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Agro-Morphological Exploitation of Genetic Diversity and Multivariate Analysis of Indigenous Chickpea (Cicer Arietinum L.) Germplasm

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Abstract

For most of the examined variables, there was significant variation between the germplasm lines. Independent of their breeding locations, the genotypes under study were grouped into seven clusters by genetic diversity analysis. Cluster I contained the most genotypes, 24 and the fewest in clusters V and VII, which were solitary. In contrast, cluster II held 10 genotypes, cluster III had 7, and clusters IV and VI could each hold 4 genotypes. Clusters III and VI had the greatest inter-cluster distance, which indicated that their genotypes had the greatest genetic diversity. According to principal component analysis (PCA), Four of the first fourteen PCs had eigenvalues greater than one. PC 1 alone accounted for the highest variance of 33.1%, followed by PC 2 with 21.7%. The outcomes of this investigation might be used as a foundation for defining and implementing subsequent chickpea breeding initiatives. Seed Yield, Number of Secondary Branches, Harvest Index, and biological yield, test weight, number of seeds per pod, and number of filled pods per plant were all found to be variable in biplot.

Keywords: *Cluster analysis, D^2 Statistics, PCA, eigenvalue, biplot*

1. Introduction

The chickpea (*Cicer arientinum* L.), one of the world's most widely farmed pulse legumes, is ranked third, in particular for tropical and subtropical areas, it is a significant food legume (Gautam *et al.* 2021). It is the first grain legume that humans have ever domesticated and one of the most significant crops for human food intake among farmed pulses (Kerem *et al.* 2007). It is a diploid self-pollinated species with the chromosomal makeup (2n=2x=16), and it may be distinguished as the Kabuli and Desi varieties based on the shape and type of seed it produces. It is also known as Bengal gram or garbanzo beans and contains 20–25% protein in the seed, making it an important pulse crop for consumption purposes worldwide, especially in developing countries like India, Pakistan, and other Asian and African countries that are struggling with a serious problem of nutritional food security (Thakur *et al.* 2022). However, in comparison, production has remained unchanged. The factors limiting the production potential can be attributed to several biotic and abiotic variables, as well as a lack of improved cultivars. Under various agroclimatic zones experiencing a shift in climate conditions, the production potential of various types varies (Sharma *et al.* 2019). The narrow genetic base of the existing cultivated varieties may also be a cause of productivity decline (Bharadwaj *et al.* 2011); this genetic base must be widened by introducing additional genetic variability (Chandra *et al.* 2013). At NBPGR in New Delhi, there are approximately 14,651 recorded chickpea accessions. However, the amount of germplasm that breeders can use to improve crops appears to be limited (Thakur *et al.* 2022). To break the production plateau and achieve long-term increases, plant breeders must incorporate a variety of germplasm into their breeding programs. Genetic variety information paves the opportunity for breeding parents to be chosen from random populations. To evaluate the potential of heterotic combinations before trying crosses, it is helpful to have a precise impression of the levels and patterns of genetic variation. This can save time and resources. Breeding between parents who have significant genetic diversity is often most conducive

to genetic progress. The ability to expand the genetic base with regard to yield and adaptation is made feasible by potential donors who have innovative features in their germplasm. Given the aforementioned information, the goal of the current study was to use multivariate analysis to determine the genetic diversity of chickpea germplasm.

2. Materials And Methods

Location of the study: At the experimental farm of the department of Genetics and Plant Breeding, Lovely Professional University, Phagwara, Kapurthala district, Punjab, India, the study titled "Agro-morphological exploitation of genetic diversity and multivariate analysis of indigenous chickpea (*Cicer arietinum* L.) germplasm" was conducted. The study area's latitude and longitude were 31.2554° N and 75.7058° E, respectively.

Year	:	2019-20
Crop season	:	rabi
Design	:	RBD
No. of genotypes	:	51
No. of replications	:	3
No. of rows/ entry	:	2
Row length	:	2m
Row to row gap	:	45cm
Plant to plant distance	:	10cm.

