

Electronic Journal of Plant Breeding



Research Note

Assessment of genetic diversity among chickpea (*Cicer arietinum* L.) genotypes

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Abstract

The experiment conducted at Bihar Agricultural University, Sabour, India, during the rabi season of 2020-21 aimed to assess genetic diversity among 35 chickpea genotypes and identify those with traits associated with terminal heat tolerance using phenotypical parameters. Based on D^2 analysis, the genotypes were classified into six clusters using the Tocher method. Clusters I and II comprised the majority of the genotypes while Clusters IV, V, and VI: were the least populated. The highest inter-cluster distance was observed between Cluster III and V, followed by Cluster III and IV and Cluster III and VI. This indicated that there was significant diversity between the genotypes belonging to these clusters. In terms of agronomic traits, all clusters showed the highest mean value for grain yield under late sown conditions, indicating their potential for better yield performance. Among the 12 traits studied, 100-seed weight exhibited the highest contribution in the manifestation of genetic divergence. The diverse clusters identified in the study can be valuable for breeding programs. They can be used to develop improved chickpea varieties that are better adapted to late-sown conditions and have enhanced productivity. This could contribute to more resilient chickpea crops, especially in regions where terminal heat stress is a concern.

Keywords: Tocher method, grain yield, genetic divergence, chickpea

Cicer arietinum (L.) is a self-pollinated legume, commonly known as 'Chickpea' or 'Channa' having diploid chromosome number, $2n=16$ with genome size 738 M bp. It is the third most important pulse crop belonging to the family *Fabaceae*. India is a leading chickpea producing nation with 10.94 m ha acreage yielding 11.08 m tonnes (FAO, 2022). Chickpea is a *rabi* season crop normally sown during October-November and harvested during March-April. It is mainly used for consumption purposes in the form of dhal, leaves are used for the preparation of saag, and seeds are also used for making sweets, snacks, or as sprouts. Its leaves are rich in malic and citric acid and serve in stomach ailments and as a blood purifier. While straw of gram is an excellent fodder, both husk and bits of dhal are considered valuable cattle feed. From the nutrition point of view, chickpea seeds are enriched with protein (18-22 %), carbohydrate (61-62 %), fat

(4.5 %), calcium, iron, and phosphorus and have a calorific value of 396 (NFSM, 2016). Chickpea requires a cool climate during the initial growth and development stages, whereas a warm climate with a higher temperature for its maturity. It is susceptible to several abiotic stresses including terminal heat stress, which is a major problem for the places where chickpea is sown after the rice crop is harvested late.

Use of genetically similar parents results in low diversity (Johnson *et al.*, 2015) among derived lines compared to using diverse parents to obtain a segregating population with greater diversity. Understanding the genetic diversity in chickpea varieties suitable for late-sown conditions is crucial for effective breeding programs. Genetic diversity provides valuable insights into the genetic structure of chickpea populations and helps breeders make

informed decisions in selecting appropriate parents for their breeding efforts. Overall, evaluating the available genotypes for their genetic diversity in relation to late-sown conditions is an essential step in chickpea breeding programs. It facilitates the development of more robust and adaptable chickpea varieties that can contribute to sustainable agriculture and food security in regions where late sowing is prevalent. The objective of the research was focused on the genetic improvement of yield and the study of genetic diversity under late sown conditions. One of the ways to measure divergence is the Mahalanobis D^2 statistic as described by Rao (1952). The relative importance of a character is determined by the percentage of variation accounted by them, avoiding bias in choice with the help of D^2 analysis.

The experiment involved 35 *desi* chickpea genotypes that were sown on 8th December 2020 under late sown conditions at the Pulses Research farm, Bihar Agricultural University, Sabour (Bhagalpur), Bihar. The cropping season experienced varying atmospheric temperatures, ranging from a minimum of 7.7°C in January to a maximum of 45°C in May. The weather conditions throughout the cropping season were favorable for this experiment, with no rainfall during the critical month of March, which was essential for evaluating chickpea genotypes against heat stress.

