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Evaluation of genetic divergence and phylogenetic relationship using sequence-tagged microsatellite (STMS) sequences in Chickpea (*Cicer arietinum* L.) genotypes

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The current investigation was carried out to analyse the genetic diversity estimates between 125 chickpea genotypes using sequence-tagged microsatellite (STMS) markers. Thirty one STMS primers generated a total of 153 loci (an average of 4.94 loci per primer) out of which 129 loci were found to be polymorphic and 24 loci were monomorphic. The value of PIC varied from 0.128 to 0.783 while the resolving power varied from 0.912 to 4.768. The UPGMA generated dendrogram showed the grouping of all the 125 chickpea cultivars into two major clusters and one small cluster. An unbiased clustering of genotypes based on STRUCTURE program, without prior knowledge about the populations, clustered all the 125 genotypes into three major groups. Percentage of polymorphic loci using POPGENE analysis was 50.98, 58.82 and 96.73 for susceptible, resistant and miscellaneous genotypes, respectively. Genetic diversity analysis in terms of Shannon's index and Nei's gene diversity for resistant, susceptible, and miscellaneous cultivars revealed higher values for miscellaneous cultivars, indicating more variability among these cultivars in comparison to resistant and susceptible cultivars. AMOVA results among groups and among cultivars were 10 and 90%, respectively, while the estimated gene flow was 6.117. The overall Nei's gene diversity (0.238) and Shannon's information index (0.372) indicated high degree of genetic polymorphism revealed by the STMS molecular markers. So, genetic divergence in chickpea can provide useful indications in understanding species relationships and may help in developing effective breeding programs.

Keywords: *Cicer arietinum,* genetic polymorphism, molecular markers, analysis of molecular variance, gene flow.

INTRODUCTION

Chickpea (*Cicerarietinum* L.) is one of the most important grain-legume crops worldwide, especially in the Indian sub-continent. It is a crop of both tropical and temperate regions. *Kabuli* type chickpea is grown in temperate regions while the *desi* type is grown in the semi-arid tropics (Dubey et al., 2010). Chickpea is an edible crop

containing large quantity of proteins and believed to be one of the most primitive cultivated grain legumes (Athar and Bokhari, 2006; Redden and Berger, 2007). Chickpea is the 4th largest grain-legume crop worldwide, with a total production of 13.1 m t from an area of 13.5 m ha and productivity of 0.97 t ha-1 (FAO STAT, 2013). Chickpea yield can vary considerably due to combination of diseases and stresses, resulting in regular crop failures in many areas (Knights and Siddique, 2002). Additionally, its importance as a food crop, it is treasured for its advantageous effects on soil fertility. Chickpea is the most economical and easily available source of calcium, magnesium, potassium, phosphorus, iron, zinc and manganese (Ibrikci et al., 2003). One of the major reasons for low productivity of cultivated chickpea is its narrow genetic base and its sexual incompatibility with other *Cicer* wild types in natural interspecific crosses (Sant et al., 1999). All over the world chickpea breeders are focusing on increasing yield by pyramiding genes for resistance/tolerance into elite germplasm (Bharadwaj et al., 2010). So, it is important to study the genetic composition of available genetic resources of chickpea (lines, cultivars, wild relatives etc.) so as to explore the chances of finding the new useful genes or alleles. As genetic diversity among chickpea cultivars is limited, chickpea breeders are keen to search for new approaches for analysis of genetic variability. The advances in molecular marker technology have speed up the progress of genome mapping and maker-assisted selection (MAS) in chickpea. The basic criterion of phylogenetic relationship is the gene homology, which in majority of cases cannot be measured directly because of reproductive barriers between species (Nisar et al., 2007).

The development and worldwide approval of molecular markers for genotyping studies have offered a unique approach for analysing genetic diversity and relationships among species. Several marker systems such as random amplified polymorphic DNA (RAPD), inter simple sequence repeats (ISSR), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), internal transcribed spacer (ITS) and microsatellites such as simple sequence repeats (SSR) have been used in chickpea for the analysis of genetic variability (Serret et al., 1997; Iruela et al., 2002; Nguyen et al., 2004; Sethy et al., 2006a; b; Rao et al., 2007; Singh et al., 2008; Aggarwal et al., 2011; 2015; Kumar et al., 2015).

