www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(8): 784-791

www.thepharmajournal.com
Received: 18-06-2021
Accepted: 24-07-2021

Deepa Singh
Department of Genetics and Plant Breeding, Prof. Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana, India

SNCVL Pushpavalli
Assistant Professor, Institute of Biotechnology, Prof. Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana, India

S Vanisri
Department of Genetics and Plant Breeding, Prof. Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana, India

G Anil Kumar
Agricultural Research Station, PJTSAU, Adilabad, Telangana, India

Corresponding Author:
SNCVL Pushpavalli
Assistant Professor, Institute of Biotechnology, Prof. Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana, India

Assessment of genetic diversity in chickpea genotypes (*Cicer arietinum* L.) using agro-morphological and SSR markers

Deepa Singh, SNCVL Pushpavalli, S Vanisri and G Anil Kumar

Abstract
The genetic diversity among thirty six chickpea genotypes was assessed by morphological and molecular studies. The Mahalanobis D^2 statistic grouped 36 genotypes into six clusters with the largest cluster comprising 20 genotypes. 100 seed weight contributed the most to genetic divergence. Out of 31 SSR markers used in the study, 28 were found to be polymorphic. A total of 107 alleles were detected across 36 chickpea genotypes with an average of 3.4 alleles per locus. Polymorphic information content ranged from 0.52 to 0.84. DICE similarity coefficient classified the genotypes into 4 clusters of which cluster I was the largest with 14 genotypes. The genetic similarity coefficient ranged from 0.03 to 0.78.

Keywords: Chickpea, D^2 analysis, genetic diversity, SSR markers, PIC

Introduction
Chickpea (*Cicer arietinum* L.) is an important Rabi legume crop. Chickpea was cultivated on 97 lakh hectares in India during 2019-20, with yield and productivity of 11.08 MT and 1142 kg/ha, respectively. (Source: Directorate of Economics and Statistics, DAC&FW 2019-20). The crop is majorly grown in the states of Madhya Pradesh, Rajasthan, Telangana, Maharashtra, Karnataka, Uttar Pradesh, and Andhra Pradesh. Chickpea meets its nitrogen requirements through biological nitrogen fixation and provides a substantial quantity of residual nitrogen for future crops, improving soil quality, long-term stability, and sustainability. It plays an important part in meeting the daily nutritional needs of the Indian populace. Chickpea production may be increased by selecting better genotypes that are directly related to seed yield and using these genotypes in breeding programs to enhance grain yield. The most commonly targeted traits for chickpea improvement programs are yield and yield contributing characters. Yield is a complicated trait that is influenced by a variety of variables and environment. The primary challenge in plant breeding is the identification and use of different germplasm. Understanding the patterns of genetic diversity and accurate and thorough descriptions of breeding materials assists the future breeding programmes. Mahalonobis D^2 statistic is widely used for analysis of genetic diversity by many researchers in crop plants (Rao, 1952) [11]. Several researchers studied genetic diversity, clustering pattern, and proportional contribution of various characteristics towards selection effectiveness and divergence. For successful selection, principal component analysis is performed to identify and convert a set of correlated variables into a set of smaller variables known as primary components.

However, it is often assumed that use of molecular markers is more trustworthy and reproducible than morphological characterization. Various marker systems have been used for chickpea characterization, including amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), and microsatellite markers like simple-sequenced repeats (SSR) or sequenced tagged microsatellite sites (STMS) (Iruela *et al*. 2002 [2]; Nguyen *et al*. 2004 [9]; Sachdeva *et al*. 2018) [13]. Because of co-dominance, multiple allelic nature, high polymorphism, locus specificity, reproducibility, and automation SSRs has been the marker of choice. The present study aims at assessment of genetic diversity among a set of 36 promising advanced breeding lines which can be the potential parents for development of high yielding varieties.

Material and Methods

Plant material and Field Experimentation

During Rabi 2020-21, 36 chickpea germplasm lines from Agricultural Research Station, Adilabad were grown in Random Block Design at PJTSAU college farm (Table 1). The entries were planted in 4m long row plots with 30 cm row spacing and 10 cm plant spacing. All the standard agronomic practices were followed to raise the crop. Five plants were chosen at random for data collection on days to 50% flowering, days to maturity, plant height (cm), number of primary branches per plant, number of pods per plant, 100 seed weight (g), and seed yield (kg/ha). To examine genetic divergence among the chosen chickpea varieties, the Mahalonobis D^2 statistic and Principal Component Analysis (PCA) (Jackson, 1991) [3] were employed.