Layout and design:

The test site had a sandy loam, was fertile, and had a regular topography. While cultivating, recommended production and safety procedures were followed.

Observations recorded: Test weight (TW), biological yield per plant (BY), harvest index (HI), days until first flowering (DFF), days until 50% flowering (D50%F), days until maturity (DM), plant height (PH), number of primary (NPB) and secondary branches (NSB) per plant, number of pods per plant (NPP), number of filled (NFP) and unfilled pods per plant (NUFP), number of seeds per pod (NSP), and days until maturity (DM) were among the 14 quantitative characters for which data were gathered. With an exception for the days to first flowering, days to 50% flowering, and days to maturity, which were tracked on a plot basis, the data for the most of the characters was gathered from a randomly chosen sample of five plants in each entry.

Statistical tools: Using the statistical tool Windostat, the recoded data was subjected to genetic distance calculations using the D^2 (Mahalanobis, 1936) methodology, and clustering was carried out using the Tocher's method (Rao C.R., 1952). The yield and yield component trait values were correlated, and the principal component analysis (PCA) was computed using these values.

3. Results and Discussion

Genetic divergence (D^2 analysis):

To measure genetic divergence or differentiation across groups of data points, which in the context of plant breeding are frequently genotypes, Mahalanobis D^2 statistics is a commonly used method in a variety of domains, including plant breeding.

Table-1.0: List of Genotypes

1	KWR 108	**18**	ICC 3525	**35**	RSG 991
2	PDE 9802 E	**19**	ICC 5434	**36**	GNG 469
3	JG-13-14-16	**20**	DCP 92-3	**37**	RSG 963
4	ICC 3020	**21**	GG-2	**38**	RSG 974
5	SUBHRA	**22**	PUSA 372	**39**	CSJ 515
6	BG 3043	**23**	ICC 5439	**40**	JG 130
7	IPC-06-77	**24**	BPM	**41**	BGD 9971
8	KAK-2	**25**	IPC 07-56	**42**	RSG 945
9	KPG-59	**26**	RSG 973	**43**	SADABAHAR
10	PUSA 547	**27**	RSG 807	**44**	PBG 5
11	K-850	**28**	RSG 931	**45**	JG 16

12	IPCK 04-29	**29**	PDG 4	**46**	RSGK 6
13	ICC 5335	**30**	NBEG 47	**47**	PBG 7
14	IPC 9767	**31**	IPC 05-28	**48**	CSJK 54
15	ICC 244-263	**32**	PUSA 3043	**49**	CSJK 6
16	BG 212	**33**	RGC 888	**50**	RGG 6
17	PUSA 72	**34**	RSG 888	**51**	PUSA 3022

Composition of Clusters:

The Tocher's approach was used to classify genotypes, as suggested by Rao C.R. in 1952. Seven clusters were formed from a total of 51 chickpea germplasm samples that were examined for the kind and degree of genetic diversity (Table-2.0). Clusters I, II, III, IV, and VI were polygenotypic, while Clusters V and VII were found to be solitary. This demonstrated the diversity of the experimented materials used in the investigation.

Table-2.0: Distribution of chickpea genotypes into different clusters

Cluster no.	No. of genotypes	List of genotypes
Cluster I	24	PUSA 372, RGC 888, GG-2, RSGK 6, NBEG 47, RSG 973, IPC 05-28, RSG 945, SADABAHAR, RSG 807, IPCK 04-29, RSG 963, KAK-2, PUSA 547, PUSA 72, BG 3043, ICC 3525, ICC 5434, DCP 92-3, ICC 244-263, PUSA 3043, RSG 974, RSG 991, RSG 888
Cluster II	10	PDE 9802 E, ICC 3020, JG-13-14-16, K-850, IPC 07-56, RVG 203, IPC-06-77, IPC 9767, BPM, CSJ 515
Cluster III	7	JG 130, BGD 9971, PBG 5, JG 16, SUBHRA, BG 212, PBG-7
Cluster IV	4	CSJK 6, RGG 6, PUSA 3022, KPG-59
Cluster V	1	ICC 5439
Cluster VI	4	GNG 469, CSJK 54, KWR 108, ICC 5335
Cluster VII	1	RSG 931

The first cluster, which had a total of 24 genotypes, was the largest of them all. It was followed by the second cluster, which held 10 genotypes, the third cluster, which held 7 genotypes, and clusters IV and VI, which each held 4 genotypes. As was previously mentioned, Clusters V and VII only contained one genotype apiece. Which means that the reason for choosing parents for a crossing program should include both genetic and geographic origin, rather than just geographic diversity.