The 35 chickpea genotypes were sown in randomized complete block design with two replications during *rabi*, 2020-21. Each plot had a size of 4 m², with two rows of 4 m length. The spacing adopted was 30 x 10 cm. The recommended packages of practices were followed to ensure the healthy growth of the chickpea crop. Data were recorded for 16 quantitative traits to observe the contribution of the traits towards total genetic divergence, which included Germination %, Days to first flowering, Days to 50% flowering, Days to first podding, Days to 50% podding, Canopy temperature at flowering and podding, Pollen viability (%), Days to physiological maturity, Primary branch per plant, Secondary branches per plant, Plant height (cm), Unfilled pods per plant, 100 seed weight (g), Grain yield (kg/ha) and Harvest

index (%). The days to 50% flowering, days to maturity and grain yield (kg/ha) were recorded on a plot basis, while plant height, number of pods per plant and 100-seed weight were recorded from a random sample of five plants in each plot. To analyze the genetic divergence among the chickpea genotypes, the Mahalanobis D^2 statistic was used, following the method described by Rao (1952). This statistic is commonly used as a measure of genetic distance or divergence between genotypes based on multiple traits. The Windostat version 9.2 software was used for the analysis of genetic divergence (D^2 static).

Based on the cluster analysis of the traits studied under late sown conditions, the genotypes were grouped into six clusters (Cluster I to VI), as shown in **Table 1**. Cluster I was the largest containing the highest number of genotypes (15), which accounted for 42.85 per cent of the total genotypes. Cluster II was the second-largest, with 11 genotypes, comprising 31.42 per cent of the total genotypes. Cluster III consisted of six genotypes, representing 17.14 per cent of the total genotypes. Clusters IV, V, and VI contained only one genotype each, making up 2.85 per cent of the total genotypes individually. The intra-cluster distance reflects the variation within each cluster, while the inter-cluster distance indicates the dissimilarity between different clusters. The highest intra-cluster distances was observed for cluster II, with a value of 270.35, followed by cluster III with a distance of 228.69 (**Table 2**). These clusters exhibited higher mean performance within their groups, indicating greater variability among genotypes within the same cluster. On the other hand, the lowest intra-cluster distances were recorded for clusters IV, V, and VI, each having a distance of zero (0.00). This implied that the genotypes within these clusters were very similar to each other in terms of the traits studied. Based on these results, genotypes from cluster II and cluster III could be considered as potential parental lines for further breeding programs due to their higher mean performance and higher variability within the group.

The inter-cluster distance indicates the dissimilarity between different clusters and the larger the inter-cluster

Table 1. Composition of clusters based on ward's minimum variance of 35 genotypes

Cluster	Number of Genotypes	Genotypes
I	15	BRHT-10, BRHT-11, BRHT-13, BRHT-9, BRHT-7, HT-38, BRHT-4, IPC 2000-25, PG-96006, HT-57, BRC-403, BRHT-1, HT-51, BRHT-5, BRHT-6
II	11	HT-64, BRC-301, IPC 2000-14, IPC 2000-02, JG-200-07, IPC 92-39, BRHT-12, BRHT-8, IPC 2000-05, IPC 05-28, HT-15
III	6	Sabour chana-2, HT-52, PDE-2, HT-44, BRC-304, PG-186
IV	1	BRHT-3
V	1	BRHT-2
VI	1	PUSA-256

