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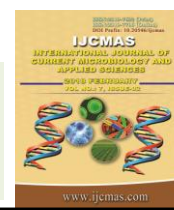

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Molecular Genetic Diversity and Population Structure Analysis in Chickpea (*Cicer arietinum* L.) Germplasm using SSR Markers

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A B S T R A C T

The genetic diversity and population structure in 51 chickpea accessions were studied using 30 chickpea specific SSR markers. Twenty eight SSR markers exhibited polymorphism producing a total of 217 alleles. The average number of alleles per locus was 7.75. The average PIC value was 0.75 and ranged from a minimum of 0.53 (TA1) to a maximum of 0.85 (TA64). The markers TA5, TA14, TA18, TA21, TA64, TA71, TA106, TR20, TR26, TR58, TS43 were considered to be highly informative (PIC ≥ 0.8), in evaluating allelic variation present in the chickpea accessions. Genetic diversity analysis resulted in the formation of two major clusters, using WARD's method of hierarchical clustering based on the dissimilarity index values. Cluster I represented a heterogeneous group with 42 genotypes representing no affiliation with the geographic regions and cluster II comprised of nine genotypes. Population structure based on allele frequency using Bayesian clustering approach identified discrete subpopulation which was similar to the dendrogram obtained using molecular data. Two groups were obtained (K=2) with the mean F_{ST} values of 0.3515 and 0.0972 respectively. However, four accessions were categorized as having admixed ancestry. The results revealed greater resolving power of SSR markers for chickpea germplasm. The availability of wide diversity in the germplasm could be effectively utilized in genetic resource conservation, association mapping as well as in breeding programmes for widening the genetic base of the cultivated chickpea.

Introduction

Chickpea (*Cicer arietinum* L.) is a self-pollinated diploid (2n=16) grain legume with a genome size of Ω740 Mb. According to Vavilov (1926), South West Asia and the Mediterranean region are the two primary centers of origin and Ethiopia is the secondary center of origin for chickpea. Chickpea is grown in more than 50 countries in the world having good wealth of alleles in the cultivated chickpea germplasm, diverse landraces, exotic and wild relatives. Identification of desirable genotypes based on genotypic and phenotypic selection and utilization in breeding programmes can help to increase the yield and stress resistance levels of agronomically superior cultivars. This warrants a knowledge on the extent of genetic diversity and population structure available in the germplasm. The study of genetic variation is the primary and foremost step in any crop

improvement programme (Allard, 1960). Diversity analysis is important because it directly alters the potential for genetic gain through selection (Kotal *et al.,* 2010). It also assists the breeders to segregate the germplasm into heterotic groups to maximize the heterosis (Menz *et al.,* 2004). Compared to morphological and biochemical markers, DNA markers are more reliable in revealing the genetic diversity existing in the germplasm collection. Molecular markers comprise of DNA sequences which can be used to identify/ generate unique tags to identify individuals in a germplasm and can give a precise resolution in assessing the diversity at gene level. They are highly polymorphic, reproducible, and are not influenced by the environment. Unlike morphological markers, these markers are not influenced by the plant ontogeny and can be analysed at different stages of plant growth. Further, these markers help in improving the efficiency of breeding program to several-folds since selection is based not directly on the trait of interest but on the molecular marker tightly linked to the trait, thereby accelerating the generation of new varieties, especially for morphological traits which are difficult to be screened visually. Hence, in addition to the morphological marker based genetic diversity assessment in chickpea, the advent of DNA based marker technology has paved the way for assessing the molecular marker based genetic diversity prevalent in chickpea germplasm.

In chickpea, extraordinarily narrow genetic diversity was portrayed by commonly used biochemical and DNA-based markers, such as isozymes (Kazan and Muehlbauer 1991), restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD), which all failed to reveal intra-specific variation (Simon and Muehlbauer 1997). But the microsatellite-fingerprinting proved that SSRs were abundant in chickpea genome and also effective in mining the genetic variation at intra-specific level (Sethy *et al.,* 2006). The study of genetic diversity and structure helps in managing the gene banks, effective tagging of germplasm and it is a prerequisite in association mapping and can be used to avoid identifying false positive correlations between markers and traits (Pritchard *et al.,* 2000). Hence this study was undertaken with the objective of assessing the genetic diversity and population structure in the chickpea germplasm.

