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Characterization of genetic diversity in chickpea (*Cicer Arietinum* L) Using SSR Markers

Mukesh Kumar, Vinay Kumar, Navneet Kumar and Satendra Kumar Yadav

Abstract
All Thirty seven cultivars of chickpea (*Cicer arietinum* L.) were collected from core collection maintained and grown CRC Chirodi of Sardar Vallabhbhai Patel University of Agriculture & Technology during rabi 2015-16 and molecular analysis was done in Molecular Biology Laboratory, Department of Genetics and Plant Breeding. Chickpea is a highly nutritious grain legume crop and is one of the cheapest sources of protein. It can be eaten raw, roasted or boiled. It can also be processed into flour or dehulled grain (dal). It contains none of the anti-nutritional or toxic compounds often present in other legumes. The aim of this study is to evaluate the genetic diversity of chickpea varieties by SSR markers. PIC values ranged from 0.053 (Primer 7) to 0.876 (primer 4) with an average of 0.497. The resolving power (RP) varies between 0.702 (Primer 4) and 1.942 (Primer 7) with an average value of 1.311. GS values ranged from 0.10 to 1.00 among all the chickpea genotypes. The UPGMA based clustering grouped 37 chickpea genotypes into six major clusters. Based upon similarity coefficient and cluster analysis of SSR primers, chickpea genotypes V5 and V33 were found to be more diverse and they can be used for their desirable characteristic in breeding programmes.

Keywords: chickpea (*Cicer arietinum* L.), SSR markers

Introduction
Pulses play an important role in Indian agriculture besides being a rich and cheapest source of dietary protein and a valuable animal feed; pulse crop also play a key role in improving and sustaining soil fertility on account of biological nitrogen fixation, deep root system, mobilization of insoluble soil nutrients and bringing qualitative changes in soil physical properties. Pulses can arrest the declining trend in productivity of cereals based cropping systems. Inclusion of pulses in intensive cereal based cropping system act as a component of integrated nutrient supply. Therefore, pulses have emerged as available option to improve soil health, conserve the natural resources and sustain the agricultural productivity. Chickpea (*Cicer arietinum* L.) is also known as garbanzo bean, Chana (north India), Indian pea, Bengal gram; is an edible legume of the family *Fabaceae* and subfamily *Faboideae;* diploid with 2n = 2x = 16. It is a highly self-pollinated crop with an out crossing rate of less than 1%. The genus *Cicer* comprises one cultivated species (*Cicer arietinum L.*) and 42 wild species. According to the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), chickpea seed contains 29% protein, 59% carbohydrate, 3% fiber, 5% oil and 4% ash. Its protein is rich in lysine and arginine but is deficient in sulfur-containing amino acids methionine and cysteine. It is also a good source of absorbable Ca, P, Mg, Fe and K.

The major PCR-based marker systems that are currently available for genetic study include Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeat (SSR) and Inter Simple Sequence Repeat (ISSR) which are generally described as dominant markers (Weising *et al.*, 2005) [2]. ISSR-PCR is a technique, which involves the use of microsatellite sequences as primers in a polymerase chain reaction to generate multi-locus markers. It is a simple and quick method that combines most of the advantages of AFLP and RAPD (Zietkiewicz *et al*., 1994) [3]. These sequences are abundantly dispersed throughout the genome and highly polymorphic in comparison with other molecular markers (Morgante and Olivieri, 1993; Wang *et al*., 1994) [4, 5]. ISSR markers were used in the present study because of their long primer repeat length (16-18bp SSR repeat) and highly polymorphic interaction at individual loci.

Materials and methods

Plant Material

The experimental material of the present investigation comprised 37 genetically diverse genotypes of chickpea. All Thirty seven cultivars of chickpea (*Cicer arietinum* L.) were collected from core collection maintained and grown at CRC, Chirodi of Sardar Vallabhbhai Patel University of Agriculture & Technology during rabi 2015-16 and molecular analysis was done in Molecular Biology Laboratory, Department of Genetics and Plant Breeding. The details of the genotypes are given in Table 1.

