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Multivariate Analysis for Elucidating Genetic Diversity of Chickpea (*Cicer arietinum* L.) Germplasm using Agro-morphological Traits

Amit Kumar[1], Hitesh Kumar[1], Chandra Mohan Singh[1], Mukul Kumar[1],
Vijay Sharma[1], Sunil Kumar[1], G.S. Panwar[2]

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ABSTRACT

Background: Chickpea is grown on a large scale in India but the productivity is very low as compared to other chickpea-growing countries. Because, in India, the crop is affected by many biotic and abiotic stresses and also has a narrow genetic base in germplasm lines, which are the prerequisite of any breeding program for developing high-yield varieties. The breeding program's success depends on genetic variation and efficient selection strategies that make it possible to exploit exist-ing genetic resources.
Methods: The agro-morphological characterization of 94 chickpea germplasm accessions including four checks *viz*., JG 14, JG 16, JAKI 9218 and Radhey was carried out during *Rabi* 2018-19. The experiment was laid out in augmented design in nine blocks. The analysis of variance was estimated with the help of statistical software SPAD and using the statistical package Windostat version 9.1.
Result: The significant variance among the germplasm lines was found for most of the studied traits. Genetic diversity analysis revealed the grouping of genotypes into eight distinct clusters irrespective of breeding centers. The maximum number of 32 genotypes was fallen in cluster II and the minimum in cluster VI which consisted of 8 genotypes, whereas clusters IV and VIII were identified as bi-genotypic clusters. Clusters VII and IV showed maximum inter-cluster distance indicating the maximum genetic diversity among the genotypes of these clusters. Principal component analysis transformed data into three variables (PCs) which contributes 97.78 percent of the total variation. The genotypes identified in this study will be utilized in chickpea improvement programs.

Key words: Agro-morphological traits, Cluster analysis, D^2-Statistics, Principal component.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the second most important legume crop after fababean and belongs to the family Fabaceae. Globally, 14.78 million tons of chickpea grain were harvested on 14.78 million ha during 2017-18 (FAOSTAT, 2017). It is a major pulse crop in India with a production of 11.23 million tons (mt), contributing to over 46% of the total pulses production (23.95mt) during 2017-18 (Dixit *et al*., 2019). More than 68% area is under rainfed cultivation in the central and southern zone of the country. Despite, the highest production in India ranking first in production, productivity is still stagnant due to various biotic and abiotic factors coupled with a lack of improved cultivar. The yield potential of varieties fluctuates in different agro-climatic regions under changing climate scenarios (Sharma *et al*., 2019). The narrow genetic base of the developed varieties is also one of the major constraints to low productivity (Bharadwaj *et al*., 2011), which needs to widen by creating genetic variability (Chandra *et al*., 2013). Genetic diversity is essential to initiating an effective breeding program to develop cultivars with high yield potential for wider agro-climatic regions. The morphological characterization of germplasm lines shows a significant role in the selection of diverse germplasm lines for desirable traits (Kumar *et al.,* 2021). The genetic diversity available in germplasm lines determines the level of improvement in crop productivity *per se* and component traits (Mohan *et al*., 2019). The crosses between the parents with more genetic divergence are generally the most responsive to genetic improvement. The potential donors with novel traits in germplasm lines are one of the possible ways to broaden the genetic base in terms of yield and adaptation. Because of the above facts, the present study was conducted to estimate genetic diversity in chickpea germplasm lines through multivariate analysis.

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[1]Department of Genetics and Plant Breeding, Banda University of Agriculture and Technology, Banda-210 001, Uttar Pradesh, India.
[2]Department of Agronomy, Banda University of Agriculture and Technology, Banda-210 001, Uttar Pradesh, India.

