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.

Keywords
Chickpea, SSR markers, Genetic diversity, Population structure

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 MgCl₂ (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, $H_j = 1 - \sum P_i^2$. 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

![Graph showing Delta K vs K](image)

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

![Population assignment chart](image)

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  | India      |
| 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      |
| S. No. | Primer Name | Sequential Information (5’ to 3’) | Tm (°C) | Linkage group | References |
|-------|-------------|----------------------------------|---------|---------------|------------|
|       | Forward     | Reverse                          |         |               |            |
| 1     | TA1         | TGAAATATGGAATGATTACTGAGTGCAC     | 52      | LG 6         | Winter et al., (1999) |
| 2     | TA5         | ATCATTTCAATTTCCTCAACTATGAAT      | 50      | LG 3         | Winter et al., (1999) |
| 3     | TA14        | TGAAGTCAATTTAAGGGAACAA           | 47      | LG 4         | Winter et al., (1999) |
| 4     | TA18        | AAAATAATCTCCTCACCTCAAAATTTTC    | 51      | LG 5         | Winter et al., (1999) |
| 5     | TA21        | GTACCTGGAAGATGAGGCGAATA          | 54      | LG 5         | Winter et al., (1999) |
| 6     | TA28        | TAATGGATCATATCTCCTACATATCGCC    | 52      | LG 5         | Winter et al., (1999) |
| 7     | TA37        | ACTTACATGAAATTTCTTCTTGTCTCC     | 50      | LG 7         | Winter et al., (1999) |
| 8     | TA43        | GGTTGTGTTCTCCAGATTAA            | 50      | LG 3         | Winter et al., (1999) |
| 9     | TA45        | ATGCCTATAAAAAACCCAGAGA          | 47      | LG 8         | Winter et al., (1999), Sabbavarapu et al., (2013) |
| 10    | TA64        | ATATATCGTAACTCATTAATCATCGC      | 51      | LG 1         | Winter et al., (1999) |
| 11    | TA71        | CGATTTAAACAAAACACAAA            | 46      | LG 3         | Winter et al., (1999) |
| 12    | TA80        | GAAATTTTACATCCGTAATG            | 47      | LG 4         | Winter et al., (1999) |
| 13    | TA89        | ATCCCTACGGTTATTAGTTTTTACA       | 54      | LG 5         | Winter et al., (1999), Bharadwaj et al., (2011) |
| 14    | TA106       | CGGATGGACTCAACTTATATTCTTAT      | 47      | LG 4         | Winter et al., (1999) |
| 15    | TA110       | ACACATAGGTATAGGGCATTAGGCAA      | 53      | LG 7         | Winter et al., (1999) |
| 16    | TA125       | TTGAATTTGAAGCTGAAACAGACTAAA     | 50      | LG 1         | Winter et al., (1999) |
| 17    | TA130       | TTCTTCTTGGCTTCCTCAATGT          | 47      | LG 2         | Winter et al., (1999) |
| 18    | TA135       | TGGTTGGAATGTTGATTTTTTCTT       | 47      | LG 1         | Winter et al., (1999) |
| 19    | TA180       | CATCCTGGAATTTGAGGGT            | 47      | LG 5         | Winter et al., (1999) |
| 20    | TR1         | CGTATGATTCTCGCTCTAT              | 50      | LG 4         | Winter et al., (1999) |
| 21    | TR19        | TCAGTATCAGCTGTGAAATTCGT         | 48      | LG 7         | Winter et al., (1999) |
| 22    | TR20        | ACCGCTCTGTTAGGACAAAT            | 50      | LG 2         | Winter et al., (1999) |
| 23    | TR26        | TCCAGCAGATGATGTTGAA            | 48      | LG 1         | Winter et al., (1999) |
| 24    | TR29        | GCCAACGTGAAAAATAAAAAAAG        | 50      | LG 3         | Winter et al., (1999) |
| 25    | TR58        | CTCATATTTTTTGGCTGTCTTTTTT       | 51      | LG 7         | Winter et al., (1999) |
| 26    | TS12        | CTATATATATATAAATCTCAAAATAAT    | 47      | LG 9         | Winter et al., (1999) |
| 27    | TS43        | AAGTTGGAAGCTGAAACTACACTACTAATA | 51      | LG 3         | Winter et al., (1999) |
| 28    | TS45        | TGACAAAAATTTTGCTCTTCTT         | 50      | LG 8         | Winter et al., (1999) |
| 29    | TS72        | CCAAAATCAATGAAATTTTGCTCT       | 51      | LG 2         | Winter et al., (1999) |
| 30    | TS83        | AAAAATCAAGGCAAAAAACTCA         | 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  | India    | 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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