



P-ISSN: 2349-8528

E-ISSN: 2321-4902

IJCS 2018; 6(4): 3177-3181

© 2018 IJCS

Received: 07-05-2018

Accepted: 13-06-2018

Lalji N Gediya

Department of Genetics and Plant Breeding, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India

Dipak A Patel

Professor and Head, Department of Genetics and Plant Breeding, B. A. College of Agriculture, AAU, Anand, Gujarat, India

Dinesh J Parmar

Associate Professor, Department of Agricultural Statistics, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India

Rumit Patel

Department of Genetics and Plant Breeding, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India

Parth Rahevar

Department of Genetics and Plant Breeding, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India

Correspondence**Lalji N Gediya**

Department of Genetics and Plant Breeding, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India

Assessment of genetic diversity of chickpea genotypes using D^2 statistics

Lalji N Gediya, Dipak A Patel, Dinesh J Parmar, Rumit Patel and Parth Rahevar

Abstract

D^2 analysis indicated wider genetic diversity among 58 genotypes of chickpea, which were grouped into sixteen clusters. In general, intra-cluster distances were lower than inter-cluster distances, indicating that genotypes included within a cluster tended to diverse less from each other. Maximum genetic divergence was observed between cluster IX and XVI, followed by Cluster IX and XIV, while closest proximity was noticed between cluster III and VIII. The characters viz., seed yield per plant, 100 seed weight, seeds per plant and pods per plant contributed much to the total genetic divergence. Hence, selection for divergent parents based on these characters would be useful for developing potential hybrids and increasing scope of isolating desirable recombinants in segregating generation in chickpea. On the basis of cluster mean values, cluster XII was superior for seed yield per plant and harvest index; cluster XV for pods per plant, seeds per plant and first pod height and cluster X for 100 seed weight. For early flowering and maturing, cluster XIV was found superior, while cluster VII was best for protein content. 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, genetic divergence, inter and intra cluster distance

1. Introduction

Chickpea (*Cicer arietinum* L, $2n=2x=16$) is the world's third most important *Rabi* food legume after dry bean and field pea (Rao *et al.*, 2010) [11]. Worldwide, it is grown on about 13.54 million ha area with the production of 13.10 million tons (FAO, 2013). In India, the area under chickpea was 94.85 lakh hectares with a production of 93.80 lakh tones and productivity of 979 kg/ha during 2016-17. In Gujarat, area under chickpea was 1.15 lakh hectares with a total production of 1.53 lakh tones and productivity of 1330 kg/ha. (Anonymous, 2016-17). 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) [10]. 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. In breeding programme, progenies derived from diverse crosses which are selected based on genetic divergence analysis are expected to show a broad spectrum of genetic variability, providing a greater scope for isolating transgressive segregants in advance generation. Mahalanobis's D^2 statistics (1936) [9] 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) [13]. Several workers studied the genetic diversity, clustering pattern and relative contribution of different characters toward divergence and effectiveness of selection (Anita *et al.*, 2015; Johnson *et al.*, 2015; Agrawal *et al.*, 2018) [2, 7, 1]. So, the present experiment was formulated to study the genetic divergence and clustering pattern of the chickpea genotypes for selection of suitable parents for utilizing in hybridization programme and to study the genetic parameters attributing to yield.

2. Materials and Methods

The present investigation comprised of 58 diverse chickpea genotypes was carried out at Genetics and Plant Breeding Farm, B.A. College of Agriculture, A.A.U., Anand during *Rabi* 2016-17.

The genotypes were evaluated in Randomized Complete Block Design (RCBD) with three replication. Each entry was grown in a single row of 4.0 m length (40 plants/genotype) with inter and intra-row spacing of 30 × 10 cm. The experiment was surrounded by guard row to avoid damage and border effect. The recommended agronomical and plant protection practices were followed for the successful raising of the crop.

Data were recorded on thirteen characters including phenological, yield and yield contributing traits. The days to 50% flowering and days to maturity were recorded on a population basis and remaining characters were recorded from a randomly selected five plants from each row of genotype. Protein content in chickpea genotypes was measured through Micro-kjeldahl method of total nitrogen estimation. Genetic divergence was estimated by using D^2 statistics of Mahalanobis (1936) ^[9] and Grouping of the genotypes in different clusters was done by using Tocher's method (Rao, 1952) ^[12]. The per cent contribution of characters towards genetic divergence was calculated according to Singh and Choudhary (1985).