Materials and Methods

The plant material for this study comprised of 51 chickpea accessions including genotypes with diverse origin obtained from ICRISAT, two nationally released varieties *viz.,* Co 4, JAKI 9218 and a local land race from Thuraiyur (Table 1). DNA was extracted from the fresh young leaves of 51 genotypes following the high salt concentration method described by Angeles *et al.,* (2005) with some modifications. The quality of DNA was checked in 0.8 per cent agarose gel electrophoresis. Fifty one chickpea germplasm accessions were subjected to microsatellite analysis. A total of 30 chickpea SSR primers (Winter *et al.,* 1999) with known linkage groups and map positions were selected in order to have a random coverage of markers distributed throughout the 16 chromosomes of chickpea (Table 2). PCR reactions, were carried out in 20 µL reactions containing 4.0 µL of genomic DNA (10ng/µL), 2.0 µL of 10X Taq buffer containing 1.5 mM $MgCl_2$ (20 mM stock), 2.0 µL of dNTP (2.5 mM of each dNTP), 1.0 µL each of forward and reverse primer (100 pmol/µL stock), 0.5 µL of Taq DNA Polymerase (3U/µL) and 9.5 µL of sterile water. PCR reaction cycles consisted of an initial denaturation step at 94°C for 5 minutes, followed by 35 cycles at 94°C for 30s for denaturation, 30 seconds at an appropriate annealing temperature (46°C - 54°C) for primer annealing, and one minute at

72°C for extension. This was followed by a final extension step at 72°C for 10 minutes. The PCR products (3μL) were then run on a six per cent denaturing Poly Acrylamide Gel Electrophoresis (PAGE) at 150 volts for 1 hour and resolved by ethidium bromide staining procedure.

Polymorphism information content (PIC) or expected heterozygosity scores for each SSR marker was calculated based on the formula, $Hj = 1\text{-}\Sigma Pi^2$. The binary marker data generated were subjected to Wards method (Ward, 1963) of hierarchical clustering using DARwin software version 6 (Perrier and Jacquemoud-Collet, 2006). The population structure was analyzed by employing a model-based approach available in Structure 2.3.2 program (Pritchard *et al.,* 2000) and the online version of Structure harvester (http://tayloro.biologyucla.edu/Struct_harvest) developed by Earl and vonHoldt (2012).

Results and Discussion

Understanding genetic relationship in germplasm collections are essential crop conservation and management strategies, for better utilization in breeding programmes. The exploration of the nature and structure of genetic diversity and relatedness among chickpea accessions provides an easy way for identification of new sources of germplasm harbouring valuable alleles for improving yield, grain quality and enhancing the level of resistance in cultivated varieties. To display the relatedness in the form of groups or clusters in the present study, the Ward's method of hierarchical clustering was employed, as it tends to form balanced clusters that could include the outlying accessions (Jobson, 1992).

Out of 30 SSR primers pairs, 28 primer pairs showed polymorphism (Plates 1-3). The 28 primer pairs detected a total of 217 alleles, with an average of 7.75 alleles per locus (Table 3) The number of alleles observed at each locus ranged from a minimum of six (TA28, TA80, TA89, TR1, TS12, TS45) to a maximum of twelve (TA71). The average PIC value was 0.75 and it ranged from a minimum of 0.53(TA1) to a maximum of 0.85 (TA64). Out of the polymorphic 28 SSR primer pairs 11 primer pairs *viz.,* TA64 (0.85), TA71 (0.84), TA106 (0.83), TA14 (0.83), TA5 (0.82), TA18 (0.82), TA21 (0.82), TR26 (0.82), TR20 (0.80), TR58 (0.80), TS43 (0.80) were highly informative and could be an effective and useful tool to determine the genetic differences among the chickpea accessions. The above results on polymorphism content revealed by SSR markers are consistent with other studies. For instance, Upadhyaya *et al.,* (2008) using 48 SSR markers detected 1683 alleles in 2915 chickpea accessions. The alleles per locus ranged from 14 to 67, which could be due to the large number of accessions surveyed. Saeed *et al.,* (2011) using 19 SSR markers in 44 chickpea genotypes reported a total of 100 alleles with PIC values ranged from 0.44 for locus NCPGR7 to 0.84 for locus NCPGR6 and TA135 with a mean of 0.68. The average number of allele per locus was 6.25 alleles and it ranged from two (locus NCPGR7) to 13 (TA135). Khamassi *et al.,* (2012) using 16 SSR primer pairs reported that PIC values ranged from 0.593 (locus NCPGR4) to 0.898 (TA116) with an average of 0.72. Zaccardelli *et al.,* (2013) identified 150 alleles ranging from two to 18 alleles per locus with an average of 9.4 alleles per locus using 16 SSR markers in 15 chickpea accessions. Ghaffari *et al.,* (2014) using 14 SSR markers detected a total of 59 alleles in 60 accessions of chickpea with a mean of 4.2 alleles per locus and the PIC value ranged from 0.31 to 0.89. De Giovann *et al.,* (2017) reported 218 alleles using 22 SSR markers in 103 chickpea accessions. The number of alleles per locus ranged from a minimum of two (CaGMS-1235