Table 1: List of chickpea germplasms and their sources.

Sr. No.	Germplasm	Sr. No.	Germplasm
1	ICCV-14510	20	Pusa-5023
2	BG-362	21	Pusa-547
3	Pusa-1053	22	Pusa-372
4	BGD-72	23	ICCV-95334
5	Pusa-2024	24	Pusa-3022
6	DCP-92-3	25	Pusa-1108
7	HC-05	26	Pusa-1105
8	C-910	27	JGK-01
9	Pusa-1003	28	GNG-1581
10	GNG-1958	29	Pusa-5028
11	PKV-4	30	C-925
12	Pusa-256	31	BGD-112
13	Pusa-1103	32	RSG-931
14	HK-4	33	ICCV-14508
15	ICCV-13309	34	GNG-1969
16	BGD-1005	35	C-905
17	C-927	36	ICCV-14512
18	Pusa-2085	37	ICCV-07102
19	JG-62		

Molecular Analysis of Chickpea Varieties

Sample Collection

Leaves were harvested from 60 days old plants during Rabi season 2015-16. Then, leaf samples were packed into sterilized plastic poly-bags and stored at -80oC in deep freezer for the purpose of isolation of genomic DNA.

DNA Isolation and Quantification

Leaf samples (0.3 g of frozen leaf) were taken and grind to fine powder with a chilled mortar and pestle using liquid nitrogen. DNA was extracted by the modified CTAB method. DNA concentration was determined spectrophotometrically, and the quality of DNA was determined by 0.8% agarose gel electro- phoresis. To measure the concentration, BIO-RAD Smart Spec TM Plus spectro-photometer was used.

Molecular marker assay

DNA amplification was performed in 20 µl reaction volume containing 3µl DNA (25ng/ml), 2ml Taq Buffer 10x with MgCl2, 4ml dNTP mix (10mM each), 2 ml primer (10 mM), 1 unit of Taq DNA polymerase (1ml) and 8 ml double distilled water. The mixture was gently mixed and centrifuged. The cycling conditions were: 1 cycle (initial denaturation) 94°C for 5 min followed by 40 cycles of 94°C for 1 min (denaturation), approx 55 °C for 1 min (for SSR) (Annealing), 72°C for 2 min (extension), and finally 1 cycle of 72°C for 7 min (final extension). The ten different primers (10 SSR) used in the study were obtained from IDT (Integrated DNA Technology). SSR -PCR products were run for electrophoretic analysis on 2% agarose gel stained with Ethidium bromide and analysis of gel was done under Gel-doc System. Ladder of 100bp was used for size estimation.

Data Analysis

Thirty seven chickpea genotypes were used to estimate genetic diversity. Polymorphic products from SSRs assays were calculated for presence (1) or absence (0) of DNA bands. The proportions of bands that have been shared between any of the two varieties averaged over loci of SSR were used as the measure of similarity. Genetic diversity was calculated using PIC and RP. The term Polymorphic Information Content (PIC) was originally introduced into human genetics by. It refers to the value of a marker for detecting polymorphism within a population, depending on the number of detectable alleles and the distribution of their frequency. In present study, PIC value of a marker was calculated according to a simplified version after, where, Pij is the frequency of the jet allele for marker, i and the summation extends over n alleles. The power of each primer to distinguish among the studied genotypes was evaluated by the resolving power (Rp). Resolving power is the capacity of any primer to distinguish among different varieties. It is defined per primer as Rp= Ó Ib where lb is the band in formativeness, that takes the values of: 1-[2x(0.5-p)], being p the proportion of the chickpea varieties containing the band. The calculation was based on the number of bands in SSR per primer. The pair-wise genetic similarities among all pairs of samples were estimated with Jaccard's coefficient (Jaccard, 1908) [7]. The statistical analysis was carried out using NTSYS-pc software (version 2.02) (Rohlf, 1993) [6]. In order to group genotypes into discrete clusters a dendrogram was constructed by employing UPGMA.

