Original Research Article

Assessment of Genetic Diversity in Quantitative Traits of Chickpea

ABSTRACT

Chickpea (*Cicer arietinum*) is the most important pulse crop providing the livelihood to framers. India is one of the topmost countries in chickpea production fallowed by middle-east. Assessment of morphological and genetic diversity will help in the increase in production of chickpea. a comprehensive evaluation of 44 distinct genotypes was conducted during 2020-21 *Rabi*season at RLBCAU, Jhansi. Utilizing Tocher's method and D^2 values, these genotypes were meticulously organized into nine non-overlapping clusters, with cluster (I) comprising the most, featuring 17 genotypes, followed by cluster (II) with 12. Significant genetic diversity was evident, especially between clusters (IV) and (VI) (2710.13) and (V) and (VI) (2565.51), highlighting the diversity among these genotypes. Key contributors to this divergence were 100-seed weight (48.8%), number of secondary branches per plant (26.5%), and seed yield per plant (9%). Cluster (VIII) exhibited higher mean values for days to maturity (140.57), while cluster (VII) had elevated values for the number of pods per plant (84.61). genotypes like Phule G 171105, RVSSG 81, RLBG 6, GL-16063 and RKG 19-2 emerged as potential contributors to future breeding programs, promising significant hybrid vigour and superior heterotic segregants to advance chickpea genetics.

Key words: Chickpea, Genotypes, Genetic diversity, heterotic segregants.

Introduction

Chickpea (*Cicer arietinum* L.) is one of the most demanding and superior leguminous crops in most of the Indian states referred to as “King of Pulses.” Chickpea is an autogamous flowering plant with a genomic dimension of 738 Mbp having a true diploid chromosomal makeup of 2n=2x=16. (Varshney *et al.* 2013). In India, chickpea occupied the highest ever

production of 13.75 million tonnes covering an area of 10.91 million hectares with a productivity of 12.61 qt/ha (Anonymous, 2022).

Legumes as preceding crops in the rotation also increases the protein content of succeeding cereals and helps in lowering mycotoxin contamination (Lengwati *et al*. 2020). Crop plant improvement is greatly impacted by genetic diversityand this knowledge has been extensively utilised for genotype selection, identification and germplasm conservation. Selecting the genotypes with desirable traits, either independently or in combinations, frequently determines how yield is enhanced. This suggests there would be adequate genetic variability in the population as well as efficient selection standards for identifying enhanced genotypes for yield and associated traits. The germplasm serves as a reservoir for genetic diversity and helpful for conserving biodiversity of agricultural crop that is designed to satisfy the changing demands for developing improved crop varieties(ref). In addition, it's essential for economic attributes in the germplasm for better utilisation after hybridization, selection or breeding methods. The D^2 analysis classifies the genotypes into relatively homogeneous groups in such a way that, with in the clusters(Dvivedi *et al*., 2023).The purpose of this research was to assess the genetic diversity of the chickpea germplasm and identify genetically divergent parents from the available germplasm before initiating a hybridization programme to improve the heterotic response for subsequent breeding efforts.

Material and Methods

The experimental material comprised of 44chickpea indigenous collectionsdeveloped under ambit of All India Coordinated Research Project (AICRP) (**Table. 1**)These genotypes showed a broad range of diversity in terms of various morphological and agronomic traits. These genotypes were evaluated in randomized block design (RBD) with three replicates under well-irrigated condition at research farm atRLBCAU, Jhansi (U.P.) during Rabi2020-

21. Each genotype was sown with 4 m row length with four rows per plot in each replication followed by row to row spaced 30 cm and intra row spacing of 10 cm under well irrigated condition with all recommended agronomic practices to raise to healthy crop stand. In present study observations were taken for quantitative traits *viz*; days to 50 % flowering on plot basis, plant height (cm), leaflet size (mm), peduncle length (mm), pod length (mm), No. of primary branches per plant, No. of secondary branches per plant, No. of pods per plant, No. of seeds per pod, 100-seed weight (g), biological yield per plant (g), harvest index (%) and seed yield per plant (g) and days to maturity were recorded on plot basis.Divergence analysis was carried out as suggested by Mahalanobis's D^2 (1936).