At present, markers generated by sequence tagged microsatellite site (STMS) primer pairs are predominantly appropriate for genetic diversity analysis. They consist of 1 to 6 bp tandem repeat regions that subsist all over the genome uniformly. Aside from being highly polymorphic, STMS markers are PCR-based, can afford single-locus detection, may be co-dominantly inherited and offer the potential for automated application in plant breeding (Mansfield et al., 1994). In recent years, STMS markers have been used broadly in genetic diversity analysis and DNA fingerprinting (Choumane et al., 2000; Abe et al., 2003; He et al., 2003). Globally, in chickpea a lot of research efforts have led to identification and characterization of a variety of microsatellite markers (Huttel et al., 1999; Winter et al., 1999; Sethy et al., 2003; Lichtenzveig et al., 2005; Choudhary et al., 2006; Sethy et al., 2006a; Castro et al., 2011) and their management for genome mapping and phylogenetic analysis (Winter et al., 2000; Sethy et al., 2006b).

Moreover, inter and intraspecific polymorphism studies were conducted to provide new dimensions for the advancement of linkage maps (Choudhary et al., 2009; Choudhary and Abhishek, 2010; Gaur et al., 2011). Therefore, use of molecular markers to access genetic diversity and genome mapping via genetic map will be valuable for identification of genes or QTLs associated with various diseases like wilt/blight resistance. Thus, the objective of present investigation was to analyze polymorphism in 125 chickpea genotypes (resistant and susceptible) by using STMS markers.

MATERIALS AND METHODS

Plant materials and DNA isolation

One hundred and twenty five chickpea genotypes (Table 1) were grown in the randomized blocks designed in three replicates at the Research Farm of the Department of Plant Breeding, CCSHAU, Hisar, India. Genomic DNA was isolated from leaves of 3 to 4 weeks old seedlings using modified CTAB (Cetyl trimethyl ammonium bromide) method of Murrayand Thompson (1980). The quality and concentration of DNA were measured on Nano-Drop spectrophotometer (ND-100) and electrophoresis using 0.8% agarose gel. The 125 genotypes are categorized into three groups/populations (Table 2) depending upon their resistance/susceptibility towards Fusarium wilt and Ascochyta blight, by using the same numbering as in Table 1.

STMS-PCR

The present study used 32 STMS markers developed by Sethy et al. (2003; 2006a) and Choudhary et al. (2006) while the primer pairs(Forward and Reverse) was purchased from Sigma-Aldrich. After optimization of PCR conditions and screening of all the primers, 31 primers provide clear and informative amplicons. STMS-PCRs were carried out in a Thermal Cycler (MJ Research) and reaction mixture was prepared in a total volume of 15µl each containing 1 × PCR Buffer, 200 µM of each dNTPs, 2.5mM $MgCl_2$, 0.4 µM of primer, 1 unit of Taq DNA polymerase (Fermentas) and about 20 ng of template DNA. Thermal profile of the PCR was: 2

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Table 1. List of chickpea cultivars used in the present study.