Results and discussion

A total of 11 bands were obtained using the 10 primers with an average 1.1 bands per primer. Out of 11 bands, no one was polymorphic. Only primer No-1 has produce 2 bands and remaining primers produce only single band. The size of fragments ranged from 120 bp to 960 bp (Table 2). The average number of polymorphic bands was 1.1 per primer. PIC values ranged from 0.053 (Primer 7) to 0.876 (primer 4) with an average of 0.497 (Table 2). The primer 4 was observed to be highly polymorphic (PIC value of 0.876). The resolving power (RP) varies between 0.702 (Primer 4) and 1.942 (Primer 7) with an average value of 1.311 (Table 2).

Significant genetic variation was found among all chickpea accessions with the GS value ranging from (0.10) to (1.00). Of the 37 pair wise combinations generated by chickpea genotypes, the smallest G.S value (0.10) was observed between Pusa 547 & ICCV 14512, RSG 931 & ICCV 14512 and JGK 1 & ICCV 14512 genotypes, which appear as the most dissimilar genotypes and distantly related. The maximum G.S value of 1.00 was obtained between GNG 1969 & C 927, JG 62 & BGD 72, Pusa 1103 & BGD 72, JG 62, HC 5 & C 905 and JGK 1 & RSG 931 genotypes, respectively. The cluster analysis based on unweighted paired group method of arithmetic means (UPGMA) with 10 SSR primers allowed the discrimination of cultivars. The UPGMA based clustering grouped 37 chickpea genotypes into six clusters (Fig. 2). Cluster I had maximum number of genotypes 12namely ICCV-14508, PKV-4, C-910, ICCV-14510, Pusa-1053, GNG-1581, Pusa-2085, Pusa-2024,

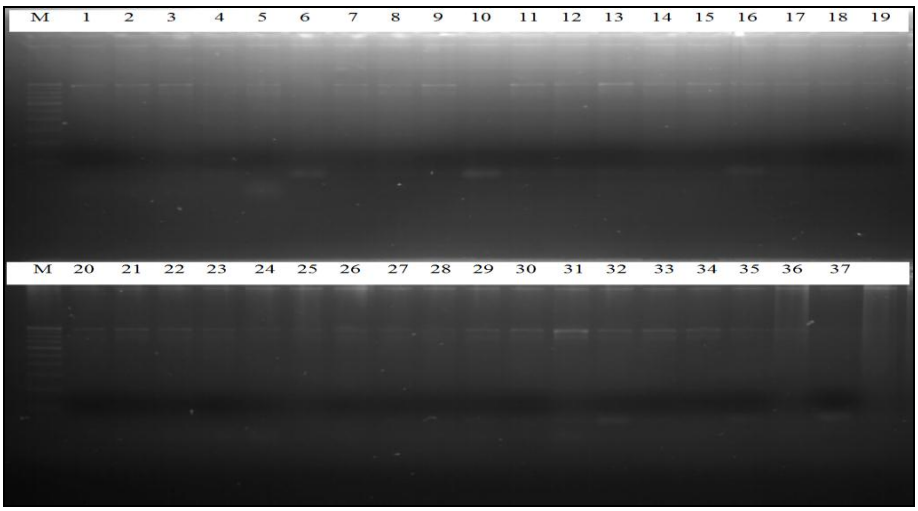

Fig 1: SSR profiling pattern of 37 chickpea varieties with Primer-1.