and NCPGR-76) to a maximum of 26 (CaGMS-13). A similar range of PIC values obtained in all these studies could also be attributed to a common source of SSR markers developed by Winter *et al.,* (1999) which has been used in all these studies to assess the genetic diversity. However, Choudhary *et al.,* (2012) reported a lower level of genetic diversity in chickpea germplasm with an average PIC value of 0.536. This could be due to the analysis carried within the primary gene pool, comprising of genotypes which are more closely related to each other compared to the secondary and tertiary gene pool.

Fig.1 Dendrogram based on SSR marker data in chickpea germplasm

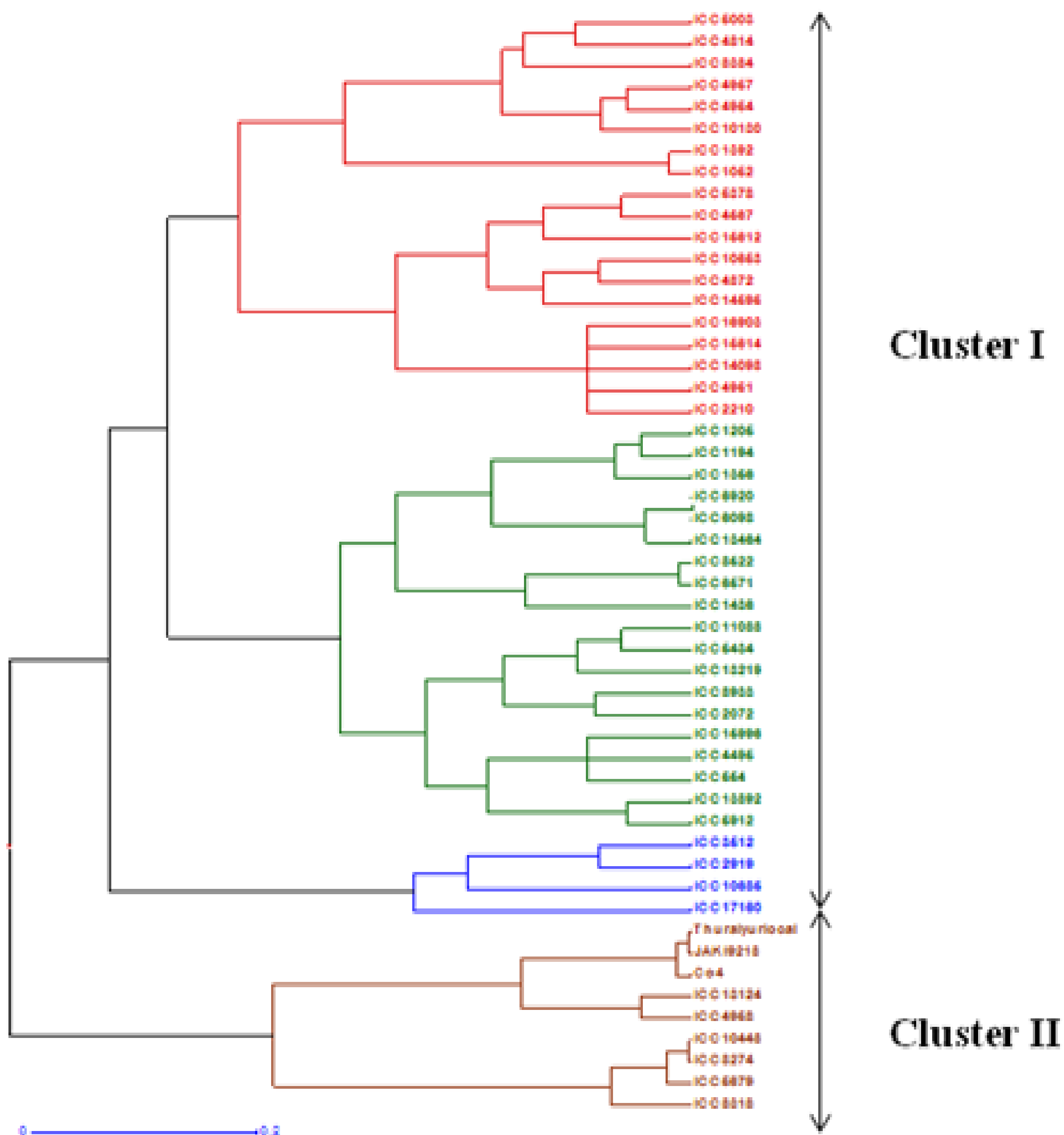


Fig.2a Determination of number of population based on secondary statistics

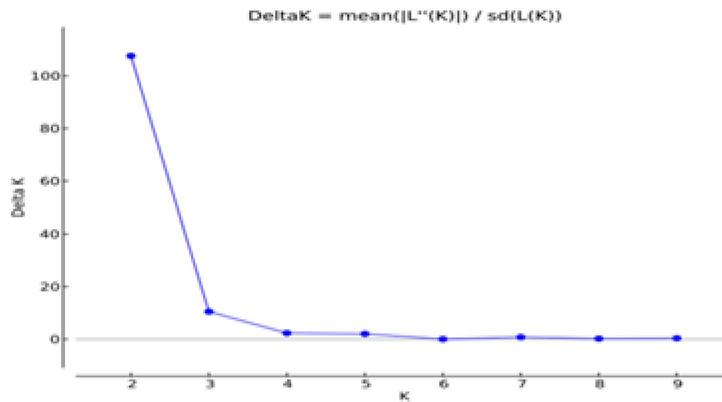
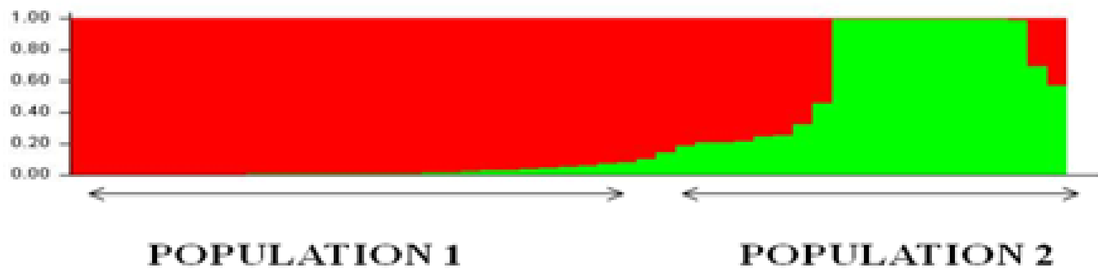


Fig.2b Population assignment for each accession at K=2 based on STRUCTURE analysis



