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Genetic diversity analysis in chickpea (*Cicer arietinum* L.) genotypes

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Abstract
Genetic diversity study was conducted in 49 chickpea (*Cicer arietinum* L.) genotypes using Mahalanobis D^2 Statistics. Based on D^2 values, 49 genotypes were grouped into 17 clusters. The cluster III consisted of maximum 13 genotypes followed by Cluster I and cluster II which both had 10 genotypes. Inter cluster values varied from 4.31 to 26.36. The maximum inter cluster distance was recorded between cluster IX and XIII (26.36). Characters 100 seed weight (62.07%), days to 50% flowering (17.94%) and canopy temperature depression (5.27%) contributed maximum towards diversity. On the basis of cluster mean values, cluster XIII was superior for yield per plot and 100 seed weight. The genotypes belonging to the clusters separated by high genetic distance could be used in hybridization programme for obtaining a wide spectrum of variation among the segregants.

Keywords: Chickpea, D^2 Statistics

Introduction
Chickpea (*Cicer arietinum* L.) is an important Rabi season legume having extensive geographical distribution. Chickpea is a diploid species with a chromosome number 2n=2x = 16. It is a self-pollinated crop and it belongs to family Leguminosae. Chickpea is the third most important pulse crop in the world after beans and peas. Chickpea seed contains 29% protein, 59% carbohydrate, 3% fibre, 5% oil and 4% ash. Chickpea protein is rich in lysine and arginine but most deficient in sulphur-containing amino acids methionine and cystine (Iqbal *et al*., 2006) [2]. A healthy crop of chickpea can fix up to 141kg nitrogen per hectare. It is cultivated on 11.89 Mha with an annual production of 11.38 Mt at 956.4 kg/ha productivity (FAOSTAT, 2018) [3]. India ranks first in terms of production and productivity, followed by Pakistan, Myanmar, Ethiopia, Mexico, Australia, Canada and United States. Maharashtra is the second largest producing state of chickpea in the country, the first being Madhya Pradesh. Maharashtra shares 12% to the country's total chickpea production (DES, Ministry of Agri. & FW, Govt. of India; 2017-18).
The knowledge of genetic diversity has a significant impact on the improvement of crop plants and this information has been successfully used for efficient germplasm management, fingerprinting and genotype selection (Rajkumar *et al*., 2015) [7]. Assessment of genetic diversity in germplasm can facilitate classification and identification of diverse heterotic group with possible breeding values in manifestation of breeding potential of genotypes in specific breeding programme. Mahalanobis's D^2 statistics (1936) [6] is a powerful tool in quantifying the degree of genetic divergence between the genotypes and relate clustering pattern with the geographic origin. The genetic distance had a definite role to play for efficient choice of parents for hybridization programme (Saha *et al*., 2018) [9]. The more diverse the parents within reasonable limits, the more are the chances of obtaining heterotic broader spectrum of variability in the segregating populations. The present study was, therefore, conducted with a view to identify divergent parents for future hybridization programmes for yield improvement of chickpea.

Materials and Methods
The experiment consisted of 49 chickpea genotypes of diverse origin developed by various research institutes/stations (Table 1) within the country. The trial was laid out following lattice square design with two replications at Agricultural Botany Farm, Mahatma Phule Krishi Vidyapeeth, Rahuri (19º39'N, 74º66'E) during Rabi 2016-17. Each entry was grown in a two rows of 3.0 m length (30 plants/genotype) with inter and intra-row spacing of 30 × 10 cm. Data were recorded on five randomly tagged plants for days to 50% flowering, days to

maturity, plant height after at one month of sowing, plant height after second month of sowing, plant height at third month after sowing, plant height at maturity, pods per plant, seeds per plant, yield per plot, 100 seed weight. In addition to these morphological traits, data on physiological traits like canopy temperature depression, growing degree days at 50% flowering and growing degree days at maturity was also recorded in the study to assess the divergence for these traits in the lines. The hand-held infrared thermometer was used to measure the canopy temperature and air temperature on three randomly selected plants and average difference was recorded as canopy temperature depression. The growing degree days (GDD) was estimated by subtracting the value of base temperature (5ºC for chickpea) from the average daily air temperature.

