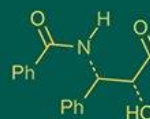
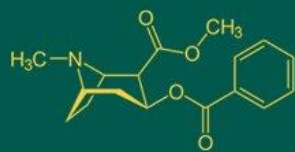


## International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693  
ISSN Online: 2617-4707  
NAAS Rating (2026): 5.29  
IJABR 2026; SP-10(5): 654-658  
[www.biochemjournal.com](http://www.biochemjournal.com)  
Received: 19-02-2026  
Accepted: 21-03-2026

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## Multi-trait evaluation and identification of superior chickpea genotypes under well-watered and water-stressed conditions

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i5Sh.8443>

### Abstract

A diverse panel of 75 chickpea genotypes was assessed under contrasting moisture regimes to identify elite genotypes using multi-trait evidence from phenology, physiology, water use, biomass, and reproductive performance. Thirteen major traits were evaluated, namely days to 50% flowering, days to maturity, pre-flowering water use, post-flowering water use, total water use, total biomass, grain yield, transpiration efficiency, water use efficiency, harvest index, pod weight, pod number, and seed number per plant. Analysis of variance revealed highly significant genotypic differences for all measured traits under both water regimes, confirming the presence of substantial exploitable variability. The highlighted G31, G139, and G8 as top performers under WW conditions, G62 and G156 as top performers under WS conditions, and G107, G165, and G135 as broadly adaptive genotypes useful for drought-resilient breeding.

**Keywords:** Chickpea, drought stress, water use efficiency, transpiration efficiency, principal component analysis, multi-trait selection, genotypic variation, drought resilience, grain yield, terminal drought, harvest index, phenology, biomass, breeding, water-use traits

### Introduction

Chickpea (*Cicer arietinum* L.), a self-pollinating diploid legume ( $2n = 2x = 16$ ) in the Fabaceae family, ranks among the world's top pulse crops for food and nutrition. It's widely grown in arid and semi-arid regions, especially South Asia, Sub-Saharan Africa, West Asia, Australia, and the Americas. Known as chana (Hindi), garbanzo (Spanish), pois chiche (French), or Bengal gram (English), it originated in southeastern Turkey with diversity centers in the Mediterranean Basin, Central Asia, Near East, Indian subcontinent, and Ethiopia (Ali *et al.*, 2005) <sup>[1]</sup>. Today, major production occurs in the Indian subcontinent, US Great Plains, Western Asia, and the Mediterranean. India dominates, producing over 75% of global output (about 13.5 million tonnes annually), with 65-90% from its farmers. In 2023, India's chickpea acreage expanded significantly, up to 50% in some regions and 25% in others.

Chickpea improvement for drought-prone environments requires selection methods that integrate phenology, water-use traits, biomass, and yield attributes rather than relying on grain yield alone. 75 chickpea genotypes were evaluated during rabi 2023-24 under well-watered (WW) and water-stressed (WS) conditions using a Lysi-Field phenotyping platform to identify superior genotypes through a multi-trait selection approach for drought-resilient cultivar development.

### Materials and Methods

A total of 75 chickpea genotypes from the ICRISAT mini-core collection were evaluated under irrigated and terminal drought conditions. The experiment was conducted at the Rain Out Shelter (ROS) lysimetric facility at ICRISAT, Patancheru. Plants were grown in PVC lysimeters (20 cm × 120 cm) filled with Vertisol soil maintained at a bulk density of  $\sim 1.2 \text{ g cm}^{-3}$ . Soil was fertilized with diammonium phosphate (100 mg  $\text{kg}^{-1}$ ). Environmental parameters were recorded using a data logger, and water extraction was monitored by periodic weighing of lysimeters using a load cell system. The study followed a Randomized Complete Block Design (RCBD) with three blocks and five replications.