Corresponding Author: Hitesh Kumar, Department of Genetics and Plant Breeding, Banda University of Agriculture and Technology, Banda-210 001, Uttar Pradesh, India.
Email: hiteshkmr25@gmail.com

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MATERIALS AND METHODS

Plant material and field experimentation

The plant material comprised 94 chickpea germplasm accessions including four checks. The experiment was carried out during *Rabi* 2018-19 at Banda University of Agriculture and Technology, Banda, Uttar Pradesh, India (24°53′ and 25°55′N and 80°07′ and 81°34′E 123 m ASL) in augmented design (Federer, 1956). A total of 10 test genotypes were planted in each block along with repeated checks to control local experimental error. The accessions were sown in paired rows of 2 m length keeping 30cm row to row and 10cm plant to plant distance. All the recommended package of practices was followed to grow a healthy crop. The total precipitation of 19.5 mm was recorded on five rainy days from sowing to harvesting duration. Throughout the crop period, temperature ranged from 7.64°C to 33.53°C and maximum relative humidity (80.00%) in January whereas, a minimum (44.00%) was recorded in March 2019 (Fig 1).

Observations recorded

A total of 17 yield contributing traits including yield were measured on the germplasm accessions. Among them, days to germination, early plant vigour, days to the first flower appearing on the plants, days to 50% flowering, days to 100% flowering, days to first pod appearance and days to maturity were observed on a plot basis. The plant height, height of first pod, inter-nodal distance, primary branches, secondary branches, pods per plant, seeds per pod and harvest index were recorded on five randomly selected plants on each accession.

Statistical analysis

The average trait values were analyzed as per the statistical procedure (Johnson *et al*., 1955) along with repeated checks to estimate adjusted mean and analysis of variance (ANOVA) with the help of statistical software SPAD (Rathore *et al*., 2004). Further, adjusted values were subjected to calculate genetic distance using D^2 (Mahalanobis, 1936) method and clustering was done by Tocher's method (Rao, 1952) using the statistical package Windostat version 9.1. The principal component analysis (PCA) was calculated with a correlation matrix of yield and yield component traits.

RESULTS AND DISCUSSION

Analysis of variance

The success of any breeding programme depends on the wide genetic variation and efficient selection strategies that make it possible to exploit exist-ing genetic resources. Thus, knowledge of genetic variability is a basic prerequisite for any crop improvement programme to develop new genotypes to meet production, protection and consumer requirements (Kumar *et al*., 2020). The genetic distance estimates form the basis for selecting parental combinations which leads to developing the new recombinants and scope for selection of transgressive segregates in early segregating generations (Singh *et al.,* 2016). The analyses of variance (ANOVA) of 17 biometrical traits are presented in Table 1. The variability in germplasm showed significant differences among all the recorded traits indicating the presence of considerable variability in the breeding material, except for the number of secondary branches, number of pods per plant and number of seeds per pod. The significant differences over the blocks for days to germination, days to flower initiation, days to 50% flowering, days to 100% flowering, days to first pod appearance, days to maturity, plant height, height of first pod and grain yield were also reported by Kumar *et al*. (2021).

Cluster analysis

Based on the seventeen quantitative characters, D^2 analysis grouped 94 germplasm accessions into eight clusters (Table 2). The maximum numbers of accessions (32) were grouped in cluster II followed by cluster I (28), cluster III (12), cluster VI (8), cluster V (7), cluster VII (3) and cluster IV and VIII consisted two accessions in each cluster. The intra-cluster distance indicates the similarities between germplasm lines under a single cluster for various yield traits. The forty genotypes of chickpea were clustered into four clusters reported by Qadeer *et al*., (2021) who found that maximum

