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Multivariate Genetic Diversity Assessment in Chickpea (*Cicer arietinum* L.) Genotypes under Different Environmental Conditions

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ABSTRACT

Background: This research assesses the genetic diversity among 55 chickpea genotypes, comprising both parental lines and F_1 hybrids, through D^2 analysis in conditions of both timely sown (TS) and late sown (LS). Conducted at the Genetics and Plant Breeding Research Farm, Acharya Narendra Deva University of Agriculture and Technology, Ayodhya, during the *Rabi* seasons of 2021-22 and 2022-23.

Methods: The experiment was laid out in a randomized block design (RBD) with three replications. Data was collected on eleven agronomic traits. Statistical analyses, including D^2 statistics, cluster analysis and principal component analysis (PCA), were conducted to assess the genetic diversity among the chickpea genotypes.

Result: Both TS and LS conditions revealed six clusters. In TS condition, the majority genotypes were found in Cluster I and Cluster II, while Cluster I was predominant under LS conditions. Highest inter-cluster distances were between Clusters I and VI in TS (158.70) and Clusters II and V in LS (314.29), indicating substantial genetic divergence. Principal component analysis (PCA) demonstrated that the first three components accounted for 81.48% of the variance in TS conditions and 80.15% in LS conditions. Significant genetic diversity exists among chickpea genotypes, with important traits like seed yield plant^{-1}, plant height and days to 50% flowering contributing to this variation. These findings are relevant for chickpea breeding programs and can aid in selecting genotypes for different sowing conditions.

Key words: Chickpea, D^2 statistics, Genetic diversity, Multivariate, Principal component analysis.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a significant pulse legume crop cultivated in dry and semi-arid region with residual soil moisture across the globe. Its genesis and domestication by humans in the Old World stretch back to 10,000 years (Yadav *et al.,* 2007). Its cultivation extended throughout the Fertile Crescent beginning in southern Anatolia around 6000 years ago (Sani *et al.,* 2018). Despite the wide range of climates and growth circumstances, chickpeas are cultivated in around 57 nations across the globe (Merga and Haji, 2019). In 2019, India accounted for 80% of the world's chickpea production, with Kabuli accounting for the remaining 20% (Merga and Haji, 2019). India is the biggest chickpea importer despite producing a lot of chickpeas (Merga and Haji, 2019), because its yields are lower than those of smaller countries like Mexico and Ethiopia (FAOSTAT, 2020). Worldwide, production in 2021 was 15.87 million metric tonnes from 15 million hectares, while in India, it was 11.91 million metric tonnes from 10.94 million hectares (FAO, 2023). Chickpea serve as an excellent source of carbohydrates, minerals and affordable dietary protein, often referred to as "poor man's meet" (Varshney *et al.,* 2019). Genetic diversity is essential for the survival of species in nature and assessing genetic material for available natural variation is crucial for developing effective breeding programs aimed at crop improvement (Mohomood *et al.,* 2018). The patterns of genetic diversity and inheritance can vary among different genetic materials, making the discovery of germplasm vital for creating breeding strategies that yield superior cultivars

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(Govindaraj *et al.,* 2015). By characterizing genetic materials, breeders can effectively classify and select the most suitable varieties from diverse population (Sharifi *et al.,* 2018). Previous studies have frequently utilized cluster analysis and principal component analysis to investigate genetic variation by analyzing the extent and pattern of diversity across various samples (Patil *et al.,* 2001; Zubair *et al.,* 2017; Farshadfar *et al.,* 2018). Additionally, D2 statistics, introduced by Mahalanobis in 1936, has proven to be an effective method for examining genetic divergence (Malik *et al.,* 2014; Chen *et al.,* 2017).

MATERIALS AND METHODS

The current study took place at the Genetics and Plant Breeding Research Farm, situated within Acharya Narendra Deva University of Agriculture and Technology, Narendra Nagar, Kumarganj, Ayodhya (U.P.) during *Rabi* 2021-22 and 2022-23. The experimental material comprised 55 genotypes, including 10 parental lines and 45 F_1 hybrids produced through half-diallel mating. These were planted using a randomized block design with three replications under both environments. The recommended agricultural practices for the crop were adhered to throughout the

Table 1: D^2 analysis of eleven characteristics in timely sown (TS) and late sown (LS) conditions clustering pattern of 10 parents and 45 half-diallel crosses.

