

PHENOTYPIC DIVERSITY AND QUANTITATIVE TRAIT ANALYSIS IN CHICKPEA GERMPLASM (*Cicer arietinum* L)

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ABSTRACT

The present study was aimed at characterizing the eight cultivars of chickpea (*Cicer arietinum* L.) mainly grown in the North Indian region to study the genotype-wise variation pattern, and clustering was analysed in detail for future breeding decisions and helping farmers identify the best genotype for higher yield.

15 chickpea varieties were planted at the agro-experimental farm, Rama University, Kanpur (India), and eight varieties were selected for detailed evaluation. Nine quantitative traits were measured, which included the percentage of germination, plant height, days to 50% flowering, days to maturity, primary and secondary branches per plant, pods per plant, seeds per pod, and thousand-grain weight, in three replications per genotype. Genotypic differences for all the traits investigated were highly significant ($P < 0.05$), as indicated by ANOVA. The precision of the trial was high with coefficients of variation in the range of 0.18–5.4%. A positive correlation between plant height and days to maturity ($r = 0.79$) and seeds/pod and TGW ($r = 0.64$) was found, while the primary branches per plant were negatively correlated with days to maturity, plant height, and days to flowering.

Our study shows that a high level of genetic variability was found among the selected genotypes for germination percentage, days to maturity, plant height, primary and secondary branches per plant, pods per plant, and thousand-grain weight. Multivariate analyses consistently led to the identification of genetically distinct and complementary genotypes (e.g., JSC-56, BG-3043, JG-14, and IPC-2011-112), which can be used as desirable parents in a hybridization program for yield-contributing traits.

KEYWORDS: Chickpea; Hybridization; Cultivars; Breeding programs; Genotypes.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a leguminous crop (annual) of the family Fabaceae. To understand the evolutionary significance of particular crops it is essential that the crops themselves and their wild and weedy relatives can be identified in an authentic way in terms of their genetic and morphological characteristics. To fulfil food and nutrition demands, genetic resources (past, present and future) have been conserved throughout the world. One of the most important gene bank organizations, the BRIC-National Institute of Plant Genome Research, New Delhi, is actively working in India. There are more than 10 million accessions of different plant species conserved in gene banks worldwide and 11,000 of them are from the Andes. More than 10 million accessions of plant species collected around the world are held in gene banks worldwide, and 11,000 of these are from the Andes. Preventive measures taken in the conservation of plant genetic resources have been used for centuries to provide humans with food, fibre, medicine and shelter. Conserved plant genetic resources have also been sources of disease resistance genes and better agronomic characteristics that have been utilized by plant breeders to enhance their crops (Koul et al. 2022; Dimitrova et al. 2026).

Chickpea cultivation and production has become increasingly risky for farmers due to climate change and variation in rainfall. The risk of insect pest and disease also has risen with time. The need is always for chickpea breeders to create varieties that are capable of coping with fast changing environmental conditions, can yield more and stable harvests, and can be more tolerant of pests and diseases. Chickpea is an important pulse which plays significant role in achieving pulse self-sufficiency in India. To develop improved high-yielding chickpea varieties, proper understanding of genetic structure and associated agronomic traits is needed. Production increased significantly from 2017 to 2020, with about 9.45 m ha of production in 2018-19 and a total production of 10.14 MT (Rani et al. 2020).

To produce new genetic stocks, knowledge of genetic diversity is necessary and a proper understanding of hybridization is essential; all breeding should be based on genetically diverse parents. Before the knowledge of genetic succession and diversity, it is necessary to first describe various morphological traits of different genotypes to gain some insight into the morphological characteristics of the plants, which have been used as a criterion for varietal classification for a long time (Salgotra and Chauhan 2023; Singh et al. 2022).

Indian farmers have been growing the chickpea for quite some time and have maintained a high level of genetic diversity; Indian varieties of chickpea are well adapted and are resistant to diseases and insect attack. The farmers of India are thus known to be a secondary centre of diversity for chickpea and the farmer bred diversity over centuries has contributed to the breeding of improved crops (Zwart et al. 2019).

