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Research Article

Screening of Chickpea (*Cicer arietinum L.*) Genotypes Under Water Stress Conditions

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ABSTRACT

Ten genotypes of chickpeas (*Cicer arietinum L.)* were examined in this study for their ability to resist drought in both normal and water stress environments through the evaluation of numerous morpho-physiological characteristics at various growth stages. Genotypes were assessed for characteristics such as plant length, root length, chlorophyll index, number of seeds per plant, pod production, and seed weight under both normal and drought conditions during field experiments at Muhammad Nawaz Sharif University of Agriculture, Multan. Factorial Randomized Complete Block Design (RCBD) with three replications were used in this experiment. The analysis of variance showed highly significant ($p<0.01$) genotypic variations across all traits. Biplot analyses explained 70.6% of the total variation, with Dim-1 representing major yield traits and Dim-2 reflecting plant and root growth, enabling clear differentiation of genotypes. NOOR-2009, TGN-2213 and TGN-2240 consistently aligned with favorable yield-related Traits arrows and showed improved root development, pod production and seed set under stress. Among these, NOOR-2009 exhibited the greatest drought resilience, with only an 11% yield reduction and performed best under normal and drought conditions. This study highlights the importance of selection of drought-tolerant genotypes for breeding programs to enhance climate resilience and to safeguard food security.

Keywords: Abiotic stress, Physiological traits, Morphological traits, Yield performance, Stress response.

INTRODUCTION

Legumes belong to the Fabaceae or Leguminosae family and are well-known for their capacity to fix nitrogen. There are four categories of legume crops: vegetable, oilseed, forage, and grain legumes or pulses (Praharaj, 2020). Legume crops give a balanced diet of carbs, minerals, proteins, and fiber. Small cereals are planted in crop rotation to reduce the frequency of weeds, diseases, and insect pests. It also improves soil fertility by fixing nitrogen for the following cereal crops (Ademe, 2023). In Pakistan, major factors responsible for low production and yield are drought, heat, salinity, and weeds.

Moreover, eroded soils with less nutrition e.g. marginal lands, alkaline soils with low organic matter and erosion), climate change, lack of crop-specific farm machinery, post-harvest losses, and marketing issues are the multiple issues that cause hurdles in chickpea productivity (Ullah, 2020).

Globally, osmotic stress has been identified as the most damaging environmental stress (Reader, 2022). Drought stress causes a 50–70% reduction in productivity. Every year, around 55 million people all over the world are impacted by drought stress (Leng, 2019). Furthermore, it is predicted that it will impact 40% of the world population by 2030 and 700 million people may have to leave their homes due to drought stress and food scarcity.

Chickpea is mostly cultivated by using soil moisture that has been retained from the previous rainy season. As a result, the crop is frequently subjected to severe heat and drought pressure (Asati, 2022). Due to production in resource-poor, semi-arid, and desert areas, drought stress may cause chickpea yields to drop by as much as 50% (Waqas et al., 2019).

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Development of drought-tolerant cultivars is crucial in relation to current scenario of climate change for sustainable agriculture.

Although conventional breeding methods have already improved the chickpea crop, there is still an abundant opportunity for further productivity gains. By applying modern genomic resources with traditional breeding approaches, it is expected that chickpea varieties with resistance to both biological and climatic challenges can be introduced within a relatively short timeframe (Fazeli-Nasab, 2025). Understanding the connections between different physiological reactions to dehydration can help create practical methods to increase chickpeas' resistance to drought stress (Rani, 2020) . Each of these variables requires a significant investment of time and resources to measure. To determine the characteristics that contribute to these genotypes' superior performance under stress conditions and their enhanced drought tolerance, the study was designed to screen chickpeas genotypes under drought stress, and selecting those genotypes that are drought-tolerant and produce better yield to enhance climate resilience and safeguard food security.

Material and methods

Ten chickpea (*Cicer arietinum L.)* genotypes (Table 1) were selected for the study due to their variations in yield performance between irrigated and non-irrigated environments. These genotypes are following, i.e., TGKN- 2203, NOOR- 2009, TGKN-2209, TGN-2202, TGN-2213, GGP-1457, THAL-2020, TGKN-2240, GGP-1482 and TGKN-2220 and listed as G1-G10 in layout design (Table 1). Muhammad Nawaz Sharif University of Agriculture, Multan, experimental field was used as the site for the experiments (Fig.1). The plot size was 300 square feet with well-loam soil properties. Seeds were hand drilled, and each genotype was sown in ten rows with a row-to-row distance of 0.3 m and a plant-to-plant distance of 0.1 m. The experiment was laid out as a Randomized Complete Block Design (RCBD) under factorial with three replications (Table 2). The sowing was performed on 29th November 2023.