Intra and Inter Cluster Distances:

As previously noted, the intra and inter cluster distances D2 between all conceivable pairs of the seven clusters were calculated, and the results are shown in table (2.0). Cluster VI recorded an intra cluster value of 9.47, cluster III revealed the highest intra cluster D2 value (D2 = 9.81), cluster II revealed the second-highest intra cluster D2 value (8.55), and cluster III revealed the lowest intra cluster distance of them all (7.96) (Table 3.0).

Table-3.0: Inter and intra cluster D^2 values for different clusters

Cluster	I	II	III	IV	V	VI	VII
I	**7.96**	15.85	11.33	10.92	14.89	33.09	13.43
II		**8.55**	20.91	11.61	10.94	20.64	13.86
III			**9.81**	16.73	17.26	38.07	14.94
IV				**8.58**	13.61	27.33	13.97
V					**0.00**	23.69	9.98
VI						**9.47**	29.37
VII							**0.00**

Regarding the intercluster distance, the genotypes of clusters III and VI had the highest value (D=38.07), followed by clusters I and VI (D=33.09). The genotypes associated with these clusters can be used in hybridization programs because crosses involving genotypes associated with the clusters showing the greatest intercluster distance may display higher heterotic response, leading to better recombinants. These clusters appear to be quite different from one another.

Cluster Means for Various Characters:

The results were sufficiently clear to demonstrate that there were variations in the cluster means of the traits presented in Table 4.0. DFF and D50%F showed the greatest cluster mean values within Cluster III, while PH, NFP, TW, BY, HI, and SY showed the highest cluster mean values in Cluster VI, and DM, NPB, NSB, and NPP displayed the highest cluster mean values in Cluster VII. As a result, parent selection for crossing programs among the entries of the aforementioned clusters would result in increased magnitudes of heterosis and segregants for various economic aspects. To use as parents in a proposed hybridization operation, potent lines must be found from these clusters. (Mihoariya *et al.* 2023), (Mahmood *et al.* 2022), (Kumar *et al.* 2022), and (Boparai and Katna, 2021) found similar findings.

Table-4.0: Cluster mean for yield and its component traits of chickpea genotypes employing Tocher's Method

	DFF	D50%F	DM	PH	NPB	NSB	NPP	NFP	NUFP	NSP	TW	BY	HI	SY
Cluster I	101.40	109.37	172.71	42.34	3.76	6.22	76.49	66.00	10.49	1.64	9.00	17.88	39.71	7.10
Cluster II	101.23	109.13	171.73	45.78	3.87	6.49	79.21	67.75	11.47	1.91	11.32	19.49	46.81	9.14
Cluster III	99.33	107.19	168.19	41.52	4.03	6.41	80.15	67.55	12.60	1.76	8.32	16.84	36.66	6.18
Cluster IV	102.67	110.58	173.83	42.52	3.80	6.50	71.15	62.47	8.68	1.90	10.03	16.65	41.51	7.00
Cluster V	97.33	105.33	162.00	39.40	4.13	6.47	78.40	66.53	11.87	2.33	11.13	19.37	44.86	8.70
Cluster VI	98.92	106.83	168.17	45.85	3.97	6.68	79.67	69.65	10.02	1.83	14.68	22.26	51.39	11.49
Cluster VII	101.00	109.00	175.00	39.00	4.13	7.20	82.00	67.00	15.00	2.73	9.97	17.04	44.18	7.47

Contribution of Various Characters towards total Genetic Divergence:

By evaluating the relative contribution made by each character on overall genetic divergence, D2 analysis's application is enhanced. The contribution of each characteristic to overall genetic diversity is shown in Figure (1.0). The results showed that the NSP, NPP, NFP, DFF, PH, DM, HI, NPB, BY per plant, number of unfilled pods, SY, and NSB contributed the most to total genetic divergence. These characteristics are responsible for expressing the greatest variability among clusters. The findings of (Vikram *et al.* 2023) and (Mihoariya *et al.* 2023) corroborated these findings.