3. Results and Discussion

The knowledge regarding the extent of variability and genetic diversity is of much importance while making improvement in a complex trait like yield. Therefore, while improving seed yield, selection of parents having wide genetic divergence for number of characters is of prime importance, which is assessed by D^2 -statistics developed by Mahalanobis (1936) ^[9]. The value of V-statistic (2749.475) which follows χ^2 distribution for 741 degrees of freedom showed highly significant differences among the genotypes for aggregate of 13 characters. Thus, one can proceed for further diversity analysis. The D^2 values between all 1653 pairs ranged from 12.20 (between Digvijay and GG-5) to 503.88 (between ICC-8151 and ICC-12866), which indicated the presence of high genetic diversity among the genotypes for all the traits (these D^2 values were taken from matrix table, which is not given here).

The clustering pattern could be utilized in hybridization programme and deciding the cross combinations, which would generate the highest variability for various characters. The superior genotypes for breeding programme could also be selected on the basis of cluster mean and inter-cluster distance.

3.1 Composition of Clusters

Grouping of the genotypes was carried-out by following the Tocher's method (Rao, 1952) ^[12] with the assumption that the genotypes within cluster have smaller D^2 -values among themselves than those from genotypes belonging to different clusters. In all, 16 clusters were formed from 58 genotypes. The composition of clusters is given in Table 1. The cluster III was the largest cluster with 14 genotypes all belonging to desi types from ICRISAT, Hyderabad. Cluster I and V was the second largest cluster with eight genotypes each. These two clusters contained varieties released from different research centers as well as germplasm from ICRISAT, Hyderabad. The cluster IV ranked third with seven genotypes, while cluster II ranked fourth with six genotypes. The clusters VI, VII, IX and XII ranked fifth with all contained two genotypes. The clusters VIII, X, XI, XIII, XIV, XV and XVI were solitary cluster with single genotype. The monogenotypic cluster indicated that genotypes belonging to these

clusters had wide diversity from the rest as well as from each other. Thus, these genotypes have entirely different genetic make-up from the others.

In a similar study, 100 diverse genotypes of chickpea were grouped into sixteen clusters by Rajkumar *et al.* (2015) ^[10]. Thirty diverse genotypes of chickpea were grouped into eight different clusters by Koinain *et al.* (2015) ^[8]. Gupta *et al.* (2016) ^[6] carried out similar type of genetic divergence study in 25 genotypes of chickpea and grouped them into seven clusters using Tocher's method.

The clustering pattern indicated that the genetic diversity was not fully associated with geographical diversity, hence there was no formal relationship between geographical diversity and genetic diversity. This could be because of there were other forces than geographical separation such as natural and artificial selection, exchange of breeding material, genetic drift and environmental variation responsible for genetic diversity.

3.2 Inter and intra-cluster distances

The inter-cluster and intra-cluster distances are shown in Table 2. The maximum inter-cluster distance ($D = 485.87$) was found between cluster IX and XVI, followed by that between IX and XIV ($D = 461.49$) and X and XVI (429.63). The minimum inter-cluster distance was observed between cluster III and VIII ($D = 62.60$). The intra-cluster distance (D) ranged from 28.25 (cluster-VII) to 66.00 (cluster-IX). The seven clusters *viz.*, VIII, X, XI, XIII, XIV, XV and XVI contained single genotype and therefore, their intra-cluster distance was zero. In general, intra-cluster distances were lower than the inter-cluster distances. Thus, the genotypes included within a cluster tended to diverse less from each other. 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. The clustering pattern could be utilized in selection of parents for crossing and deciding the best cross combinations which may generate the highest possible variability for various traits.