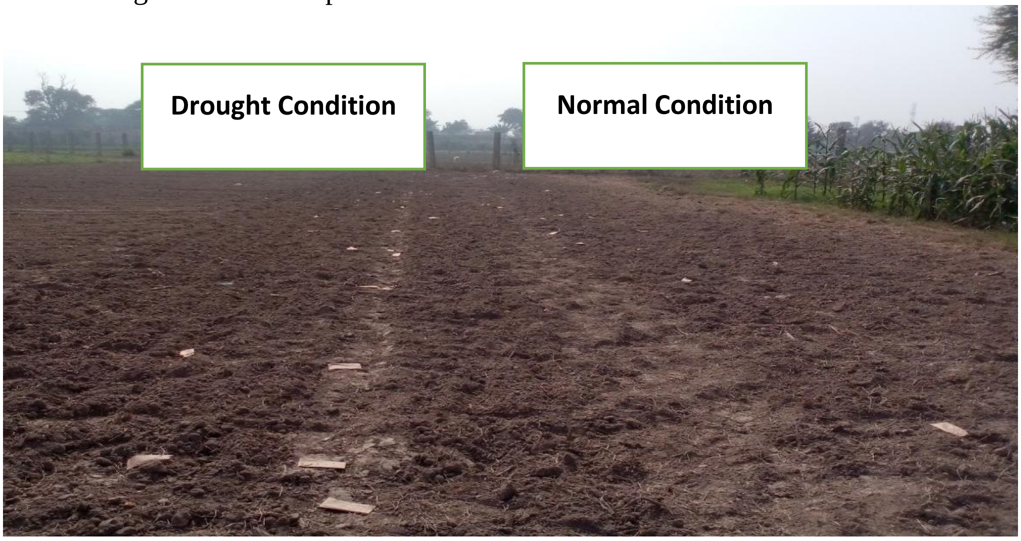


Figure 1. The field area divided into Normal and Drought Conditions at C block MNSUAM.

Light irrigation was applied to support seed germination in both treatments. Irrigation was applied on normal treatment at regular intervals of time, but in stress conditions, no further irrigation was applied. All measurements were done at podding, ripening, and maturation stages in both normal and stress treatments. All genotypes were selected randomly from every replication in each plot to measure the number of seeds per plant, number of pods per plant, plant length, root length, chlorophyll index, and number of seeds per pod. The number of seeds per plant was measured by harvesting at crop maturity. The plant length was measured with a measuring tape from the level of the ground to the plant shoot tip, while root length was measured from the tip to the end of the root. SPAD chlorophyll meter was used for measuring the chlorophyll index (CI).

The number of seeds per plant, the number of seeds per pod, and the number of pods per plant were counted manually for every genotype from both plots. Seed weight was measured by using a weight balance after harvesting. All plants per replication were selected, and readings were taken in the afternoon. The temperature range was 21-26°C at the time of sowing, while 31°C at the time of harvesting.

Table 1. Arrangement of Genotypes according to the field experiment layout. that was Factorial under RCBD. C1 refers to drought condition while C2 is for Normal condition. G refers to genotypes and total 10 genotypes was used in the field experiment. R refers for Replication and r is denoted for rows.

Rows	Drought Condition= C1			Normal Condition= C2		
r 1	C1 G1	C1 G3	C1 G5	C2 G2	C2 G3	C2 G10
r 2	C1 G2	C1 G7	C1 G6	C2 G4	C2 G6	C2 G7
r 3	C1 G8	C1 G4	C1 G9	C2 G6	C2 G8	C2 G4
r 4	C1 G6	C1 G5	C1 G7	C2 G10	C2 G10	C2 G6
r 5	C1 G9	C1 G2	C1 G8	C2 G1	C2 G2	C2 G5
r 6	C1 G4	C1 G10	C1 G10	C2 G3	C2 G1	C2 G8
r 7	C1 G5	C1 G9	C1 G4	C2 G8	C2 G5	C2 G2
r 8	C1 G3	C1 G1	C1 G3	C2 G9	C2 G9	C2 G1
r 9	C1 G7	C1 G6	C1 G1	C2 G3	C2 G7	C2 G9
r 10	C1 G10	C1 G8	C1 G2	C2 G7	C2 G4	C2 G5
Replication	**R1**	**R2**	**R3**	**R2**	**R1**	**R3**

