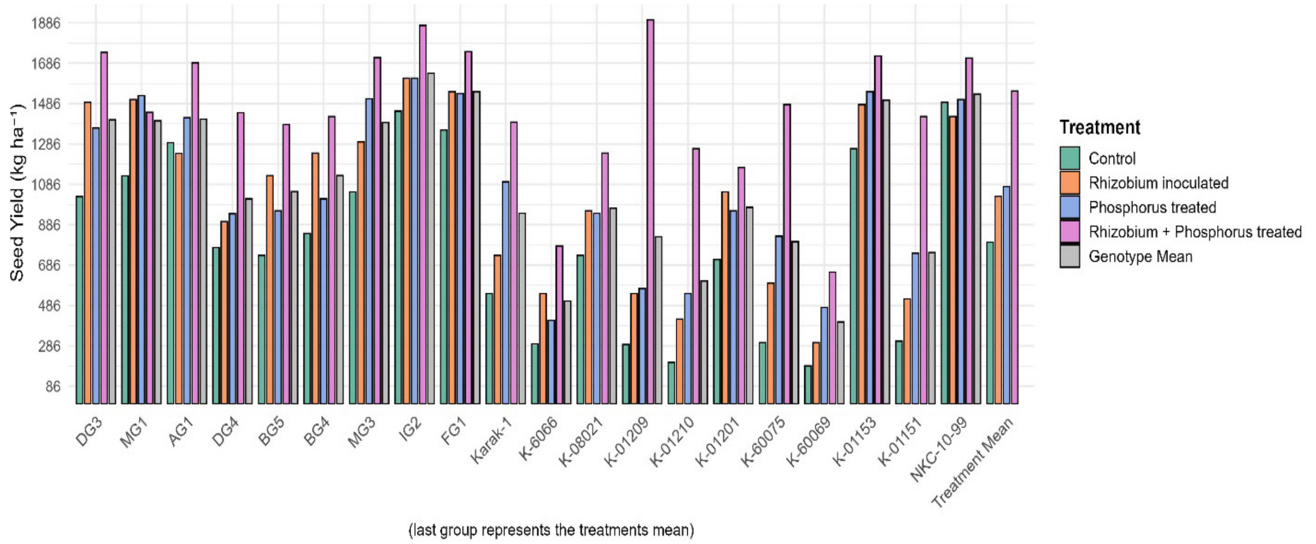

Figure 7: *Mean values of chickpea genotypes for Seed yield Kg ha^{-1} under four different growing conditions at The University of Agriculture Peshawar during 2023-24*

from 404.1 to 1636.1 kgha^{-1}. The genotype IG2 showed maximum seed yield followed by genotype FG1 i.e. 1636.1 and 1544.8 kgha^{-1} respectively. Genotype K-60069 showed the lowest seed yield followed by K-6066 i.e. 404.1 and 508.5 respectively. The genotype × environment interaction means of chickpea genotypes for seed yield varied from 186.6 to 1900.7 kgha^{-1}, the lowest seed yield was noted for genotype K-60069, whereas genotype K-01209 showed maximum seed yield. Environment means across genotypes ranged from 799.4 to 1548.3. Maximum seed yield was observed for combined rhizobium and phosphorus treated environment i.e. 1548.3, while control showed the least seed yield i.e. 799.4 (Figure 7).

Conclusions and Recommendations

The study highlighted the significant impact of rhizobium inoculation, phosphorus application, and their combined effects on growth, yield, and nutrient uptake. Results demonstrated that chickpea traits are significantly influenced by genotype and environmental factors, with notable improvements observed in growing condition with combined application of rhizobium and phosphorus as compared to the other studied environments in this experiment. The combined application of rhizobium inoculum and phosphorus fertilizer showed a synergistic effect, significantly enhancing growth parameters, yield, and nutrient uptake across various genotypes. Genotype K-01209, K-01210, K-08021, NKC-10-99, FG, IG2, AG1, FG1, K-6066 performed better in all the studied growing conditions and are recommended as potential candidates for breeding programs aimed at sustainable agricultural practices.

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Novelty Statement

This study demonstrated the significant impact of rhizobium inoculation, phosphorus application, and their combined effects on chickpea growth, yield, nutrient uptake and biological nitrogen fixation.

Author's Contribution

Hamayoon Khan: Conceptualization, Supervision, visualization
Rozina Gul: Conceptualization, Investigation, Methodology, Supervision
Tahreem Shah: Data collection, writing original draft
Amina Khan: Writing editing and review
Maaz Khan: Formal analysis, validation
Tahir Nadeem: Investigation, Data curation

Generative AI or AI assisted technology statement
The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have no conflict of interest.

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