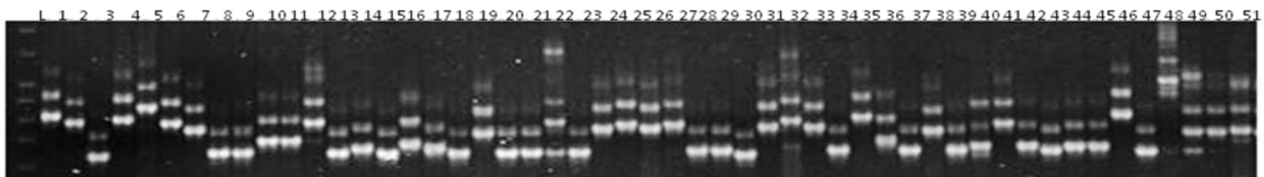


Plate 1 . SSR marker profile generated by the primer TA18

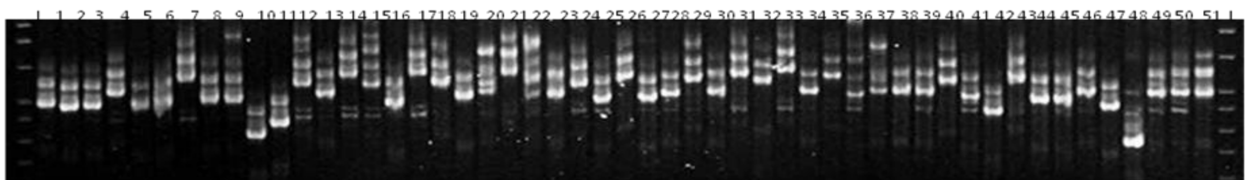


Plate 2 . SSR marker profile generated by the primer TA71

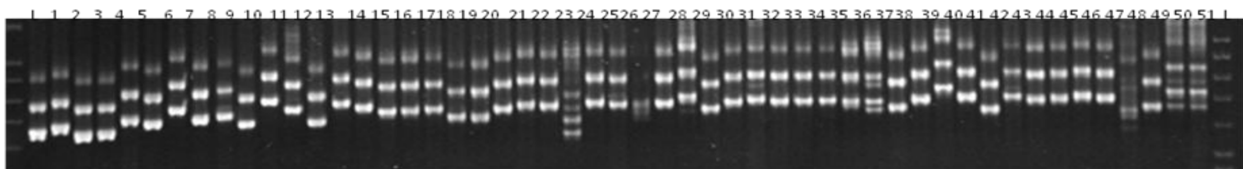


Plate 3 . SSR marker profile generated by the primer TS72

Table.1 List of chickpea accessions, other names and their source of origin

S. No.	Accessions/ Genotypes	Other names	Origin
1	ICC 554	P 436 – 2	India
2	ICC 1052	P 886, PI 217520 – 2	Pakistan
3	ICC 1194	P 1115	India
4	ICC 1205	P 1120	India
5	ICC 1356	P 1217	India
6	ICC 1392	P 1240	India
7	ICC 1436	P 1261 – 4	India
8	ICC 2072	P 1670, PB 22	India
9	ICC 2210	P 1781, Algeria 444	Algeria
10	ICC 2919	P 3318 – 1	Iran
11	ICC 3512	P 4216 – 1	Iran
12	ICC 4495	P 6002	Turkey
13	ICC 4814	P 6540	Iran
14	ICC 4567	P 6112 – 2	India
15	ICC 4872	P 9667 – 1	India
16	ICC 4951	G 62 - 404, JG 62	India
17	ICC 4954	P 9623; Mexican sel.2, H 208	India
18	ICC 4957	Hima	India
19	ICC 4958	JGC 1	India
20	ICC 5003	K 850	India
21	ICC 5378	NP 56	India
22	ICC 5434	Ponaflar 2	India
23	ICC 5679	Annigeri 1	India
24	ICC 5912	T 39 – 1	India
25	ICC 6098	JG 74	India
26	ICC 6571	P 542, PI 359366, NEC 647	Iran
27	ICC 6920	P 4204, PI 360348, NEC 1154	Iran
28	ICC 8274	Annigeri 1	India
29	ICC 8318	Chandpur 2	India
30	ICC 8384	PB 1 – 8	India
31	ICC 8522	JM 552	Italy
32	ICC 8933	K 315, WR 315	India
33	ICC 10130	CPS 1	India
34	ICC 10448	RPSP 182	India
35	ICC 10653	RS 11, SP 1	India
36	ICC 10685	CRIC 34849	Turkey
37	ICC 11088	BG 212, P340 x G 130	India
38	ICC 13124	P 1390, PI 450831	India
39	ICC 13219	P 3046, PI 450953, Ardabil 169	Iran
40	ICC 13892	RAM 4 – 3	Ethiopia
41	ICC 13464	P 4204, PI 360348	Turkey
42	ICC 14098	RBA 95	Ethiopia
43	ICC 14595	RSW 1	India
44	ICC 15612	AMF 237 – 1	Tanzania
45	ICC 15614	AMF 428 – 1	Tanzania
46	ICC 15996	ICCV 10/ICCL 83228, P 1231 x P 1265, Bharti	India
47	ICC 16903	KP 5388	India
48	ICC 17160	ICCW 45, No. 205, ATC 42236, PI 489777	Turkey
49	Co 4	-	India
50	JAKI 9218	-	India
51	Thuraiyur local	-	India