Data Analysis

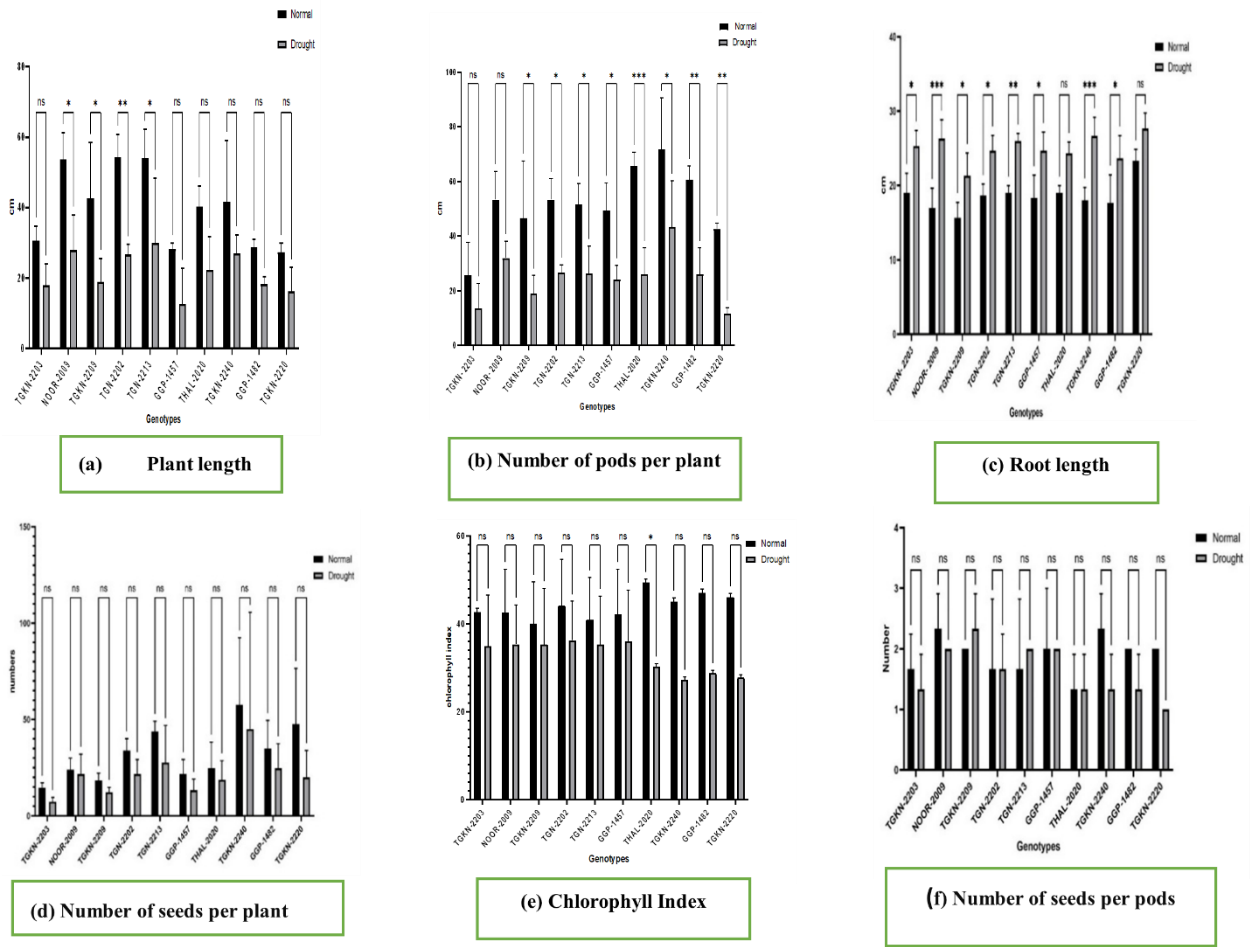
Analysis of variance (ANOVA) was computed for each parameter using GraphPad Prism v2024 to assess variations in genotypes and how they interact. Several traits i.e., plant height, chlorophyll index, number of seeds per plant, number of seeds per pod, number of pods per plant, root length, and seed weight, were recorded for all ten genotypes at the podding, ripening, and maturation stages under normal conditions and drought conditions. Duncan 's Multiple Range test (DMRT) was applied to check the significant differences among pairs of genotypes (Supplementary data). Biplot analyses were computed by using the R language in R Studio software to analyze the relationships among genotypes and traits.

RESULTS AND DISCUSSION

Drought stress at Podding stage

This ANOVA (Fig.2) analyses several key parameters at the podding stage for chickpea genotypes (TGKN-2203 to TGKN-2220) under both normal and drought conditions. ANOVA was utilized to assess the importance of variations among situations, with p-values displayed on connecting lines. In case of plant height, more significant variation was observed TGN-2202 while TGKN-2203, TGKN-2220, GGP-1482, TGKN-2240, THAL-2020 and GGP-1457 behaved non-significantly in both stress and normal conditions. For number of pods per plant, Noor 2009 behaved non-significantly in both conditions however TGKN 2240 developed more pods (70 pods/plant) as compared to other genotypes. In case of drought, THAL-2020 and TGKN-2220 showed more sensitivity to drought and produced less pods as compared to others. Root length is an important parameter in case of drought. It was observed that NOOR-2009 and TGKN-2240 produced longer roots in stress condition as compared to other genotypes. For number of seed, TGKN-2240 produced higher number of seeds. While most genotypes had shorter plants and fewer pods under drought, a few genotypes, like TGKN-2240 and NOOR-2009, showed better drought resilience.