Plant spacing was 30 cm × 10 cm, maintaining a density of ~25 plants m<sup>-2</sup>. The traits evaluated in the study were grouped into four major categories based on their functional significance and application in selection. Phenological traits, including days to 50% flowering and days to maturity, are important for identifying genotypes with drought escape mechanisms and suitable crop duration for different environments. Water-use traits, such as pre-flowering water use, post-flowering water use, and total water use, help distinguish between conservative and high water-extracting genotypes, particularly under stress conditions. These traits are crucial for understanding how plants utilize available soil moisture throughout their growth stages. Productivity traits, including total biomass, grain yield, pod weight, pod number, and seed number per plant, capture the overall yield potential and reproductive success of genotypes under both well-watered and water-stressed environments. Efficiency

traits, such as transpiration efficiency, water use efficiency, and harvest index, indicate how effectively plants convert water and accumulated biomass into grain yield, making them essential indicators for selecting high-performing and resource-efficient genotypes.

## Results and Discussion

Analysis of variance showed highly significant genotypic variation for all 13 traits under both WW and WS environments, demonstrating strong scope for selection. The thesis also reported that drought stress reduced mean grain yield by 55 percent and total biomass by 47 percent, while pod and seed number per plant declined markedly under WS conditions. In contrast, traits such as water use efficiency and seed weight increased under stress in selected entries, indicating adaptive plasticity and better resource allocation under drought.

**Table 1:** Analysis of variance (One-way ANOVA) for chickpea genotypes under well-water (WW) conditions

| SV  | DOF     | DM      | PRE-FLW-WU | POST-FLW-WU | TWU     | TBM      | GY      | TE     | WUE    | HI       | POD WT  | POD NO   | SEED NO   |
|-----|---------|---------|------------|-------------|---------|----------|---------|--------|--------|----------|---------|----------|-----------|
| GMS | 49.51** | 49.51** | 0.41ns     | 16.19**     | 18.15** | 145.17** | 30.62** | 0.61** | 0.16** | 207.42** | 59.91** | 1490**   | 1446.21** |
| EMS | 3.05**  | 3.05**  | 0.45ns     | 10.52**     | 11.98** | 21.24**  | 4.59**  | 0.32** | 0.04** | 33.61**  | 59.91** | 201.95** | 237.66**  |
| SD  | 4.23    | 4.23    | 0.66       | 3.51        | 3.73    | 7.82     | 3.60    | 0.65   | 0.28   | 9.47     | 4.97    | 24.85    | 25.08     |
| CV% | 3.92    | 1.85    | 23.55      | 30.96       | 25.95   | 16.83    | 26.55   | 26.78  | 32.57  | 20.11    | 22.12   | 23.68    | 28.35     |
| DF  | 74      | 74      | 74         | 74          | 74      | 74       | 74      | 74     | 74     | 74       | 74      | 74       | 74        |

Note: \*\* =  $p < 0.01$ , \* $p < 0.05$ , ns =  $p \geq 0.05$

**Table 2:** Analysis of variance (One-way ANOVA) for chickpea genotypes under water-stress (WS) conditions

| SV  | DOF     | DM      | PRE-FLW-WU | POST-FLW-WU | TWU    | TBM     | GY     | TE     | WUE    | HI       | POD WT  | POD NO   | SEED NO  |
|-----|---------|---------|------------|-------------|--------|---------|--------|--------|--------|----------|---------|----------|----------|
| GMS | 58.26** | 58.26** | 0.12**     | 0.85**      | 0.80** | 15.85** | 7.64** | 0.47** | 0.29** | 318.38** | 12.01** | 430.63** | 452.91** |
| EMS | 2.20**  | 2.20**  | 0.04**     | 0.3**       | 0.3**  | 3.91**  | 0.84** | 0.13** | 0.03** | 32.28**  | 1.38**  | 48.20**  | 43.31**  |
| SD  | 4.50    | 4.50    | 0.25       | 0.69        | 0.68   | 2.98    | 1.74   | 0.49   | 0.34   | 11.15    | 2.19    | 13.08    | 13.23    |
| CV% | 3.39    | 1.77    | 16.58      | 13.37       | 0.11   | 13.57   | 25.12  | 12.73  | 24.05  | 22.71    | 18.16   | 25.52    | 24.47    |
| DF  | 74      | 74      | 74         | 74          | 74     | 74      | 74     | 74     | 74     | 74       | 74      | 74       | 74       |

