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Genetic Diversity Studies for Yield, and Quality Traits using PCA and Cluster Analysis in Timely Sown Chickpea (*Cicer arietinum L.*) Genotypes

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Abstract

Chickpea (*Cicer arietinum* L.) is an important pulse crop that contributes substantially to nutritional security because of its high protein content and adaptability to diverse environments. The present study assessed the extent of genetic diversity among 31 chickpea genotypes evaluated in a randomized block design (RBD) with

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three replications using morphological, physiological and biochemical traits. Multivariate techniques, including principal component analysis (PCA) and cluster analysis, were used to identify the key traits contributing to genetic divergence. PCA revealed that the first three principal components collectively explained 66.79% of the total phenotypic variation, indicating the effectiveness of dimensionality reduction. Traits related to yield components, phenology and physiological responses, such as days to flowering, number of pods per plant, proline content, malondialdehyde (MDA) and protein content, contributed substantially to genetic variability. Cluster analysis grouped the genotypes into three distinct clusters, reflecting considerable genetic heterogeneity, with greater divergence observed between clusters C1 and C3. Genotypes exhibiting higher proline accumulation coupled with lower MDA levels indicated better physiological efficiency, while wide variation in protein content revealed significant nutritional diversity among the genotypes. Based on overall performance across yield, physiological and quality traits, RKGM 20-2, ICCV 191611 and GNG 1958 were identified as promising genotypes for use in future chickpea breeding programmes. The study demonstrates that the combined use of PCA and cluster analysis is an effective approach for identifying genetically diverse and superior genotypes, thereby providing useful information for selecting parents aimed at improving yield, nutritional quality and adaptability in chickpea.

Keywords: Genetic diversity; principal component analysis (PCA); proline; malondialdehyde (MDA); cluster analysis; stress resilience; nutritional quality.

1. Introduction

Chickpea (*Cicer arietinum* L.) is one of the world's most important pulse crops, valued for its high nutritional quality, wide adaptability to diverse agro-climatic conditions and major contribution to global food and nutritional security. It serves as a primary source of dietary protein, particularly in developing countries where plant-based proteins dominate human diets (Jha et al., 2024). Assessment of phenotypic diversity for key morphological and agronomic traits is considered a fundamental approach for identifying superior and elite genotypes in chickpea improvement programmes (Upadhyaya et al., 2003). In addition, chickpea has the unique ability to fix atmospheric nitrogen biologically through symbiotic association, thereby improving soil fertility and contributing to the sustainability of different cropping systems (Gaur et al., 2012). India is the largest producer and consumer of chickpea, contributing nearly 70% of global production and accounting for a major share of the Rabi pulse area in the country (Directorate of Economics and Statistics, 2023). Recent estimates indicate that in India,chickpea is cultivated over approximately 11 million hectares, with a total production of about 11.34 million metric tonnes and an average productivity of around 1180 kg ha^{-1} (ICAR-Indian Institute of Pulses Research [IIPR], 2025). Trade statistics further reveal that India imported about 295 thousand metric tonnes and exported nearly 159 thousand metric tonnes of chickpea during recent years, reflecting its growing importance in domestic and international markets (Directorate of Pulses Development, 2022). Rajasthan is one of the major chickpea-producing states of India and ranks second after Madhya Pradesh, contributing around 18% of the total area and 19% of the national production. In Rajasthan, chickpea is cultivated over nearly 2.0-2.1 million hectares, with a total production of about 2.0-2.1 million metric tonnes and an average productivity of around 1000-1050 kg ha^{-1} (ICAR-IIPR, 2025). Furthermore, in the Humid South Eastern Plain Zone (Zone V) of Rajasthan, chickpea productivity has been reported to be around 1800-1860 kg ha^{-1} under improved production technologies, which is considerably higher than the state and national averages.

Chickpea yield and its component traits are complex, polygenically controlled and significantly influenced by environmental factors. Saxena (2003) reported that timely sowing is crucial because it exposes the crop to favourable climatic conditions across different developmental stages, thereby optimising yield. However, late sowing is frequently encountered because of delayed harvesting of the preceding kharif crop due to delayed onset of the monsoon or specific cropping sequences. This delay exposes the crop to sub-optimal temperature regimes during critical reproductive stages, often resulting in reduced yield and related attributes compared with optimal sowing dates, as late-sown chickpea experiences heat and moisture stresses that limit pod set and grain development. Recent field studies also indicate that high temperature stress and shortened reproductive periods can reduce seed yield in chickpea (Danakumara et al., 2024). Addressing this challenge requires a clear understanding of genetic diversity among chickpea genotypes and the key traits determining yield under varying sowing times. Recent studies emphasise that identifying diverse agronomic traits in germplasm lines is essential for crop improvement (Kumar et al., 2023). Genetic diversity, particularly among yield-contributing characters, is commonly evaluated using principal component analysis (PCA) and diversity analysis. PCA is a robust

multivariate technique for reducing data dimensionality while preserving the most significant variation (Jolliffe, 2002; Jolliffe&Cadima, 2016).