In terms of root length, drought stress led to considerable increases in root length for several genotypes, indicating potential drought resistance, although some genotypes showed no significant difference. The chlorophyll index also demonstrated a clear reduction under drought stress, with the degree of reduction varying by genotype. The two-way ANOVA highlighted significant interactions between genotypes and treatment, showing that different genotypes respond variably to drought stress, suggesting that some genotypes are more resilient than others. Overall, these findings emphasize the importance of genotype selection in breeding programs to improve drought resistance in chickpeas.



(a) Plant length

(b) Number of pods per plant

(c) Root length

(d) Number of seeds per plant

(e) Chlorophyll Index

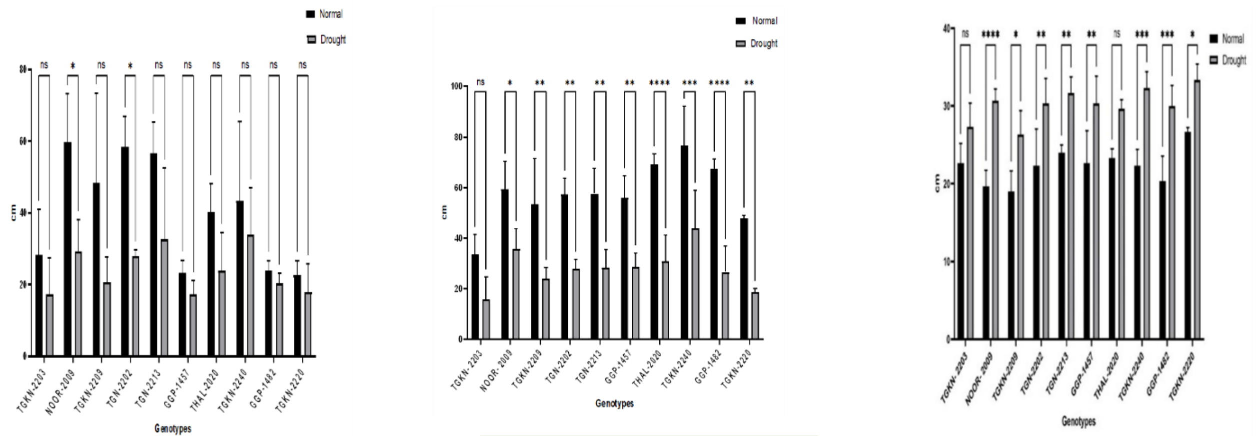
(f) Number of seeds per pods

Figure 2. Graphical representation of ANOVA results of several key parameters at the podding stage for chickpea genotypes (TGKN-2203 to TGKN-2220) under both normal and drought conditions. At the podding stage, **(a)** plant length, **(b)** pod production, **(c)** root length, **(d)** seeds per plant, **(e)** chlorophyll index, and **(f)** seeds per pod were all assessed across 10 genotypes under two treatments: normal (black bars) and drought (gray bars). While ns is denoted for non-significance. While no. of stars shows the level of significance.

Drought Stress at the Ripening Stage

The findings (Fig.3) showed significant variations in several traits, including plant length, number of pods per plant, root length, number of seeds per plant, chlorophyll index, and number of seeds per pod. Drought stress consistently reduced plant length and pod production across all genotypes, with some genotypes, like TGKN-2220, NOOR-2009, and TGKN-2203, demonstrating greater resilience.

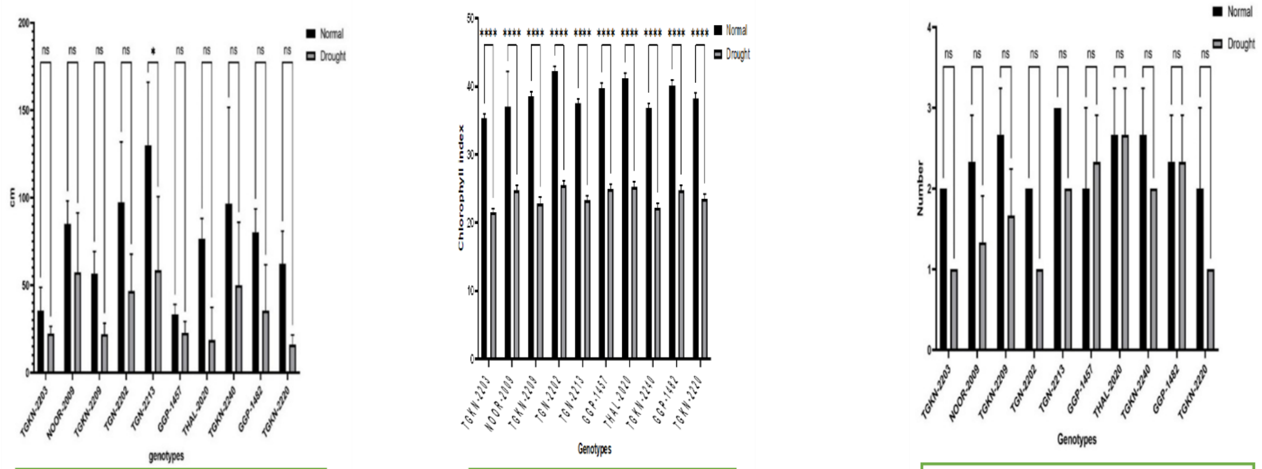
Notably, drought also affected root length, with several genotypes showing significant increase, indicating potential drought resistance. The number of seeds per plant was significantly influenced by both genotype and drought, with some genotypes exhibiting better performance under drought stress. For the chlorophyll index, drought stress led to significant reductions, and the interaction between genotype and drought was also significant, emphasizing the variability in drought responses among genotypes. However, there were no significant differences in the number of seeds per pod between normal and drought conditions, suggesting that drought stress did not affect seed integrity. Overall, the statistical analysis confirmed that drought significantly impacts chickpea traits, and different genotypes show varying levels of resistance, which could inform breeding programs for improved drought tolerance.