Fig-1.0: Contribution of various traits towards clustering in chickpea genotypes

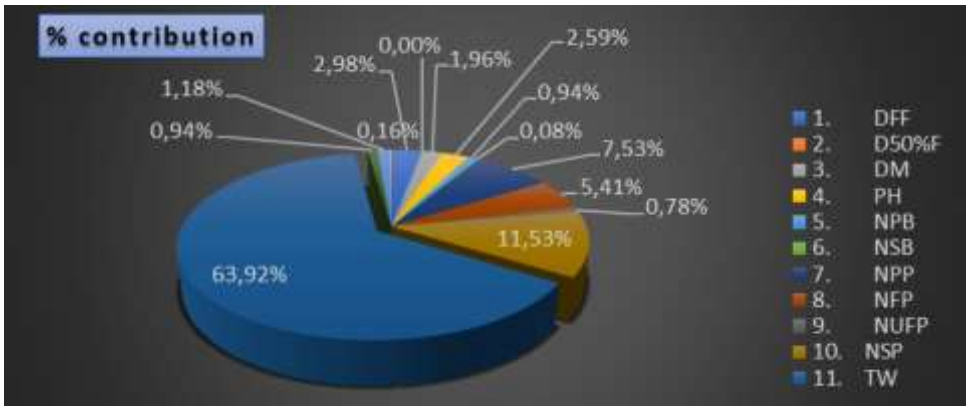


Fig-2.0: Clustering of genotypes by torcher method

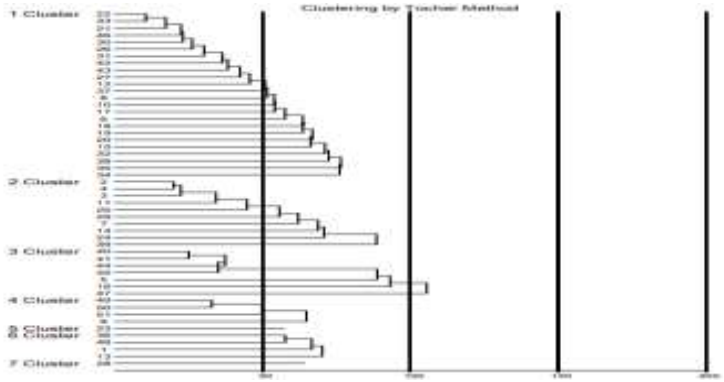
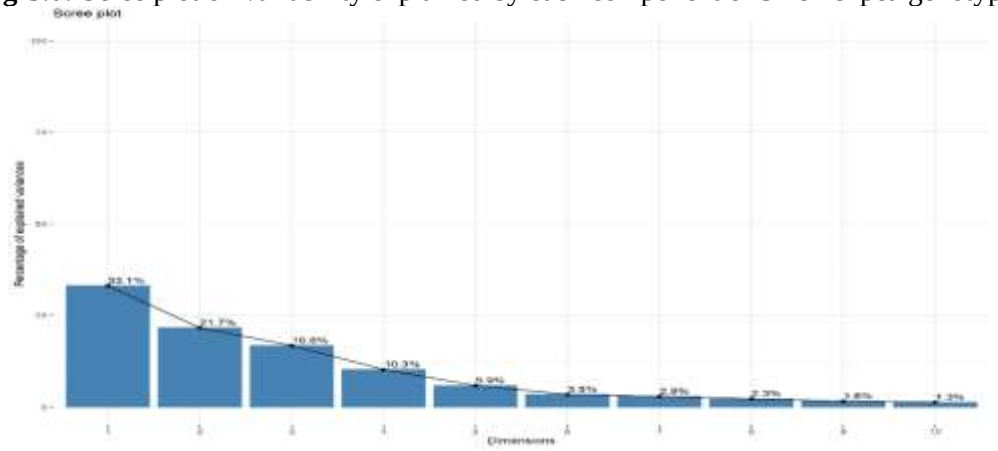