Diversity analysis, conversely, quantifies the extent of genetic variation within and between chickpea varieties, which is essential for effective parent selection in breeding programmes. Recent molecular studies using SSRs, SNPs and genomic tools have highlighted substantial intra- and inter-varietal variability that can be exploited for targeted improvement (Varshney et al., 2021). These statistical and analytical approaches, often supported by multivariate analysis frameworks, are vital for elucidating the mechanisms of abiotic stress tolerance and for developing chickpea genotypes with contrasting and desirable agronomic or physiological/biochemical traits, such as proline and malondialdehyde (MDA) content, particularly under stress conditions such as heat and drought (Hair et al., 2010). Proline accumulation is a general plant response to abiotic stress, acting as an osmotic adjuster and cellular protectant (Ashraf &Foolad, 2007). MDA, which serves as an index of lipid peroxidation, is frequently used as a biomarker for measuring oxidative stress in plants (Del Río et al., 2002). These physiological metrics collectively offer a comprehensive view of genotypic responses to different stress conditions. Phenotypic diversity and cluster analysis remain standard approaches for evaluating yield-related traits in germplasm (Kumar et al., 2023).

Besides yield components, protein content is a paramount quality trait in chickpea that directly determines its nutritional value. As with many crops, chickpea seed protein content is influenced by both genotype and the prevailing environmental conditions during growth (Gowda et al., 2011; Verma et al., 2024). Comprehensive studies on genetic structure and allelic richness help identify these variations (Upadhyaya et al., 2008). Therefore, breeding efforts must simultaneously target the improvement of yield potential, desirable agronomic characteristics and high protein content. Despite the availability of diverse chickpea germplasm, integrated evaluation of yield, physiological and quality traits under timely sown conditions remains limited for identifying parents suited to the Humid South Eastern Plain Zone of Rajasthan. The overall objective of this study was to comprehensively investigate yield, morpho-physiological and quality traits across various chickpea genotypes under timely sown conditions using PCA and phenotypic diversity analysis. This research aims to provide a deeper understanding of the critical factors associated with high yield and stress resilience, including agronomic traits, proline content, MDA content as an indicator of membrane lipid integrity and protein levels, which are instrumental in strengthening future chickpea breeding programmes.

2. Materials and Methods

The field experiment was conducted during Rabi 2022-2023 at the Research Farm, Agricultural Research Station, Ummedganj, Kota, Agriculture University, Kota. The experimental site represents clay loam soil (Vertisols) located in the South Eastern Humid Plain Zone (Zone V) of Rajasthan, with average rainfall of 700 mm. The experimental material comprised 31 diverse chickpea (*Cicer arietinum* L.) genotypes procured from the All India Coordinated Research Project (AICRP) on Chickpea, Agricultural Research Station, Ummedganj, Kota. These genotypes were selected to represent a broad genetic background for comprehensive evaluation. The experiment was sown on 15 November 2022 following a randomized block design (RBD) with three replications. Each genotype was planted in four rows of 4 m length, with crop geometry of 30 × 10 cm. All recommended package of practices were followed to raise a healthy crop. Observations were recorded on five randomly selected competitive plants from each plot in each replication for morpho-physiological traits, namely number of nodules per plant (NP), nodule weight per plant (NWP, g), plant height (PH, cm), total branches per plant (BP), total pods per plant (PP), pod length (PL, cm), number of seeds per pod (NSP), 100-seed weight (SW, g), biological yield per plant (BY, g), seed yield per plant (SY, g) and harvest index (HI, %). Data on days to 50% flowering (DF) and days to maturity (DM) were recorded on a plot basis, while protein content (PC, %), malondialdehyde (MDA) and proline content were estimated from seed after harvest. Protein content was estimated following the methodof Lowry et al. (1951) ; proline content was determined using the method described by Bates et al. (1973); and lipid peroxidation was assessed by estimating MDA content using the procedure outlined by Goyal and Asthir (2010). Statistical analysis was performed using SPSS version 25.0 (or R version 4.2.1) and Indostat software. To account for the different units of measurement across morphological and biochemical traits, the data were standardised (mean = 0, variance = 1) before performing PCA to ensure that each variable contributed equally to the analysis.