Table.2 Details of SSR primer pairs used in the present study

S. No.	Primer Name	Sequential Information (5' to 3')		Tm (^{0}C)	Linkage group	References
		Forward	Reverse			
1	TA1	TGAAATATGGAATGATTACTGAGTGAC	TATTGAAATAGGTCAGGCTTATAAAAA	52	LG 6	Winter *et al.,* (1999)
2	TA5	ATCATTTCAATTTCCTCAACTATGAAT	TCGTTAACACGTAATTTCAAGTAAAGAT	50	LG 3	Winter *et al.,* (1999)
3	TA14	TGACTTGCTATTTAGGGAACA	TGGCTAAAGACAATTAAAGTT	47	LG 4	Winter *et al.,* (1999)
4	TA18	AAAATAATCTCCACTTCACAAATTTTC	ATAAGTGCGTTATTAGTTTGGTCTTGT	51	LG 5	Winter *et al.,* (1999)
5	TA21	GTACCTCGAAGATGTAGCCGATA	TTTTCCATTTAGAGTAGGATCTTCTTG	54	LG 5	Winter *et al.,* (1999)
6	TA28	TAATTGATCATACTCTCACTATCTGCC	TGGGAATGAATATATTTTTGAAGTAAA	52	LG 5	Winter *et al.,* (1999)
7	TA37	ACTTACATGAATTATCTTTCTTGGTCC	CGTATTCAAATAATCTTTCATCAGTCA	50	LG 7	Winter *et al.,* (1999)
8	TA43	GGTTGTGTTCTCCAGATTTT	AAGAGTTGTTGGAGAGCAA	50	LG 3	Winter *et al.,* (1999)
9	TA45	ATGCGTATAAAACCCAGAGA	TGTTTTTATTGGATTTTCAGTTTCA	47	LG 8	Winter *et al.,* (1999), Sabbavarapu *et al.,* (2013)
10	TA64	ATATATCGTAACTCATTAATCATCCGC	AAATTGTTGTCATCAAATGGAAAATA	51	LG 1	Winter *et al.,* (1999)
11	TA71	CGATTTAACACAAAACACAAA	CCTATCCATTGTCATCTCGT	46	LG 3	Winter *et al.,* (1999)
12	TA80	CGAATTTTTACATCCGTAATG	AATCAATCCATTTTGCATTC	47	LG 4	Winter *et al.,* (1999)
13	TA89	ATCCTTCACGCTTATTTAGTTTTTACA	CAAGTAAAAGAGTCACTAGACCTCACA	54	LG 5	Winter *et al.,* (1999), Bharadwaj *et al.,* (2011)
14	TA106	CGGATGGACTCAACTTTATC	TGTCTGCATGTTGATCTGTT	47	LG 4	Winter *et al.,* (1999)
15	TA110	ACACTATAGGTATAGGCATTTAGGCAA	TTCTTTATAAATATCAGACCGGAAAGA	53	LG 7	Winter *et al.,* (1999)
16	TA125	TTGAAATTGAACTGTAACAGAACATAAA	TAGATAGGTGATCACAAGAAGAGAATG	50	LG 1	Winter *et al.,* (1999)
17	TA130	TCTTTCTTTGCTTCCAATGT	GTAAATCCCACGAGAAATCAA	47	LG 2	Winter *et al.,* (1999)
18	TA135	TGGTTGGAAATTGATGTTTT	GTGGTGTGAGCATAATTCAA	47	LG 1	Winter *et al.,* (1999)
19	TA180	CATCGTGAATATTGAAGGGT	CGGTAAATAAGTTTCCCTCC	47	LG 5	Winter *et al.,* (1999)
20	TR1	CGTATGATTTTGCCGTCTAT	ACCTCAAGTTCTCCGAAAGT	50	LG 4	Winter *et al.,* (1999)
21	TR19	TCAGTATCACGTGTAATTCGT	CATGAACATCAAGTTCTCCA	48	LG 7	Winter *et al.,* (1999)
22	TR20	ACCTGCTTGTTTAGCACAAT	CCGCATAGCAATTTATCTTC	50	LG 2	Winter *et al.,* (1999)
23	TR26	TCATCGCAGATGATGTAGAA	TTGAACCTCAAGTTCTCTGG	48	LG 1	Winter *et al.,* (1999)
24	TR29	GCCCACTGAAAAATAAAAAG	ATTTGAACCTCAAGTTCTCG	50	LG 3	Winter *et al.,* (1999)
25	TR58	CTCTATATTTGTTTGTTTTTCGTTTTG	TAAAATGTGTAGGGTGCAGAATAAATA	51	LG 7	Winter *et al.,* (1999)
26	TS12	CTTAAATAATAAAATCCTAAATAAT	TAATCATATGAGAATCTTAGAATATCAC	47	LG 9	Winter *et al.,* (1999)
27	TS43	AAGTTTGGTCATAACACACATTCAATA	TAAATTCACAAACTCAATTTATTGGC	51	LG 3	Winter *et al.,* (1999)
28	TS45	TGACACAAAATTGTCTCTTGT	TGTTCTTAACGTAACTAACCTAA	50	LG 8	Winter *et al.,* (1999)
29	TS72	CAAACAATCACTAAAAGTATTTGCTCT	AAAAATTGATGGACAAGTGTTATTATG	51	LG 2	Winter *et al.,* (1999)
30	TS83	AAAAATCAGAGCCAACCAAAAA	AAGTAGGAGGCTAAATTATGGAAAAGT	50	LG 10	Winter *et al.,* (1999)