Note: \*\* =  $p < 0.01$ , \* $p < 0.05$ , ns =  $p \geq 0.05$

(DOF = days to 50% flowering, DM = days to maturity, PRE-FLW-WU = pre flowering water use, POST-FLW-WU = post flowering water use, TWU = total water use, TBM = total biomass, GY = grain yield, TE = transpiration efficiency, WUE = water use efficiency, HI = harvest index, POD WT = pod weight, POD NO = pod number, SEED NO = seed number per plant, GMS = Grand mean square, EMS = Error mean square, SD = Standard deviation, CV% = coefficient of variance percent)

The significant variation across genotypes in both water regimes highlights the differential adaptive strategies and plasticity among the bean accessions. For instance, substantial variation in water-use traits reflects the capacity of certain genotypes to modulate water uptake and utilization in response to soil moisture availability. Similar findings were reported by Sirohi *et al.*, (2008), where significant genotype × environment interactions were observed for drought-related traits in legumes, reinforcing the value of evaluating germplasm across contrasting conditions.

Phenological traits such as days to 50% flowering and days to maturity also showed significant differences, indicating genotypic variation in heat or terminal drought escape potential. Early flowering and maturity are well-recognized drought avoidance mechanisms in common bean and other grain legumes (Polania *et al.*, 2016; Singh *et al.*, 2019) [15, 20]. Meanwhile, biomass and reproductive output (Total biomass, pod weight, seed number per plant, and seed weight) varied markedly among genotypes, supporting the existence of both yield potential and drought resilience sources in the panel.

In summary, the presence of significant genetic variation across all measured traits under well watered and water

stress conditions confirms the potential for targeted selection of genotypes possessing either high productivity under optimal conditions or resilience under stress. The observed variation further supports the integration of physiological and agronomic traits into breeding pipelines to develop ideotypes suited to diverse agro-ecological contexts. Under water stress, grain yield reduced by 55%, as drought strongly constrained final productivity. Total biomass decreased by 47%, reflecting substantially restricted vegetative growth. Pod number and seed number per plant declined markedly, indicating that reproductive sink size was highly sensitive to moisture deficit. Water use efficiency increased in selected genotypes, showing that some used limited water more productively. Seed weight increased in selected entries, suggesting better assimilate partitioning partly compensated under drought.

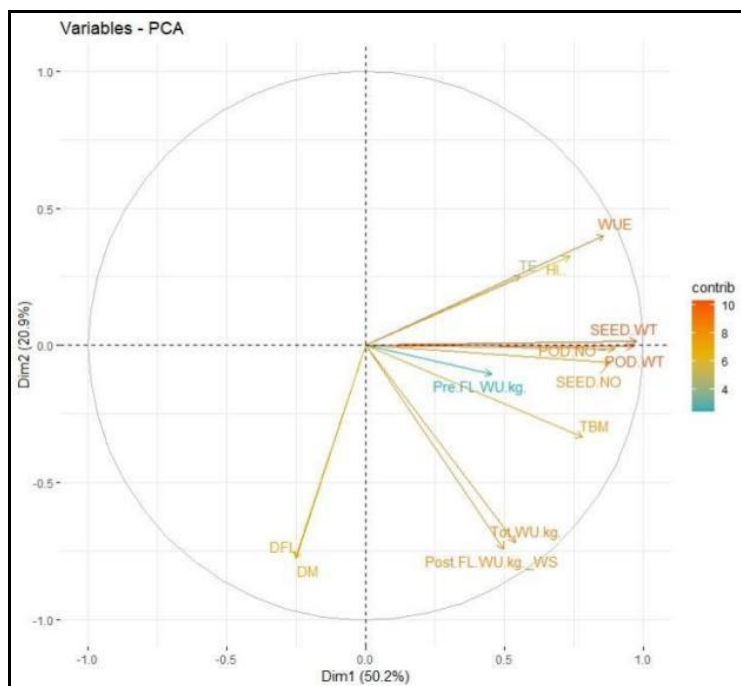
## Principal component analysis

PCA is a strong statistical method for feature extraction, data visualization, and dimensionality reduction is principal component analysis, or PCA. As much of the original variance as feasible is preserved when converting a high-dimensional data set into a lower-dimensional format (Pearson *et al.*, 1901) [14]. In domains where datasets