Fig-3.0: Scree plot on variability explained by each component of 51 chickpea genotypes



Principal component analysis:

A statistical method called principal component analysis (PCA) can be used to evaluate high-dimensional data and extract the most crucial information from it. This is achieved by combining strongly linked variables while changing the original data into a lower-dimensional space. A total of fourteen principal components have been generated (PC 1 to PC 14), which also correspond to the number of traits in the data, where each component explains a percentage of the total variance in the data set.

Choosing the Principal Components

With a suggested threshold of only keeping components with eigenvalues larger than or equal to 1, Kaiser's Rule for PCA is a technique for figuring out the best number of principal components to keep depending on the eigenvalues. First four of the fourteen PCs had eigenvalues greater than one (Table-5.0), similar results were reported by (Hailu, 2021; Jakhar *et al.* 2018). The variation explained by each primary component is quantified by the eigenvalue, with bigger eigenvalues indicating more variance explained.

Table-5.0: Eigen analysis of the Correlation Matrix

PCs	PC1	PC2	PC3	PC4
Eigenvalue	4.6405	3.036	2.3571	1.4384
Proportion	0.331	0.217	0.168	0.103
Cumulative	0.331	0.548	0.717	0.819

Table-6.0: Eigenvectors showing Principal Components for 14 yield contributing traits of chickpea

Variable	PC1	PC2	PC3	PC4
DFF	-0.223	0.207	-0.464	-0.242
D50%F	-0.232	0.219	-0.454	-0.226
DM	-0.259	0.115	-0.409	-0.116
PH	0.313	-0.107	-0.248	-0.131
NPB	0.012	0.441	0.289	-0.149
NSB	0.052	0.502	0.204	-0.083

NPP	0.293	-0.199	0.029	-0.558
NFP	0.324	-0.192	-0.085	-0.371
NUFP	-0.021	-0.049	0.268	-0.52
NSP	0.017	0.448	0.205	-0.184
TW	0.347	0.178	-0.097	0.216
BY	0.385	0.007	-0.176	0.118
HI	0.328	0.298	-0.164	0.074
SY	0.4	0.195	-0.187	0.104

Graphical Presentation of the scree plot

The first principal component accounts for around 33.1% of total variation, followed by PC2 (21.7%), PC3 (16.8%) and PC4 (10.30%) contributing 81% of the overall variability (Tsehaye *et al.* 2020). These PCs can be used to demonstrate the number and contribution of top-performing genotypes and factors to total variability.

According to the loading matrix, the first principal component exhibited high positive values for yield related characters SY (0.4), BY (0.385), TW (0.347), HI (0.328), NFP (0.324), PH (0.313), NPP (0.293), NSB (0.052), NSP (0.017), and NPB (0.012) and was negative for chronological characters *i.e.*, DM (-0.259), days to 50% flowering (-0.232), DFF (-0.223), and NUFP (-0.021).

The NSB (0.502) had the highest positive weight in the second principal component, followed by the NSP (0.448), the NPB (0.441), the HI (0.298), the D50%F (0.219), the days to the first flowering (0.207), the SY (0.195), the TW (0.178), the DM (0.115), and the BY (0.007). PH (-0.107), NFP (-0.192), number of empty pods per plant (-0.049), and NPP (-0.199) all yielded negative findings.