Cluster number	Environmental condition	Number of genotypes	Genotypes
I	TS	19	BG 256 × BG 3043, BG 256 × Pant G 186, BG 256 × GNG 2207, BG 256 × SAKI 9516, BG 362 × RSG 888, BG 362 × GNG 2207, BG 362 × SAKI 9516, BG 362 × Pant G 186, BG 256 × RSG 888, BG 256 × JAKI 9218, BG 362 × JAKI 9218, BG 256 × BG 362, BG 362 × GCP 105, BG 256 × GCP 105, BG 256 × IPC 2004-52, BG 362 × IPC 2004-52, IPC 2004-52 × JAKI 9218, IPC 2004-52 × GCP 105, BG 362 × BG 3043
	LS	27	BG 256 × GNG 2207, BG 256 × Pant G 186, BG 256 × SAKI 9516, BG 256 × BG 3043, BG 3043 × JAKI 9218, BG 362 × Pant G 186, BG 362 × SAKI 9516, BG 362 × RSG 888, BG 362 × GNG 2207, BG 362 × BG 3043, BG 256 × RSG 888, IPC 2004-52 × RSG 888, IPC 2004-52 × GCP 105, JAKI 9218 × GCP 105, GNG 2207 × JAKI 9218, BG 256 × JAKI 9218, GNG 2207 × GCP 105, IPC 2004-52 × GNG 2207, JAKI 9218 × RSG 888, JAKI 9218 × SAKI 9516, IPC 2004-52 × Pant G 186, GCP 105 × Pant G 186, IPC 2004-52 × SAKI 9516, GNG 2207 × SAKI 9516, IPC 2004-52 × BG 3043, GNG 2207 × RSG 888
II	TS	23	JAKI 9218 × SAKI 9516, JAKI 9218 × RSG 888, JAKI 9218 × Pant G 186, IPC 2004-52 × GNG 2207, IPC 2004-52 × Pant G 186, IPC 2004-52 × SAKI 9516, IPC 2004-52 × BG 3043, JAKI 9218 × GCP 105, IPC 2004-52 × RSG 888, RSG 888 × GCP 105, BG 256, BG 362, IPC 2004-52, GCP 105 × Pant G 186, GNG 2207 × SAKI 9516, BG 3043 × SAKI 9516, GNG 2207 × JAKI 9218, GNG 2207 × GCP 105, GNG 2207 × RSG 888, BG 3043 × RSG 888, GNG 2207 × BG 3043, GNG 2207 × Pant G 186, BG 3043 × JAKI 9218
	LS	6	BG 256 × IPC 2004-52, BG 362 × IPC 2004-52, BG 256 × BG 362, BG 362 × JAKI 9218, BG 256 × GCP 105, BG 362 × GCP 105
III	TS	1	BG 3043 × GCP 105
	LS	1	GNG 2207 × Pant G 186
IV	TS	1	BG 3043 × Pant G 186
	LS	14	BG 256, BG 3043 × Pant G 186, BG 3043 × SAKI 9516, GNG 2207 × BG 3043, JAKI 9218 × Pant G 186, GCP 105, BG 362, IPC 2004-52, SAKI 9516 × Pant G 186, JAKI 9218, SAKI 9516, RSG 888 × Pant G 186, SAKI 9516 × RSG 888, BG 3043 × RSG 888
V	TS	6	SAKI 9516 × Pant G 186, RSG 888 × Pant G 186, SAKI 9516, RSG 888, SAKI 9516 × RSG 888, SAKI 9516 × GCP 105
	LS	4	BG 3043, Pant G 186, GNG 2207, RSG 888
VI	TS	5	BG 3043, Pant G 186, GNG 2207, JAKI 9218, GCP 105
	LS	3	SAKI 9516 × GCP 105, RSG 888 × GCP 105, BG 3043 × GCP 105

experimental period to ensure the cultivation of healthy and robust crops. Each genotype was planted in a single row having four meters, with 30 cm between rows and 10 cm between individual plants. Observations on eleven traits was recorded from five randomly chosen competitive plants in each plot. The averages of data collected from these selected plants in each plot for various traits were utilized for statistical analyses. Data were recorded for the traits *viz.,* days to 50% flowering, days to maturity, primary branches plant^{-1}, secondary branches plant^{-1}, plant height (cm), number of pods plant^{-1}, number of seeds pod^{-1}, 100 seed weight (g), biological yield plant^{-1} (g), harvest index (%) and seed yield plant^{-1} (g). Statistical analysis was performed using the Windostat Version 9.2 statistical package.