Table 3: SSR Primer Product size, PIC and RP values of chickpea by using SSR Markers

S. No.	Primer code	Product size (bp)	PIC	RP
1.	Primer 1	960	0.616	1.215
2.	Primer 2	420	0.543	1.35
3.	Primer 3	120	0.835	0.81
4.	Primer 4	135	0.876	0.702
5.	Primer 5	260	0.763	0.972
6.	Primer 6	280	0.109	1.89
7.	Primer 7	285	0.053	1.942
8..	Primer 8	180	0.646	1.188
9.	Primer 9	670	0.427	1.152
10.	Primer 10	130	0.105	1.89
	Total			
	Average		0.497	1.311

Pusa-1003, BG-362, Pusa-372 and Pusa-256 followed by cluster III comprises 10 genotypes namely C-927, GNG-1969, BGD-72, JG-62, Pusa-1103, C-925, HK-4, Pusa-3022, BGD-112 and GNG-1958.Cluster IV comprises 8genotypes namely ICCV-14512, C-905, HC-5, ICCV-95334, Pusa-1105, Pusa-5028, Pusa-1108 and Pusa-547.Cluster II comprises 4 genotypes namely ICCV-07102, DCP-92-3, BGD-1005 and Pusa-5023.Cluster VI comprises only 2 genotypes namely RSG-931 and JGK-1 and cluster V had only 1 genotype namely ICCV-13309.

PIC values ranged from 0.053 (Primer 7) to 0.876 (primer 4) with an average of 0.497. The primer 4 was observed to be highly polymorphic (PIC value of 0.876). The resolving power (RP) varies between 0.702 (Primer 4) and 1.942 (Primer 7) with an average value of 1.311. GS values ranged from 0.10 to 1.00 among all the chickpea genotypes. Similar results also shown by Rajesh *et al.* (2002) [8], Rakesh *et al.* (2002) [9], Sudupak *et al* (2004) [10], Rao *et al.* (2007) [11], Bhagyawant and Srivastava (2008) [12], Tahir and Karim (2011) [13], Aggarwal *et al.* (2011) [14], Amirmoradi *et al.* (2012) [16] and Aggarwal *et al.* (2015) [1, 15].

Conclusion

PIC values ranged from 0.053 (Primer 7) to 0.876 (primer 4) with an average of 0.497. The primer 4 was observed to be highly polymorphic (PIC value of 0.876). The resolving power (RP) varies between 0.702 (Primer 4) and 1.942 (Primer 7) with an average value of 1.311. GS values ranged from 0.10 to 1.00 among all the chickpea genotypes. The UPGMA based clustering grouped 37 chickpea genotypes into six major clusters. Cluster I had maximum number of genotypes (12) and cluster V had single genotype. Many of the chickpea germplasm used in the present study are members of more restricted germplasm pool than would be found in a random sampling as a core collection of cultivars from diverse geographical origin. Genotypes namely RSG-931, JGK-1 and ICCV-13309 were placed separately from remaining accessions when analysed through SSR markers. Therefore, these distinct accessions can be used for desirable characteristics in chick pea breeding programmes. SSR markers could be used to trace the flow of genes or quantitative trait loci of interest in chickpea and to make predictions about the outcome of the crossing and selection programs that will help in improving high yielding varieties.

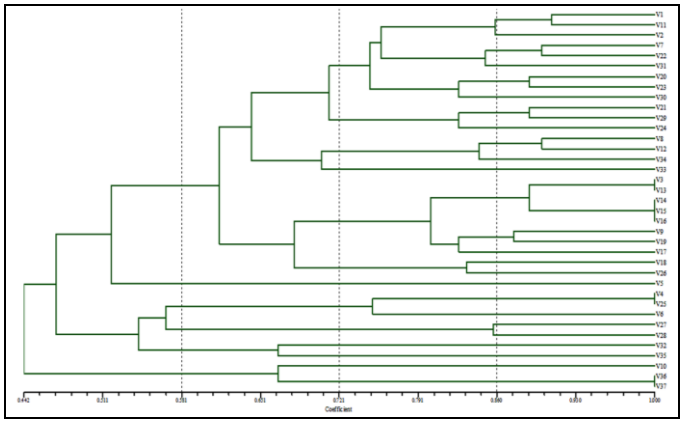

Fig 2: Dendrogram showing clustering of 37 chickpea varieties constructed using UPGMA based on Jacquard's similaritycoefficient obtained from SSR marker analysis.