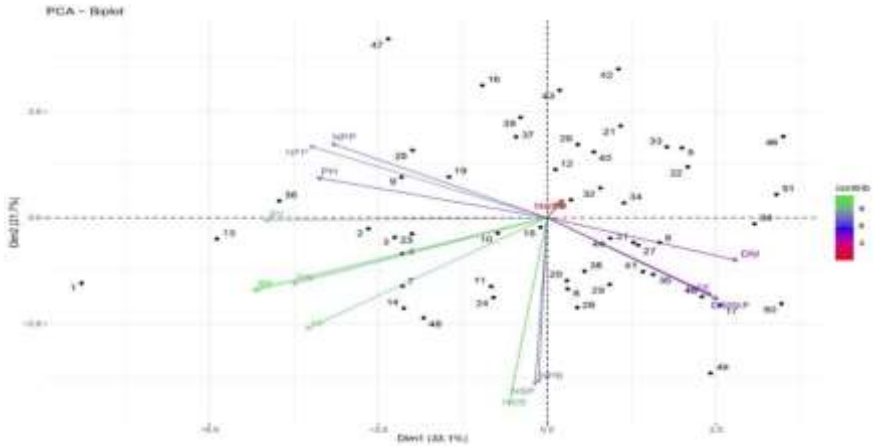
The NPB per plant (0.289) had the highest positive values in the third principal component, followed by the number of empty pods per plant (0.268), the NSP (0.205), the NSB per plant (0.204), and the NPP (0.029). D50%F (-0.454), DM (-0.409), PH (-0.248), SY (-0.187), BY (-0.176), HI (-0.164), and NFP (-0.085) all indicated negative values.

In the fourth principal component, the highest positive weights were obtained for TW (0.216), BY (0.118), SY (0.104), and HI (0.074). The lowest weights were for the NPP (-0.558), the number of empty pods per plant (-0.52), the NFP (-0.371), the NSP (-0.184), the plant's height (-0.131), the number of days until maturity (-0.116), and the NSB (-0.083).

PCA-Biplot Analysis

The first two major components of the dataset's biplot analysis, which revealed a 54.80% divergence, indicate the relationship between various features and genotypes (Figure 4.0). In the biplot, the two initial main components were represented by four coordinates along the x- and y-axes.

Fig-4.0: Biplot for the 51 chickpea genotypes and 14 traits along the first 2 principal components



Coordinate-1 comprised fourteen genotypes with positive values for the first and second PCs: 5, 12, 18, 21, 22, 26, 32, 33, 34, 40, 42, 43, 46, and 51. For this position, the variable Number of empty pods per plant was connected to genotypes. Genotype 46, which is located far from the origin, is the most diversified. Genotypes 42, 51, and 43 follow, with genotype 43 being the most diverse.

Coordinate-2 is made up of eight genotypes: 9, 16, 19, 25, 36, 37, 39, and 47. These genotypes are exhibited in the negative direction of PCA1, with genotype 9 directly associated to the number of filled pods and genotype 19 closely related to the NPP. The number of full pods per plant was the longest vector, followed by the NPP and PH. (ref)

The thirteen genotypes that make up Coordinate-3 are 1, 2, 3, 4, 7, 10, 11, 13, 14, 15, 23, 24, and 48. With them, the first two primary components were adverse and were located at coordinate 3. Important variables like the BY, SY, TW, HI, and NSB were linked to this coordinate's treatments. The most diverse genotype is genotype 1, which is positioned far from the origin and is followed by genotype 13. All other genotypes are situated close to the center. The longest vectors were seen in the SY and NSB, followed by the HI, which showed the greatest volatility. While genotypes 10 and 4 had a substantial correlation with SY and TW, genotypes 7 and 15 had a strong correlation with HI and the NSB.

The sixteen genotypes that make up Coordinate-4 are 6, 8, 18, 20, 27, 28, 29, 30, 31, 35, 38, 41, 44, 45, and 49, respectively. These genotypes have positive and negative values for the first and second PCs. The genotypes for this coordinate were related to significant factors including the DFF, D50%F, and DM. While genotypes 17, 35, 41, and 45 were strongly related with DFF and D50%F, respectively, genotype 6 was strongly connected with DM. The biplot's genotype distribution indicated that there was some genetic variation among the various genotypes of chickpea.

Contribution along with the Quality of Variables and Genotypes

The SY among the 14 variables demonstrated the highest percentage contribution and quality of representation for the overall variability, followed by the NSB, HI, and BY, TW, NSP, NFP, NPB per plant to principal components 1-2 (Figures 5a and 5b). Findings of (Hailu, 2021; Tsehaye *et al.* 2020) supported these results.