RESULTS AND DISCUSSION

Genetic divergence analysis

The D^2 analysis elucidated the clustering patterns of chickpea, providing insights into genetic diversity among the parental lines and their crosses (Table 1). Under TS conditions, six distinct clusters were identified. Cluster I, containing 19 genotypes and Cluster II, with 23 genotypes, were the most populated, indicating substantial genetic diversity. Notable genotypes in Cluster I included BG 256 × BG 3043, BG 362 × SAKI 9516 and IPC 2004-52 × JAKI 9218. Cluster II comprised genotypes like JAKI 9218 × SAKI 9516 and IPC 2004-52 × GNG 2207, reflecting diverse genetic backgrounds.

Under LS conditions, six clusters were identified, with Cluster I being the most populated with 27 genotypes, including BG 256 × GNG 2207, BG 362 × SAKI 9516 and IPC 2004-52 × RSG 888, indicating wide genetic variation. Clusters II and IV displayed considerable genetic variability, containing six and fourteen genotypes, respectively. Cluster II included combinations like BG 256 × IPC 2004-52 and BG 362 × GCP 105, while Cluster IV contained genotypes such as BG 3043 × Pant G 186 and GNG 2207 × BG 3043. Clusters III, V and VI had fewer genotypes, indicating more genetic homogeneity.

The notable inter-cluster distances, particularly between Clusters I and VI under TS (158.70) and between Clusters II and V under LS (314.29), underscore significant genetic divergence. The estimation of intra- and inter-cluster distances provided insights into the genetic diversity within and between clusters of chickpea genotypes (Table 2).

In TS conditions, inter-cluster distances varied significantly, with the highest distance noted between Clusters I and VI (158.70), indicating significant genetic divergence and the shortest distancebetween clusters was seen between III and IV (29.35), indicating closer genetic relationships. Intra-cluster distances ranged from 17.38 in Cluster I to 29.7 in Cluster V, reflecting differing degrees of genetic variability within the clusters. Notably, Clusters III and IV exhibited zero intra-cluster distance, indicating genetic uniformity within these clusters.

Under LS conditions, inter-cluster distances also varied significantly, with the highest distance between Clusters II and V (314.29), highlighting pronounced genetic divergence. The lowest inter-cluster distance was between Clusters III and IV (35.3), suggesting genetic similarity. The intra-cluster distances varied from 18.08 in Cluster II to 50.58 in Cluster VI. Cluster III exhibited no intra-cluster distance, reflecting genetic uniformity, whereas Cluster VI had the greatest intra-cluster distance, suggesting significant genetic variability.

The genetic divergence analysis of chickpea genotypes revealed significant variability in agronomic traits across clusters (Table 3). Under TS conditions, Cluster I demonstrated early flowering at 58.53 days and reached maturity in 123.42 days. This cluster also achieved a high biological yield of 58.04 g. plant^{-1} and a seed yield of 23.55 g. plant^{-1}. In contrast, Cluster II exhibited later flowering at

Table 2: Estimates of average intra and inter-cluster distance for six clusters in half-diallel crosses of chickpea in timely sown (TS) and late sown (LS) c onditions.

Cluster number	Environmental condition	I	II	III	IV	V	VI
I	TS	17.38	59.45	43.97	94.16	88.64	158.70
	LS	**24.6**	73.45	46.81	65.59	141.72	41.89
II	TS		**27.26**	40.85	44.42	63.07	61.77
	LS		**18.08**	160.07	192.72	314.29	84.97
III	TS			**0**	29.35	103.86	82.46
	LS			**0**	35.3	56.71	88.25
IV	TS				**0**	115.34	39.02
	LS				**33.69**	57.24	85.49
V	TS					**29.7**	104
	LS					**42.47**	178.37
VI	TS						**19.9**
	LS						**50.58**

Note: Bold figures indicate intra-cluster distance.