(a) **Plant length**

(b) **Number of pods per plant**

(c) **Root length**



d) Number of seeds per plant

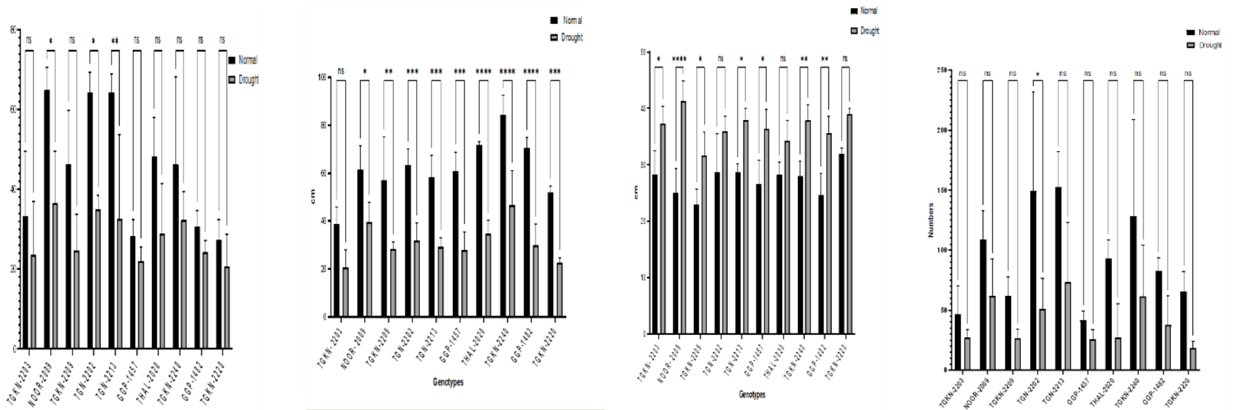
(e) **Chlorophyll Index**

(f) Number of seeds per pods

Figure 3. Graphical representation of ANOVA results of several key parameters at the podding stage for chickpea genotypes (TGKN-2203 to TGKN-2220) under both normal and drought conditions. At the podding stage, **(a)** plant length, **(b)** pod production, **(c)** root length, **(d)** seeds per plant, **(e)** chlorophyll index, and **(f)** seeds per pod were all assessed across 10 genotypes under two treatments: normal (black bars) and drought (gray bars). While ns is denoted for non-significance. While no. of stars shows the level of significance.

Drought stress at the maturation stage

Drought stress consistently reduces plant height, pod production, and seed weight in most genotypes, with some genotypes like NOOR-2009 and TGKN-2203 showing greater resilience. Significant differences were observed (Fig.4) in root length under drought stress, with several genotypes, including NOOR-2009 and TGKN-2203, developing longer roots. For the number of seeds per plant, drought stress significantly decreased seed production in TGKN-2202 but had less effect on other genotypes. The chlorophyll index showed notable reductions under drought conditions, with significant interactions between genotype and condition, indicating different drought responses. Seed weight was reduced under drought for some genotypes, reflecting sensitivity, while others remained resilient, maintaining seed weight despite stress. Overall, these results demonstrate how drought affects chickpea traits, with responses varying across genotypes, highlighting the importance of considering both main effects and genotype-by-condition interactions in breeding drought-resistant chickpea varieties.