Table 1: List of chickpea genotypes used in the present study

S. No.	Genotype	S. No.	Genotype
1	ADBG 1	19	ADBG 503
2	ADBG 2	20	ADBG 504
3	ADBG 377	21	ADBG 509
4	ADBG 487	22	ADBG 510
5	ADBG 488	23	ADBG 511
6	ADBG 490	24	ADBG 512
7	ADBG 491	25	ADBG 513
8	ADBG 492	26	ADBG 514
9	ADBG 493	27	ADBG 515
10	ADBG 494	28	ADBG 516
11	ADBG 495	29	ADBG 517
12	ADBG 496	30	ADBG 519
13	ADBG 497	31	ADBG 520
14	ADBG 498	32	ADBG 522
15	ADBG 499	33	ADBG 523
16	ADBG 500	34	NBeG 47
17	ADBG 501	35	JG 16
18	ADBG 502	36	JG 11

Statistical analysis

Mahalanobis' D^2 analysis

D^2 values between the i_{th} and the j_{th} genotypes for 'p' characters were calculated as per Mahalanobis (1936) [7]

$$D_{ij}^2 = \sum_{t=1}^{p} (\overline{Y}_{it} - \overline{Y}_{jt})^2$$

Where,

Y_{it} = Uncorrelated mean value of i_{th} genotype for the character 't',

Y_{jt} = Uncorrelated mean value of j_{th} genotype for the character 't'.

D^2_{ij} = D^2 between the i_{th} and j_{th} genotypes

The following are the measures involved in calculating D^2 values.

Test of significance

A comparative test for significance of deviations in the mean values with respect to the pooled effect of characters was performed using the 'Λ' statistic.

The calculation of 'Λ' (Wilk's criterion) was done by using the following equation.

$$\Lambda = W/S$$

Where,

Λ = Wilk's criterion

W = determinant of error matrix

S = determinant of error variety matrix

The significance of 'Λ' was tested by:

$$\lambda^2 pq = V\,(stat) = -m \log_e \text{'}\Lambda\text{'} = -\left[n - \frac{p+q+1}{2}\right] \log_e \text{'}\Lambda\text{'}$$

Where,

m = n-(p+q+1)/2

p = Number of variables

q = Number of variables -1 (or degree of freedom for the population)

n = Degrees of freedom for error + varieties

Loge Λ = 2.3026 log10 'Λ'

For testing the significance, the tabulated value of $\lambda^2 pq$ for degrees of freedom at the 5% level is compared to the above values obtained.

Transformation of correlated variables

D^2 values were simplified to simple summation values of the discrepancies in mean values of different characters of the two genotypes, i.e., Σdi^2. Correlated variables were transformed into uncorrelated variables. The pivotal consideration approach was used to convert the results.

Testing for significance of D^2 values

The values obtained of D^2 for a pair of populations, which are taken as calculated values are compared to the tabulated value of λ^2 for 'p' degrees of freedom, where 'p' is the number of characters that are taken into account.

Contribution of the individual characters towards divergence

Every character was graded in each combination based on their contribution to the divergence between the two entries (di = Yit - Yjt). The highest mean difference is given rank 1, and the lowest receives rank 'p,' where 'p' is the total number of characters being evaluated. The following formula was used to measure the percentage of contribution of each character to the genetic divergence:

Percentage contribution of the characters = X/Y x 100

Where,
X = Number of genotype contribution where the character was ranked first
Y = All the possible combinations of number of genotypes considered

Grouping of the genotypes into different clusters
Tocher's system, as defined by Rao (1952) [11], was used to group the populations into various clusters. This clustering criteria of this method is that any two varieties in a same to the same cluster should have a comparable D^2 value on average than those belonging to other clusters.
D^2 values of all the genotype combinations were organized in a tabular form in increasing order of magnitude for this reason.

Average intra cluster distance
For estimating the intra cluster distance, the given formula was used

$$\text{Average inter cluster distance} = \frac{\Sigma D_i^2}{n}$$

Where, $\Sigma D^2 i$ = Sum of distances between all the possible combinations (n) of the populations present
in a cluster
n = Number of genotypes included in a cluster

Average inter cluster distance
Clusters were examined one by one, and distances between them and other clusters were measured. The distance between two clusters was calculated by dividing the total of D^2 values between members of one cluster members of the other cluster by the product of the number of genotypes in both clusters. The genetic distance between the clusters was calculated by taking the square root of the average value of D^2.

$$\text{Average inter cluster distance} = \frac{D^2}{n_1 x\, n_2}$$

Where, n1 and n2 are the number of genotypes present in cluster 1 and cluster 2, respectively.

Cluster diagram
The clusters and their interrelationships were depicted in a diagram. The gap was calculated using the square root of average D^2, which was an estimated calculation of group divergence.

Principle Component Analysis
The Eigen values and associated Eigen vectors of correlation matrix were determined by solving the given equation.