Table 1. List of thirtyone chickpea genotypes and details of their source

S. No.	Genotypes	Pedigree	Source
1	RKGM 20-1	GL 28143 X GJG 0918-2	AICRP on Chickpea (ARS, Kota)
2	RKGM 20-2	GJG 0731 X Phule G 00108	AICRP on Chickpea (ARS, Kota)
3	ICCV 191611	JG 11 X ICCV 05013	ICRISAT, Hyderabad (T.S.)
4	RKG 21-10	ICCC 37 X K 1189	ICRISAT, Hyderabad (T.S.)
5	RKG 22-5	PC 1 X RKG 143	AICRP on Chickpea (ARS, Kota)
6	RKGD 21-2	Pusa 547 X (JG 11 X Pusa 372)	IARI, New Delhi
7	RKG 13-378	BGD 112 X BG 1105	AICRP on Chickpea (ARS, Kota)
8	RKG 21-3	ICCV 95333 X ICC 5270	AICRP on Chickpea (ARS, Kota)
9	RKG 13-501	ICCV 06109 X Phule G 00110	AICRP on Chickpea (ARS, Kota)
10	RKGD 17-7	ICCV 14103 X BGD 72	IARI, New Delhi
11	ICCV 201203	ICCV 93954///ICCV 93954// ICCV 96029/ICCV 93954	ICRISAT, Hyderabad (T.S.)
12	ICCV 191612	JG 16 X ICCV 05013	ICRISAT, Hyderabad (T.S.)
13	RKG 22-13	PC 1 X JG 2000-87	AICRP on Chickpea (ARS, Kota)
14	RKG 21-1	JG 11////JG 11///JG 11//ICC 5810/JG 11	ICRISAT, Hyderabad (T.S.)
15	ICCV 15111	(ICCV 37 X ICCV 94954) X ICC 4958	ICRISAT, Hyderabad (T.S.)
16	RVG 202 (C)	(JAKI 9226 X DCP 20) X JC 412	RVSKVV, Gwalior (M.P.)
17	RVG 203 (C)	(ICCV 91902 X ICCV 10) X ICCV 89230	RVSKVV, Gwalior (M.P.)
18	GNG 1958 (C)	GNG 1365 X SAKI 9516	SKRAU, Bikaner (Raj.)
19	GNG 2171 (C)	GNG 63 X BG 1044	SKRAU, Bikaner (Raj.)
20	GNG 2144 (C)	CSJG 901 X CSG 8962	SKRAU, Bikaner (Raj.)
21	Phule G 0405 (C)	Digvijay X WCG 2000-2	MPKV, Rahuri (M.H.)
22	GCP 101 (C)	GCP 2 X ICCV 2	JAU, Junagadh (Guj.)
23	JG 16 (C)	ICCV 4 X ICCV 10	JNKVV, Jabalpur (M.P.)
24	CSJ 515 (C)	FG 712 X CSJ 146	SKNAU, Jobner (Raj.)
25	RKG 13-515 (C)	GNG 469 X IPC 2729	AICRP on Chickpea (ARS, Kota)
26	RKG 22-26	Mutant of PC 1	AICRP on Chickpea (ARS, Kota)
27	RKG 22-27	Mutant of PC 1	AICRP on Chickpea (ARS, Kota)
28	RKG 22-28	GAG1107 X NBeG 738	AICRP on Chickpea (ARS, Kota)
29	RKG 22-29	ICC 16232 X GJG 0814	ICRISAT, Hyderabad (T.S.)
30	RKG 22-30	GNG 1581 X RKG 13-180	AICRP on Chickpea (ARS, Kota)
31	RKG 22-31	GNG 1581 X RKG 13-180-7	AICRP on Chickpea (ARS, Kota)

3. Results

ANOVA: The analysis of variance (ANOVA) revealed highly significant differences ($p < 0.01$) among all 31 genotypes for all the studied traits, indicating substantial genetic variability in the experimental material and justifying the choice of experimental material.