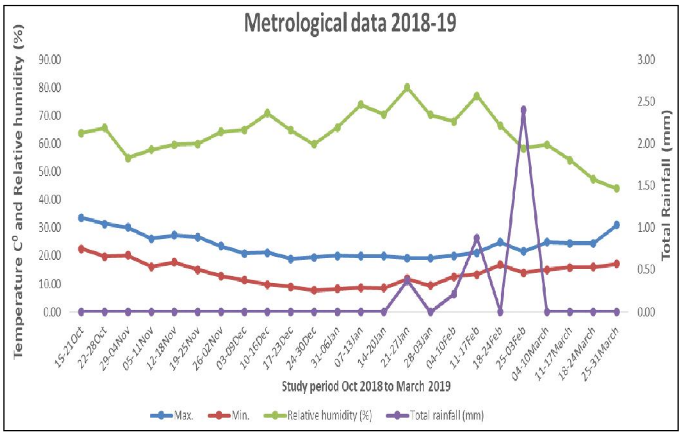

Fig 1: Weekly temperature (Max. Min.), air humidity and rainfall during study period *rabi* 2018-19.

genotypes consisted of cluster III (14). Similarly, fifteen genotypes of chickpea were also grouped into three clusters by Mahmood *et al*. (2018). The intra-cluster distance of clusters ranged from 279.88 to 3860.74 is presented in Table 3. Inter-cluster distance shows the dissimilarities and diversity among the clusters. The maximum inter-cluster distance (179096.9) was recorded between clusters VII and IV followed by clusters IV and III (140587.5), clusters VIII and VII (128935.5) and clusters IV and I (96556.05). Cluster II and I showed minimum inter-cluster distance (3639.5). Similar results were reported with intra and inter-cluster distance among seven groups for thirty-six genotypes (Agarwal *et al*., 2018). Clusters with maximum inter-cluster distances were indicating the highest genetic diversity among the genotypes which were grouped in these clusters and could be useful for recombination breeding. The lowest inter-cluster distance shows the closeness between clusters with low diversity. The diversity analysis based on agro-

Table 1: Analysis of variance (ANOVA) for the seventeen quantitative characters in 94 genotypes of chickpea.

Source	Mean sum of square					
	Block (Adj.)	Treatments (Adj.)	Error	Among control	Among test genotypes	Test-vs control
d.f.	8	93	24	3	89	1
GR	8.81**	4.90*	2.61	5.13	4.90*	0.00079
EPVS	1.98	3.88*	1.91	6.70*	3.78*	4.34
FI	41.18*	102.49***	14.51	487.74***	90.48***	15.11
F50	49.12**	101.00***	11.76	437.62***	89.82***	86.42*
F100	76.12**	40.31*	17.78	177.65***	35.93*	18.09603
FPA	106.87***	75.99***	7.75	225.29***	71.81***	0.38
PH	95.27*	79.05**	30.82	314.48***	70.39*	143.08*
HFP	155.02***	58.13***	8.74	120.11***	56.65***	3.92676
DTP	0.04	0.15***	0.03	0.09	0.16***	0.03
PB	0.56	1.49***	0.39	3.18***	1.41***	2.85*
SB	8.15	5.53	4.37	25.65**	4.9	1.2
PPP	168.61	108.49	104.71	369.87*	100.89	0.97
SPP	0.15	0.27	0.18	1.13**	0.23	0.76
DM	62.87***	24.71***	6.52	14.91667	25.32***	0.03889
HI	6.75784	39.53***	6.74	161.24***	34.87***	88.79**
GW	1.4	42.44***	1.02	14.22***	39.13***	422.4***
TGY	5959.93	8936.34**	3237.75	17961.22**	8338.19**	35097.22**

*Indicates significance at P=0.05; **Indicates significance at P=0.01; ***Indicates significance at P=0.001; GR- Days to germination; EPVS- Early plant vigour; FI- Flower initiation; F50- Days to 50% flowering; F100- Days to 100% flowering; FPA- Days to first pod appearance; PH- Plant height; HFP- Height of first pod; DTP- Internodes distance; PB- Primary branches; SB- Secondary branches; PPP- Pods per plant; SPP- Seeds per pod; DM- Days to maturity; HI- Harvest index; GW- 100 seed weight; TYP- Total yield per plot.

Table 2: Grouping of 96 chickpea germplasm lines based on D^2 statistics clusters.