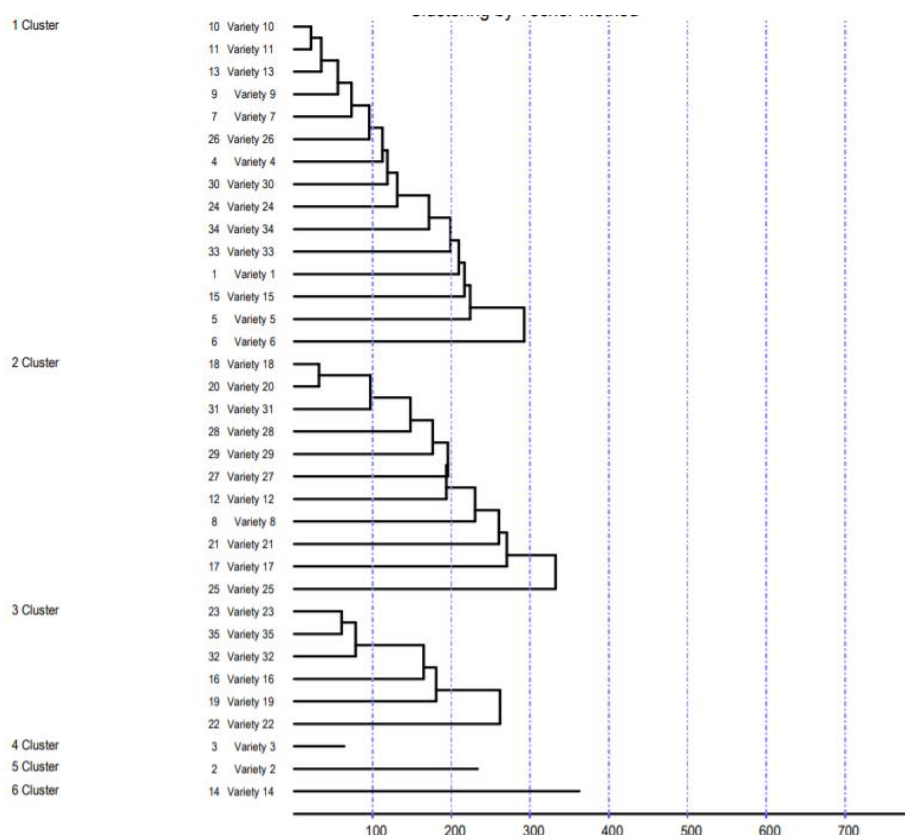
Table 2. Average of intra (diagonal) and inter cluster distance (Euclidean)²: Cluster: Ward) in chickpea genotypes

Cluster distance	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Cluster I	199.71	759.97	3266.24	474.49	613.56	571.94
Cluster II		270.35	1268.93	1881.68	2172.18	1352.98
Cluster III			228.69	5496.98	6105.11	4021.69
Cluster IV				0	233.27	478.12
Cluster V					0	1051.83
Cluster VI						0

distance, the greater the diversity between the genotypes of those clusters. The inter-cluster distance ranged from 474.49 – 6105.11 between clusters V and IV, respectively. The highest inter-cluster distance was observed between cluster- III and V (6105.11), followed by cluster- III and IV (5496.98). The lowest inter- cluster distance was observed between clusters IV and V (**Table 2 and Fig. 2**). Selecting parents for future breeding programmes based on their large inter-cluster distance is a sound strategy for isolating useful recombinants in segregating generations. When parents with high inter-cluster distances are crossed, it increases the chances of bringing together

diverse genetic traits, resulting in more diverse and potentially superior offspring. The minimum inter-cluster recorded between clusters IV and V and clusters I and IV showed that these clusters were genetically least diverse and possessed genotypes of almost the same genetic architecture (Haddad, 2004; Santosh *et al.*, 2017; Jayalakshmi *et al.*, 2023).

