Archives of Current Research International

Volume 24, Issue 5, Page 51-58, 2024; Article no.ACRI.115485
ISSN: 2454-7077

Assessment of Genetic Diversity among Quantitative Traits of Chickpea Genotypes

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ACRI/2024/v24i5681

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/115485

Original Research Article

Received: 05/02/2024
Accepted: 10/04/2024
Published: 12/04/2024

ABSTRACT

Chickpea (*Cicer arietinum* L.) is the most important pulse crop providing the livelihood to farmers. India is one of the topmost countries in chickpea production followed by middle-east. Assessment of morphological traits and their genetic diversity will help in increasing production of chickpea. A comprehensive evaluation of 44 distinct chickpea genotypes was conducted during 2020-21 *Rabi* season at RLBCAU, Jhansi. Utilizing Tocher's method and D^2 values, the studied genotypes were meticulously organized into nine non-overlapping clusters, with cluster (I) comprising the most, featuring 17 genotypes, followed by cluster (II) with 12 genotypes. Significant genetic diversity was evident, especially between clusters (IV) and (VI) (2710.13) followed by (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

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(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.

Keywords: Chickpea; genotypes; genetic diversity; heterotic segregants.

1. INTRODUCTION

"Chickpea 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" [1]. "In India, chickpea recorded 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" [2].

Legumes as preceding crops in the rotation also increases the protein content of succeeding cereals and helps in reducing mycotoxin contamination [3]. Crop plant improvement is greatly impacted by genetic diversity and 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 the importance of 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. In addition, it's essential for economic attributes in the germplasm for better utilisation after hybridization, selection or breeding methods. To overcome the genetic bottlenecks and create superior gene pools, broadening the genetic base through pre-breeding is required to enhance the utility of germplasm [4]. Genetic diversity is crucial for a germplasm collection as it primarily assists to plant breeders to maintain superior hybrids/crossbred varieties and various genes that could lead to resistance to pests, diseases, or other stress conditions [5]. The D^2 analysis classifies the genotypes into relatively homogeneous groups within the clusters [6]. 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.

2. MATERIALS AND METHODS

The experimental material comprised of 44 chickpea genotypes indigenous collections developed 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 with three replicates under well-irrigated condition at research farm of RLBCAU, Jhansi (U.P.) during Rabi 2020-21. Each genotype was sown with 4 m row length with four rows per plot in each replication spaced by inter rows (30 cm) and intra rows (10 cm) with all recommended agronomic practices. In present study observations were taken for quantitative traits *viz* plant height "PH" (cm), leaflet size "LS" (mm), peduncle length "PEDL" (mm), pod length "PODL" (mm), No. of primary branches per plant "PBP", No. of secondary branches per plant "SBP", No. of pods per plant "PPP", No. of seeds per pod "SPP", 100-seed weight "100SW" (g), biological yield per plant "BYP" (g), harvest index "HI" (%) and seed yield per plant "SYP" (g). While days to flowering "DF" and days to maturity "DM" were recorded on plot basis. Divergence analysis was carried out as suggested by Mahalanobis's [7].

3. RESULTS AND DISCUSSION

To test the significance of trait contribution in making of clusters (I to IX) among the genotypes analysis of variance (ANOVA) was performed [8]. The results indicating that the major 7 traits were namely DF, DM, PH, 100SW, PPP, SBP, and SYP had the significant value ($p \leq 0.005$) over all the traits. Means the above significant traits have the crucial role in clustering the current material (Table. 2).