Cluster no.	Frequency	Name
I	28	GNG 2372, CSJ 868, JG 16, RG 2015-08, KGD 2013-2, GNG 2391, JG 36, JG 218, JG 11, JG 24, JG 315, Vijay, ICCV181107, IPK 16-103, EC-556270, ICP-08-103, GNG 1581, GNG 469, GNG 2226, GNG 2171, GNG 1999, GNG 1969, GNG 1107, BG 11-1, DC 2012-13, DC 2012-5, KDG 12-49, JG 14
II	32	BDNG 2010-1, JG 322, DBGV 215, PG 184, BRC 5, KGD 2017-1, JG 412, JG 12, JG 130, GPF 2, ICCV-181108, ICCV-181105, ICCV-181101, HC 5, ICCV-181110, ICCV-181117, GNG 1947, ICC-495468, PUSA-1053, CSJ-515, BDG 3038, BG 5051, BDNG 804, GNG 2127, GNG 1958, DC 2012-14, GNG1854, ICCV-181312, ICCV-181310, JG 16, Radhey, JAKI 9218
III	12	BG 3087, JG 2017-47, PBG 7, L 552, PBG-5, GNG 1929, BDG 1059, GNG 312, GNG 2081, DG 1012-3, GNG 1926, BKD 876
IV	2	ICVT-181103, ICCV-181102
V	7	PDG-4, ICCV-181113, ICCV-181114, ICCV-181115, ICCV-181116, IC 244263, ICCV-181118
VI	8	ICVT-181106, ICCV-181104, ICCV-181111, K-850, ICCV-205, BG-212, KGD 11-1
VII	3	CSJ 867, PDG 3, ICCV-181112
VIII	2	RLBG-2, BG 5030

Table 3: Intra and inter-cluster distances amongst 8 clusters.

Cluster no.	I	II	III	IV	V	VI	VII	VIII
I	1540.84							
II	8484.58	1679.06						
III	3639.5	18642.41	1189.92					
IV	107139.3	60164.68	140587.5	279.88				
V	5488.19	4514.51	12443.89	79923.38	2408.26			
VI	30805.06	9719.79	49236.2	26381.47	18498.05	3390.47		
VII	11743.07	34582.83	4812.72	179096.9	23029.14	73138.8	3860.74	
VIII	69199.91	32898.04	96556.05	5339.7	48025.07	10452.82	128935.5	1430.1

Diagonal- Intracluster distance; Non-diagonal- Inter-cluster distance.

Table 4: Cluster mean values of seventeen traits and percent contribution of each trait towards genetic divergence.

Characters	Clusters								Contribution
	I	II	III	IV	V	VI	VII	VIII	%
GR	9.39	8.38	9.50	7.50	9.86	9.25	9.33	11.00	0.02
EPVS	6.04	6.08	6.10	3.97	4.11	4.44	4.47	4.72	0.00
FI	76.96	74.50	77.67	74.00	55.29	73.38	65.00	72.50	2.81
F50	83.54	82.97	84.33	78.00	61.86	81.13	70.33	80.50	2.43
F100	102.64	102.28	101.33	101.00	97.29	100.63	96.67	106.50	0.50
FPA	90.29	89.78	93.75	89.50	71.00	89.50	78.00	83.50	1.49
PH	47.78	51.75	46.36	50.79	45.70	50.37	44.00	53.22	0.02
HFP	21.36	28.72	15.58	28.50	25.43	32.25	11.33	48.00	1.81
DTP	1.51	1.77	1.34	2.10	1.82	1.69	1.54	1.83	0.27
PB	3.07	3.59	3.00	4.00	3.43	4.13	1.67	5.00	0.00
SB	5.04	6.03	3.00	8.50	5.29	6.25	2.67	8.50	0.00
PPP	16.85	18.46	18.37	18.32	11.91	17.98	16.24	17.95	0.00
SPP	1.32	1.22	1.67	1.50	1.57	1.13	1.33	1.50	1.44
DM	124.29	125.06	126.75	123.00	114.57	123.25	119.00	125.50	0.00
HI	52.50	50.59	51.73	63.83	53.34	53.07	57.38	61.01	1.72
GW	17.17	21.82	16.15	24.53	20.24	21.27	13.91	23.59	0.66
TGY	159.54	242.13	111.72	485.14	206.64	328.08	64.56	418.64	86.82