Table 3: Jaccard's similarity coefficient obtained from 37 chickpea genotypes using 10 SSR markers based on UPGMA cluster analysis.

	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19	V20	V21	V22	V23	V24	V25	V26	V27	V28	V29	V30	V31	V3	V33	V34	V35	V36	V37
V1	1.00																																				
V2	0.90	1.00																																			
V3	0.72	0.63	1.00																																		
V4	0.40	0.30	0.44	1.00																																	
V5	0.54	0.60	0.45	0.22	1.00																																
V6	0.30	0.20	0.33	0.75	0.11	1.00																															
V7	0.72	0.63	0.63	0.44	0.77	0.33	1.00																														
V8	0.81	0.72	0.72	0.40	0.54	0.30	0.72	1.00																													
V9	0.63	0.54	0.70	0.50	0.66	0.37	0.88	0.63	1.00																												
V10	0.60	0.50	0.66	0.66	0.30	0.50	0.50	0.45	0.55	1.00																											
V11	0.90	0.81	0.81	0.36	0.63	0.27	0.81	0.90	0.72	0.54	1.00																										
V12	0.72	0.63	0.80	0.44	0.45	0.33	0.63	0.90	0.54	0.50	0.81	1.00																									
V13	0.72	0.63	1.00	0.44	0.45	0.33	0.63	0.72	0.70	0.66	0.81	0.80	1.00																								
V14	0.63	0.54	0.88	0.50	0.50	0.37	0.70	0.63	0.77	0.75	0.72	0.70	0.88	1.00																							
V15	0.63	0.54	0.88	0.50	0.50	0.37	0.70	0.63	0.77	0.75	0.72	0.70	0.88	1.00	1.00																						
V16	0.63	0.54	0.88	0.50	0.50	0.37	0.70	0.63	0.77	0.75	0.72	0.70	0.88	1.00	1.00	1.00																					
V17	0.63	0.54	0.88	0.50	0.50	0.37	0.70	0.63	0.77	0.55	0.72	0.70	0.88	0.77	0.77	0.77	1.00																				
V18	0.45	0.36	0.66	0.66	0.44	0.50	0.66	0.60	0.75	0.50	0.54	0.66	0.66	0.75	0.75	0.75	0.75	1.00																			
V19	0.54	0.45	0.77	0.57	0.55	0.42	0.77	0.54	0.87	0.62	0.63	0.60	0.77	0.87	0.87	0.87	0.87	0.85	1.00																		
V20	0.80	0.70	0.70	0.50	0.50	0.37	0.70	0.63	0.77	0.75	0.72	0.54	0.70	0.77	0.77	0.77	0.60	0.55	0.66	1.00																	
V21	0.80	0.70	0.54	0.50	0.50	0.37	0.70	0.80	0.60	0.55	0.72	0.70	0.54	0.60	0.60	0.60	0.45	0.55	0.50	0.77	1.00																
V22	0.81	0.72	0.72	0.40	0.70	0.30	0.90	0.81	0.80	0.60	0.90	0.72	0.72	0.80	0.80	0.80	0.63	0.60	0.70	0.80	0.80	1.00															
V23	0.90	0.80	0.63	0.44	0.60	0.33	0.80	0.72	0.70	0.66	0.81	0.63	0.63	0.70	0.70	0.70	0.54	0.50	0.60	0.88	0.88	0.90	1.00														