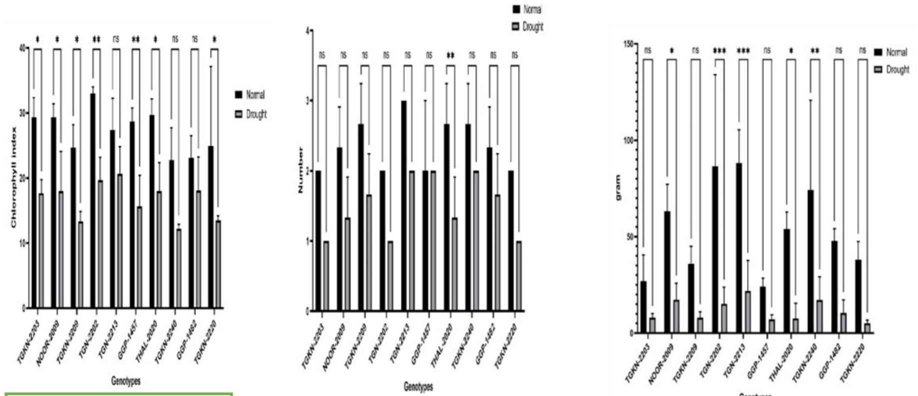


(a) Plant length

(b) Number of pods per plant

(c) Root length

(d) Number of seeds per plant



(e) Chlorophyll index

(f) Number of seeds per pods

(g) Seed weight

Figure 4. Graphical representation of ANOVA results of several key parameters at the podding stage for chickpea genotypes (TGKN-2203 to TGKN-2220) under both normal and drought conditions. At the podding stage, **(a)** plant length, **(b)** pod production, **(c)** root length, **(d)** seeds per plant, **(e)** chlorophyll index, **(f)** seeds per pod, and **(g)** seed weight were all assessed across 10 genotypes under two treatments: normal (black bars) and drought (gray bars). While ns is denoted for non-significance. While no. of stars shows the level of significance.

Biplot Analysis at the Podding Stage

Ten chickpea genotypes were analyzed under both normal and drought conditions using PCA biplot analysis, which highlights the relationships between plant traits and genotypes. 61.9% and 15.7 % of the variance are explained by the two main components, Dim 1 (Horizontal axis) and Dim 2 (Vertical axis) respectively (Fig. 5). Genotype clustering illustrates how closely genotypes positioned on the plot respond to the same conditions. For example, genotypes sharing similar traits may cluster together under normal conditions.

Plant characteristics such as root length and the number of seeds per plant are represented by the red lines. The length and direction of these lines indicate how each parameter influences the genotypes. Parameters that point in the same direction as a genotype suggest a significant correlation. The separation of genotypes along principal components can reveal how different conditions, like normal versus drought stress, impact the genotypes.

In this biplot (Fig.5), Most yield related traits i.e., Number of pods per plant**,** Number of seeds per plant, Number of seeds per pod**,** Chlorophyll index**,** Plant length are contributing in component one. Genotypes on the right side show higher yield components and greater plant length and these are TGN-2209, NOOR-2009, TGN-2213, TGN-2202**.** The Trait contributing more towards vertical axis is root length and Genotypes at the top have longer roots**.** Which showed their drought resilience feature. i.e., TGN-2209 and NOOR-2009 are showing stress tolerance and productivity in terms of longer roots.

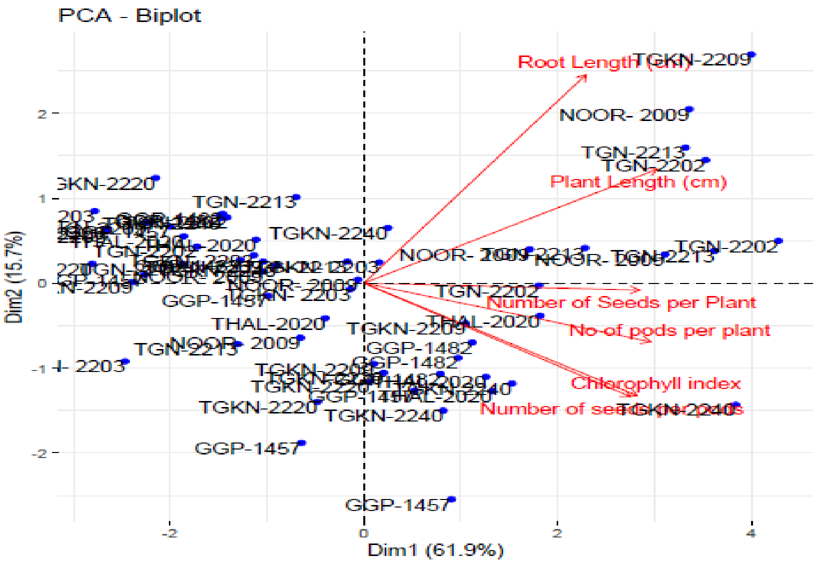


Figure 5. Biplot analyses of 10 chickpea genotypes at podding stage under normal and drought conditions. Number of pods per plant**,** Number of seeds per plant, Number of seeds per pod**,** Chlorophyll index**,** Plant length (cm) these traits strongly contribute in Dim-1 while root length is contributing more towards dim-2.