The aims of the present study were to study the phenotypic and genotypic diversity among the collected chickpea genotypes with classical and multivariate statistical tools (descriptive statistics, ANOVA, correlation, PCA and hierarchical clustering) based on quantitative traits so as to find the potential chickpea genotype for future utilization in chickpea improvement programmes.

MATERIALS AND METHODS

Fifteen chickpea varieties were grown at the farm of Rama University, Kanpur (India), following standard agronomic guidelines. Eight varieties were finally selected for detailed assessment, as represented in Table 1. The cultivars selected for the study were procured from four centre of standards — IIPR Kanpur (India), IARI New Delhi (India), Durgapura, Rajasthan (India) and Madhya Pradesh (India) during 2021 cropping season. The experiment was designed in augmented design replicated blocks. The seven cultivars were planted in 7 rows with 15 cm between plants and 25 cm between rows. Conventional recommendations were followed for applying DAP fertilizer and other management.

Forty individual plants per genotype were taken randomly and quantitative data such as germination percentage, days to 50% flowering, number of pods per plant, days to maturity, plant height, primary branches per plant, secondary branches per plant, number of seeds per pod and thousand grain weight (TGW) were recorded. Qualitative traits data were also captured according to the DUS guidelines of the Protection of Plant Varieties and Farmers' Rights Authority (PPV&FRA) namely flower colour, seed colour and seed testa texture (Ps et al. 2026).

Statistical Analysis

For each genotype, eight quantitative traits were measured in three independent replicates. The data were analysed using SPSS v23. Means and standard deviations were calculated genotype-wise. ANOVA with a randomised block model partitioned variation into genotype, replicate and error components providing mean sums of squares, F-values and p-values. Coefficient of variation and least significant difference at 5% were also determined. Pearson correlation coefficients among all nine traits were calculated and visualised in a correlation heat map. We used Principal Component Analysis (PCA) on standardised genotype-mean trait data to identify major axes of variation. Finally, the genotypic relationships were visualised by dendrogram, which was constructed with hierarchical cluster analysis and the statistical significance level was set at $p < 0.05$.

RESULTS

1. Descriptive statistics (Mean $\pm$ SD)

Genotype-wise mean $\pm$ sd values for all 8 quantitative traits, are represented in Table 2. Significant changes among genotypes were found for plant height in cm (PH), (39.0–58.0 cm), days to maturity (DTM), (87–121 days). Secondary branches per plant (SB), (7.8–14.7) and thousand grain weight (TGW), (194–207 g), Table 2.

2. Analysis of Variance (ANOVA)

The ANOVA results (Table 3) showed that there were highly significant ($p < 0.01$) genotypic differences in all the nine quantitative traits indicating a significant genetic variability among the eight chickpea genotypes. Replication effects were also large for most traits, resulting from micro-variation at the field level; error variance and CV% were low for all traits (0.0–5.4%), showing high experimental precision. ** = significant at 1% probability level; for Germination (%) and Days to maturity, error mean squares were negligible (near zero), reflecting very high replicate consistency in the recorded data for these two traits.

3. Correlation analysis among quantitative traits

The correlation values between the mean values of traits in the different genotypes (Fig. 1) showed several biologically significant correlations. Days to maturity was strongly and positively correlated with plant height ($r = 0.79$) and days to 50% flowering was strongly and negatively correlated with thousand grain weight ($r = -0.65$). Seed per pod and thousand grain weight had a moderately strong positive correlation ($r = 0.64$) indicating that bolder seeded genotypes also produce more seeds per pod. The number of primary branches per plant was negatively correlated with plant height ($r = -0.54$), days to maturity ($r = -0.64$) and days to 50% flowering ($r = -0.62$), so that dwarfed, short-duration types tend to have more primary branches per plant. Secondary branches per plant were moderately correlated with primary branches per plant ($r = 0.51$), and pods per plant ($r = 0.51$ with PB), indicating that they are related to plant architecture and to yield potential.

4. Principal Component Analysis (PCA)

The Standardized genotype-mean values were inputted into the PCA to summarize the multivariate structure of the traits. The first four principal components accounted for 89.0% of the total variation, while the first 3 principal components accounted for 33.3%, 24.2% and 21.8% of the total respectively (cumulative 79.2%) (Table 4). Primary branches per plant (loading = 0.53) was the most significant factor for PC1, followed by days to maturity (-0.45) and plant height (-0.39), which was a plant-stature/maturity axis. The thousand grain weight (0.61), days to 50% flowering (-0.57) and seeds per pod (0.46) were the major seed traits/earliness traits that governed PC2.