65.26 days and maturity at 132.71 days, along with fewer primary branches (1.95 plant^{-1}) and pods (44.71 plant^{-1}). However, it maintained a high harvest index of 41.21% and a moderate seed yield of 18.48 g. plant^{-1}. Cluster III showed a higher number of primary (2.33 plant^{-1}) and secondary branches (11.53 plant^{-1}), with intermediate flowering (69.00 days) and maturity (127.00 days) and a high seed yield of 25.86 g. plant^{-1} and biological yield of 57.90 g. plant^{-1}. Cluster IV exhibited the latest flowering 73.67 days and reached maturity at 134.33 days, with a moderate primary branch (2.00) and secondary branches (9.93) plant^{-1}, along with a biological yield of 52.92 g. plant^{-1}, but a low seed yield of 19.50 g. plant^{-1} was recorded. In contrast, Cluster V showed early flowering at 57.72 days and moderate maturity at 131.28 days, the lowest primary branches at 1.90 plant^{-1} and the lowest seed yield at 12.83 g plant^{-1}. Cluster VI showed the latest flowering (74.80 days) and maturity (137.87 days), with the lowest primary (1.60) and secondary branches plant^{-1} (8.71) and low biological (33.52 g) and seed yield (14.00 g).

Under LS conditions, Cluster I had early flowering (51.33 days) and maturity (115.36 days) and achieved biological yield of 45.67 g. plant^{-1} and a moderate seed yield of 18.05 g. plant^{-1}. Cluster II exhibited the earliest flowering (44.5 days) and maturity (107.5 days), with the highest primary branches (2.07 plant^{-1}), secondary branches (10.04 plant^{-1}), biological yield of 61.86 g. plant^{-1} and seed yield of 24.67 g. plant^{-1}. Cluster III had later flowering (62.67 days) and maturity (122.33 days), with lower primary (1.93 plant^{-1}) and secondary branches (8.47 plant^{-1}) and moderate biological yield of 39.67 g. plant^{-1} and seed yield of 14.24 g. plant^{-1}. Cluster IV showed intermediate flowering (57.31 days) and maturity (121.24 days), with the lowest primary (1.62 plant^{-1}) and secondary branches (8.36 plant^{-1}) and low biological yield of 32.14 g. plant^{-1} and seed yield of 12.81 g. plant^{-1}. Cluster V had the latest flowering (65.83 days) and maturity (128.92 days), with low primary (1.6 plant^{-1}) and secondary branches (7.59 plant^{-1}) and the lowest biological (27.26 g. plant^{-1}) and seed yield (10.37 g. plant^{-1}). Cluster VI exhibited early flowering (46.22 days) and early maturity (112.22 days), with moderate values for most traits, including high biological yield of 44.59 g. plant^{-1} and moderate seed yield of 16.76 g plant^{-1}.

The early flowering and maturity traits in certain clusters under LS conditions suggest potential candidates for breeding programs targeting late sowing scenarios. Conversely, genotypes in clusters with high yield and biomass under TS conditions could be prioritized for optimal planting schedules.

The analysis of genetic divergence among chickpea genotypes revealed significant contributions from various quantitative traits (Table 4). In the TS condition, days to 50% flowering emerged as the most significant contributor to genetic divergence, accounting for 50.44% of the total variation. This was succeeded by the 100-seed weight at 13.87% and days to maturity at 13.60%, emphasizing the

Table 3: Cluster means for nine clusters in chickpea in half diallel crosses of chickpea in timely sown (TS) and late sown (LS) conditions.