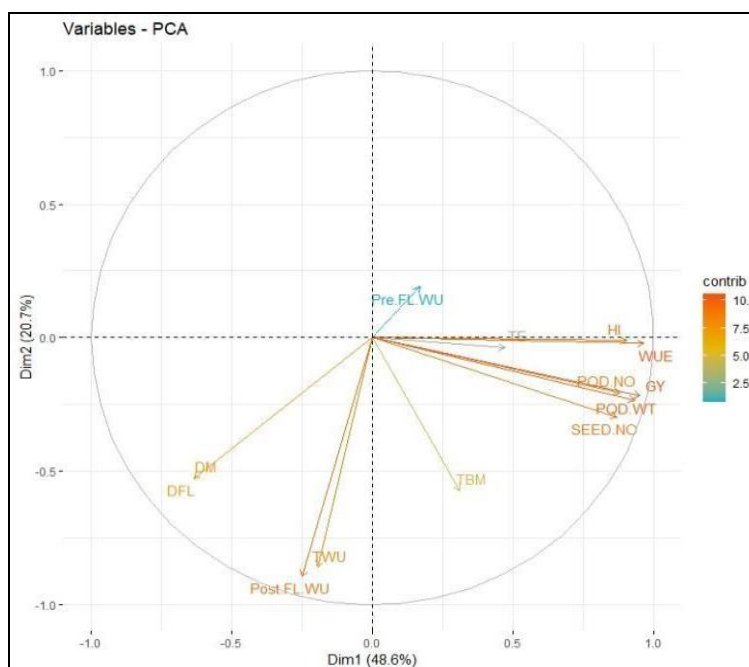
frequently contain numerous connected variables, like machine learning, image processing, genomics, and finance, PCA is frequently utilized. Finding principal components new, uncorrelated variables that are linear combinations of the original variables is the fundamental concept of principal component analysis (PCA). These elements identify the main directions of variation in the data

Principal component analysis reinforced the multi-trait selection framework by showing that the first three principal components explained 75 percent of total variation under

WW and 70 percent under WS conditions. Under drought stress, PC1 explained 48.6 percent of the variation and contrasted delayed flowering and maturity with water use efficiency, pod weight, and harvest index, suggesting that earlier and more efficient genotypes had an adaptive advantage. Under WW conditions, the first three components also captured the majority of trait variability, indicating that a relatively small set of integrated traits was sufficient to discriminate genotype performance.



PCA biplot of chickpea genotypes under Well-Watered conditions



PCA biplot of chickpea genotypes under Water-stress conditions

The principal component analysis (PCA) revealed distinct patterns of variation under different environmental conditions. Under well-watered conditions, the first three principal components explained 75% of the total variation among genotypes. This indicates that most of the variability

was driven by coordinated differences in growth, water use, and efficiency-related traits when moisture was not limiting. Under water-stressed conditions, the first three principal components accounted for 70% of the total variation, suggesting that a relatively smaller set of adaptive traits

governed the majority of differences in drought response among genotypes. Specifically, the first principal component (PC1) alone explained 48.6% of the variation, highlighting the importance of early phenology, improved harvest index, and enhanced water-use efficiency in supporting drought adaptation.

The second principal component (PC2), which explained 20.7% of the variation under water stress, was associated with higher late-stage water use and total water use. These traits were linked to comparatively weaker biomass performance under terminal drought stress, indicating that excessive water use during later growth stages may negatively impact overall plant productivity under such conditions.

Comparing the findings with previous studies such as (Shah *et al.*, 2020) <sup>[19]</sup>, who analyzed chickpea physiological traits under heat stress, a similar pattern emerges. Their PCA indicated that 77% of the genetic variation was explained by traits like seed number per plant, pod number, flowering time, and plant yield—many of which overlap with the current drought-focused analysis. In the present study, under both well-watered and drought-stress environments, a substantial proportion of the total trait variability (75% and 70%, respectively) was captured by the top three components, reaffirming the critical importance of a small set of phenology, water use and yield-related traits in determining genotype performance across contrasting environments (Sivasakthi *et al.*, 2018) <sup>[18]</sup>. These results further emphasize the need to target early flowering, moderate biomass accumulation, and high harvest index in breeding programs aiming for drought resilience. In water-limited environments, selecting genotypes that can efficiently partition available resources towards reproductive structures, rather than merely maximizing vegetative growth or total water use, can enhance productivity and adaptation.