The mean data are presented in **Table 3 and 4** for 12 traits excluding days to 50% podding, canopy temperature at flowering and podding as well as primary branch per plant. As significant differences in cluster mean values

**Fig.1. Cluster analysis based on dendrogram of 35 chickpea genotypes**

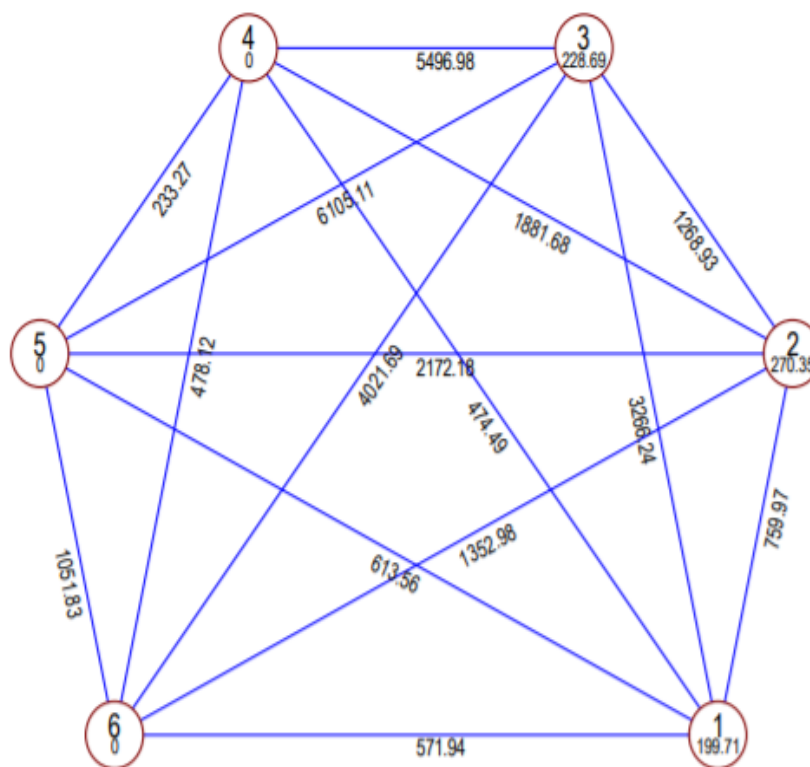


Fig. 2. Cluster diagram showing Euclidean² distance

Table 3. Average value of pre- harvest parameters of 35 genotypes pooled in six clusters

Cluster Mean: Tocher Method	Germination (%)	Days to first flowering	Days to 50% flowering	Days to first podding	Physiological maturity (days)	Secondary branches (no.)	Pollen viability (%)	Plant height (cm)
Cluster 1	83.04	45.93	68.47	74.03	111.97	3.47	10.67	53.47
Cluster 2	86.93	45.82	68	74.73	104.86	3.18	10.09	51.45
Cluster 3	88.33	46.67	69.58	74.67	109.92	3.17	10.33	47.67
Cluster 4	97.51	48	67.01	75	112.01	4.02	12	49.01
Cluster 5	94.38	46.5	68.02	74	111.5	3	10	62
Cluster 6	76.25	48	69	75.5	110.03	5.21	14	54.03
Average	87.74	46.82	68.34	74.66	110.04	3.64	11.18	52.93

Table 4. Average value of post-harvest parameters of 35 genotypes pooled in six clusters

Cluster Mean: Tocher Method	Grain yield (kg/ha)	Harvest Index (%)	100 Seed weight (g)	Unfilled pods per plant (%)
Cluster 1	2408.75	51.8	26.7	19.51
Cluster 2	2311.17	52	21.95	20.09
Cluster 3	2160.07	50.44	14.92	17.25
Cluster 4	1922.92	42.85	31.11	28.88
Cluster 5	2718.75	54.81	30.75	17.51
Cluster 6	1250	53.21	28.02	26.58
Average	2128.61	50.85	25.55	21.64