Table.3 Measures of genetic diversity based on SSR markers

S. No.	SSR Locus	Total No. of alleles	PIC Value
1	TA1	7	**0.53**
2	TA5	8	0.82
3	TA14	8	0.83
4	TA18	8	0.82
5	TA21	7	0.82
6	TA28	6	0.75
7	TA37	7	0.66
8	TA64	9	**0.85**
9	TA71	12	0.84
10	TA80	6	0.55
11	TA89	6	0.60
12	TA106	9	0.83
13	TA110	7	0.77
14	TA125	7	0.76
15	TA130	7	0.78
16	TA135	7	0.70
17	TA180	8	0.75
18	TR1	6	0.72
19	TR19	11	0.76
20	TR20	8	0.80
21	TR26	9	0.82
22	TR29	9	0.79
23	TR58	8	0.80
24	TS12	6	0.68
25	TS43	9	0.80
26	TS45	6	0.66
27	TS72	7	0.77
28	TS83	9	0.67
Total		217	20.93
Mean		**7.75**	**0.75**

Table.4 Model based cluster membership coefficients of 51 chickpea accessions as determined by structure

Accession	Origin	Q1	Q2	Population
ICC 554	India	0.006	0.994	P2
ICC 1052	Pakistan	0.013	0.987	P2
ICC 1194	India	0.028	0.972	P2
ICC 1205	India	0.118	0.882	P2
ICC 1356	India	0.351	0.649	Admixed
ICC 1392	India	0.011	0.989	P2
ICC 1436	India	0.007	0.993	P2
ICC 2072	India	0.007	0.993	P2
ICC 2210	Algeria	0.010	0.99	P2
ICC 2919	Iran	0.009	0.991	P2
ICC 3512	Iran	0.003	0.997	P2
ICC 4495	Turkey	0.005	0.995	P2
ICC 4814	Iran	0.010	0.99	P2
ICC 4567	India	0.037	0.963	P2
ICC 4872	India	0.993	0.007	P1
ICC 4951	India	0.006	0.994	P2
ICC 4954	India	0.005	0.995	P2
ICC 4957	India	0.025	0.975	P2
ICC 4958	India	0.997	0.003	P1
ICC 5003	India	0.167	0.833	P2
ICC 5378	India	0.182	0.818	P2
ICC 5434	India	0.590	0.41	Admixed
ICC 5679	India	0.997	0.003	P1
ICC 5912	India	0.240	0.76	P2
ICC 6098	India	0.007	0.993	P2
ICC 6571	Iran	0.009	0.991	P2
ICC 6920	Iran	0.005	0.995	P2
ICC 8274	India	0.997	0.003	P1
ICC 8318	India	0.975	0.025	P1
ICC 8384	India	0.029	0.971	P2
ICC 8522	Italy	0.012	0.988	P2
ICC 8933	India	0.049	0.951	P2
ICC 10130	India	0.178	0.822	P2
ICC 10448	India	0.997	0.003	P1
ICC 10653	India	0.045	0.955	P2
ICC 10685	Turkey	0.126	0.874	P2
ICC 11088	India	0.117	0.883	P2
ICC 13124	India	0.995	0.005	P1
ICC 13219	Iran	0.114	0.886	P2
ICC 13892	Ethiopia	0.032	0.968	P2
ICC 13464	Turkey	0.004	0.996	P2
ICC 14098	Ethiopia	0.003	0.997	P2
ICC 14595	India	0.566	0.434	Admixed
ICC 15612	Tanzania	0.017	0.983	P2
ICC 15614	Tanzania	0.009	0.991	P2
ICC 15996	India	0.006	0.994	P2
ICC 16903	India	0.295	0.705	Admixed
ICC 17160	Turkey	0.002	0.998	P2
Co 4	India	0.996	0.004	P1
JAKI 9218	India	0.995	0.005	P1
Thuraiyur Local	India	0.995	0.005	P1