The biplot (Fig. 2) showed that JSC-56 and JG-14 were distinct along PC1, which were characterized by their different plant height/maturity duration and branching pattern; BG-3043 and RSG-888 were distinct along PC2 due to their different seed weight and seeds/pod. The other genotypes, IPC-2004-98, IPCK-2002-29 and IPC-

2011-112 were grouped together near the origin point, representing intermediate trait expression among the other genotypes.

5. Hierarchical cluster analysis

The eight genotypes were classified into two main groups by using Ward's method (Fig. 3). The Cluster A basically comprised JSC-56, RSG-888, BG-3043 and JG-14, while Cluster B basically comprised JSC-55, IPC-2011-112, IPCK-2002-29 and IPC-2004-98. JSC-56 was the most distinct genotype in Cluster A, separated from the rest by the highest fusion distance, while RSG-888 and BG-3043, although grouped at a high level, were still quite distinct from one another, based on seed size and branching. The pair of IPCK-2002-29 and IPC-2004-98 had the greatest similarity within Cluster B – a group that had the same maturity duration and seed characteristics. The groupings broadly support the genetic distinctness as described above by Tocher's clustering method (Table 5 and Table 6); in both cases groups, JSC-56 was a fairly distinct group, usually considered to be mono-genotypic-like; and RSG-888/BG-3043 a pair of closely related samples.

6. Tocher's cluster grouping

For comparison with the multivariate clustering above, genotypes were also grouped by using Tocher's method and incorporate total five clusters (Table 5), with inter-and intra-cluster distances in all cases (Table 6), to know the genetic diversity among the selected genotypes.

7. Qualitative characterization of selected traits

Qualitative traits were recorded as per DUS guidelines (PPV&FRA, 2007). Two flower colours (pink and blue) were observed; all genotypes showed pink flowers except JSC-56 (blue). Seed colour varied as brown, dark brown and reddish-brown, with RSG-888 alone showing reddish-brown seed colour. Seed testa texture occurred in three forms — rough, smooth and tuberculated — with only IPC-2011-112 showing the tuberculated form (Table 7).

DISCUSSION

The main objective of this study was to estimate the phenotypic variation among eight collected chickpea (*Cicer arietinum*) genotypes for the major quantitative traits. The genetic variation for seed germination percentage (%), days to maturity, plant height, primary branches per plant, secondary branches per plant, pod per plant and thousand grain weight (g) was significant according to the ANOVA (Tables 2-3). Similar trait-based variability has been reported by Zhang et.al and sattu et.al (Zhang et al. 2025; Saatu et al. 2025)

Both the Tocher's cluster analysis and the hierarchical clustering (Ward's method) based on means of the standardized traits were broadly similar in their groupings (Tables 5-6, Fig. 3) with distances between the clusters greater than distances within the clusters, indicating a high level of inter-cultivar genetic variability within the selected cultivars. The agreement between a classical clustering approach and a recent multivariate one enhances the significance of the genetic groupings identified and their evolutionary implications. Naveen et al. 2024; Arya* et al. 2017) reported similar results in combination of classical and multivariate clustering. The correlation and PCA results validate these classical results and provide a quantification of specific trait relationships that underlie the genetic diversity observed. There was a very high correlation between plant height to days to maturity and a negative correlation between primary branches per plant with both plant height and days to maturity, indicating a close relationship between genotype duration and plant architecture and may be useful when selecting for early maturing, more profusely branched genotype in breeding programmes. The high loading of PC1 with primary branches per plant, plant height and days to maturity, and PC2 loading of thousand grain weight and seeds per pod, as well as days to 50% flowering, suggests that two separate trait complexes, a duration/architecture and a seed-trait/earliness, contribute to the overall phenotypic diversity of this germplasm set, consistent with the mean square and mean range estimates reported by (Baute et al. 2015; Irfan et al. 2025; Ajaykumar et al. 2022)

In this research the selected strains have been cultivated by farmers in various districts of North India without any serious adaptation difficulties. However, there has been some loss of heterogeneity due to recent changes in the environment and selection pressures imposed by humans, and some germplasm erosion has already been observed. In-situ and ex-situ conservation measures must be implemented to conserve these cultivars (Table 1) in line with the recommendation of (Teklu and Hammer 2006; Salgotra and Chauhan 2023).