$| R - \lambda_1 | v = 0$

Being (λ_1, v_1), (λ_2, v_2),……$(\lambda p, vp)$ the eigen values and eigen vector pairs of R With $\lambda 1 \geq \lambda_2 \geq$……..$\geq \lambda p$, then the first principal component Y; was calculated as

$Y_1 = v_1 \; X = V_{1\,1} \; X_1 + V_{1\,2} \; X_2 +$…… $+V_1 \, pXp$

Such that the variance of Y, was maximized subject to the constraint that the sum of squared weights was equal to one (i.e. $\Sigma vij^2=1$). The second principal component was calculated as:

$Y_2 = v_2 \; X = V_{2\,1} \; X_1 + V_{2\,2} \; X_2 +$…… $+V_2 \, pXp$

This was uncorrelated with first principal component and $\Sigma vij_2 = 1$
The principal components were uncorrelated with each other and their variances were equal to the eigen values λ_1, λ_2 ,……..$\geq \lambda p$, i.e.
Kaiser's (1958) [6] proposal of removing principal components of correlation matrices with eigen roots <1 one was followed in determining the number of principal components to be maintained.

DNA Extraction and PCR amplification
Genomic DNA was isolated from young leaves following the CTAB (cetyl trimethyl ammonium bromide) procedure as described by Saghai-Maroof *et al.* (1984) [14]. The DNA was quantified using 0.8% agarose gel stained with ethidium bromide and comparing it to lambda Phage DNA as a standard. For PCR, the genomic DNA was diluted to 50ng/ml. A total of 31 SSR markers were employed. The following cycling conditions were used to amplify DNA in a total reaction volume of 10µl: Initial denaturation at 94°C for 5 min, followed by denaturation at 94°C for 1 min, annealing at 48-58°C (based on primer Tm) for 30 sec and extension at 72°C for 45 sec. This cycle was repeated 35 times, with a final extension of 7 min at 72°C. Ethidium bromide stain was used to detect the amplified products, which were separated on a 3% MetaPhor agarose gel. The gel was photographed utilizing gel documentation system (Biorad).

SSR allele scoring and data analysis
The PCR products were analyzed by scoring qualitatively for presence (1) or absence (0). Polymorphism Information Content (PIC) was calculated according to Anderson *et al.* (1993) [1] using the following equation:

$PIC_j = 1 - \sum_{i=1}^{n} P_i^2$

Where, i = the i^{th} allele of the j^{th} marker,
n=the number of alleles at the j^{th} marker and
P = allele frequency.

Genetic similarity (GS) was estimated for all genotype pairs using the equation $GS_{i,j} = 2N_{i,j}/(2N_{i,j} + N_i + N_j)$ (Nei and Li, 1979) [8], where $GS_{i,j}$ represents the similarity estimate between the genotypes *i* and *j*, based on SSR data, $N_{i,j}$ is the total number of bands common to *i* and *j*, and N_i and N_j correspond to the number of bands found in genotypes *i* and *j*. The matrix generated with the GS estimates was used to cluster the genotypes in a dendrogram based on unweighted pair group method using arithmetic averages (UPGMA). The mean of the similarity matrix would be the cut off line position on the dendrogram to identify the number of clusters. To test the goodness of fit of clustering to a set of data, copheneic correlation or cophenetic value was estimated using the COPH and MXCOM options in NTSYS-pc2.02 program (Rholf, 1993) [12].

Results and Discussion
Mahalanobis D^2 statistic divided the 36 genotypes into six clusters, given in table 2, with cluster I (the biggest)

comprising 20 genotypes, followed by cluster IV (9) and cluster II (4). Clusters III, IV, and VI were all monogenotypic, suggesting that these genotypes are diverse. In earlier studies, Jeena *et al.* (2005) [4] grouped 80 genotypes into 11 clusters, with cluster I containing a maximum of 60 genotypes. Nimbalkar (2000) [10] divided 40 chickpea genotypes into 16 clusters, 10 of which were monogenotypic. The genotypes ADBG 493, ADBG 500, and ADBG 519 reported higher seed yield per plant. It was observed that genotypes with comparable pedigrees were clustered together. ADBG 499 and ADBG 501, both descended from the same parental lines (NBeG 3 x ICCV 05103), were found in the same cluster I, as were ADBG 516 and ADBG 517 with pedigree (JG 11 x ICCV 05103) grouped in cluster IV. The genotypes ICCV 05103, ADBG 497 and ADBG 498, with a single common parent were placed together in cluster I. Due to differences in genetic architecture, single genotype clusters were observed. Cluster II and VI had the greatest inter-cluster distance of 30.08, followed by cluster V and VI (25.42), while cluster III and cluster V had the least inter cluster distance of 5.61 (Table 3). In a hybridization procedure, genotypes from diverse clusters, such as II and VI, may be used as parental lines to produce highly heterotic hybrids. Cluster IV had most of the erect genotypes such as ADBG 517, ADBG 513, ADBG 511, ADBG 494, and ADBG 515 while Cluster I (ADBG 504), cluster II (ADBG 503), cluster V (ADBG 512), and cluster VI (ADBG 519) had one erect genotype. Cluster II had early maturing genotypes (96.92 days), genotypes in cluster IV had highest number of pods per plant (40.56) and the maximum seed yield per plant (12.66g) and cluster VI had the highest 100 seed weight (32.67g) as shown in table 4. The genotypes from these promising clusters can be exploited as prospective parental lines in a chickpea enhancement breeding programme. The attribute 100-seed weight contributed the most to genetic divergence (51.11%), followed by days to 50% flowering (17.94%) and number of major branches per plant (8.89%). In the chickpea genotypes, the parameters 100-seed weight and days to 50% flowering account for roughly 69% of the overall genetic divergence (Table 5). Earlier Jivani *et al.* (2013) [5] reported that seed yield per plant, 100 seed weight, and number of pods per plant contributed more than 63 percent of overall genetic divergence. Days to maturity and days to 50% flowering contributed the most to divergence according to Upadhyaya *et al.* (2017) [16]. According to Shivwanshi *et al.* (2019) [15], pods per plant were the most important factor in total divergence, followed by biological yield per plant and plant height. The first three principal components explained around 79.75 percent of the variability in this investigation. Table 6 shows that PC1 which included 100 seed weight and seed yield per plant accounted for 51.22% of the variability. These characteristics can be used to select elite lines for chickpea crop improvement. The positive contribution of PC2 is phenological traits such as days to 50% flowering and number of primary branches per plant while PC3 included plant height.