The data recorded were subjected to D^2 statistics to know the genetic diversity among the germplasm as suggested by Mahalanobis (1936) [6]. Grouping of genotype into different clusters was done as per the method described by Rao (1952) [8]. The statistical data were analysed using software programme INDOSTAT version 8.5.

Results and Discussion

Based on the D^2 values the 49 genotypes were grouped into 17 clusters (Table 2; Figure 1) which revealed that the genotypes varied significantly for all the characters studied indicating considerable variation in the germplasm. Gediya *et al*., (2018) [5] grouped 58 chickpea genotypes in 16 clusters. Shivwanshi *et al*., (2017) [10] grouped 434 germplasm lines of chickpea into 14 clusters. The cluster III consisted of maximum 13 genotypes, followed by cluster I and cluster II (10 genotypes). The cluster III consisted of maximum 13 genotypes indicating that the genotypes had narrow genetic divergence among them. The similarity in the base population, from which they had been evolved, might be the cause of genetic uniformity. Clusters IV, V, VI, VII, VIII, IX, X, XI, XII, XIV, XV, XVI and VXII are solitary, consisting single genotype in each cluster. The monogenotypic cluster indicated that genotypes belonging to these clusters had wide diversity from the rest as well as from each other showing its uniqueness. Thus, these genotypes have entirely different genetic make-up from the others.

The per cent contribution of 13 characters studied, towards total divergence is presented in Table 3. It was observed that, 100 seed weight (62.07%) contributed highest for divergence, it was followed by days to 50% flowering (17.94%) and canopy temperature depression (5.27%). These characters were responsible for expressing maximum diversity between the clusters, and therefore should be given due weightage during selection. Remaining characters exhibited very low or negligible contribution towards divergence. Gediya *et al*., (2018) [5] also observed highest contribution towards divergence in case of 100 seed weight The maximum contribution towards divergence was observed by Thakur *et al*., (2018) [11] in days to 50% flowering (22.89 %) followed by number of seeds per plant (14.16 %) and plant height (13.80 %).

Intra cluster and inter cluster D^2 values were computed for the 17 clusters (Table 4). The maximum genetic distance was found between the clusters IX and XIII (26.36) while the minimum genetic distance was found between the clusters X and XI (4.31). It has been well established fact that more the genetically diverse parents used in hybridization programme, the greater will be the chances of obtaining high heterotic hybrids and broad spectrum variability in segregating generations (Arunachalam, 1981) [1]. The genotype JG 14 (ICCV92944) which grouped into cluster XIV was already identified as a heat tolerant genotype which escapes heat stress through early maturity (Gaur *et al.,* 2010) [4] and preferably used under late sown conditions of cereal based cropping system in India. The results of present investigation indicated that the genotypes grouped in these clusters were highly divergent from each other. Assessment of chickpea germplasm and its utilization in selection of diverse genotype from above clusters would produce a range of genetic variability for quantitative traits which might enable further selection and improvement.

The cluster mean values for 13 characters are presented in Table 5. Wide range of mean values among the clusters were recorded for different traits. The cluster mean for days to 50% flowering varied from 44.00 days (XVII) to 69.00 days (IX). The cluster mean for days to maturity varied from 116.00 days (IV) to 130.50 days (VI). The highest cluster mean for plant height at first month after sowing was 21.00 cm, which was observed in cluster (V) and lowest for (VIII) 10.30 cm. The cluster mean for plant height at second month after sowing ranged from 25.30 cm (VIII) to 50.20 cm (V). The highest cluster mean plant height at third month after sowing was 55.30 cm (XII) and lowest was 30.20 cm (XVII). The highest cluster mean plant height at maturity was 56.00 cm (XII) and lowest was 36.00 cm (XVII). The highest cluster mean for number of pods per plant was 144.10 (XI) and lowest was 31.30 (XVII). The genotypes in cluster XI were found highest mean for seeds per plant (90.10), while lowest in cluster XVII (22.70). Genotypes in cluster XIII showed highest mean for yield per plot (600.07 g) and lowest (234.85 g) in cluster XVII. Cluster XIII (35.76 g) performed better for trait 100 seed weight while canopy temperature depression recorded highest mean in cluster XVI (8.23). Genotypes in cluster XVII (672.78) performed better for trait growing degree days at 50% flowering while genotypes in cluster IV (1851.05) performed better for trait growing degree days at maturity.