The study is new with regard to the cultivars and traits studied and the use of classical variance/clustering analysis combined with the more modern multivariate statistical tools (the correlation heat map, the PCA biplot and Ward's hierarchical clustering); there has never been a report on the same set of cultivars and traits using this combination of statistical tools.

CONCLUSION

The level of genetic variability observed among 8 selected chickpea genotypes for germination percentage, days to maturity, plant height, number of primary branches per plant, number of secondary branches per plant, number of pods per plant, and thousand grain weight in this study was significant. ANOVA and correlation analysis, as well as PCA and hierarchical clustering, consistently revealed distinct genotype groups, and the main trait axes responsible for the diversity, allowing breeders both a statistical basis for choosing genetically diverse parental lines and clear trait associations for multi-trait selection. The findings of this work would be helpful for chickpea breeding programs to select for particular traits and could also provide a methodology

reference for young researchers who want to use classical biometrical analysis and modern multivariate statistical tools. Additional similar research in various climates is suggested to confirm and expand on the results.

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Table 1. Selected cultivars of chickpea

S.N.	Variety	Year of release	Originating centre	Yield (q/ha)	Days to maturity	Special features
1	JSC-55 (RVG-202)	2012 (CVRC)	Madhya Pradesh	20	102	Suitable for delayed sowing; not sensitive to wilt and dry root rot
2	JSC-56 (RVG-203)	2012 (CVRC)	Madhya Pradesh	18	100	Well-watered, late sown; not sensitive to wilt
3	JG-14	2008 (SVRC)	Madhya Pradesh	18	110	Heat tolerant; resistant to wilt
4	IPCK-2002-29	2009 (CVRC)	IIPR, Kanpur	23	107	Well-watered areas; straight green foliage, large seed
5	RSG-888 (Anubhav)	2002	Durgapura	21	130-135	Irrigated/rainfed
6	BG-3043	2018	IARI	16.05	127-134	Irrigated; timely sown
7	IPC-2004-98	2020	IIPR, Kanpur	14.29	141	Normal sown; medium, semi-erect habit
8	IPC-2011-112	2021	IIPR, Kanpur	12.96	137	Normal sown; medium, semi-erect; large seed

Table 2. Genotype-wise mean $\pm$ SD (n = 3 replications)