morphological traits in chickpeas is a useful method to divide genotypes into different groups which could be utilized for further improvement programs (Mohammed and Tesso, 2019).

Cluster mean and trait contribution toward divergence

The mean values of clusters of yield and component traits are presented in Table 4. The mean value for days to flower initiation was highest in cluster III (77.67days) and lowest in cluster V (55.29days). The mean value for days to 50% flowering ranged from 62 to 84 days represented in clusters V and III respectively. The mean value for plant height was highest (53.22 cm) in cluster VIII and lowest (44.00 cm) in cluster VII. The lowest mean value for days to maturity (114.57) was found in cluster V and the highest (126.75) in cluster III. Cluster IV shows the highest mean value for harvest index (63.83) and the lowest (50.59) in cluster II. The highest mean values for grain yield per plant, harvest index, biological yield per plant, primary and secondary branches per plant, plant height and days to maturity showed in cluster VII (Agarwal *et al*., 2018). Cluster II out of four clusters showed the maximum mean value for number of pods per plant (83), 100 seed weight (31 gm), plant height (55 cm) and yield (1732 kg/ha) reported by Qadeer *et al*., (2021). The contributions of characters toward the genetic divergence are presented in Table 5. The maximum contribution towards total divergence was recorded for the seed yield per plant (86.82%) followed by the days to flower initiation (2.81%), days to 50% flowering (2.43%), the height of first pod (1.81%), harvest index (1.72%), days to first pod appearance (1.49%), number of seeds per pod (1.44%) and rest of the traits were very low contribution towards genetic divergence. Under the present results, previous studies have demonstrated that maximum contribution toward total divergence for seed yield per plant, 100 seed weight, number of seeds per plant and number of secondary branches (Akhil *et al*., 2019).

Principal component analysis

The principal components analysis (PCA) transformed the large set of data into a small number of variables (PCs) which contribute to the maximum proportion of variance of the experimental data (Sharifi *et al*., 2018). The principal

Table 5: Eigenvector, eigenroot and associated variation for principal components analysis.

Characters	Eigenvector		
	1	2	3
Days to germination	0.00281	0.01417	0.04927
Early plant vigour	0.00383	-0.0233	-0.0206
Days to flower initiation	0.00915	-0.5664	0.07234
Days to 50% flowering	0.00408	-0.5516	-0.0109
Days to 100% flowering	-0.00481	-0.2349	0.08717
Days to first pod appearance	0.00673	-0.4963	-0.046
Plant height	0.00031	-0.2393	0.0459
Height of first pod	-0.02125	-0.0262	-0.577
Internodes distance	-0.00344	-0.1032	-0.1743
Number of primary branches	-0.00172	0.00571	-0.0118
Number of secondary branches	-0.00526	0.00299	0.00775
Number of pods per plant	-0.0146	0.00275	0.09478
Number of seeds per pod	-0.06958	0.01983	0.51316
Days to maturity	0.00083	-0.001	-0.0059
Harvest index	-0.01088	0.03891	0.42955
Seed index	-0.03047	0.04508	-0.3992
Seed yield per plant	-0.9966	-0.0121	-0.0169
Eigen value (Root)	676547.9	26487.3	8591.69
% Var. exp.	92.96106	3.63948	1.18054
Cum. var. exp.	92.96106	96.6005	97.7811