Table 2: Clustering of 36 chickpea genotypes into different clusters by Tocher's Method

Cluster No.	Number of genotypes included	Genotypes
I	20	ADBG 502, JG 16, ADBG 514, ADBG 520, ADBG 501, ADBG 491, ADBG 377, ADBG 487, ADBG 2, ADBG 498, ADBG 497, ADBG 522, ADBG 504, ADBG 499, JG 11, NBeG 47, ADBG 510, ADBG 490, ADBG 488, ADBG 496
II	4	ADBG 503, ADBG 523, ADBG 509, ADBG 495
III	1	ADBG 492
IV	9	ADBG 493, ADBG 517, ADBG 500, ADBG 513, ADBG 511, ADBG 494, ADBG 1, ADBG 515, ADBG 516
V	1	ADBG 512
VI	1	ADBG 519

Table 3: Average inter and intra cluster distances (D^2) of six clusters of chickpea genotypes

Cluster	I	II	III	IV	V	VI
I	4.55	10.06	7.84	10.49	8.30	13.44
II		3.88	10.77	25.09	10.64	30.08
III			0.00	12.90	5.61	21.33
IV				7.50	14.27	11.22
V					0.00	25.42
VI						0.00

Table 4: Mean performance of clusters for seven characters in chickpea

Clusters	Days to 50% flowering	Plant height (cm)	No. of primary branches per plant	No. of pods per plant	Days to maturity	100 seed Wt (g)	Seed yield per plant (g)
I	44.83	57.10	6.23	39.92	99.07	26.51	10.38
II	46.83	59.96	6.75	40.25	96.92	21.50	8.66
III	54.00	58.83	7.67	40.33	105.00	25.33	10.17
IV	47.41	58.96	6.04	40.56	97.30	31.31	12.66
V	54.67	60.67	4.33	38.33	97.33	25.83	9.87
VI	37.67	60.50	7.33	37.67	103.67	32.67	11.90

Table 5: Percent contribution of seed yield and its attributing characters to divergence among 36 genotypes of chickpea

S. No.	Characters	Times ranked first	Per cent contribution
1	Days to 50% flowering	113	17.94
2	Plant height (cm)	38	6.03

3	Number of primary branches per plant	56	8.89
4	Number of pods per plant	41	6.51
5	Days to maturity	23	3.65
6	100 seed weight (g)	322	51.11
7	Seed yield per plant (g)	11	1.75
	Total	604	100

Table 6: Eigen value and contribution of variability for the principal component axes in chickpea genotypes

	PC 1	PC 2	PC 3
Eigene Value (Root)	84.65	33.00	14.17
% Var. Exp.	51.22	19.96	8.57
Cumulative Var. Exp.	51.22	71.19	79.76
Days to 50% flowering	0.01	0.86	0.00
Plant height (cm)	-0.02	0.15	0.47
Number of primary branches per plant	-0.04	0.23	-0.72
Number of pods per plant	0.08	-0.37	-0.21
Days to maturity	0.04	0.09	-0.30
100 seed weight (g)	0.95	0.07	0.09
Seed yield per plant (g)	0.26	-0.13	-0.30