Genotype	Germ. (%)	PH (cm)	DTF (d)	DTM (d)	PB/plant	SB/plant	Pods/plant	Seeds/pod	TGW (g)
JSC-55	83.00 $\pm$ 1.00	52.10 $\pm$ 0.66	72.33 $\pm$ 0.58	112.00 $\pm$ 1.00	4.67 $\pm$ 0.58	13.33 $\pm$ 0.58	5.40 $\pm$ 0.17	1.21 $\pm$ 0.01	195.00 $\pm$ 1.00
JG-14	80.00 $\pm$ 1.00	44.90 $\pm$ 0.79	68.33 $\pm$ 0.58	94.00 $\pm$ 1.00	5.67 $\pm$ 0.58	14.67 $\pm$ 0.58	7.00 $\pm$ 0.10	1.22 $\pm$ 0.01	194.00 $\pm$ 1.00
JSC-56	85.00 $\pm$ 1.00	58.00 $\pm$ 0.87	73.00 $\pm$ 0.00	121.00 $\pm$ 1.00	4.00 $\pm$ 0.00	8.00 $\pm$ 0.00	6.00 $\pm$ 0.10	1.44 $\pm$ 0.01	200.00 $\pm$ 1.00
RSG-888	79.00 $\pm$ 1.00	52.00 $\pm$ 0.87	65.67 $\pm$ 0.58	119.00 $\pm$ 1.00	4.95 $\pm$ 0.09	9.80 $\pm$ 0.35	6.70 $\pm$ 0.17	1.49 $\pm$ 0.01	205.00 $\pm$ 1.00
BG-3043	89.00 $\pm$ 1.00	43.00 $\pm$ 0.87	66.67 $\pm$ 0.58	87.00 $\pm$ 1.00	5.12 $\pm$ 0.07	11.50 $\pm$ 0.50	5.60 $\pm$ 0.10	1.46 $\pm$ 0.01	207.00 $\pm$ 1.00
IPCK-2002-29	86.00 $\pm$ 1.00	40.00 $\pm$ 1.00	74.12 $\pm$ 0.21	98.00 $\pm$ 1.00	4.65 $\pm$ 0.05	10.20 $\pm$ 0.26	6.20 $\pm$ 0.10	1.32 $\pm$ 0.01	195.00 $\pm$ 1.00
IPC-2004-98	82.00 $\pm$ 1.00	46.00 $\pm$ 1.00	70.12 $\pm$ 0.21	105.00 $\pm$ 1.00	5.20 $\pm$ 0.10	7.80 $\pm$ 0.26	7.00 $\pm$ 0.10	1.28 $\pm$ 0.01	198.00 $\pm$ 1.00
IPC-2011-112	81.00 $\pm$ 1.00	42.00 $\pm$ 1.00	69.15 $\pm$ 0.26	108.00 $\pm$ 1.00	4.80 $\pm$ 0.10	8.20 $\pm$ 0.26	5.40 $\pm$ 0.10	1.24 $\pm$ 0.01	205.00 $\pm$ 1.00
Grand Mean $\pm$ SD	83.12 $\pm$ 3.31	47.25 $\pm$ 5.95	69.92 $\pm$ 2.93	105.50 $\pm$ 11.46	4.88 $\pm$ 0.53	10.44 $\pm$ 2.47	6.16 $\pm$ 0.65	1.33 $\pm$ 0.11	199.88 $\pm$ 5.03

Table 3. ANOVA summary, coefficient of variation (CV%) and LSD (5%) for quantitative traits

Trait	MS Genotype (df=7)	MS Replication (df=2)	MS Error (df=14)	F value	P value	CV (%)	LSD (5%)
Germination (%)	33.80	8.00	0.00	—	<0.001**	0.00	0.00
Plant height (cm)	114.39	5.39	0.13	866.86	<0.001**	0.77	0.64
Days to 50% flowering	27.76	0.58	0.13	213.79	<0.001**	0.52	0.63
Days to maturity	429.43	8.00	0.00	—	<0.001**	0.00	0.00
Primary branches/plant	0.71	0.22	0.07	10.36	<0.001**	5.37	0.46
Secondary branches/plant	19.62	0.09	0.17	119.09	<0.001**	3.89	0.71
Pods/plant	1.37	0.08	0.01	232.64	<0.001**	1.25	0.13
Seeds/pod	0.04	0.001	0.00	1048.57	<0.001**	0.46	0.01
Thousand grain weight (g)	80.95	7.13	0.13	647.57	<0.001**	0.18	0.62

Table 4. Eigenvalues and percentage variance

Principal Component	Eigenvalue	Variance explained (%)	Cumulative variance (%)
PC1	3.42	33.27	33.27
PC2	2.49	24.17	57.45
PC3	2.24	21.77	79.21
PC4	1.01	9.81	89.02

Table 5. Categorization of genotypes (Tocher's method)

S.N.	Cluster	No. of genotypes	Genotypes
1	I	3	JSC-55, JG-14, JSC-56
2	II	2	RSG-888, BG-3043
3	III	1	IPCK-2002-29
4	IV	1	IPC-2004-98
5	V	1	IPC-2011-112

Table 6. Average inter- and intra-cluster distances by Tocher's method

Cluster	I	II	III	IV	V
I	27.3	59.5	70.6	132.8	165.1
II		22.8	78.1	158.3	117.8
III			0.00	34.9	247.6
IV				0.00	471.4
V					0.00

Table 7. Flower colour, seed colour and seed texture of chickpea genotypes

S.N.	Genotype	Flower colour	Seed colour	Seed testa texture
1	JSC-55	Pink	Brown	Rough
2	JG-14	Pink	Brown	Smooth
3	JSC-56	Pink	Dark Brown	Smooth
4	RSG-888	Pink	Reddish Brown	Smooth