From the results of present study, it can be concluded that the traits 100 seed weight, days to 50% flowering and canopy temperature depression contributed maximum towards divergence. Therefore, the emphasis should be given to these characters for improvement of chickpea yield. Narrow genetic base of the material is the major limiting factors for initiating the breeding programme aimed at increasing the yield potential of any crop. It is generally observed that the genetically diverse parents show maximum heterosis and provide scope for the selection of the transgressive segregants. The hybridization between the genotypes of the same cluster thus, may not provide good segregants. Therefore, in the present investigation, based on large inter-cluster distances, it is advisable to attempt crossing of the genotypes from clusters VIII and IX, which may lead to broad spectrum of favourable genetic variability for yield improvement in chickpea.

Table 1: Details of chickpea genotypes used in the investigation

Sr. No.	Name of genotype	Source	Pedigree
1	JSC 55	Sehore, MP	(JAKI 9226 x DCP 20) x JG 412
2	JSC 56	Sehore, MP	(ICCV 91962 x ICCV 10) x ICCV 892330
3	JSC 40	Sehore, MP	-
4	RV SSG 29	Sehore, MP	FG 712 x CSG 9505
5	RV SSG 28	Sehore, MP	BGD 112 x BG72
6	JG 6	Jabalpur, MP	(ICCV10 x K850) x (H208 x RS11)
7	JG 315	Jabalpur, MP	Selection from Kanpur Germplasm
8	JG 12	Jabalpur, MP	(N. Bold x PG5) x PG 5
9	JG 14	Jabalpur, MP	(GW 5/7 x P327) x ICCV 83149
10	JG 63	Jabalpur, MP	Single plant selection from JG 62
11	JG 322	Jabalpur, MP	Caffa x BG1
12	JG 16	Jabalpur, MP	ICCC 42 x ICCV 10
13	JG 11	Jabalpur, MP	(Phue G 5 x Narsingpur bold) x (ICCC37 x ICCC 860265-BPB 29P- BP)
14	JG 42	Jabalpur, MP	[CJM 1 x IPC 9239) JG 7] 14-11-2011-42
15	ICCV 10	ICRISAT, Hyderabad	PI 231 x PI 265
16	ICC 4958	ICRISAT, Hyderabad	Germeplasm collection
17	NBeG 780	ANGRAU, AP	ICCC 37 x K 1189
18	NBeG 03108	ANGRAU, AP	ICCV 92065 x ICCV 88202) x KW 118
19	NBeG 47	ANGRAU, AP	ICCV 2 x PDG 84-16
20	CSJ 513	Durgapura, Rajasthan	FG712 x CSJ 146
21	BG 256	IARI, New Delhi	(JG 62 x 850-3/27) x (L550 x H208)
22	BG 372	IARI, New Delhi	P 1231 x P 1265
23	BG 391	IARI, New Delhi	ICC3935 x BG 256
24	BGD 72	IARI, New Delhi	(BG 256 x E 100 Ym)
25	BG 3062	IARI, New Delhi	ICCV 10 X ICCL 87
26	BG 3061	IARI, New Delhi	ICCCV 03112 x JAKI 9218
27	BG 3056	IARI, New Delhi	ILC 3279 x RSG 143-1
28	BGM 547	IARI, New Delhi	Mutant of BG 256
29	Phule Vikram	MPKV, Rahuri, MS	ICC 4958 x Annegiri
30	Phule G 0405	MPKV, Rahuri, MS	Digvijay x WGC 2000-2
31	Phule G 12107	MPKV, Rahuri, MS	ICCV 04112 x ICCV 88202
32	Phule G 12110	MPKV, Rahuri, MS	ICCV 03112 x JAKI 9218
33	Phule G 0302	MPKV, Rahuri, MS	PGC x Phule G 96006
34	GCP 101	Junagadh, Gujrat	GCRZ x ICCV 2
35	GJG 1205	Junagadh, Gujrat	ICCV 04112 x JG 130
36	GJG 1207	Junagadh, Gujrat	ICCV 04112 x JG 130
37	IPC 2010-09	IIPR, Kanpur, UP	RSG 143-1 x IPC 2000-33
38	IPC 2009-91	IIPR, Kanpur, UP	JGK 1 x JG 315
39	IPC 2011-28	IIPR, Kanpur, UP	HC 5 x GL 23138
40	IPC 2011-112	IIPR, Kanpur, UP	IPC 94 x IPC 2002-120
41	PBC 506	PAU, Ludhiana, Punjab	ICCV 5107 x ICCC 37
42	DBGV 101	UAS, Dharwad, Karnataka	-
43	H-11-22	Hisar, Haryana	HC 1 x WR 315
44	BDNG 797	Badnapur, MS	-
45	JAKI 9218	PDKV, Akola, MS	(ICCC 37 x GW 517) x ICCV 10
46	SAKI 9516	PDKV, Akola, MS	ICCC 4 x ICCV 10
47	Digvijay	MPKV, Rahuri, MS	Phule G 9108 x Bheema
48	Vishal	MPKV, Rahuri, MS	K 850 x ICCL 80074
49	Vijay	MPKV, Rahuri, MS	P127 x Annegiri