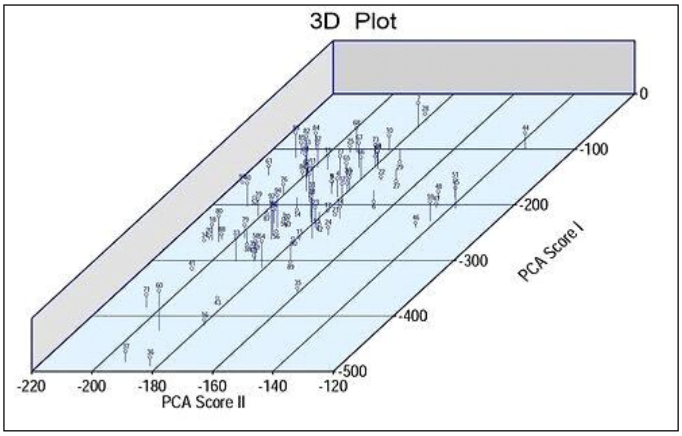

Fig 2: The three-dimensional PCA plot of 94 germplasm lines of chickpea.

component analysis of 90 germplasm lines of chickpea-based yield and contributing traits correlation matrix which yielded the eigenroots, eigenvectors and associated percentage of variation explained by eigenroot has been presented in Table 5. The recorded data was transformed into three principal components (PCs) which explained 97.78 percent of the total variation in which PC1, PC2 and PC3 accounted for 92.96%, 3.63% and 1.18% of the total variation, respectively (Fig 2). PCA provides a better way to understand the source of variation among the germplasm lines and is also responsible for the highest percentage of variation governed by the lower number of traits (Sharifi *et al*., 2018). The first principal component had the largest eigenroot values 676547.9 of total variation followed by 26487.3 and 8591.69 for the second and third principal components, respectively. Out of six principal components first two PCs with more than 1 eigenvalue were contributing 77.67% and 79.54% of the total variance (Rafiq *et al*., 2018; Rafiq *et al*., 2020).

The first principle component had the largest positive weight to days to flower initiation (0.00915) followed by days to first pod appearance (0.00673), days to 50% flowering (0.00408), early plant vigour (0.00383) and the high negative weight for seed yield per plot (-0.996), number of seeds per pod (-0.0695), seed index (-0.0304) and height of first pod (0.0212). The positive significant values for yield kg per ha, harvest index, pods per plant and 100 seed weight are exhibited by PC_1 (Mahmood *et al.,* 2018). The second principle component had the highest positive weight to seed index (0.0450) followed by harvest index (0.0389), number of seeds per pod (0.0198), days to germination (0.0141) and the high negative weight for days to flower initiation (-0.566), days to 50% flowering (-0.551), days to first pod appearance (-0.496), plant height (-0.293) and days to 100% flowering (-0.234). Similarly, Qadeer *et al*., (2021) reported that the PC_1 and PC_2 show the highest positive significant value for number of pods per plant and 100 seed weight. The traits which indicate significant eigenvalue among the categorized components should be considered for selection of parents in a hybridization program (Qadeer *et al*., 2021; Zubair *et al.,* 2017).

CONCLUSION

The significant differences in agro-morphological traits revealed the presence of a wide range of variability among germplasm accessions. Accessions were grouped into eight clusters indicating wider genetic diversity. The accessions grouped into different clusters showed high genetic diversity compared to those grouped under a single cluster. Similarly, the distance between the clusters shows more diversity between genotypes that came under these clusters. The genetic diversity and grouping information is useful to develop improved progenies by using the diverse parental line. The characters *namely* seed yield, days to flower initiation, days to 50% flowering, the height of first pod, harvest index, days to first pod appearance and number of seeds per pod were responsible for the highest diversity among the germplasm lines. Thus, it can be concluded that the genotypes which showed desire and useful diversity for studied parameters especially 50% flowering, seed and seed yield can be utilized further for hybridization programmes to develop transgrassive sergeants to increase chickpea yield and increase farmer income.

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Conflict of interest: None.

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