The results clearly show that a multi-trait selection approach because superior genotypes under drought were characterized by coordinated expression of phenology, water-use behavior, reproductive ability, and partitioning efficiency rather than by grain yield alone. Under WS conditions, early or moderate flowering and maturity appear advantageous because delayed genotypes tended to show poorer efficiency and weaker reproductive performance in the PCA interpretation. Likewise, harvest index, pod weight, seed number, and water use efficiency emerged as practical selection indicators because they captured the capacity of genotypes to convert limited water and biomass into economic yield.

The contrast between WW and WS rankings also suggests that environment-specific and broad-adaptation breeding strategies should be pursued simultaneously. G31, G139 and G8 are better suited as donors for favorable-moisture productivity, whereas G62 and G156 are more relevant for drought-prone target environments. Among the most valuable entries, G107, G165 and G135 stand out because they offer broad adaptation across water regimes and thus provide strong parental value for breeding cultivars with stable performance under rainfall uncertainty.

### Identified superior genotypes

The thesis summary reported distinct superior genotypes for WW and WS environments, along with broadly adapted lines valuable across both conditions. These entries are

important for direct selection, crossing programs, and development of stable drought-resilient chickpea cultivars.

The genotypes evaluated in the study were categorized based on their performance under different moisture conditions and their breeding value. Under well-watered conditions, G31, G139 and G8 were identified as the best performers, exhibiting high productivity along with favorable trait expression in non-limiting moisture environments. In contrast, under water-stress conditions, G62 and G156 showed superior performance, indicating strong adaptation and resilience to drought stress. Additionally, G107, G165 and G135 were classified as dual-adaptive genotypes, as they performed consistently well across both well-watered and water-stressed conditions, making them valuable parental lines for improving stability in variable environments.

The study highlighted several key traits and associated genotypes that are important for multi-trait selection in chickpea improvement programs. For grain yield, G165, G135 and G110 were identified as superior genotypes, indicating their strong productivity under contrasting environmental conditions. In terms of water use efficiency, G165 and G60 stood out, demonstrating their ability to utilize water effectively under both drought and irrigated situations.

Total biomass was notably higher in G107 and G44, reflecting their greater vigor and capacity for dry matter accumulation. Similarly, G95 and G107 exhibited higher total water use, indicating differences among genotypes in soil water extraction ability. For post-flowering water use, which is critical for grain filling under terminal drought conditions, G111 and G107 were identified as important genotypes.

Reproductive traits also played a significant role in selection. Pod number per plant was higher in G173, G174 and G107, supporting better reproductive success and ultimately contributing to higher yield. Pod weight was greater in G86 and G107, representing stronger sink capacity. Harvest index, an indicator of efficient biomass partitioning into grain, was higher in G86 and G69. Lastly, transpiration efficiency was notable in G90 and G60, making them useful physiological indicators for drought adaptation.

### Conclusion

For drought-resilient chickpea improvement, the thesis supports selecting genotypes that combine suitable phenology, stronger reproductive sink traits, higher harvest index, and efficient water use under stress. Significant genotypic differences across all 13 traits under both well-watered (WW) and water-stressed (WS) conditions confirm exploitable variability for breeding. Drought reduced grain yield by 55%, total biomass by 47%, and pod/seed numbers markedly, while water use efficiency and seed weight increased in adaptive genotypes, indicating better resource allocation.

Principal component analysis captured 75% variation under WW and 70% under WS, with PC1 (48.6% under WS) linking early phenology, high harvest index, pod weight, and water use efficiency to drought adaptation. Excessive late-stage water use correlated with poorer biomass under stress. The Superior genotypes identified in WW top performers: G31, G139, G8 (high productivity in favorable conditions). WS top performers: G62, G156 (strong drought

resilience). Broadly adaptive: G107, G165, G135 (stable across regimes, ideal parents). Other notables include G165 (grain yield, WUE), G107 (biomass, water use, pods), and G60 (WUE, transpiration efficiency).

Breeding Recommendations are Pursue environment-specific for example G62 for drought and broad-adaptation strategies simultaneously, prioritizing early/moderate flowering, reproductive sinks, harvest index, and efficient water use for ideotype development in variable agro-ecologies. These findings support integrating traits into breeding pipelines for stable, drought-resilient cultivars.

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