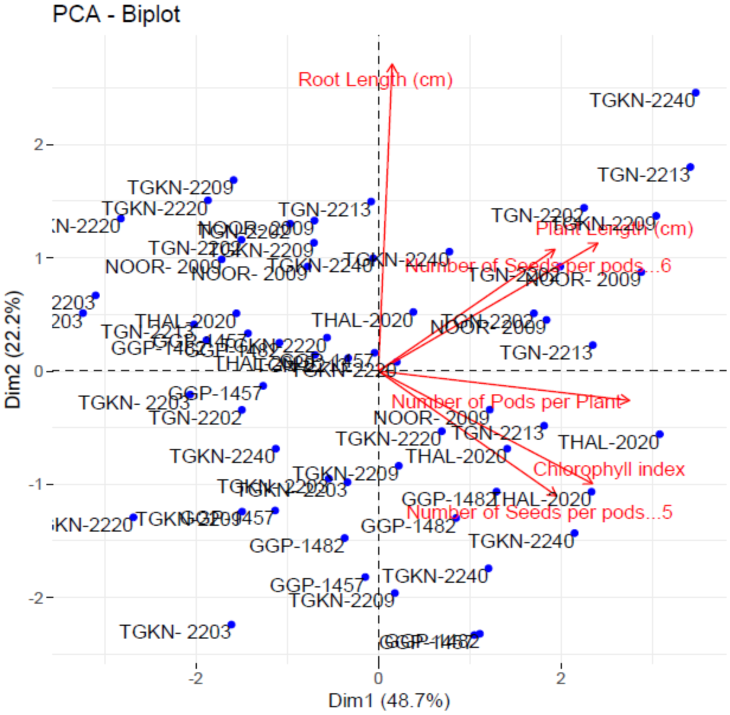


Figure 6. Biplot analyses of 10 chickpea genotypes at ripening stage under both normal and drought conditions.

During the chickpea ripening stage, the PCA biplot (Fig.6) highlights the relationships between plant traits and genotypes in both normal and drought-affected environments. Dim 1 and Dim 2 are the main components, explaining 48.47% and 22.22% of the variance, respectively. Which arrows are close together these show strong positive correlation e.g., Yield related traits are pointing towards more in dim-1 and these are number of pods per plant, number of seeds per pod, chlorophyll index, plant length and number of seeds per plant

Then the genotypes TGN-2213, TGN-2240, TGN-2202, NOOR-2009, TGN-2209 present on the right have higher yield potential and chlorophyll content. While genotypes present on the above top show strong relation with root length arrow and show better drought tolerance. Theses genotypes are TGKN-2240, TGN-2213 and TGN-2209.

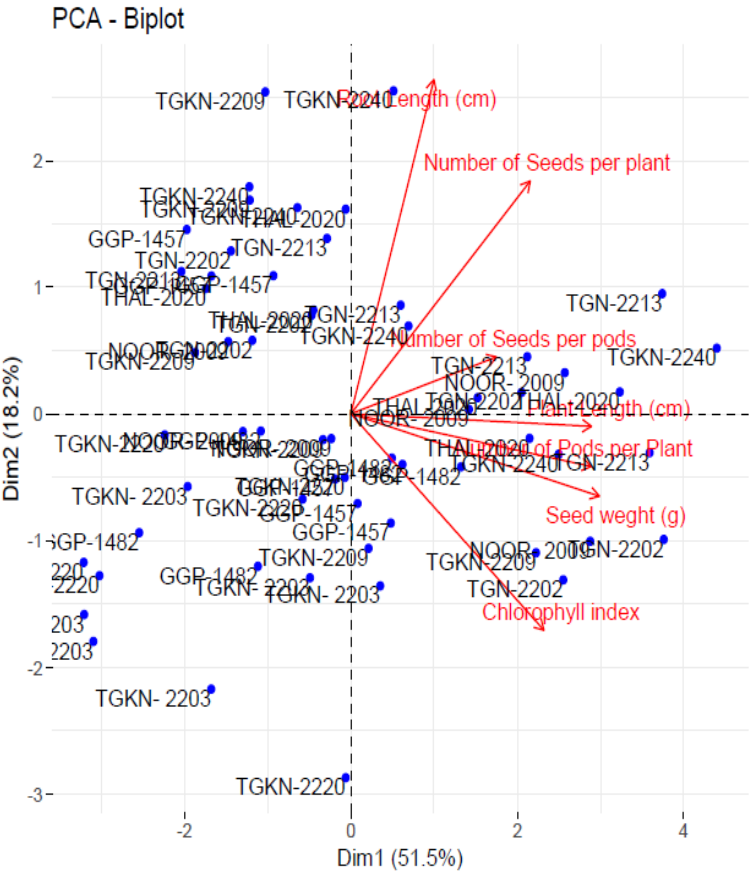


Figure 7. Biplot analyses of 10 chickpea genotypes at ripening stage under both normal and drought conditions

During the chickpea ripening stage, the PCA biplot (Fig. 7) reveals the relationships between plant traits and genotypes in both normal and drought-prone environments. Dim 1 accounts for 51.1% of the variation, while Dim 2 accounts for 18.2%. which traits strongly pointed towards horizontal direction are Number of pods per plant, Number of seeds per pod, Number of seeds per plant, Chlorophyll index, Seed weight (g). these are the yield components and genotypes present on the right side are high yielding. While root length and plant are pointing more towards vertical axis. These genotypes are TGKN-2209, TGKN-2241**,** TGKN-2240 and TGN-2213.