3.1 Principal Component Analysis

Table 2. Eigen values and explained variance of principal components (PC1–PC16)

Principal Component	Eigen Value	Percentage of Variance (%)	Cumulative Percentage (%)
PC1	4.6473	29.05	29.05
PC2	3.3174	20.73	49.78
PC3	2.7224	17.02	66.79
PC4	1.2194	7.62	74.42
PC5	1.1182	6.99	81.40
PC6	0.9183	5.74	87.14
PC7	0.7001	4.38	91.52

Principal Component	Eigen Value	Percentage of Variance (%)	Cumulative Percentage (%)
PC8	0.4559	2.85	94.37
PC9	0.2950	1.84	96.21
PC10	0.2531	1.58	97.79
PC11	0.1263	0.79	98.58
PC12	0.0822	0.51	99.10
PC13	0.0592	0.37	99.47
PC14	0.0451	0.28	99.75
PC15	0.0217	0.14	99.88
PC16	0.0184	0.12	100.00

Table 3. Importance of principal components (PCA)

Component	Standard Deviation	Proportion of Variance	Cumulative Proportion
PC1	2.1558	0.2905	0.2905
PC2	1.8214	0.2073	0.4978
PC3	1.6500	0.1701	0.6680
PC4	1.1043	0.0762	0.7442
PC5	1.0574	0.0699	0.8141
PC6	0.9583	0.0574	0.8714
PC7	0.8367	0.0438	0.9152
PC8	0.6752	0.0285	0.9437
PC9	0.5431	0.0184	0.9621
PC10	0.5030	0.0158	0.9779
PC11	0.3554	0.0079	0.9858
PC12	0.2866	0.0051	0.9910
PC13	0.2434	0.0037	0.9947
PC14	0.2124	0.0028	0.9975
PC15	0.1474	0.0014	0.9989
PC16	0.1358	0.0012	1.0000

Table 4. Factor loadings of traits for the first three principal components (PC1–PC3)

Trait	PC1	PC2	PC3
DF	0.0558	-0.4355	0.3092
NP	-0.3081	0.1564	0.3002
WNP	-0.3216	0.1307	0.3669
DM	0.0339	-0.4090	0.3131
PH	-0.1502	-0.1334	0.2655
BP	-0.2056	-0.3692	-0.1433
PP	-0.2608	-0.3126	-0.2009
PL	-0.0719	0.3970	0.0181
NSP	-0.0427	-0.1299	-0.3279
SW	-0.2285	0.3711	0.0289
BY	-0.3751	-0.0278	-0.2848
SY	-0.3753	-0.0517	-0.2987
HI	0.2912	-0.0308	-0.4000
PC	0.0398	-0.1576	0.0881
PROLINE	0.3482	0.0701	0.0341
MDA	-0.3408	-0.0578	-0.0491

DF - Days to 50% flowering, NP - No. of nodules/plant, NWP – Nodule Weight/plant (g), DM - Days to maturity, PH - Plant height (cm), BP - Total branches/plant, PP - Total pods/plant, PL - Pod length(cm), NSP - Number of Seeds/pod, SW - 100-seed weight (g), BY - Biological yield/plant(g), SY - Seed yield/plant(g), HI - Harvest Index(%),PC - Protein content(%).Proline and Malondialdehyde (MDA).

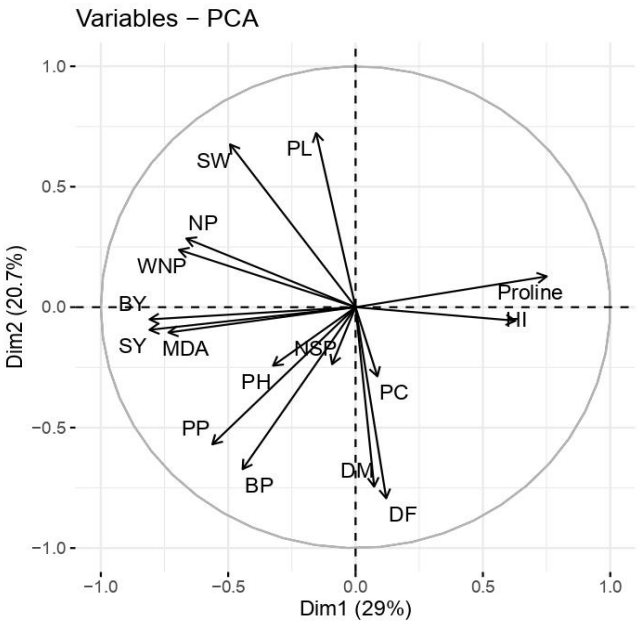

Fig. 1.Principal component analysis (PCA) biplot of yield and quality traits in chickpea genotypes under timely sown conditions