Table 1: List of chickpea genotypes used for present study

S.N.	**Genotypes**	**Pedigree**	**Developed at centres**
1	GL-16063	GPF 2 x [PBG 1 x (ICCV 96030 x C. *pinnatifidum 188)* x ICCV 96030]	Ludhiana, Punjab
2	BRC 9-14	SAKI 19516 x GNG 1958	Dholi, Bihar
3	GNG 2462	GNG1958 x BG1064	Sriganganagar, Rajasthan
4	GJG 1707	GJG 0107 x GJG 0207	Junagadh, Gujarat
5	BG 4010	JG 11 / BG 1098	IARI, New Delhi
6	PG 227	M 35 (selection from MAGIC cross involving 8 parents)	Pantnagar, Uttarakhand
7	NBeG 690	ICCV 03112 x JAKI 9218	Nandyal, Andhra Pradesh
8	IPC 2015-12	IPC 2009-50 x IPC 2007-88	IIPR, Kanpur, Uttar Pradesh
9	NBeG 698	ICCV 03112 x JAKI 9218	Nandyal, Andhra Pradesh
10	ADBG 487	ICC4958 TM x JAKI9218	Adilabad, Telangana
11	RVSSG 79	JG 11 x JSC52	Sehore, Madhya Pradesh
12	RKG 19-1	JAKI 9218 x ICCV 00108	Kota, Rajasthan
13	DC 18-1107	ICC 4958 TM/JG 130	Dholi, Bihar
14	BAUG 106	JG 11 x ICC 4958	Ranchi, Jharkhand
15	PG 237	M 47 (Selection from MAGIC cross involving 8 parents)	Pantnagar, Uttarakhand
16	GJG 1708	GJG 0727 x GCP 101	Junagadh, Gujarat
17	JG 2019-155-118	ICCV 05530 x ICCV 88510	Jabalpur, Madhya Pradesh

18	Phule G 171103	(JG 11 x ICC 4552) x (ICCC 37 x ICC 5683)	Rahuri, Maharashtra
19	H 13-36	PDG84-16 x H04-31	Hisar, Haryana
20	RLBG 6	JG 14/ICCV 96836	Jhansi, Uttar Pradesh
21	DC 18-1104	Genesis 836/JAKI 9218	Dholi, Bihar
22	DBGC 1	BGD256 x WR315	CER Patna, Bihar
23	Phule G 171105	(ICC 4958 x ICCV 97105) x (ICCV 10 x ICCV 00108)	Rahuri,Madhya Pradesh
24	BG 4011	F1[F1(ICC4958 x ICCV10) x F1(Pusa372 x Pusa 256)] x F1(Pusa 547 x JAKI 9218)	IARI, New Delhi
25	H 12-22	HC1 x (HC1 x ICCV96030	Hisar, Haryana
26	IPC 2016-107	IPC 2009-50 x IPC 2007-88	IIPR Kanpur, Uttar Pradesh
27	NDG 18-2	MPJGK6 x BG 2058) x BGD 112	Faizabad, Uttar Pradesh
28	RSGD 1071	RSG-931 x JG-11	Jaipur, Rajasthan
29	BDNG 2017-44	Digvijay x ICC 4533	Badnapur, Maharashtra
30	RSGD 1057	JG-11 x RSG 973	Jaipur, Rajasthan
31	GL 17020	ICCX 04147 x ICCX 040126	Ludhiana, Punjab
32	BDNG 2017-49	BDNG 804 x BDNG 797	Badnapur, Maharashtra
33	BUC-1	Genesis 836 x JAKI 9218	Banda, Uttar Pradesh
34	IPCD 2016-44	IPC 2008-57 x WR 315	IIPR Dharwad, Karnataka
35	RKG 19-2	ICCV-14103 x BGD 72	Kota, Rajasthan
36	NDG 18-9	(MPJGK 6 x BG 2058) x BGD 112	Faizabad, Uttar Pradesh
37	GNG 2477	GNG 1581 x ICC1 2951	Sriganganagar, Rajasthan
38	AKG 1506	JAKI 9218 x AKG 46	Akola, Maharashtra
39	RVSSG 81	JAKI 9218 x JSC 52	Sehore, Madhya Pradesh
40	GCP 101	GCP 2 x ICCV 2	Junagadh, Gujarat
41	RVG 202	(JAKI 9226 x DCP 20) x JG 412	Sehore, Madhya Pradesh
42	JG 315	Selection from WR 315	Jabalpur, Madhya Pradesh
43	JG 16	ICCV 4 x ICCV 10	Sehore, Madhya Pradesh
44	Phule G 0405	Digvijay x WCG 2002-2	Rahuri, Maharashtra

Results and Discussions

To test the significance of trait contribution in making of clusters (I to IX) among the genotypes analysis of variance (ANOVA) was performed. The results indicating that the

major 7 traits out of 14 studied traits were namely days to 50% flowering, days to maturity, plant height, 100 seed weight, number of pods per plant, number of secondary branches and seed yield per plant had the significant value ($p \leq 0.005$) over all the traits. Means the above significant traits have the crucial role in making of clusters among the test material(**Table. 2**).