A set of 31 Polymorphic SSR markers enlisted in table 8 were used to examine genetic diversity in 36 chickpea genotypes. A total of 107 alleles were observed with number of alleles ranging from 3 to 5, with an average of 3.4. The values of polymorphic information content (PIC) ranged from 0.52 to 0.84. The highest PIC value was 0.84 for the marker CaM0803. All of the polymorphic markers utilized in the study were found to be informative (PIC>0.5), implying that they might be used for cultivar identification**.** Based on the Dice similarity coefficient, genetic similarity across 36 chickpea genotypes ranged from 0.03 (between genotypes ADBG 488 and ADBG 503) to 0.78 (between genotypes ADBG 496 and ADBG 498), with an average of 0.36. UPGMA clustering grouped the genotypes into four clusters based on SSR data (NTSYS Pc-2.0). Among the four clusters, it is seen in table 7 that cluster I was the largest with 39% percent of genotypes followed by cluster II and cluster III. Cluster IV had least number with 5 genotypes. The cophenetic value of 0.98 obtained using the SSR data indicated a very good fit of clustering based on SSR data.

Table 7: Grouping of 36 chickpea genotypes based on SSR analysis using DICE similarity coefficient

Cluster	Sub Cluster	No. of genotypes	Cluster composition
I	A	9	ADBG 1, ADBG 2, ADBG 491, ADBG 492, ADBG 493, ADBG 377, ADBG 487, ADBG 488, ADBG 490
	B	5	ADBG 494, ADBG 495, ADBG 496, ADBG 497, ADBG 498
II	A	5	ADBG 515, ADBG 516, ADBG 517, ADBG 519, ADBG 520,
	B	5	ADBG 522, ADBG 523, NBeG 47, JG 16, JG 11
III	A	5	ADBG 499, ADBG 500, ADBG 501, ADBG 502, ADBG 503,
	B	2	ADBG 504, ADBG 509
IV	A	4	ADBG 510, ADBG 511, ADBG 512, ADBG 513
	B	1	ADBG 514

Table 8: List of SSR markers used for genotyping of 36 chickpea genotypes along with their product size, number of alleles and PIC

S. No	SSR locus	Allele size range (bp) (approximate)	No. of alleles	PIC
1	GA6	220-235	Monomorphic	-
2	NCPGR147	320-335	5	0.78
3	TS29	325-340	3	0.57
4	CaM1903	210-225	3	0.62
5	CaM1502	205-220	4	0.69
6	NCPGR74	280-310	5	0.75
7	NCPGR103	210-230	4	0.61
8	TR58	335-350	2	0.79
9	TA136	195-215	4	0.70
10	H3A10	320-340	Monomorphic	-
11	TR43	220-235	4	0.62
12	TA25	205-220	4	0.7
13	NC81	200-230	4	0.65
14	GAA47	210-270	5	0.77
15	NCPGR69	175-195	Monomorphic	-
16	TA130	170-190	4	0.67
17	ICCM0249	280-300	5	0.69
18	TAA170	200-220	3	0.52
19	STMS11	195-220	3	0.7

20	CaGM00495	100-130	4	0.74
21	CaGM00515	210-240	3	0.63
22	ICCeM0050	210-235	4	0.62
23	CaM1577	220-240	4	0.63
24	NCPGR223	230-290	4	0.71
25	NCPGR138	175-190	3	0.52
26	ICCM0105	220-245	4	0.68
27	ICCM0228	120-145	4	0.67
28	1CCM0301	345-370	4	0.67
29	CaM0040	230-250	4	0.69
30	CAM0489	130-170	4	0.63
31	CaM0803	132-155	3	0.84

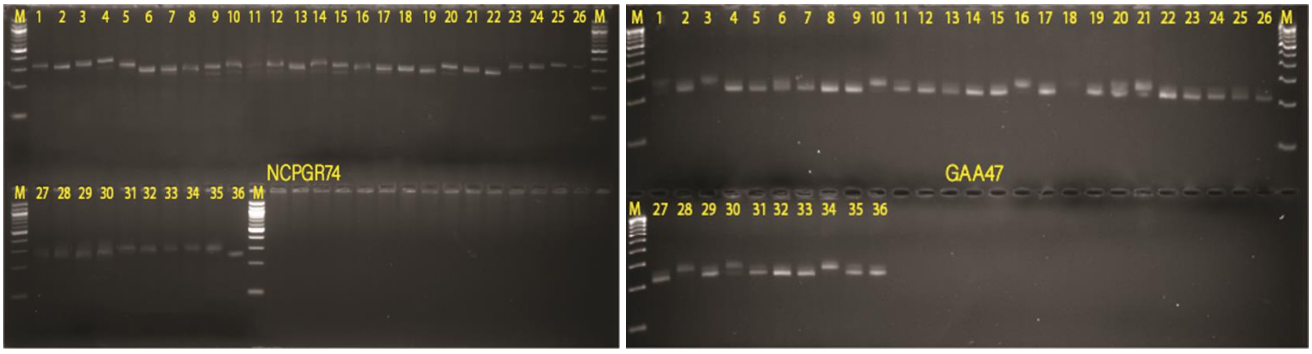

Fig 1: PCR amplification profile generated with SSR markers (GAA47 & NCPGR74) in 36 chickpea genotypes