Table 2: Grouping of 49 chickpea genotypes into 17 clusters by Tocher's method

Cluster	Genotypes
Cluster I	JG 11, Vishal, BG 3062, Phule G 0302, Phule G 12107, NBeG 03108, Phule G 0405, Digvijay, BDNG 797, JG 16
Cluster II	BG 256, RV SSG 29, BGM 547, BGD 72, BG 391, GJG 1205, BG 3061, Phule G 12110, Phule Vikram, GJG 1207
Cluster III	JG 2, H-11-22, JG 322, GCP 101, CHJ 513, ICCV 10, BG 3056, JG 63, IPC 2011-28, JAKI 9218, JG 315, JG 42, JSC 55
Cluster IV	JSC 40
Cluster V	NBeG 47
Cluster VI	IPC 2010-09
Cluster VII	RV SSG 28
Cluster VIII	IPC 2011-2112
Cluster IX	BG 372
Cluster X	Vijay
Cluster XI	SAKI 9516
Cluster XII	IPC 2009-91
Cluster XIII	ICC 4958, PBC 506, NBeG 780
Cluster XIV	JG 14
Cluster XV	JG 6
Cluster XVI	DBGV 101
Cluster XVII	JSC 56

Table 3: Percent contribution of 13 characters for diversity in chickpea

Sr. No	Characters	Times ranked 1st	Contribution (%)
1	Days to 50% flowering	211	17.94
2	Days to maturity	8	0.68
3	Plant height at first month after sowing	34	2.89
4	Plant height at second month after sowing	14	1.19
5	Plant height at third month after sowing	4	0.34
6	Plant height at maturity	3	0.26
7	Pods per plant	28	2.38
8	Seeds per plant	10	0.85
9	Yield per plot	44	3.74
10	100 seed weight	730	62.07
11	Canopy temperature depression	62	5.27
12	Growing degree days at 50% flowering	13	1.11
13	Growing degree days at maturity	15	1.28
	Total	1176	100