Table 2: ANOVA table for cluster analysis in different yield attributing traits of chickpea genotypes

Traits	Cluster mean square	df	Error mean square	df	F	P value
DF	38.237**	8	8.525	35	4.485	0.001
DM	80.973**	8	18.43	35	4.394	0.001
PH	185.36**	8	20.803	35	8.91	0
LS	1.915	8	2.054	35	0.933	0.503
PEDL	5.684	8	9.239	35	0.615	0.759
PODL	10.517	8	8.568	35	1.228	0.312
PB	1.05	8	0.358	35	2.933	0.013
SB	51.928*	8	15.006	35	3.46	0.005
PPP	1690.29**	8	39.923	35	42.339	0
100 SW	71.821**	8	16.392	35	4.381	0.001
NSP	0.063	8	0.04	35	1.588	0.164
BYP	46.582	8	14.564	35	3.198	0.008
SYP	19.157*	8	5.752	35	3.331	0.005
HI	0.003	8	0.005	35	0.563	0.801

* and ** correlation significant at 0.05 and 0.01 level of significance

DF-Days to 50% flowering, **DM**-Days to maturity, **PH**-Plant height (cm), **LS**-Leaflet size (mm), **PEDL**-Peduncle length (mm), **PODL**-Podlength (mm), **PB**-Number of primary branches, **SB**- Number of secondary branches, **PPP**- Number of pods per plant, 100 **SW**-Weight of 100 seeds (g), **NSP**- Number of seeds per pods, **BYP**- Biological yield per plant (g), **SYP**- Seed yield per plant (g), **HI**-Harvest index (%).

Genetic divergence using Mahalanobis's D^2 analysis

The germplasm is a collection of available genetic variation that is used to produce better crop varieties to get more yield in responding to emerging demands Shivwanshi*et al.*(2019) It's also essential that the germplasm have ample amount of genetic diversity for

economic characteristics to be used commercially after hybridization during selection. The importance of ideal parental variety for obtaining improved genotypes for recovering transgressive segregants has also been stressed. Previously, researchers utilised distance in place of origin as a measure of genetic variety and used it to choose parents for hybridization. However, factors such as genetic diversity, place of release and ploidy level do not necessarily influence the genetic variety of selected parents. Thus, reliable statistical techniques such as D^2 statistics should be used to characterise germplasm for genetic divergence selecting appropriate and varied genotypes. To evaluate genetic divergence, these methods employ resemblance or distinction criteria based on the combined effect of a variety of agronomically related attributes. Characterization and quantification of genetic diversity has long been a predominant goal in crop improvement programs. Mahalanobis' D^2 statistic for yield and its component characteristics was used to measure genetic divergence quantitatively.

Grouping of genotypes into various clusters

44 genotypes were grouped into nine clusters based on D^2 values using Tocher's method (Rao, 1952) such that the genotypes belonging to same cluster had an average smaller D^2 values than those belonging to different clusters. The base to selecting parents for a targeted breeding effort is offered by creating clusters and finding of intra- and inter-cluster divergence. Pandey *et al*. (2015). The distribution of genotypes into various clusters has been presented in **Table 3**. Out of 9 clusters, cluster I was the largest comprising of 17 genotypes followed by cluster II with 12 genotypes, cluster III with 4 genotypes, cluster IV with 3 genotypes, cluster V with 3 genotypes, cluster VI with 2 genotypes, clusters VII, VIII and IX were mono-genotypic clusters, suggesting the existence of high degree of heterogeneity

among the genotypes. Grouping of genotypes was not related with the geographical distribution and were mainly grouped based on morphological differences.