M – 100 bp Ladder

1. ADBG 1
2. ADBG 2
3. ADBG 377
4. ADBG 487
5. ADBG 488
6. ADBG 490
7. ADBG 491
8. ADBG 492
9. ADBG 493
10. ADBG 494
11. ADBG 495
12. ADBG 496
13. ADBG 497
14. ADBG 498
15. ADBG 499
16. ADBG 500
17. ADBG 501
18. ADBG 502
19. ADBG 503
20. ADBG 504
21. ADBG 509
22. ADBG 510
23. ADBG 511
24. ADBG 512
25. ADBG 513
26. ADBG 514
27. ADBG 515
28. ADBG 516
29. ADBG 517
30. ADBG 519
31. ADBG 520
32. ADBG 522
33. ADBG 523
34. NBeG 47
35. JG 16
36. JG 11

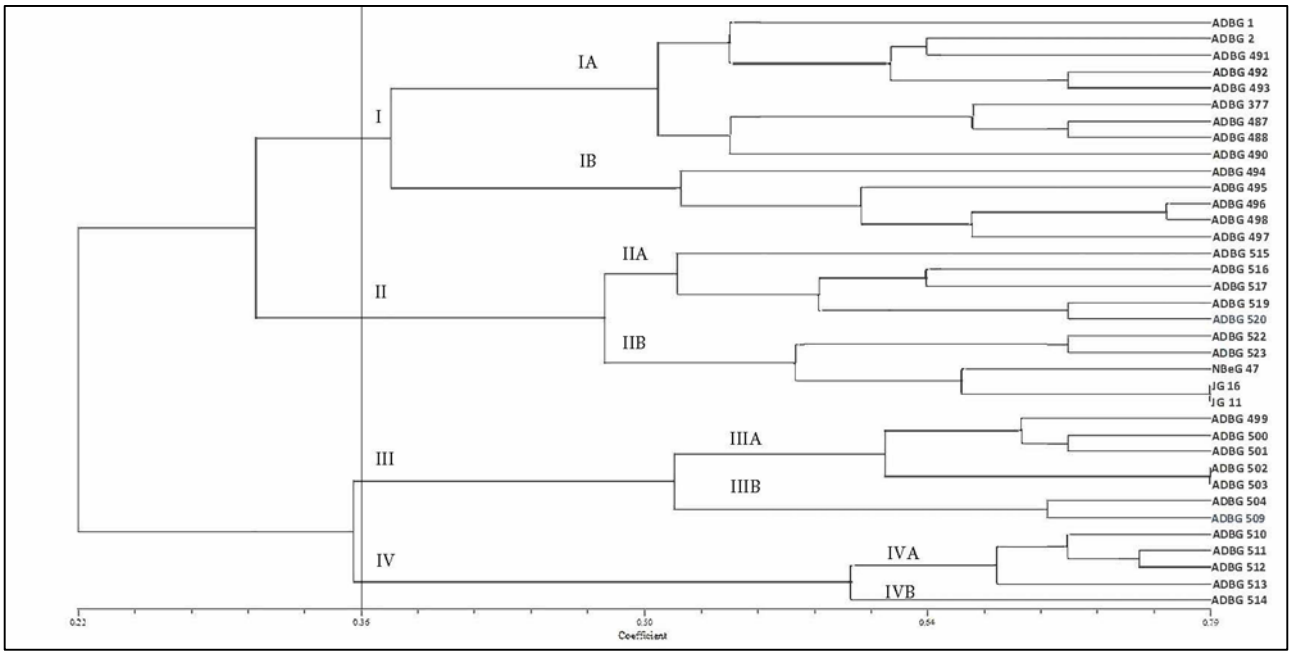

Fig 2: Phylogram of 36 chickpea germplasm constructed using UPGMA method based on DICE similarity coefficient based on SSR data

Two dimensional and 3-dimensional plots based on SSR data is in accordance with UPGMA based clustering (Fig. 3 and Fig. 4)