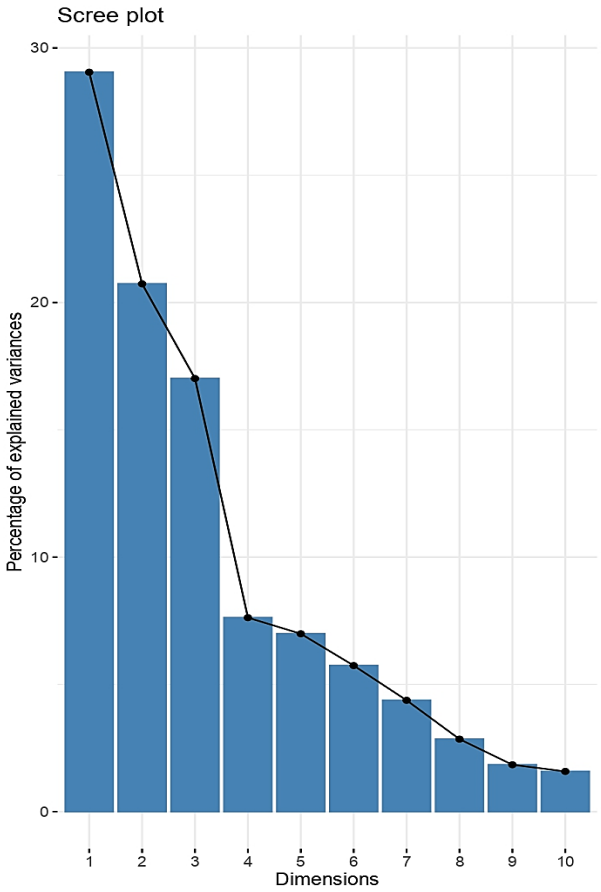

Fig. 2.Scree Plot Showing Percentage of Explained Variance by Principal Components in Chickpea Genotypes Analysis

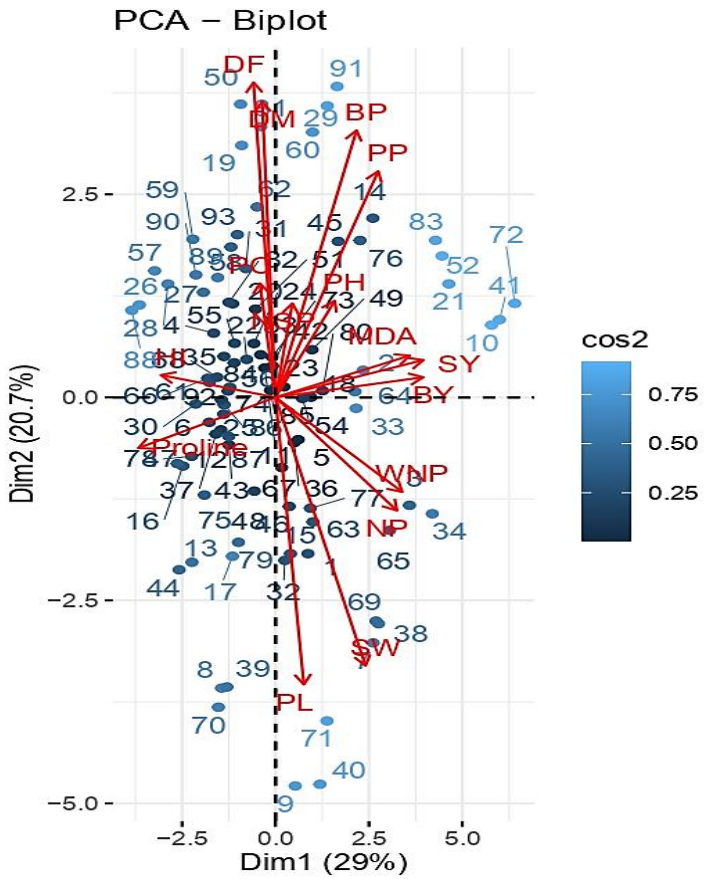

Fig. 3. Principal Component Analysis (PCA) Biplot for Morpho-Physiological and Nutritional Traits in Chickpea Genotypes