3.3 Cluster means of various characters

The cluster mean value for 13 characters are presented in Table 3. Wide range of mean values among the clusters were recorded for different traits. The cluster XV (ICC-13357) manifested highest mean values for pods per plant (136.00) and seeds per plant (201.87) and lowest mean value for first pod height (33.87 cm). The cluster XII (ICC-2919 and ICC-12028) showed highest mean values for seed yield per plant (38.77 g) and harvest index (54.89%). The cluster X (ICC-5037) had highest cluster mean value for 100 seed weight (42.34 g). The cluster XVI (ICC-13219) exhibited highest mean value for plant height (105.87 cm). The cluster XIV showed lowest mean value for days to 50 per cent flowering (44.00 days) and day to maturity (107.33 days), while it manifested highest mean value for seeds per pod (1.93). The cluster VII depicted highest mean value for protein content (26.60%). The cluster I showed highest mean value for primary branches per plant (3.13), while cluster VI manifested highest mean value for secondary branches per plant (12.70). Therefore, intercrossing of such genotypes involved in these clusters would be useful for generating variability for the respective characters, and their rational improvement for increasing the seed yield.

3.4 Characters contribution towards genetic divergence

The analysis of variance for each character was carried out using mean of the 58 genotypes. Estimation of inter and intra cluster variances, along with ratio of inter cluster variance to the total variance (R^2) and inter cluster coefficient of variation (CV_b) for 13 characters were worked out and presented in Table 3. Maximum value of R^2 was observed for 100 seed weight (0.893) followed by days to maturity (0.844) and seeds per plant (0.808), while minimum value of R^2 was observed for primary branches per plant (0.074). From inter cluster coefficient of variation (CV_b), it was observed that the seed yield per plant contributed maximum (50.53%) towards the total divergence in yield. The next major contribution came from the 100 seed weight (46.90%) followed by seeds per plant (44.81%), pods per plant (36.45%) and harvest index (23.38%).

The % contribution towards genetic divergence by 13 quantitative characters (Figure 1) revealed that, protein content contributed most towards genetic divergence followed

by 100 seed weight, days to maturity and days to 50 % flowering. These characters were responsible for expressing maximum diversity between the clusters therefore, should be given due weightage during selection. Remaining characters exhibited very low or negligible contribution towards divergence.

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) [5]. It has also been observed that the most productive hybrids may come from high yielding parents with a high genetic diversity. Therefore, in the present investigation, based upon high yielding genotypes and large inter-cluster distances, it is advisable to attempt crossing of the genotypes from clusters IX and XVI, which may lead to produce broad spectrum of favorable genetic variability for yield improvement in chickpea.

Table 1: Grouping of 58 genotypes of chickpea in various clusters on the basis of D^2 statistics

S. No	Clusters	No. of genotypes	Name of the genotypes	Type	Source
1	I	8	GG-2, GG-5, GJG-6	Desi	JAU, Junagadh
			Vishal		MPKV, Rahuri (MH)
			GNG-28		RAU, Sriganaganagar (RJ)
			ICC-13124		ICRISAT, Patancheru (AP)
			JG-16 (SAKI 9516)		JNKVV, Jabalpur (MP)
			Digvijay		MPKV, Rahuri (MH)
2	II	6	GNG-14	Desi	RAU, Sriganaganagar, (RJ)
			ICC-8350		Kabuli
			ICC-2277, ICC-8621, ICC-13283, ICC-15264		
3	III	14	ICC-231, ICC-1431, ICC-3230, ICC-4639, ICC-8058, ICC-9942, ICC-10399, ICC-12492, ICC-12928, ICC-13524, ICC-13764, ICC-14799, ICC-14831, ICC-15888	Desi	ICRISAT, Patancheru
4	IV	7	ICC-157, ICC-1164, ICC-2210, ICC-4463, ICC-8195, ICC-12155	Desi	ICRISAT, Patancheru
			ICC-13441	Kabuli	
5	V	8	ICC-4872, ICC-8318, ICC-8855, ICC-10945, ICC-14669	Desi	ICRISAT, Patancheru
			GG-1, Dahod Yellow		JAU, Junagadh
			Vijay		MPKV, Rahuri (MH)
6	VI	2	ICC-7255, ICC-15333	Kabuli	ICRISAT, Patancheru
7	VII	2	ICC-8522, ICC-13219	Desi	ICRISAT, Patancheru
8	VIII	1	GNG-27	Desi	RAU, Sriganaganagar, (RJ)
9	IX	2	ICC-8151, ICC-12328	Kabuli	ICRISAT, Patancheru (AP)
10	X	1	ICC-5037	Desi	ICRISAT, Patancheru
11	XI	1	JG-130	Desi	JNKVV, Jabalpur (MP)
12	XII	2	ICC-2919, ICC-12028	Kabuli	ICRISAT, Patancheru
13	XIII	1	GJG-3	Desi	JAU, Junagadh
14	XIV	1	ICC-10393	Desi	ICRISAT, Patancheru
15	XV	1	ICC-13357	Kabuli	ICRISAT, Patancheru
16	XVI	1	ICC-12866	Desi	ICRISAT, Patancheru