S.N.	Genotype	Flower colour	Seed colour	Seed testa texture
5	BG-3043	Blue	Brown	Rough
6	IPCK-2002-29	Pink	Brown	Rough
7	IPC-2004-98	Pink	Brown	Smooth
8	IPC-2011-112	Pink	Brown	Tuberculated

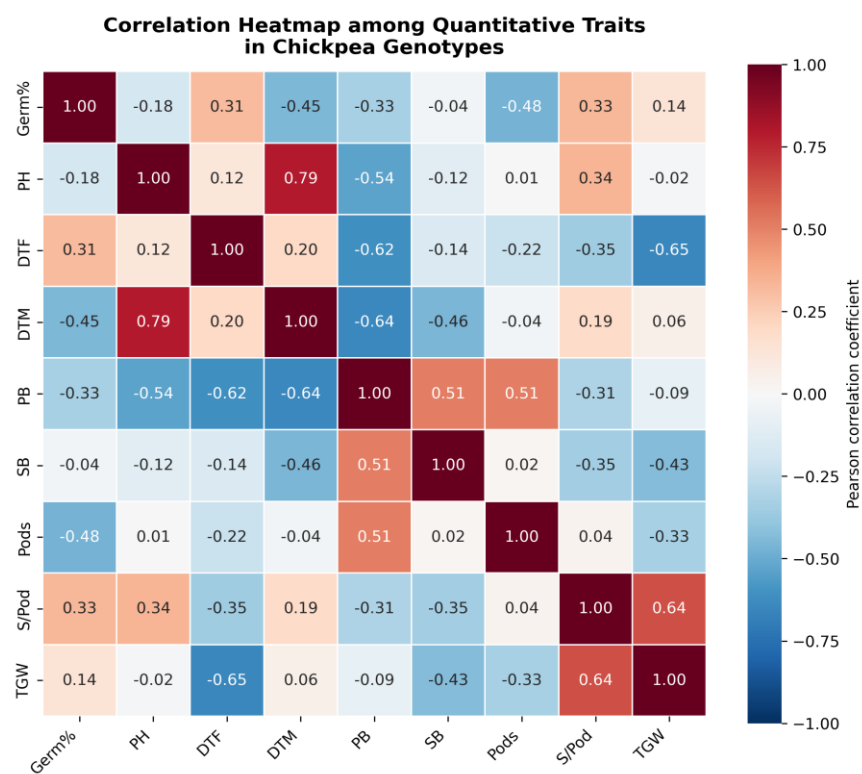


Fig. 1. Pearson correlation heat map among nine quantitative traits of chickpea genotypes.