DISCUSSION

Using information from many studies, the discussion is brought in the context of climate change impacts on food crops and sustainable agriculture (Abdullahi, 2024). Chickpeas have a high nutritional value because they include essential dietary such as 15 to 25% protein, fiber, vitamins, minerals and proteins and rich in essential amino acids such as arginine, leucine, isoleucine, lysine, valine, threonine, cysteine, phenylalanine and tyrosine, that provide full nutrition (Kakaei, 2024). Around the world where meat consumption is restricted, chickpea are particularly significant as a source of plant-based protein in low-income source and surroundings (Yan, 2024).

Bioactive compounds in chickpea are also included in chickpea nutritional composition which have the ability to treat chronic illnesses such as diabetes, hypertension and some types of cancer (Haji, 2024). Therefore, chickpeas offer tremendous work as a functional food and as a dietary standby with memorable nutraceutical advantages (Ajay, 2024). Chickpea yield is affected by one important abiotic factor that is drought stress (Hubbard, 2025). According to various studies, chickpeas are primarily grown in rainfed areas are very susceptible to soil moisture deficiency and provide suitable yield under this type of water deficit areas (Sadeque, 2025).

Recent studies from 2023 to 2025 have provided significant understanding into drought stress tolerance mechanism in chickpea through physiological, metabolomic, transcriptomic, and breeding approaches (Slishchuk, 2025). Research revealed key metabolites such as proline, polyamines, and flavonoids associated with osmotic adjustment and oxidative stress defense (Khan, 2025), while tolerant genotypes exhibit higher antioxidant enzyme activity, improved membrane stability, and greater water holding under stress (Fiza, 2025). Field studies identified drought tolerant genotypes maintaining yield stability through efficient resource use and prolonged grain filling (Zhao, 2025). These findings shows that in case of chickpea, drought tolerance is controlled by complex biochemical, physiological, and genetic mechanisms offering a strong foundation for breeding climate-resilient cultivars (Prado, 2025).

This research highlights many important advantages for chickpea improvement and sustainable agriculture. It identifies drought-tolerant genotypes such as NOOR-2009 and TGKN-2203 which maintain higher pod production, yield, and root growth under drought stress. By analyzing morphological and physiological traits the study increase understanding of how chickpeas respond to drought at different growth stages. The findings also support the development of climate resilient and water efficient chickpea varieties, offering to food security in drought prone areas. The use of ANOVA and PCA biplot analysis and DMRT strengthens breeding strategies by identifying key traits and genotype environment interactions, offering valuable guidance for future breeding programs aimed at improving drought tolerance in chickpeas.

In all three biplots during podding ripening and maturation stages, the first component explained almost half of the total variability that was dominated by yield related traits i.e., pods per plants, seeds per pods, total seeds per plant and chlorophyll content. Genotypes including TGN-2213, TGN-2240, TGN-2202, TGN-2209 and NOOR-2009 showed alignment with these yield traits indicated these are high yielding lines. Other component of biplot (Dim-2) contributed 16-22 % variation which was significantly aligned with root length and plant length. It indicated that these genotypes are more stress responsive. The genotypes present near the center of plot showed average performance while those present on negative side of PC1 were associated with low yielding traits. However, the pattern of variability among all three biplots remained consistent that provided the support to identify those lines having good yield potentials along with stress tolerance attributes.

CONCLUSION

By examining various morpho-physiological traits at different growth stages under both normal and drought stress conditions, this study evaluated the performance of ten chickpea genotypes in water-stressed environments. Significant genotype-by-environment interactions were observed, with genotypes such as NOOR-2009 emerging as promising drought-tolerant options, demonstrating an 18% higher seed yield than TGKN-2202, and recommended for cultivation in water-scarce areas. Resilient genotypes maintained better root elongation and chlorophyll content under drought conditions, while most genotypes experienced reductions in plant height, pod production, seed weight, and chlorophyll index. The biplot (70.6%) and Analysis of Variation ($p<0.01$) revealed relationships between genotypes, as well as genotype and trait performance under both normal and drought conditions, indicating that root length and seed production are key traits for screening drought-resistant genotypes. The study underscores the importance of trait screening and genotype selection in developing drought-tolerant chickpea varieties, offering valuable insights for breeding programs aimed at improving chickpea productivity in water-limited environments amidst climate change and water scarcity.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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