Table 4: Average intra and inter cluster distance D ^{2} values

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII	Cluster IX	Cluster X	Cluster XI	Cluster XII	Cluster XIII	Cluster XIV	Cluster XV	Cluster XVI	Cluster XVII
Cluster I	4.43																
Cluster II	6.20	5.27															
Cluster III	8.01	10.32	5.67														
Cluster IV	7.14	10.80	7.21	0.00													
Cluster V	6.20	6.25	10.99	10.85	0.00												
Cluster VI	7.49	7.17	7.38	10.65	8.49	0.00											
Cluster VII	9.64	7.24	14.90	14.10	8.65	11.68	0.00										
Cluster VIII	6.38	6.90	9.19	7.82	9.01	8.18	8.12	0.00									
Cluster IX	13.84	16.28	8.37	11.67	16.89	11.62	21.52	15.29	0.00								
Cluster X	6.79	10.08	7.32	4.47	10.09	9.64	13.18	7.27	11.76	0.00							
Cluster XI	5.26	8.10	6.68	5.93	8.80	7.80	11.81	7.28	11.51	4.31	0.00						
Cluster XII	9.27	6.37	14.33	14.41	8.29	10.32	4.64	8.69	20.71	13.81	11.95	0.00					
Cluster XIII	14.04	11.67	19.85	19.15	11.09	16.33	7.40	14.27	26.36	17.97	16.47	8.25	5.55				
Cluster XIV	5.47	7.42	10.19	6.48	7.35	10.31	8.53	5.11	16.56	7.03	7.15	9.21	13.36	0.00			
Cluster XV	7.69	7.52	13.23	10.68	8.47	12.10	5.21	6.25	19.92	10.27	9.48	6.92	10.02	5.01	0.00		
Cluster XVI	6.87	7.81	10.87	9.64	8.59	10.48	9.02	6.71	16.70	8.01	7.67	8.97	12.97	6.97	6.28	0.00	
Cluster XVII	10.71	14.06	11.27	5.23	13.98	14.57	16.23	9.90	15.21	7.83	10.05	16.85	21.05	8.19	12.15	12.33	0.00

Table 5: Cluster means performance for 13 characters of 49 chickpea genotypes

	D50%F	DM	H1M	H2M	H3M	HatM	PPP	SPP	YPPl	100SW	CTD	GDDF	GDDM
Cluster I	57.70	120.60	15.00	39.39	49.39	51.59	82.75	56.16	460.54	21.69	5.14	870.44	1941.25
Cluster II	62.10	123.05	13.56	37.69	50.37	52.73	68.77	48.38	414.21	25.34	4.38	925.15	1980.24
Cluster III	62.96	122.35	12.48	34.08	42.85	45.52	58.51	39.03	294.49	16.45	4.84	931.99	1998.01
Cluster IV	52.50	116.00	13.20	33.00	43.10	43.20	59.50	38.46	281.25	15.96	4.92	791.38	1851.05
Cluster V	57.00	120.50	21.00	50.20	53.80	53.80	51.40	37.15	426.99	24.89	3.25	855.25	1935.95
Cluster VI	68.00	130.50	12.70	31.60	46.90	48.10	72.70	37.15	426.99	24.89	3.25	855.25	1935.95
Cluster VII	59.00	117.50	11.10	32.10	42.10	44.20	43.30	45.07	302.60	21.21	3.05	999.85	2117.05
Cluster VIII	58.00	122.50	10.30	25.30	31.40	40.00	31.60	27.62	318.68	30.87	3.25	883.20	1880.28
Cluster IX	69.00	124.50	11.10	30.10	36.60	39.50	66.10	44.24	415.72	10.13	4.99	1015.95	2005.68
Cluster X	55.50	118.00	15.50	31.10	36.00	38.00	119.40	82.08	391.31	18.11	4.93	836.10	1888.20
Cluster XI	58.50	122.50	12.10	34.10	43.20	45.70	144.10	90.10	575.00	19.14	5.45	857.80	1963.85
Cluster XII	61.50	127.50	12.80	34.70	55.30	56.00	47.00	34.20	400.59	30.40	4.97	918.43	2056.78
Cluster XIII	55.83	120.00	18.37	44.63	52.33	54.77	78.07	48.74	600.09	35.76	4.10	840.07	1926.10
Cluster XIV	51.50	117.50	14.60	33.70	44.80	45.00	45.20	33.79	352.28	22.39	4.10	778.58	1880.28
Cluster XV	52.00	118.50	12.30	35.00	42.10	48.00	60.70	38.81	336.31	27.19	5.50	784.45	1898.75
Cluster XVI	56.50	121.50	15.50	36.60	42.60	43.90	51.20	36.85	305.86	26.41	8.23	850.83	1954.50
Cluster XVII	44.00	120.50	12.30	26.90	30.20	36.00	31.30	22.70	234.85	14.16	4.02	672.78	1930.08

D50%F- days to 50% flowering, DM- days to maturity, PPP- numbers of pod per plant, SPP - numbers of seeds per plant, YPPl-yield per plot, 100SW - 100 seed weight, H1M - plant height at first month after sowing, H2M - second month after sowing, H3M - third month after sowing, HatM - plant height at maturity, CTD - canopy temperature depression, GDDF – growing degree days at 50% flowering, GDD- growing degree days at maturity.