Table 2: Average intra-cluster and inter-cluster distance for 58 genotypes of chickpea

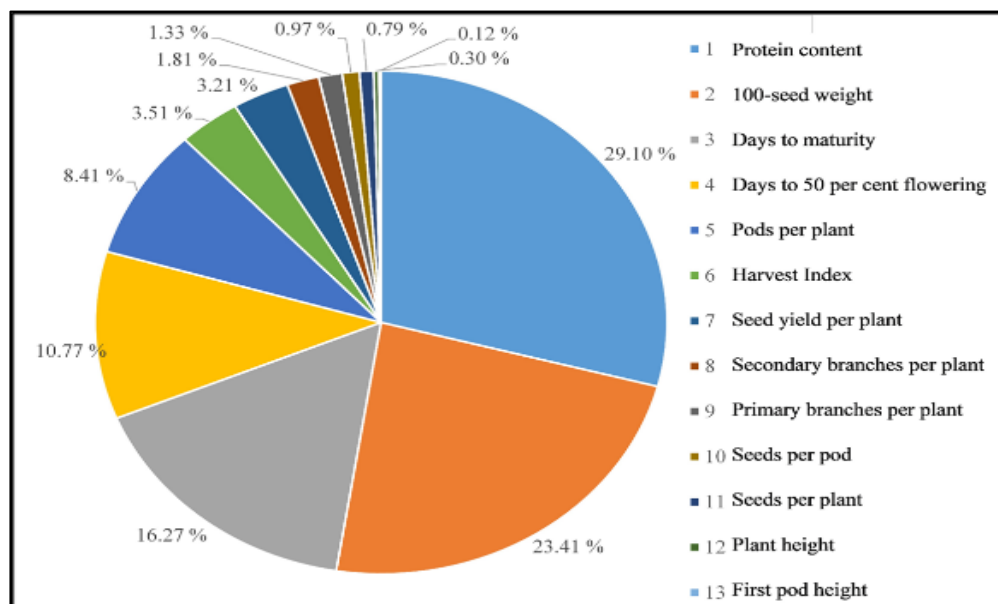
Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
I	45.72															
II	115.62	44.25														
III	160.02	100.38	46.08													
IV	100.38	121.72	85.39	53.60												
V	89.29	100.21	85.41	103.12	50.97											
VI	97.98	65.25	181.20	171.50	137.80	39.73										
VII	203.94	165.91	85.92	169.44	74.13	245.71	28.25									
VIII	170.94	75.58	62.60	100.22	122.91	131.38	154.02	0.00								
IX	259.18	96.99	247.94	305.29	251.23	96.65	326.67	186.44	66.00							
X	150.05	90.48	134.31	100.69	161.65	148.09	273.44	87.81	188.43	0.00						
XI	113.70	189.04	257.59	187.81	203.17	128.84	367.15	218.19	318.41	278.29	0.00					
XII	159.61	105.85	129.63	162.86	141.58	104.63	202.86	87.45	197.94	220.18	106.95	58.02				
XIII	90.21	111.54	166.31	179.66	76.45	90.44	127.79	181.42	219.61	262.75	164.50	135.83	0.00			
XIV	170.94	241.79	134.27	114.01	149.25	261.74	189.76	175.72	461.49	297.36	169.32	158.18	215.06	0.00		

XV	249.14	99.75	161.52	199.62	227.89	116.71	305.88	95.22	121.72	128.93	249.88	116.93	273.90	293.76	0.00	
XVI	183.96	250.96	137.43	138.07	117.31	316.95	87.18	207.80	485.87	282.01	377.94	285.27	193.38	152.05	429.63	0.00