were observed for 12 traits only. These considerable differences in trait mean values among the clusters suggest that each cluster represents genotypes with distinct phenotypic characteristics. The above findings highlight the variation in mean values for various traits among the different clusters of chickpea genotypes under late-sown conditions. Cluster I showed the highest mean value for grain yield (2408.75 kg/ha) and the lowest mean value for the number of secondary branches (3.47), pollen viability (10.67 %), unfilled pod per plants per cent (19.51), 100-seed weight (26.70 g) and days to first flowering (45.93). Cluster II had the highest mean value for grain yield (2311.17 kg/ha), although slightly lower grain yield potential than Cluster I but still exhibiting desirable values for vegetative and reproductive traits such as the lowest mean value for the number of secondary branches (3.18), pollen viability (10.09 %), unfilled pod per plants per cent (20.09), 100 seed weight (21.95 g.). Cluster III had the highest mean value for grain yield (2160.07 kg/ha), and the lowest mean value for number of secondary branches (3.17), pollen viability (10.33%), unfilled pod per plants per cent (17.25), 100 seed weight (14.92 g). Cluster IV had the highest mean value for grain yield (1922.92 kg/ha), and the lowest mean value for number of secondary branches (4.02), pollen viability (12.00 %), unfilled pod per plants percent (28.88), 100 seed weight (31.11 g) and harvest index (42.85 %). Cluster V had highest mean value for grain yield (2718.75 kg/ha), and lowest mean value for number of secondary branches (3.00), pollen viability (10.00 %), unfilled pod per plants percent (17.51), 100 seed weight (30.75 g.), days to first flowering (46.50). Cluster VI shows highest mean value for grain yield (1250.00 kg/ha), and lowest mean value for number of secondary branches (5.21), pollen viability (14.00), unfilled pod per plants percent (26.58), 100 seed weight (28.02). Overall, the study indicated that Cluster V showed the highest grain yield potential under late-sown conditions, while Cluster VI had the lowest mean yield. Additionally, clusters with higher grain yield potential tend to have lower values for traits related to vegetative growth (e.g., number of secondary branches) and reproductive performance (e.g., pollen viability and unfilled pod per plant per cent). These findings can be valuable for breeding programs as they help identify genotypes with specific trait combinations that could be used as parents to develop high-yielding chickpea varieties suitable for late sown conditions.

By selecting parents from clusters with contrasting mean values for specific traits, breeders can target specific traits of interest and facilitate the development of improved chickpea varieties with desired characteristics. This approach allows breeders to capitalize on the natural genetic diversity present in the different clusters and strategically combine desirable traits through hybridization and subsequent selection. The availability of diverse genetic material through these clusters can potentially lead to the development of more resilient and

high-performing chickpea varieties suited for late sown conditions.

The study suggests that using diverse genotypes from different clusters, along with other desirable attributes, in breeding programs can lead to the development of better segregants with high seed yield and yield-contributing traits, primarily due to non-allelic interactions. Non-allelic interactions refer to the interactions between genes at different loci, which can result in unique trait combinations in the offspring. Interestingly, the clustering of genotypes in the study was found to cut across geographical boundaries, indicating that genetic diversity is not solely influenced by geographical isolation. This suggests that the genotypes within a cluster may have some degree of ancestral relationship, indicating that genetic diversity can be influenced by various factors beyond geographical location. Similar findings of non-relationship between geographical and genetic diversity have been reported in other studies by Santosh *et al.* (2017), Sewak *et al.* (2012), Sachin *et al.* (2014), Vijayakumar *et al.* (2017). The genetic diversity observed is attributed to factors like changes in breeding material, genetic drift, natural variation, and artificial selection, in addition to ecological and geographical diversification (Willoughby, 2015). Based on these findings, the study recommends selecting parents for hybridization based on genetic diversity rather than geographic diversity to obtain more heterotic recombinants and desired transgressive segregants. Heterotic recombinants are offspring that exhibit superior traits compared to their parents, and transgressive segregants possess traits that exceed the range of variation observed in the parents. However, the study also cautions that while selecting divergent genotypes for crosses can lead to increased genetic diversity, it may not always result in proportionate heterotic responses. This means that not all crosses between divergent genotypes will necessarily lead to improved traits in the offspring. Careful consideration and evaluation of the specific traits of interest are important in the selection of parental lines to achieve the desired breeding outcomes (Shin *et al.*, 2019).