Number of clusters	Environmental condition	Days to 50% flowering	Days to maturity	Primary branches plant^{-1}	Secondary branches plant^{-1}	Plant height (cm)	Pods plant^{-1}	Seeds pod^{-1}	100-seed weight (g)	Biological yield plant^{-1} (g)	Harvest index (%)	Seed yield plant^{-1} (g)
I	TS	58.53	123.42	2.12	10.75	58.04	49.42	2.04	23.31	58.04	40.54	23.55
	LS	51.33	115.36	1.88	8.97	53.71	43.44	1.92	21.67	45.67	39.68	18.05
II	TS	65.26	132.71	1.95	9.69	57.60	44.71	2.00	20.65	44.96	41.21	18.48
	LS	44.5	107.5	2.07	10.04	56.86	47.9	2.13	24.19	61.86	40.04	24.67
III	TS	69.00	127.00	2.33	11.53	53.33	51.20	2.33	21.65	57.90	44.64	25.86
	LS	62.67	122.33	1.93	8.47	56.4	40.83	1.73	20.15	39.67	35.94	14.24
IV	TS	73.67	134.33	2.00	9.93	48.53	43.80	2.13	20.92	52.92	36.80	19.50
	LS	57.31	121.24	1.62	8.36	43.65	38.47	1.81	18.38	32.14	39.99	12.81
V	TS	57.72	131.28	1.90	9.11	46.77	39.57	1.99	16.20	32.83	39.41	12.83
	LS	65.83	128.92	1.6	7.59	41.02	34.67	1.77	16.89	27.26	38.3	10.37
VI	TS	74.80	137.87	1.60	8.71	47.67	38.04	1.92	19.21	33.52	41.81	14.00
	LS	46.22	112.22	1.96	8.93	44.66	44.47	1.98	19.06	44.59	38.38	16.76

significance of flowering time and seed traits in assessing genetic diversity. These findings align with previous studies by Reddy *et al.* (2021) and Richards *et al.* (2020) which also emphasized the critical roles of flowering time and seed traits in shaping genetic variation in chickpea.

Under LS conditions, days to 50% flowering remained the primary contributor to genetic divergence, though its contribution decreased to 39.8%. Biological yield plant^{-1} (25.66%) and 100-seed weight (20.07%) also showed substantial contributions, indicating their importance in adaptation to different sowing conditions and their influence on yield potential and maturity in chickpea varieties.

The significant contribution of days to 50% flowering underscores its role in the adaptability and genetic differentiation of chickpea genotypes. The significant impact of 100-seed weight and biological yield plant^{-1} under LS conditions indicates that these traits are essential for sustaining productivity in less favourable sowing environments. The relatively lower contributions from traits like primary branches plant^{-1}, harvest index and secondary branches plant^{-1}, indicate their lesser impact on genetic divergence compared to flowering time and seed-related traits.

By focusing on traits such as days to 50% flowering, 100-seed weight and biological yield plant^{-1}, breeders can enhance the genetic diversity and adaptability of chickpea genotypes, ultimately improving yield stability under varying environmental conditions.

Principal component analysis

The principal component analysis (PCA) revealed the genetic structure and associations among chickpea parental lines and F_1 hybrids under both environmental conditions (Table 5).

In the TS condition, the first three principal components (PCs) accounted for 81.48% of the total variance, with eigen values of 6.06, 2.05 and 0.85, respectively. PC1 explained the highest variance (55.10%), with significant contributions from days to 50% flowering, plant height and days to maturity. These traits were critical in distinguishing the genetic diversity among the genotypes, underscoring their importance in breeding programs aimed at enhancing yield and maturity. PC2, which accounted for 18.67% of the variance, was primarily influenced by seed yield plant^{-1}, harvest index, plant height and days to 50% flowering. PC3, which accounted for 7.71% of the variance, was influenced by factors such as days to 50% flowering, plant height and harvest index.

Similarly, in LS condition, first three PCs explained 80.15% of the total variance, with eigenvalues of 6.59, 1.28 and 0.95, respectively. PC1 contributed the most to the variance (59.90%), with plant height and days to 50% flowering as major contributors. PC2, explaining 11.62% of the variance, was influenced by the seed yield plant^{-1} and harvest index. PC3, accounting for 8.63% of the variance, was primarily driven by plant height. The consistent patterns of trait associations across different sowing

Table 4: Contribution of 11 quantitative characters towards divergence in chickpea in half diallel crosses of chickpea in timely sown (TS) and late sown (LS) conditions.