Table 3: Clustering pattern of 44 genotypes into different 9 clusters

Cluster Number	**Number of genotypes**	**Genotypes**
Cluster I	17	GJG 1707, BG 4010, PG 227, NBeG 690, IPC 2015-12, NBeG 698, RKG 19-1, DC 18-1107, BAUG 106, DC 18-1104, DBGC 1, BG 4011, BDNG 2017-44, GL 17020, BUC-1, IPCD 2016-44, AKG 1506
Cluster II	12	BRC 9-14, PG 237, H 13-36, NDG 18-2, RSGD 1071, RSGD 1057, BDNG 2017-49, NDG 18-9, GNG 2477, GCP 101, JG 315, JG 16
Cluster III	4	GNG 2462, ADBG 487, GJG 1708, RVG 202
Cluster IV	3	Phule G 171105, RVSSG 81, RLBG 6
Cluster V	3	RVSSG 79, JG 2019-155-118, Phule G 171103
Cluster VI	2	GL-16063, RKG 19-2
Cluster VII	1	H 12-22
Cluster VIII	1	IPC 2016-107
Cluster IX	1	Phule G 0405

Contribution of characters towards divergence of the genotypes

The characters which showed more contribution (%) towards the divergence should be given prime importance during selection Shivwanshi*et al.* (2019). The percentage of individual contribution towards genetic divergence by all the studied traits is presented in **Table 4**. The results showed that 100 seed weight (g) (48.8%) contributed maximum to genetic divergence followed by number of secondary branches per plant (26.5%), seed yield per plant (g) (9%) indicated that these characteristics might be offered great scope for effective selection of the desired genotypes for further breeding improvement program of chickpea. Kumar *et al*. (2018) also found similar findings of 100 seed weight (g) trait contribution (46%) in total divergence of chickpea genotypes.Similarly, negligible

contribution towards genetic divergence was recorded for traits pod length (mm) (0.9%), days to 50% flowering (0.5%), days to maturity (0.5%) and harvest index (0.4%). Bapurao*et al.* (2018), Thakur *et al.* (2018), Pandey *et al.* (2013) also found that 100 seed weight (g) contributed greatest in the genetic divergence into the chickpea genotypes. Malik *et al.* (2014) also reported that seed yield per plant (g) had the greater impact forthe genetic diversity in chickpea.

Table 4: Relative contribution of traits in total divergence of chickpea genotypes

Sl. No.	**Traits as a source of divergence**	**Contribution (%)**
1.	Days to 50% flowering	0.5
2.	Days to maturity	0.5
3.	Plant height (cm)	1.3
4.	Leaflet size (mm)	5.4
5.	Peduncle length (mm)	1.1
6.	Pod length (mm)	0.9
7.	Number of primary branches per plant	1
8.	Number of secondary branches per plant	26.5
9.	Number of pods per plant	1.3
10.	100 seed weight (g)	48.8
11.	Number of seeds per pod	1.1
12.	Biological yield per plant (g)	2.1
13.	Seed yield per plant (g)	9
14.	Harvest index	0.4

Average inter and intra cluster distances among clusters

D^2 statistics measures the forces of differentiation at two levels namely, intra cluster and inter cluster levels. It might be easier for making crosses between genotypes separated by high estimates of statistical distance, as D^2 values indicate the cluster's genetic diversity index (Parhe*et al.*2014). The average intra and inter cluster D^2 values are presented in **Table 5** and statistical distance among 44 genotypes. Intra cluster D^2 values ranged from zero (cluster VII, VIII and IX) to 353.18 (cluster I). Maximum intra cluster distance was observed in cluster I (353.18), followed by cluster III (279.40), in cluster VI (275.46), cluster II (273.12), cluster V (263.49), cluster IV (201.86).

Table 5: Intra and Inter cluster distances among the 9 different clusters

Clusters	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**
Cluster I	353.18	652.6	646.73	959.82	878.21	935.07	784.86	706.93	917.67
Cluster II		273.12	945.01	1712.39	1969.16	548.85	598.41	864.2	1115.2
Cluster III			279.40	812.78	735.34	1836.66	1344.07	1027.13	871.05
Cluster IV				201.86	981.04	2710.13	1058.42	2366.07	468.66
Cluster V					263.49	2565.51	2277.17	1162.39	1622.59
Cluster VI						275.46	967.16	763.67	2045.69
Cluster VII							0	1874.81	524.98
Cluster VIII								0	2180.48
Cluster IX									0

The inter cluster distance was greater than intra cluster distance **(Table5)**, indicating the presence of wide genetic diversity among the different genotypes. Maximum inter cluster distance was recorded between cluster IV and VI (2710.13) followed by cluster V and VI (2565.51),which indicated that the genotypes found in above clusters might produce high heterotic response and thereby superior segregants (Lal *et al.* 2001). Distance is directly proportional to the wider genetic diversity between two clusters. Highly divergent genotypes would be of great use in recombination breeding programme in order to make highly

desirable recombinants. The lowest inter cluster divergence was recorded between cluster VII and IX (524.98) indicating that the genotypes included in these clusters were closely related. Selection should be performed in genetically diversified clusters to maintain relatively broad genetic base. Similar results were also revealed by Qudeer*et al.* (2021),Janghel*et al.* (2020), Thakur *et al.* (2018), Bapurao*et al.* (2018)**,** Malik *et al.* (2014), Pandey *et al.* (2013), Syed *et al.* (2012) in chickpea genetic divergence analysis.