Table 1. List of chickpea genotypes used for present study [24]

S.N.	Genotypes	Pedigree	Origin
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

Table 2. ANOVA for cluster analysis of yield and its attributing traits

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
PBP	1.05	8	0.358	35	2.933	0.013
SBP	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
SPP	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, LS-Leaflet size, PEDL-Peduncle length, PODL-Pod length, PBP-Number of primary branches, SBP- Number of secondary branches, PPP- Number of pods per plant, 100 SW- Weight of 100 seeds, SPP- Number of seeds per pods, BYP- Biological yield per plant, SYP- Seed yield per plant, HI- Harvest index

3.1 Genetic Divergence Using Mahalanobis's D^2 Analysis

The germplasm is a collection of genotypes which were used to improve crop varieties for yield increment to cope emerging demands [9]. Divergent parents were used for selection of improved genotypes for obtaining transgressive segregations has also been addressed. Previously, researchers used it for the spatial origin as a measure of genetic variety and to choose elite parents for hybridization programs. However, factors such as genetic diversity, place of release and ploidy level, do necessarily, influence on the variety of selected parents. Thus, reliable statistical techniques such as D^2 statistics could be used to characterise germplasm for genetic divergence for selecting appropriate genotypes. Characterization and estimation 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.

In that view, studied genotypes were grouped into nine clusters based on D^2 values using Tocher's method [10]; accordingly, the genotypes belonging to same cluster had an average smaller D^2 values than those belonging to different clusters. The base to select parents for a targeted breeding goal can be achieved by creating clusters and finding of intra- and inter-cluster divergence [11]. The distribution of genotypes into various clusters has been presented in Table 3. Out of 9 clusters, cluster I was the largest cluster which it comprises 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, and cluster VI with 2 genotypes. while clusters VII, VIII, and IX were mono-genotypic clusters. These results suggest the existence of high degree of heterogeneity among the genotypes. Grouping of genotypes was not related to the geographical distribution and were mainly grouped based on morphological differences.

3.2 Contribution of Characters towards Divergence of the Genotypes

The characters with high contribution (%) towards the divergence should be given prime importance during selection [9]. 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%) has the maximum contribution to genetic divergence followed by number of secondary branches per plant (26.5%), seed yield per plant (g) (9%); these results indicated that these traits offer great scope for effective selection of the desired genotypes for further breeding improvement program of chickpea. Similar findings were reported by Kumar et al. [12], where he found that 100 seed weight (g) trait contribution (46%) in total divergence of chickpea genotypes. In contrast, negligible

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

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	1.3
4.	Leaflet size	5.4
5.	Peduncle length	1.1
6.	Pod length	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	48.8
11.	Number of seeds per pod	1.1
12.	Biological yield per plant	2.1
13.	Seed yield per plant	9
14.	Harvest index	0.4

contribution towards genetic divergence was recorded for traits pod length (0.9%), days to 50% flowering (0.5%), days to maturity (0.5%) and harvest index (0.4%). Bapurao et al. [13], Thakur et al. [14], Pandey et al. [15] found that 100 seed weight has the greatest contribution in the genetic divergence in chickpea genotypes. Malik et al. [16] also reported that seed yield per plant had a great impact for genetic diversity in chickpea.

3.3 Average Inter and Intra Cluster Distances among Clusters

D^2 statistic 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 [17]. The average intra and inter cluster D^2 values are presented in Table 5 and statistical distance among the studied 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).

The inter cluster distance was greater than intra cluster distance (Table 5), 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 [18]. Distance is directly proportional to the wider genetic diversity between two clusters. Highly divergent genotypes would be of great use in 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. [19], Janghel et al. [20], Thakur et al. [14], Bapurao et al. [13], Malik et al. [16], Pandey et al. [15], Syed et al. [21] in chickpea genetic divergence analysis.

3.4 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).

Utilizing parents from the most divergent clusters are expected to manifest greatest heterosis in crossing and wide variability in genetic makeup of a crop [22,23]. 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 (11.67 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.

Table 5. Intra and Inter cluster distances among the 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

Table 6. Mean performance of agronomic traits in nine 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
PBP	4.27	4.34	4.08	4.70	3.81^{L}	5.08^{H}	4.91	4.52	3.99
SBP	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
SPP	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*

4. CONCLUSION

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/115485