Source	Environmental condition	Times ranked 1st	Contribution (%)
Days to 50% flowering	TS	749	50.44
	LS	591	39.8
Days to maturity	TS	202	13.60
	LS	89	5.99
Primary branches plant^{-1}	TS	1	.07
	LS	4	0.27
Secondary branches plant^{-1}	TS	1	.07
	LS	1	0.07
Plant height (cm)	TS	61	4.11
	LS	51	3.43
Pods plant^{-1}	TS	17	1.14
	LS	8	0.54
Seeds pod^{-1}	TS	30	2.02
	LS	15	1.01
100 seed weight (g)	TS	206	13.87
	LS	298	20.07
Biological yield plant^{-1}	TS	177	11.92
	LS	381	25.66
Harvest index (%)	TS	13	.86
	LS	9	0.61
Seed yield plant^{-1} (g)	TS	28	1.89
	LS	38	2.56

Table 5: Loadings of the first three principal components of genetic divergence among 10 parental lines and 45 F1s of chickpea for 11 quantitative characters in timely sown (TS) and late sown (LS) conditions.

Characters	Timely sown			Late sown		
	PC1	PC2	PC3	PC1	PC2	PC3
Days to 50% flowering	0.20618	0.31065	0.73994	0.27418	0.25899	0.36261
Days to maturity	0.33278	0.17826	-0.1629	0.33504	0.12692	0.21891
Primary branches plant^{-1}	-0.359	-0.0444	-0.0084	-0.3595	-0.1209	-0.1687
Secondary branches plant^{-1}	-0.3666	0.09675	0.06773	-0.3275	0.03669	-0.0242
Plant height (cm)	-0.1979	0.44865	-0.4519	-0.2137	0.12666	0.79703
Pods plant^{-1}	-0.3089	0.35212	0.29281	-0.3451	0.00783	0.16584
Seeds pod^{-1}	-0.3661	0.12717	-0.0648	-0.3748	0.02221	0.05202
100 seed weight (g)	-0.368	0.0652	-0.0091	-0.3624	-0.0284	0.16722
Biological yield plant^{-1}	-0.3427	0.03686	0.16605	-0.3394	0.03005	-0.0552
Harvest index (%)	-0.1929	-0.4892	0.30437	-0.023	0.736	-0.0896
Seed yield plant^{-1} (g)	-0.1576	-0.5235	-0.083	-0.1542	0.58384	-0.2951
Components eigen value (Root)	6.06148	2.05328	0.84839	6.58917	1.27822	0.94919
% variance explained	55.1043	18.6662	7.71266	59.9015	11.6202	8.62896
Cum. variance explained	55.1043	73.7705	81.4832	59.9015	71.5217	80.1507

conditions highlight their relevance in genetic diversity and adaptability.

The PCA results demonstrate that plant height, days to 50% flowering and yield components are pivotal in driving genetic differentiation among chickpea genotypes. These findings are consistent with previous studies (Kumari *et al.,* 2020; Kushwah *et al.,* 2021; Danakumara *et al.,* 2023; Devi *et al.,* 2021) and suggest that these traits are critical for selecting and breeding chickpea varieties with improved yield potential and adaptability to different environmental conditions.

Understanding the genetic basis of trait variation through PCA provides valuable insights for breeding programs. By focusing on key traits such asplant height, days to 50% flowering and yield components, breeders can develop improved chickpea varieties that are better suited to diverse environments and changing climatic conditions. Future research should aim to validate these findings across various environments and integrate genomic tools to enhance targeted trait improvement in chickpea.

CONCLUSION

The study revealed significant genetic diversity among chickpea genotypes. Six distinct clusters were identified in each condition, with notable genetic divergence between clusters. Essential characteristics like flowering time, maturity and seed yield were the primary contributors to this genetic diversity. Principal component analysis highlighted seed yield plant^{-1}, plant height, days to maturity and days to 50% flowering as critical contributors to genetic differentiation. These insights provide valuable guidance for chickpea breeding programs aimed at developing high-yielding, adaptable varieties.

Conflict of interest

All authors declared that there is no conflict of interest.

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