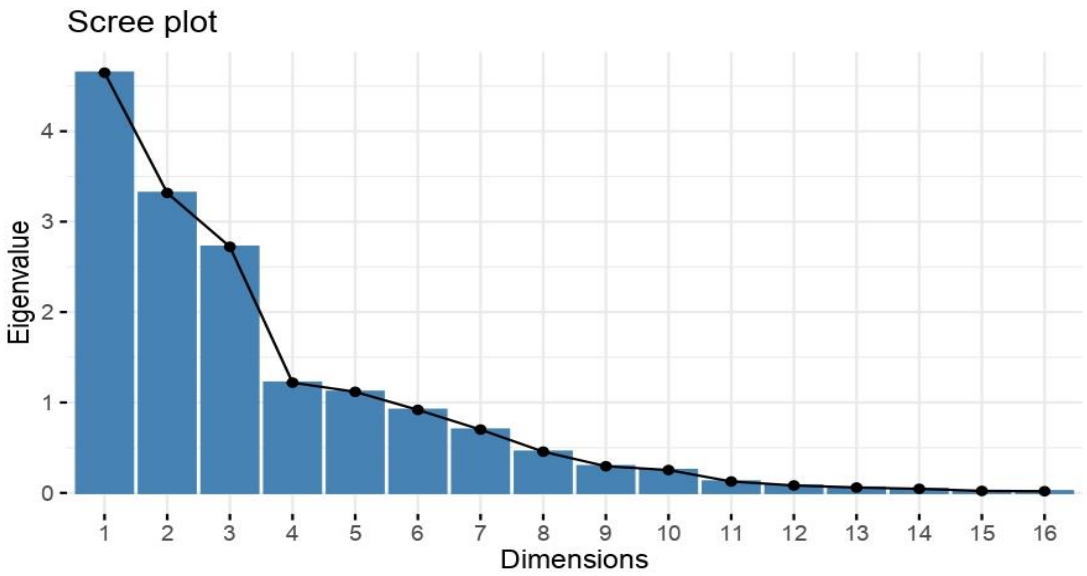

Fig. 4. Scree Plot Showing Eigenvalues of Principal Components for Chickpea Traits Analysis