Figure 5a: Graphical representation of percent contribution of variables to Dim-1-**2**

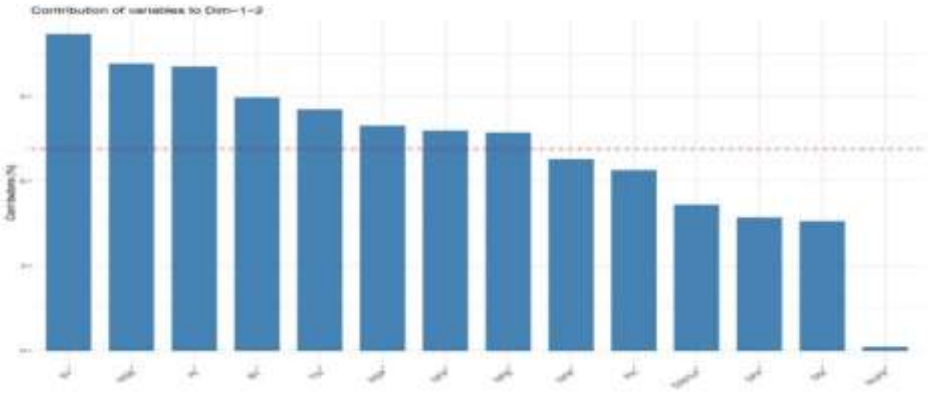
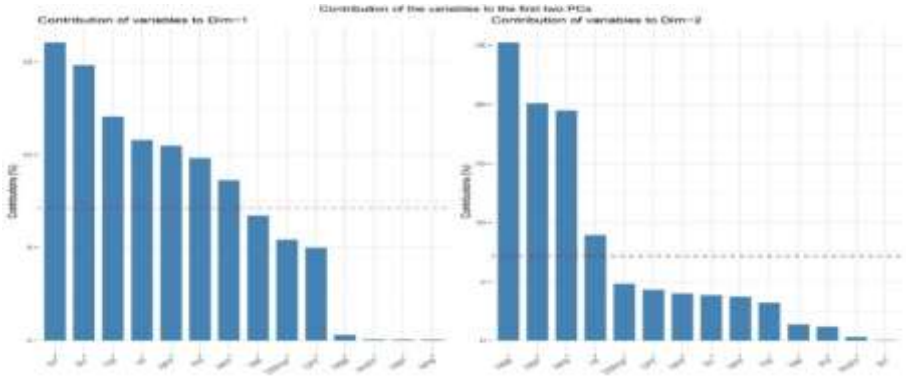


Figure 5b: Graphical representation quality of contribution of variables



The genotype 1 of the 51 chickpea genotypes contributed the highest proportion to the total diversity, followed by genotypes 3, 47, 49, 50, 36, 46, 42, 51, 17, 16, 30, 14, 43, 48, and 45, to main components 1-2, respectively. However, genotype 1, followed by genotypes 49, 13, 43, 47, 42, 17, 16, 50, 51, 5, 22, 7, 21, 46, 14, 30, 33, and 2 in that order, revealed the greatest quality divergence (Figures 6a & 6b).

Figure 6a: Graphical representation of percent contribution of each genotype to the total variance