The study found significant variation among the cluster means for different characters, further supporting the genetic diversity observed among the chickpea genotypes under late sown conditions. Similar results highlighting appreciable variation among cluster mean values have been reported in other studies conducted by Tomar *et al.* (2011), Puri *et al.* (2013), Kuldeep *et al.* (2015), Naveed *et al.* (2015) and Babbar *et al.* (2015). This consistency across multiple studies emphasizes the importance of genetic diversity in chickpea breeding programs. In this investigation, the selection of genotypes as parents for crop improvement was based on D^2 analysis in the inter-cluster distance. Genotypes with the maximum inter-cluster distance are considered genetically more divergent, implying that they possess greater genetic

Table 5. Percent contribution of the traits, towards total genetic divergence of chickpea genotypes

S. No.	Traits	Contribution of the traits (%)
1	Germination (%)	7.10
2	Days to first flowering	1.00
3	Days to 50 % flowering	1.20
4	Avg. CT at flowering (°C)	2.70
5	Avg. CT at podding (°C)	3.10
6	Days to first podding	1.31
7	Days to 50% podding	2.44
8	Physiological maturity (days)	2.33
9	Primary branches (no.)	4.67
10	Secondary branches (no.)	5.51
11	Pollen Viability (%)	2.50
12	Plant height (cm.)	8.22
13	Unfilled pods per plant (%)	2.33
14	100 seed weight (gm.)	47.88
15	Grain yield (Kg/ha)	2.68
16	Harvest index (%)	5.03
	Total	100.00

Where, CT=canopy temperature

variation and are likely to contribute unique and desirable traits to their offspring.

The study also analyzed the per cent contribution of 16 traits towards total genetic divergence, as shown in **Table 5**. The results revealed that 100-seed weight made the highest contribution (47.88 %) to the manifestation of genetic divergence, indicating its significance in differentiating the genotypes. Other traits with substantial contributions included plant height (8.22 %), germination percentage (7.10 %), number of secondary branches (5.51 %), harvest index (5.03 %), number of primary branches (4.67 %), average canopy temperature at podding (3.10 %), average canopy temperature at flowering (2.70 %), grain yield (2.68 %), days to 50% podding (2.44 %), unfilled pods per plant percent (2.33 %), and pollen viability (2.50 %). These findings imply that different clusters of genotypes exhibited superiority or distinctiveness for specific traits. Therefore, the choice of parents for hybridization should be based on the contribution of individual characters towards genetic divergence. By selecting parents with diverse and desirable trait combinations, breeders can aim to develop chickpea varieties with improved performance and adaptability to late sown conditions.

The hybridization program involving genotypes HT-52, IPC 92-39, BRHT-2, BRHT-4, BRHT-8 and IPC 2000-25 under late sown conditions can be undertaken to isolate high yielding segregants since these genotypes have high yielding potential, more number of primary branches,

more number of secondary branches, high per cent of pollen viability, low canopy temperature, high plant height, high harvest index, high 100-seed weight and early days to physiological maturity with more genetic distances. In chickpea breeding program, the choice of parents is a critical step to improve specific traits and overall performance. By selecting parents with diverse and desirable trait combinations, breeders can increase the chances of obtaining progeny with improved performance and adaptability to late sown conditions. This approach aims to bring together favorable traits from different genotypes to create hybrids or lines that exhibit enhanced yield potential, resilience to environmental stresses, and other desirable agronomic traits.

Selecting genetically diverse parents with desirable trait combinations is crucial for developing chickpea varieties that excel in late sown conditions. By understanding the genetic diversity within the chickpea population and carefully selecting parents, breeders can make significant progress in developing high-performing, adaptable, and resilient chickpea varieties to meet the challenges of changing agricultural environments. By incorporating advanced breeding techniques, such as marker-assisted selection and genomic selection, breeders can further improve the efficiency of selecting parents with specific trait combinations. These tools allow for the identification of specific genes or markers associated with desirable traits, streamlining the breeding process and accelerating the development of improved chickpea varieties.

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