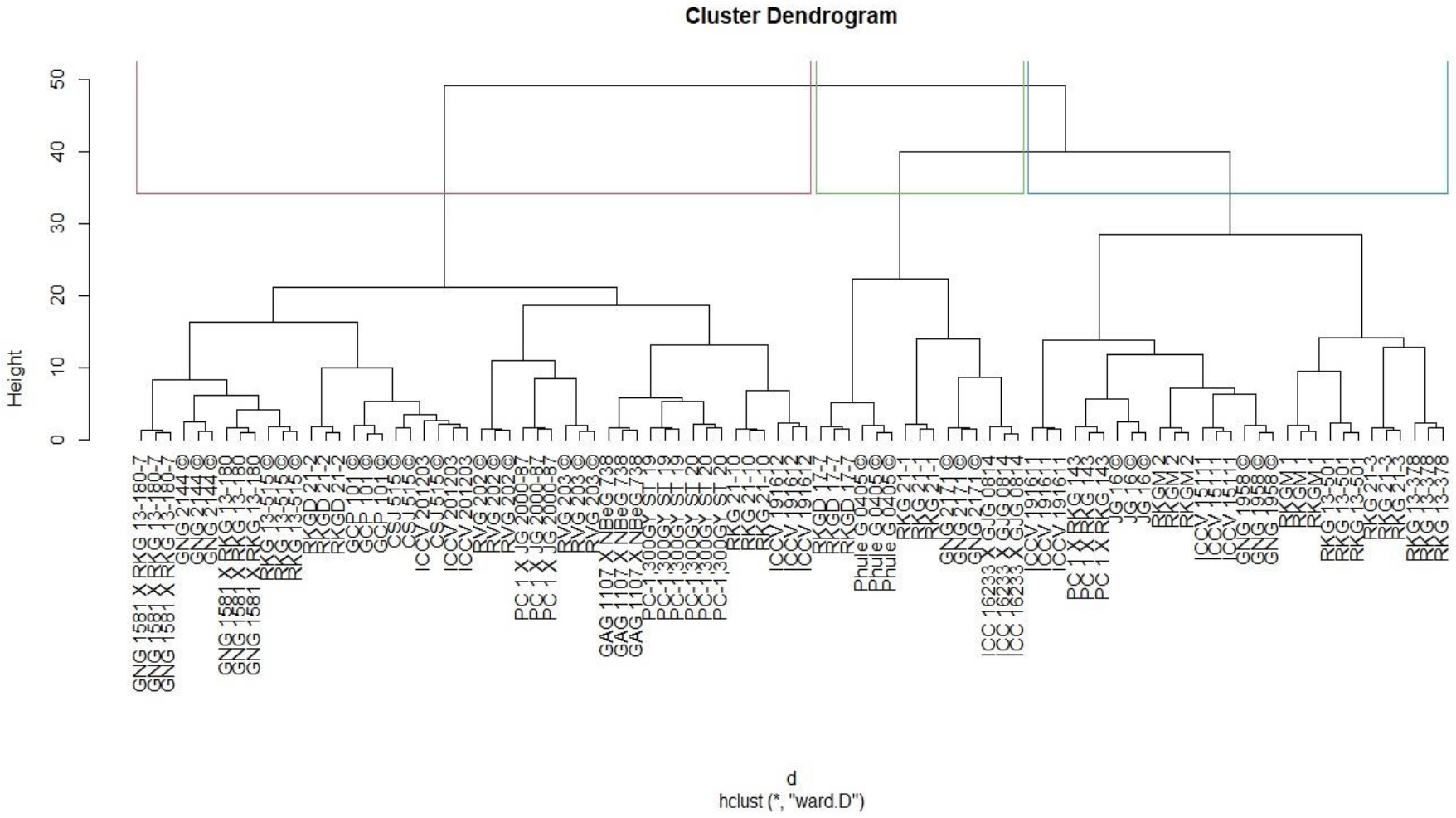

Fig 5. Hierarchical Cluster Analysis of Chickpea Genotypes Based on Morphological and Physiological Traits

3.2Results and Analysis of Diversity

Table 5. Intra-cluster distances of clusters

Cluster	Complete distance	Average distance	Centroid distance
C1	8.2856	4.9411	3.4942
C2	7.0533	4.2398	3.0018
C3	9.0774	4.8028	3.4185

Note: Intra-cluster distances indicate variability within each cluster.

Table 6. Inter-cluster distances (average linkage method)

Cluster	C1	C2	C3
C1	0.0000	5.6249	6.6276
C2	5.6249	0.0000	6.5006
C3	6.6276	6.5006	0.0000

Note: Inter-cluster distances show divergence between clusters. Higher value = more distinct clusters.

Table 7. Cluster sizes (based on 31 genotypes)

Cluster	Number of genotypes
C1	11
C2	10
C3	10
Total	31

Note: Number of genotypes in each cluster is based on the total sample size of 31 genotypes.

4. Discussion

4.1 Principal Component Analysis (PCA)

Principal component analysis revealed substantial multivariate variation among the 31 chickpea genotypes evaluated under timely sown conditions. Out of sixteen, five components exhibited more than 1.00 Eigen value (Table 2). However, only the first three principal components (PCs) were retained and given due importance for further explanation based on the Scree plot analysis (Fig. 4). The scree plot displayed a distinct semi-curve line which showed that after the third PC, very little variation was observed in each subsequent component. Retaining these three components successfully maintained statistical parsimony while explaining a substantial 66.79% of the total phenotypic variation, indicating that a limited number of components were sufficient to explain most of the variability present in the experimental material. Similar proportions of variance explained by the first few components have been reported in recent chickpea diversity studies, highlighting the effectiveness of PCA in summarising complex trait relationships (Kumar et al., 2023; Jha et al., 2024).

The factor loading matrix (Table 4) was evaluated to identify the individual trait contributions, considering loading values greater than 0.300 as significant. The high contribution of yield-related traits (pods per plant, seed yield and biological yield), phenological traits (days to flowering and maturity) and physiological attributes (proline and MDA) to the major principal components suggests that these traits play a decisive role in governing genetic divergence among genotypes. This indicates that yield performance in chickpea is not regulated by a single trait but by an integrated response of morphological, physiological and biochemical parameters. Recent studies have similarly emphasised the combined role of yield and adaptive traits in determining the performance of chickpea genotypes under field conditions (Thapa et al., 2022; Verma et al., 2023).

4.2 Genetic Diversity and Cluster Analysis

Cluster analysis was performed following Mahalanobis D^2 statistics to assess genetic divergence among the chickpea genotypes (Mahalanobis, 1936). Cluster analysis grouped the 31 chickpea genotypes into three distinct clusters, indicating considerable genetic divergence within the studied material. The existence of multiple

clusters suggests that the genotypes originated from diverse genetic backgrounds and possess differential adaptive potential. Similar clustering patterns have been reported in recent chickpea diversity assessments using multivariate techniques (Kumar et al., 2023; Jha et al., 2024).

4.3 Intra-cluster Diversity

Intra-cluster distance analysis revealed variable levels of genetic heterogeneity within clusters. Cluster C3 exhibited the highest intra-cluster distance, indicating greater variability among its constituent genotypes, whereas clusters C1 and C2 showed relatively lower but substantial internal variation. Higher intra-cluster distances reflect the presence of diverse genotypes within a cluster, offering scope for selecting superior lines even without inter-cluster hybridisation. This observation aligns with recent findings that clusters with higher internal variability often harbour genotypes with contrasting yield attributes (Verma et al., 2023).