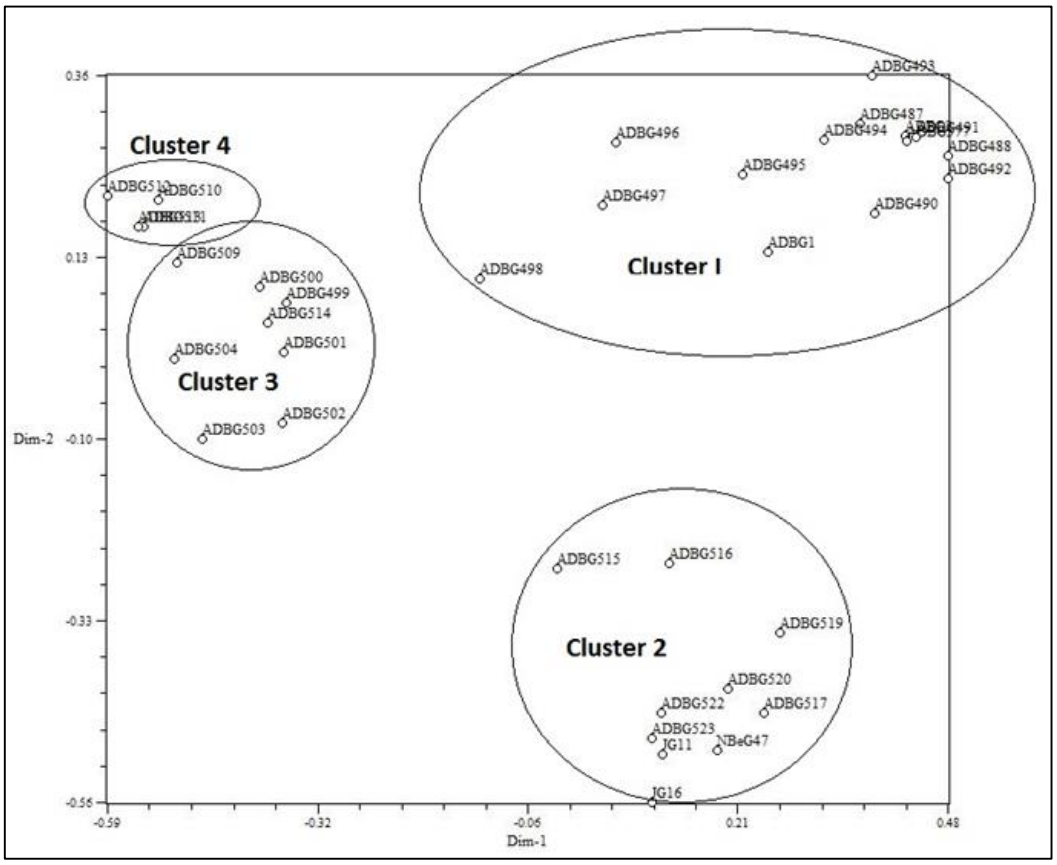

Fig 3: Two dimensional plot based on SSR marker data of chickpea genotypes

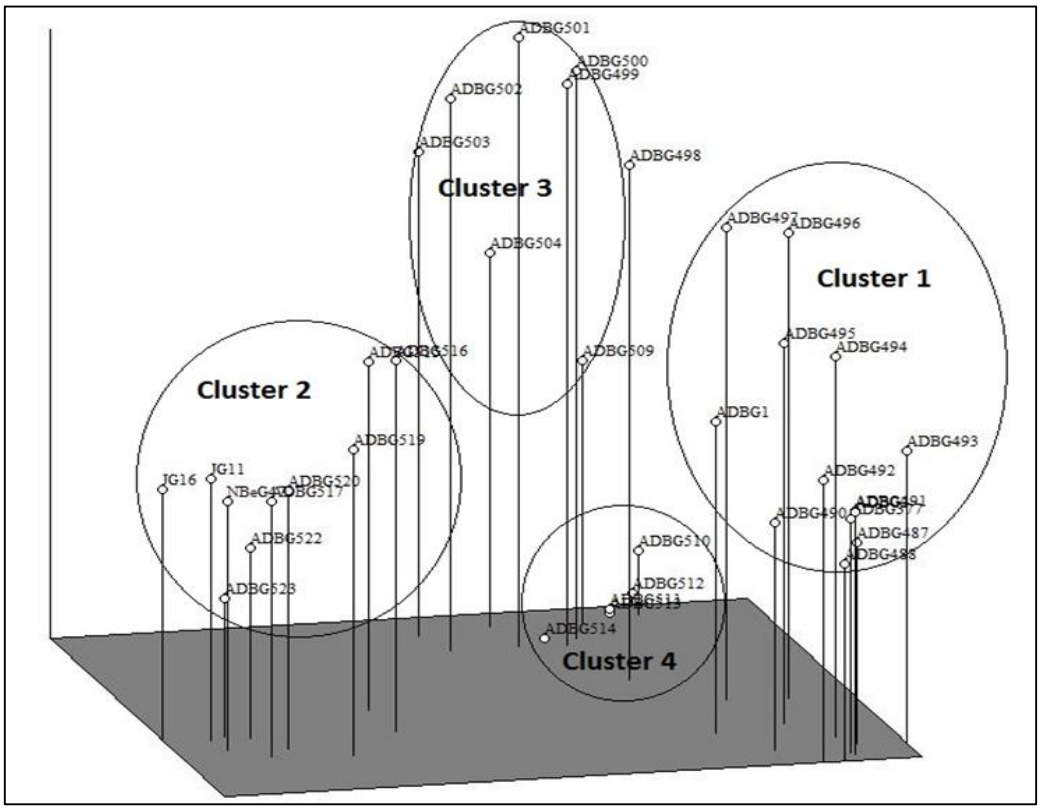

Fig 4: Three dimensional plot based on SSR marker data of chickpea genotypes

A comparison of clustering based on agro-morphological features and SSR data indicated that genotypes ADBG 2, ADBG 491, ADBG 377, ADBG 487, ADBG 488, ADBG 496, ADBG 497, and ADBG 498 were grouped together in both approaches. In both methods, the released varieties NBeG 47 and JG 11 were present in one group. The clustering of genotypes based on SSR data was also shown to be in agreement with their pedigree and genotypic similarities. Cluster III included ADBG 499, ADBG 500, and ADBG 501, which all had the same pedigree (NBeG 3 x ICCV 05103), as

well as ADBG 502, ADBG 503, and ADBG 504, which had the pedigree (JAKI 9218 x ICCV 05103). Cluster II comprised of genotypes ADBG 516, ADBG 517 and ADBG 519 with common parents (JG 11 x ICCV 05103). Since SSR markers may not always sample genomic areas that regulate the phenotypic traits being evaluated, these findings are not surprising. The SSR represent genotype relationships based on DNA sequences, whereas the morphological features represent genotype associations in general depending on their growing environment. SSR data on diversity can be used to correlate with pedigree relationships and morphological traits for genetic improvement in the chickpea crop.

Conclusion

The present investigation indicated the existence of significance variance with respect to days to 50% flowering, days to maturity, plant height (cm), number of primary branches per plant, number of pods per plant, 100 seed weight (g), and seed yield (kg/ha)**.** Clustering of genotypes based on morphological characteristics has also aided in the identification of promising parental lines for producing superior recombinants in chickpea breeding program. The current work has revealed the utility of SSR markers for characterization of the genotypes at DNA level.

Acknowledgements

The authors are thankful to Prof. Jayashankar Telangana State Agricultural University, Hyderabad for providing the facilities and financial assistance for carrying out the present study. We wish to present our special thanks to Agricultural Research Station, Adilabad for providing seeds of chickpea genotypes. This study is part of M.Sc. student thesis work of first author.