V24	0.70	0.60	0.45	0.57	0.40	0.42	0.60	0.70	0.50	0.62	0.63	0.60	0.45	0.50	0.50	0.50	0.36	0.44	0.40	0.66	0.87	0.70	0.77	1.00													
V25	0.40	0.30	0.44	1.00	0.22	0.75	0.44	0.40	0.50	0.66	0.36	0.44	0.44	0.50	0.50	0.50	0.50	0.66	0.57	0.50	0.50	0.40	0.44	0.57	1.00												
V26	0.50	0.40	0.55	0.80	0.33	0.60	0.55	0.50	0.62	0.57	0.45	0.55	0.55	0.62	0.62	0.62	0.62	0.83	0.71	0.62	0.62	0.50	0.55	0.50	0.80	1.00											
V27	0.60	0.50	0.50	0.66	0.30	0.50	0.50	0.60	0.40	0.50	0.54	0.66	0.50	0.40	0.40	0.40	0.55	0.50	0.44	0.40	0.55	0.45	0.50	0.62	0.66	0.57	1.00										
V28	0.70	0.60	0.60	0.57	0.27	0.42	0.45	0.70	0.36	0.62	0.63	0.77	0.60	0.50	0.50	0.50	0.50	0.44	0.40	0.50	0.66	0.54	0.60	0.75	0.57	0.50	0.85	1.00									
V29	0.90	0.80	0.63	0.44	0.45	0.33	0.63	0.90	0.54	0.50	0.81	0.80	0.63	0.54	0.54	0.54	0.54	0.50	0.45	0.70	0.88	0.77	0.80	0.77	0.44	0.55	0.66	0.77	1.00								
V30	0.70	0.60	0.60	0.37	0.40	0.42	0.60	0.54	0.66	0.62	0.63	0.45	0.60	0.66	0.66	0.66	0.50	0.44	0.55	0.87	0.66	0.70	0.77	0.55	0.37	0.50	0.30	0.40	0.60	1.00							
V31	0.72	0.63	0.63	0.30	0.60	0.33	0.80	0.72	0.70	0.50	0.81	0.63	0.63	0.70	0.70	0.70	0.54	0.50	0.60	0.70	0.70	0.90	0.80	0.60	0.30	0.40	0.36	0.45	0.63	0.77	1.00						
V32	0.60	0.50	0.50	0.42	0.30	0.50	0.50	0.60	0.40	0.50	0.54	0.66	0.50	0.55	0.55	0.55	0.40	0.50	0.44	0.55	0.75	0.60	0.66	0.62	0.42	0.57	0.50	0.62	0.66	0.62	0.66	1.00					
V33	0.63	0.54	0.70	0.33	0.50	0.37	0.70	0.63	0.60	0.40	0.72	0.70	0.70	0.60	0.60	0.60	0.77	0.55	0.66	0.45	0.45	0.63	0.54	0.36	0.33	0.44	0.55	0.50	0.54	0.50	0.70	0.55	1.00				
V34	0.63	0.54	0.70	0.33	0.36	0.37	0.54	0.80	0.45	0.40	0.72	0.88	0.70	0.60	0.60	0.60	0.60	0.55	0.50	0.45	0.60	0.63	0.54	0.50	0.33	0.44	0.55	0.66	0.70	0.50	0.70	0.75	0.77	1.00			
V35	0.40	0.30	0.44	0.60	0.10	0.75	0.30	0.40	0.33	0.66	0.36	0.44	0.44	0.50	0.50	0.50	0.33	0.42	0.37	0.50	0.50	0.40	0.44	0.57	0.60	0.50	0.42	0.57	0.44	0.57	0.44	0.66	0.33	0.50	1.00		
V36	0.40	0.30	0.44	0.33	0.10	0.40	0.30	0.27	0.33	0.66	0.36	0.30	0.44	0.50	0.50	0.50	0.33	0.25	0.37	0.50	0.33	0.40	0.44	0.37	0.33	0.28	0.25	0.37	0.30	0.57	0.44	0.42	0.33	0.33	0.60	1.00	
V37	0.40	0.30	0.44	0.33	0.10	0.40	0.30	0.27	0.33	0.66	0.36	0.30	0.44	0.50	0.50	0.50	0.33	0.25	0.37	0.50	0.33	0.40	0.44	0.37	0.33	0.28	0.25	0.37	0.30	0.57	0.44	0.42	0.33	0.33	0.60	1.00	1.00

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