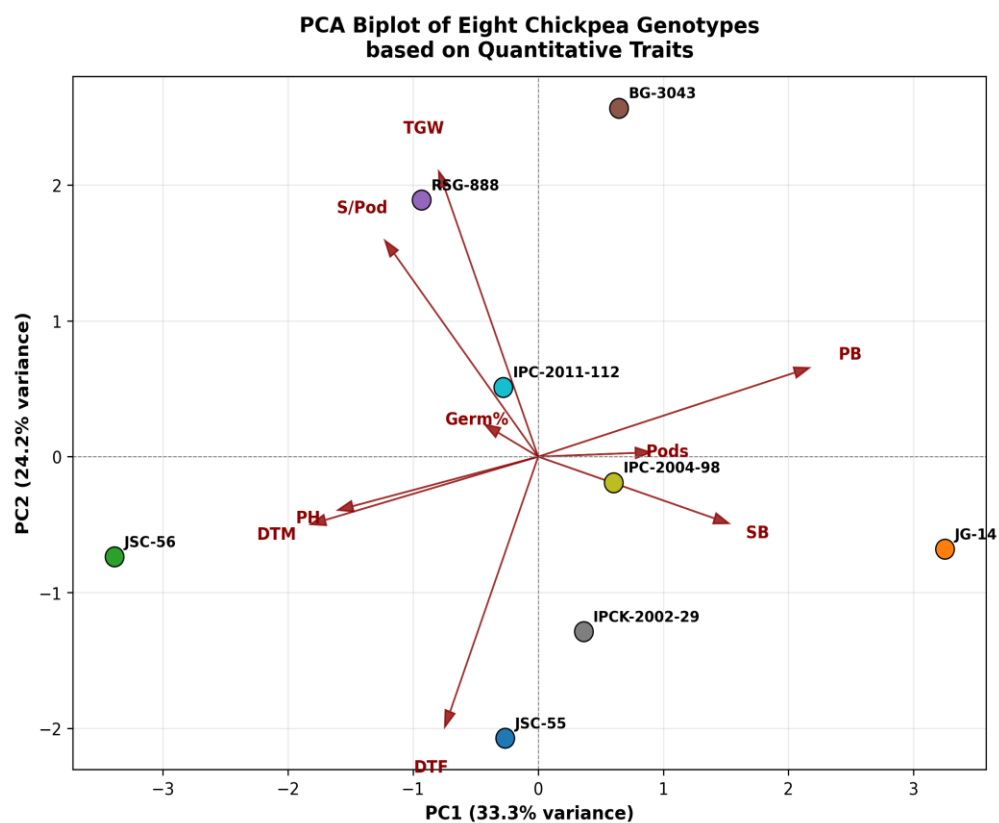


Fig. 2. PCA biplot showing distribution of eight chickpea genotypes and loading vectors of nine quantitative traits (PC1 vs PC2).

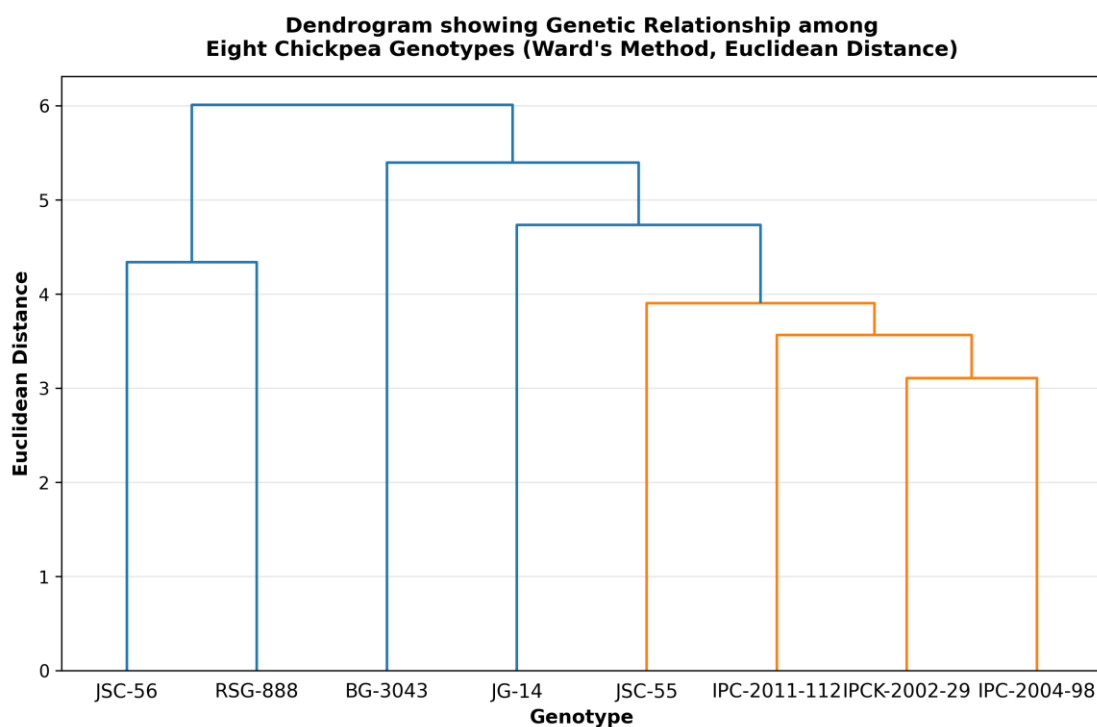


Fig. 3. Dendrogram (Ward's method, Euclidean distance) depicting genetic relationships among eight chickpea genotypes based on quantitative traits.