Cluster mean values of different characters

The mean value of the 14 quantitative traits in each cluster was presented in **Table 6**. Cluster mean value for days to 50% flowering was highest in Cluster IX (83.29) and lowest in cluster V (80.11). Days to maturity were showed the highest and lowest in cluster VIII (140.57) and cluster III (131.58) respectively. Cluster VI exhibited highest number of primary branches (5.08) while in cluster V it was lowest (3.81). Similarly, in cluster IV was showed highest number of secondary branches (26.59) while cluster VI displayed lowest number of secondary branches (14.05). The number of pods per plant was highest observed in cluster VII (84.61) and lowest pods were found in cluster V (52.07).

Table 6: Mean performance of agronomic traits in 9 different clusters

Clusters / **Traits**	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**
DF	80.88	82.92	81.50	81.56	80.11^{L}	81.37	83.23	82.52	83.29^{H}
DM	133.45	137.19	131.58^{L}	134.56	138.78	132.15	136.05	140.57^{H}	140.41
PH (cm)	60.88^{H}	53.45	59.11	59.22	56.39	54.57	52.04^{L}	54.02	57.23
LS (mm)	8.98	8.66	10.97^{H}	9.63	9.96	9.21	8.29^{L}	8.98	9.18
PEDL (mm)	14.15	12.17	13.19	11.70	11.66^{L}	15.39	16.16^{H}	12.79	11.93
PODL (mm)	23.35	22.50	23.67	24.92^{H}	24.55	22.63	22.21^{L}	22.99	23.94
PB	4.27	4.34	4.08	4.70	3.81^{L}	5.08^{H}	4.91	4.52	3.99

SB	17.38	14.36	14.32	26.59^{H}	15.92	14.05^{L}	18.24	16.37	15.68
PPP	71.39	68.47	62.79	78.55	52.07^{L}	79.50	84.61^{H}	71.72	65.20
100SW (g)	23.24	17.06	26.23	27.71	32.59^{H}	20.60	17.00^{L}	18.58	20.69
NSP	1.32	1.32	1.27	1.09^{L}	1.40	1.47^{H}	1.27	1.16	1.16
BYP (g)	17.41	11.67^{L}	14.20	20.49	19.67	18.11	17.64	17.28	21.18^{H}
SYP (g)	10.73	7.48^{L}	9.41	13.08	12.23	10.97	11.12	11.00	13.71^{H}
HI (%)	0.63	0.60	0.53^{L}	0.57	0.63	0.66	0.68^{H}	0.67	0.64
*Where, H- Highest and L- Lowest									

Utilizing parents from the most divergent clusters are expected to manifest greatest heterosis in crossing and wide variability in genetic makeup of a crop. (Chowdhury *et al*., 2002; Kumar *et al*., 2016). Highest 100 seed weight was observed in cluster V (32.59 g) and lowest was recoded for cluster VII (17.0 g). Highest biological yield per plant was observed for cluster IX (21.18 g) and lowest value observed for cluster II (7.48 g). Similarly, for seed yield per plant cluster IX had highest value (13.71 g) and lowest seed yield observed for cluster II (7.48 g). The investigation into genetic divergence among the 44 chickpea genotypes involved D^2 cluster analysis. Emphasizing selection within genetically diverse clusters is pivotal to maintain a broad genetic base. The classification into nine clusters, primarily highlighted by substantial representation in cluster I with 17 genotypes, underscores the diversity within these groups. Notably, clusters IV and VI exhibited the most significant inter-cluster distance (2710.13), housing promising genotypes like Phule G 171105, RVSSG 81, RLBG 6, GL-16063, and RKG 19-2. These diversified genotypes hold significant promise for future experimentation, breeding programs, and hybridization efforts. Their potential as parent genotypes in breeding programs could yield superior heterotic segregants, contributing to the development of more adaptable and robust chickpea varieties.

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