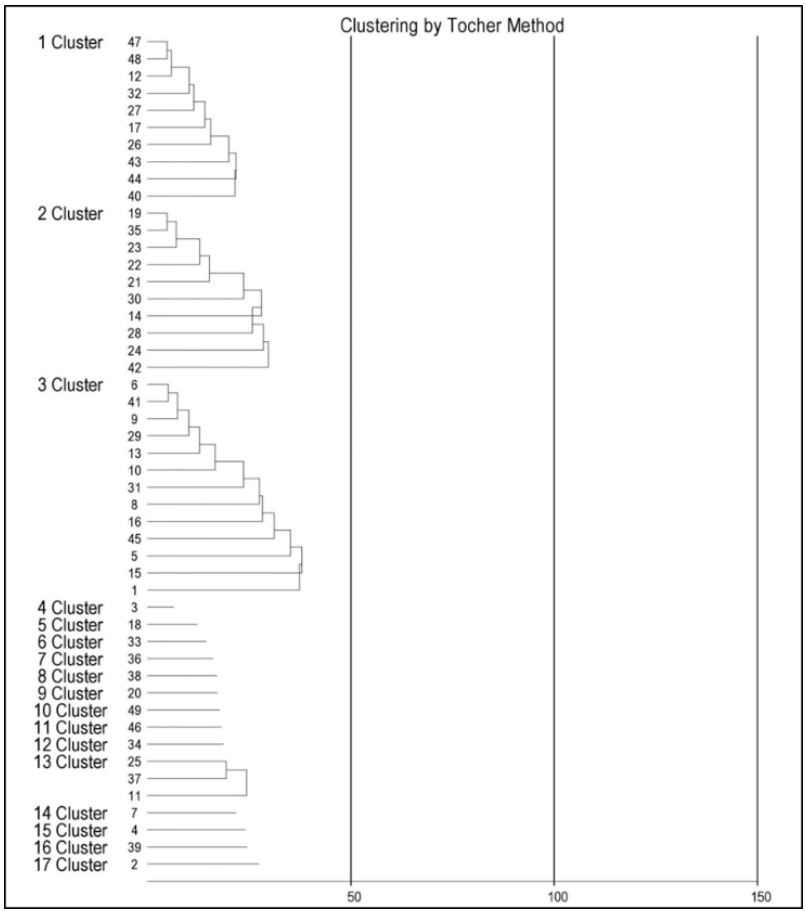

Fig 1: Grouping of 49 chickpea genotypes into 17 clusters by Tocher's method

No	Genotype	No	Genotype	No	Genotype	No	Genotype	No	Genotype
1	JSC 55	11	NBeG 780	21	BG 391	31	BG 3056	41	H-11-22
2	JSC 56	12	BG 3062	22	BGD 72	32	Phule G 0302	42	GJG 1207
3	JSC 40	13	CHJ 513	23	BGM 547	33	IPC 2010-09	43	Digvijay
4	JG 6	14	BG 3061	24	Phule Vikram	34	IPC 2009-91	44	BDNG 797
5	JG 315	15	JG 42	25	ICC 4958	35	RV SSG 29	45	JAKI 9218
6	JG 2	16	IPC 2011-28	26	Phule G 0405	36	RV SSG 28	46	SAKI 9516
7	JG 14	17	NBeG 03108	27	Phule G 12107	37	PBC 506	47	JG 11
8	JG 63	18	NBeG 47	28	Phule G 12110	38	IPC 2011-2112	48	Vishal
9	JG 322	19	BG 256	29	GCP 101	39	DBGV 101	49	Vijay
10	ICCV 10	20	BG 372	30	GJG 1205	40	JG 16		

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