SSR marker based cluster analysis using DARwin.6.0 program based on the dissimilarity index values divided the 51 chickpea accessions into two distinct groups (Figure 1). Cluster I represented a heterogeneous group with 42 genotypes representing different geographic regions. Cluster I was sub divided into three sub clusters, in which, sub cluster I and sub cluster II had 19 genotypes each and the sub cluster III had a minimum of four genotypes. Cluster II included nine genotypes, all belonging to India. This situation implies no parallelism between genetic diversity and geographical distribution. Similar trend was also reported by earlier workers in chickpea (Arora, 1990; Kumar and Arora, 1992). A lesser extent of relationship with geographical origin was also reported in lentil by Mekonnen *et al.,* (2016). Similar findings from Murthy and Arunachalam (1996), suggested that the diversity can be obtained by genetic drift and selection in different environments rather than geographic distance. Further, dispersal of seed material and subsequent adaptation to various agro climatic conditions may also be responsible for such variation. Hence selection of genotypes for hybridization should be made only based on genetic diversity rather than geographical diversity. This is in concurrence with the findings of Bhattacharya and Ganguly (1998) and Harisatyanarayana and Reddy (2000). On contrary, Hajibarat *et al.,* (2014) depicted a close relationship between genetic diversity and geographical origin.

A total of 30 SSR markers were used to understand the population structure in the panel of 51 accessions of chickpea employing a model-based approach of Structure. Fifty data sets were obtained by setting the number of possible clusters (K) from 1 to 10 with five replications each. The results were then permuted for each K value using CLUMPP software. The LnP(D) value for each given K increased with the increase of K, but since there was no abrupt change in LnP(D), the probable K value could not be inferred. However, applying the second-order statistics (ΔK) developed by Evanno *et al.,* (2005), there was a sharp peak of ΔK at K=2, suggesting two major populations (Fig. 2a). The values of membership coefficient for each genotype are presented in Table 4. The genotypes were assigned to specific population group based on the threshold value of membership coefficients (≥0.75). Out of fifty one genotypes, forty seven genotypes had the membership coefficients more than the threshold value of 0.75 and could be assigned unambiguously to either of the populations. However four genotypes showed admixtures. The STRUCTURE plot for K=2 is presented in Figure 2b. The mean F_{ST} values within the population 1 and 2 were 0.3515 and 0.0972 respectively. The F_{ST} values are crucial, since population differentiation is relatively weaker if the F_{ST} is less than 0.08, and determining the correct number of clusters becomes difficult irrespective of the methods used (Odong *et al.,* 2011).

The present study revealed a structured population in chickpea, and was divided into two groups. The first group consisted of genotypes from different geographic regions where as the second group consisted of genotypes from India, which was similar to the dendrogram obtained by the WARD's method of hierarchical clustering based on molecular data. Population structure analysis by Keneni *et al.,* (2011) revealed 5 clusters in the Ethiopian chickpea population comprising of 155 entries (139 Ethiopian germplasm accessions, eight nationally released varieties and eight breeding lines from ICARDA using 33 SSR primer pairs. The population structure indicated there existed relationship between geographical origins and genetic diversity. Teshome *et al.,* (2012) also reported a

structured population in chickpea with strong subpopulation fixation and differentiation indicating allele fixation in each subpopulation by analyzing a set of 999 chickpea accessions using SNP markers. Hajibarat *et al.,* (2015) studied population structure of 48 chickpea genotypes comprising of 19 Iranian landrace and 29 international lines and cultivars using 38 SSR markers. This study also showed the presence of two distinct populations, one comprising of landraces and the other with cultivated types. This could be attributed to the introduction of exotic materials which could have broadened the genetic base of the chickpea.

While the level of genetic relatedness between the chickpea accessions provides scope for germplasm exploitation in breeding programmes because of its allelic richness, the presence of a structured population in chickpea as revealed by the present study, also indicates that care should be taken to utilize this information in forming core collections and in association mapping studies to avoid false positive associations.

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