4.4 Inter-cluster Diversity

Inter-cluster distance analysis demonstrated pronounced genetic divergence among clusters, with the maximum distance observed between clusters C1 and C3, followed by C2 and C3. High inter-cluster distances indicate wide genetic divergence, suggesting that crosses between genotypes belonging to these clusters may produce transgressive segregants and broaden the genetic base. This principle has been supported in modern chickpea breeding literature, where inter-cluster hybridisation is considered a key strategy for genetic improvement (Thapa et al., 2022; Kumar et al., 2023).

The fairly balanced distribution of genotypes across clusters further suggests that the experimental material adequately represents the existing genetic diversity, thereby strengthening the reliability of the diversity estimates obtained in the present study.

4.5 Physiological and Biochemical Basis of Diversity

The inclusion of physiological (proline and MDA) and biochemical (protein content) traits provided deeper insight into the adaptive and nutritional diversity among the genotypes. Proline accumulation is widely recognised as a stress-responsive trait associated with osmotic adjustment and cellular protection, while MDA content serves as an indicator of lipid peroxidation and oxidative damage.

Genotypes such as ICCV 191611, RKGM 20-2, GNG 1958 and RKG 13-378 exhibited comparatively higher proline accumulation coupled with moderate to lower MDA levels, indicating superior physiological stability even under normal timely sown conditions. Such genotypes are considered physiologically efficient and better equipped to tolerate environmental fluctuations, as supported by recent chickpea stress physiology studies (Negussu et al., 2023; Jha et al., 2024).

Protein content varied significantly among genotypes, highlighting notable biochemical diversity. Genotypes such as RKGM 20-2, ICCV 191611 and GNG 1958 recorded higher protein content along with good seed yield, suggesting the possibility of simultaneous improvement of nutritional quality and productivity. Recent reports have emphasised the importance of integrating quality traits into chickpea breeding programmes to meet nutritional security goals (Jha et al., 2024; Verma et al., 2024).

4.6 Implications for Chickpea Improvement

The combined interpretation of PCA, cluster analysis and physiological-biochemical traits clearly demonstrates that genetic diversity in chickpea is multidimensional. Genotypes showing superiority across multiple traits and belonging to divergent clusters, particularly RKGM 20-2, ICCV 191611 and GNG 1958, emerged as promising parental lines. These genotypes can be used effectively in hybridisation programmes to develop high-yielding, nutritionally rich and physiologically resilient chickpea cultivars.

5. Conclusion

The present investigation revealed substantial genetic diversity among the evaluated chickpea genotypes, as evidenced by multivariate analyses involving principal component analysis and cluster analysis. The first three principal components together accounted for 66.79% of the total variation, indicating that a limited number of traits were sufficient to explain most of the phenotypic variability present in the material.

Cluster analysis grouped the genotypes into three distinct clusters, confirming wide genetic divergence. Higher inter-cluster distances, particularly between clusters C1 and C3, suggested that genotypes belonging to these clusters are genetically diverse and may serve as potential parents for hybridisation programmes aimed at generating superior recombinants.

Physiological and biochemical traits such as proline, MDA and protein content contributed significantly to genetic differentiation. Genotypes exhibiting higher proline accumulation with relatively lower MDA levels indicated better physiological efficiency, while wide variation in protein content highlighted nutritional diversity. The concurrent expression of high yield, physiological stability and protein content in genotypes such as RKGM 20-2, ICCV 191611 and GNG 1958 underscores their potential utility in chickpea improvement programmes.

Overall, the integrated assessment of morphological, physiological and biochemical traits through multivariate approaches proved effective in identifying genetically diverse and superior genotypes. These findings provide a valuable foundation for future breeding strategies aimed at enhancing yield, nutritional quality and adaptive potential of chickpea under diverse agro-climatic conditions.

6. Limitation

The study was conducted in a single season and location under timely sown conditions, which may limit broader environmental interpretation. The evaluation was based on phenotypic, physiological and biochemical traits; therefore, multi-location, multi-season testing and molecular marker validation would strengthen the reliability of genotype selection and confirm the stability of the identified promising lines.

Declaration of AI Use

This manuscript was prepared through the combined contributions of all author(s), including contributions to the study design, data, content development, results, interpretation, and related scholarly work. The author(s) acknowledge the use of Grammarly and ChatGPT to assist with grammar checking, language refinement, reference formatting. These AI-assisted tools were not used as authors and did not replace the intellectual contributions or scholarly judgment of the author(s). All AI-assisted outputs, including content, references, and interpretations, were carefully reviewed, revised, verified, and approved by the author(s). The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

Authors have declared that they have no known competing financial interests OR non-financial interests, OR personal relationships that could have appeared to influence the work reported in this paper.

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