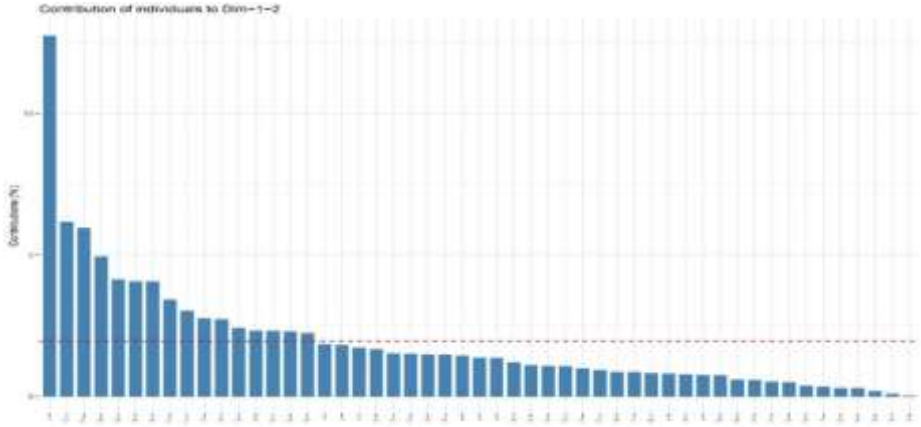
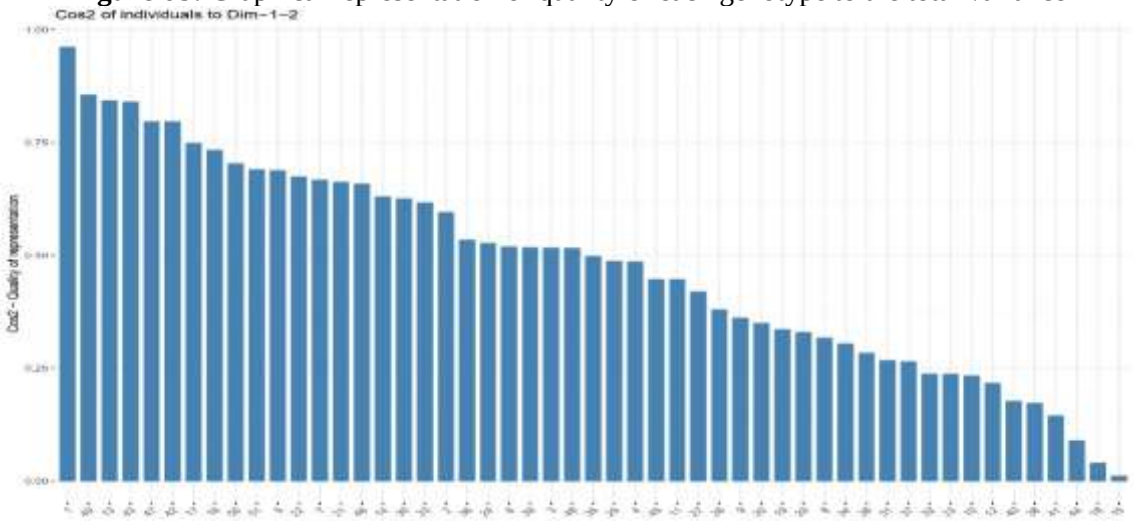


Figure 6b: Graphical representation of quality of each genotype to the total variance



4. Conclusion

The significant variances in agro-morphological features revealed that there was a wide range of variability among the germplasm accessions. The accessions formed seven clusters, indicating more genetic diversity. When compared to accessions placed in a single cluster, those placed in many clusters showed a considerable level of genetic divergence. Similarly, greater genotypic heterogeneity within these clusters is indicated by the distance between them. It is feasible to improve progenies by harnessing genetic variety and grouping information by employing a varied parental line. Further hybridization studies to generate transgressive sergeants to boost chickpea production and farmer revenue can benefit from genotypes that indicated desired and helpful diversity for examined parameters, particularly the quantity of pods per plant and days to 50% flowering. Researchers can learn how much variance each genotype and study feature contribute using PCA. Every breeder's breeding program should include extremely variable genotypes. Biplot, on the other hand, ties genotypes to traits to ensure that there is a link between them in variability. Seed Yield, Number of Secondary Branches, Harvest Index, and biological yield, test weight, number of seeds per pod, and number of filled pods per plant were all found to be variable using biplot analysis. These are critical in terms of contributing to total yield. According to the data, the top five performing genotypes with quality divergence were KWR-108, CSJK 54, ICC 5335, SADABAHAR and PBG 7. Further hybridization studies to generate transgressive sergeants to boost chickpea production and farmer revenue can benefit from genotypes that indicated desired and helpful diversity for examined parameters.

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