Table 3: Cluster means of different characters in chickpea

Cluster	Days to 50 per cent flowering	First pod height (cm)	Days to maturity	Plant height (cm)	Primary branches per plant	Secondary branches per plant	Pods per plant	Seeds per pod	Seeds per plant	100-seed weight (g)	Seed yield per plant (g)	Harvest Index (%)	Protein content (%)
I	49.00	40.38	108.71	78.09	3.23	10.89	72.47	1.38	87.48	24.74	18.74	41.84	19.14
II	65.89	45.76	117.89	85.72	2.76	9.91	52.61	1.40	62.36	26.51	15.23	31.72	21.33
III	62.86	39.74	119.00	77.24	2.95	10.16	74.19	1.52	103.27	15.55	14.27	33.88	22.48
IV	63.00	41.49	114.91	75.92	2.84	12.27	79.10	1.50	103.82	15.17	14.52	36.94	18.71
V	48.71	38.83	111.38	78.68	2.82	10.25	68.83	1.48	79.14	18.89	14.83	35.72	22.79
VI	55.00	46.30	118.00	91.80	2.67	12.70	66.53	1.43	77.03	34.51	23.40	41.86	20.46
VII	48.17	38.34	113.67	78.43	3.03	11.07	64.67	1.67	87.97	14.88	11.98	37.62	26.60
VIII	69.00	43.87	124.67	77.20	2.47	8.00	75.40	1.87	114.93	19.06	25.13	52.71	20.86
IX	70.00	59.67	125.00	103.20	2.67	7.07	44.00	1.07	43.20	38.51	14.67	24.09	20.83
X	68.67	42.33	122.00	84.13	2.47	10.13	27.47	1.40	27.87	42.34	9.27	23.67	24.85
XI	72.00	44.07	122.33	76.87	2.93	7.60	35.53	1.40	36.27	20.92	7.87	34.43	17.30
XII	54.17	40.20	113.83	76.73	2.83	10.97	123.03	1.23	157.63	25.99	38.77	54.89	19.12
XIII	67.33	47.40	121.00	94.07	3.20	12.07	105.40	1.07	127.80	28.00	30.37	39.87	22.85
XIV	44.00	42.07	107.33	88.53	2.93	12.53	60.93	1.93	103.33	26.36	24.87	42.95	23.27
XV	46.33	33.87	115.00	74.07	2.33	12.60	136.00	1.53	201.87	16.23	25.77	41.48	20.43
XVI	68.33	52.93	131.00	105.87	2.80	11.87	77.40	1.00	76.07	26.49	19.03	24.07	19.76
Mean	58.22	41.60	115.75	80.34	2.89	10.67	72.09	1.45	92.22	21.06	16.96	36.77	21.26
S.Em.	2.91	2.64	1.45	3.99	0.26	0.95	7.27	0.14	9.24	1.58	2.10	3.36	0.70
CD @ 5%	8.07	7.32	4.03	11.06	NS	2.62	20.15	0.38	25.62	4.37	5.82	9.31	1.94
CV %	10.89	13.81	2.73	10.81	19.23	19.28	21.94	20.37	21.81	16.28	26.96	19.89	7.11
R ²	0.780	0.470	0.844	0.519	0.074	0.353	0.734	-	0.808	0.893	0.778	0.580	0.799
CV _b %	20.44	12.97	6.36	11.24	5.43	14.24	36.45	-	44.81	46.90	50.53	23.38	14.20

*R²: Ratio of inter-cluster variance to the total variance, NS: Non-Significant, -: Not estimated due to -ve variance, CV_b %: Inter-cluster coefficient of variation

**Fig 1:** Contribution of different characters towards genetic divergence of 58 chickpea genotypes

4. Conclusion

The genetic divergence assessed by Mahalanobis D²-statistic grouped 58 genotypes into sixteen clusters. Maximum genetic divergence was observed between cluster IX and XVI and cluster IX and XIV. The characters, viz., seed yield per plant, 100 seed weight, seeds per plant and pods per plant contributed much to the total genetic divergence. On the basis of cluster mean values, cluster XII (ICC-2919 and ICC-12028) was superior for seed yield per plant and harvest index, whereas cluster XV (ICC-13357) was good for pods

per plant and seeds per plant and cluster X (ICC-5037) was good for 100 seed weight. From the D² analysis of genetic diversity, based upon high yielding genotypes and large inter-cluster distances, it is advisable to attempt crossing of the genotypes from clusters IX (ICC-8151 and ICC-12328) and XVI (ICC-12866) and cluster IX (ICC-8151 and ICC-12328) and XIV (ICC-10393), which may lead to broad spectrum of favorable genetic variability for yield improvement in chickpea.

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