References

1. Anderson 6A, Churchill GA, Autrique JE, Tanksley SD, Sorrells ME. Optimizing parental selection for genetic linkage maps. Genome 1993;36(1):181-186.
2. Iruela M, Rubio J, Cubero JI, Gil J, Millan T. Phylogenetic analysis in the genus Cicer and cultivated chickpea using RAPD and ISSR markers. Theoretical and Applied Genetics 2002;104(4):643-651.
3. Jackson JE. A user's guide to principal components. Wiley: New York 1991,569p.
4. Jeena AS, Arora PP, Ojha OP. Variability and correlation studies for yield and its components in chickpea. Legume Research-An International Journal 2005;28(2):146-148.
5. Jivani JV, Mehta DR, Pithia MS, Madariya RB, Mandavia CK. Variability analysis and multivariate analysis in chickpea (*Cicer arietinum* L.). Electronic Journal of Plant Breeding 2013;4(4):1284-1291.
6. Kaiser HF. The varimax criterion for analytic rotation in factor analysis. Psychometrika 1958;23(3):187-200.
7. Mahalanobis BC. On the generalized distance in statistics. Proceed. Nat. Acad. Sci 1936;2:49-55.
8. Nei M, Li WH. Mathematical models for studying genetic variation in terms of restriction endonucleases. Proceedings of National Academy of Sciences USA 1979;76:5269-5273
9. Nguyen TT, Taylor PW, Redden RJ, Ford R. Genetic diversity estimates in Cicer using AFLP analysis. Plant Breeding 2004;123(2):173-179.
10. Nimbalkar RD. Genetic variability and heritability studies and scope for improvement in chickpea. Journal of Maharashtra Agricultural Universities 2000;25(1):109-110.
11. Rao CR. Advanced statistical methods in biometric research. John Wiley & Sons, Inc., New York 1952,390.
12. Rholf FJ. NTSYS-PC, numerical taxonomy and multivariate analysis system. Version 2.02h, Applied Bio statistical Inc. New York 1993.
13. Sachdeva S, Bharadwaj C, Sharma V, Patil BS, Soren KR, Roorkiwal M *et al*. Molecular and phenotypic diversity among chickpea (*Cicer arietinum*) genotypes as a function of drought tolerance. Crop and Pasture Science 2018;69(2):142-153.
14. Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proceedings of the National Academy of Sciences 1984;81(24):8014-8018.
15. Shivwanshi R, Babbar A. Genetic divergence analysis in chickpea germplasm. Legume Research-An International Journal 2019;42(5):715-718.
16. Upadhyaya HD. AVIB. Genetic diversity for yield and its component traits in chickpea (*Cicer arietinum* L.). Electronic Journal of Plant Breeding 2017;8(1):89-95.