S/N	Genotype	S/N	Genotype	S/N	Genotype	S/N	Genotype	S/N	Genotype
1	ICCV 4958	26	Avrodhi	51	RSGK-6(k)	76	GPF-2	101	Pusa 391
2	Katila	27	CSG 8962	52	JG-64	77	JGG-1	102	SAKI9516
3	PDG 84-16	28	Pusa 372	53	ICCV-10	78	PG 12	103	GCP 105
4	BG 276	29	HK 98-155	54	BushyMutant	79	RSG-2	104	RAU 52
5	Tyson	30	RSG 973	55	Hima	80	Chaffa	105	Pusa 240
6	H-208	31	RSG 888	56	BG 396	81	PDG-3	106	Sadabahar
7	HC-3	32	HC-1	57	BG 1006	82	GNG 1292	107	RSG-11
8	E 100 Ym	33	Pusa 256	58	IPC 92-39	83	JG 11	108	Pusa 329
9	GNG 663	34	Pusa 362	59	IPC 98-12	84	KWR 108	109	Dohadyellow
10	C-235	35	Vishal	60	ICCV14880	85	JG 218	110	Pusa 1003
11	DCP 92-3	36	H04-45	61	IPC 99-18	86	Phule G-5	111	JG 130
12	Radhey	37	HC-5	62	IPC2000-33	87	Pant G114	112	B 108
13	RSG 963	38	H03-56	63	IPC 2001-2	88	Pusa 312	113	BGD 75
14	Pusa 261	39	Gaurav	64	IPC 95-1	89	K 850	114	C 214
15	Annegiri	40	ICC 4958	65	PG 96006	90	GCP 101	115	C 15
16	RSG 931	41	Amethyst	66	IPC 97-67	91	BGM 413	116	C 20
17	GNG 146	42	WR-315	67	IPC 94-94	92	Virat(k)	117	C 16
18	BGM 408	43	ICCV92944	68	IPC2000-41	93	PBG-5	118	M 1
19	Pusa 267	44	ICCV96030	69	IPC2000-45	94	PDG 4	119	M 2
20	Vijay	45	L 551	70	RSG 807	95	RSG 44	120	H04-57
21	HK 94-134	46	Pusa 1053	71	Pusa 209	96	Pusa 212	121	H04-44
22	GNG 469	47	L 550(k)	72	CSJD-844	97	GL 769	122	H04-87
23	JG 315	48	ICCV-2	73	GG-2	98	Vaibhava	123	H04-11
24	BGD 72	49	JG 74	74	RS-10	99	KPG 59	124	Digvijay
25	PBG-1	50	JKG-1(k)	75	Pusa 244	100	ICCV 37	125	PantG186

Table 2. Grouping of chickpea cultivars in response to Fusarium wilt/Ascochyta blight.

S/N	Groups	Response to *Fusarium* wilt/Ascochyta blight
1-42	Group 1	Resistant
43-55	Group 2	Susceptible
56-125	Group 3	Miscellaneous

min of initial denaturation at 94°C, followed by 35 cycles; each containing a denaturation segment of 30 s at 94°C, an annealing step of 50 s at 42 to 60°C depending on the primer and an extension segment of 50 s at 72°C, but in case of some primers,that is, Cp14, 18, 20, 24, 25, 26, 28, 29, 30 and 31, the extension segment is lowered to 60°C, due to their high AT content. Reaction was terminated with a final extension phase of 10 min at 72°C and amplified PCR products were separated on 2.8% (w/v) metaphor agarose (Amerseco) in 1 × Tris-Borate EDTA (TBE) buffer at 90 V for 1 h, stained with ethidium bromide and photographed under ultraviolet light using Gel Documentation System (SynGene, Germany). 100 and 50bp ladders were used as molecular size markers. All PCR reactions were run in duplicate and only reproducible and clear bands were scored.

Data analysis

Band patterns for each of the microsatellites markers were recorded for each genotype. Bands which were either diffused or highly faint or those that were hard to score due to multiple bands were considered as 'missing data' and was not considered during analyzing the genetic diversity. The bands were scored in such a format where '0' represents absence of band while '1' represents presence of band. Dice dissimilarity coefficient (D) was calculated, subjected to cluster analysis by bootstrapping and neighbour-joining method using the program DARWIN (version 5.0.158). Statistically unbiased clustering of collected genotypes was performed using STRUCTURE (version 2.3.1) (Evann et al., 2005). The STRUCTURE program was run 10 times for each number of subpopulation (K) values, ranging from 1 to 10, using the admixture model with 50,000 replicates for burn-in and 5000 replicates during analysis. POPGENE software was used to calculate Nei's unbiased genetic distance among genotypes. Data for observed number of alleles (Na), effective number of alleles (Ne), Nei's genetic diversity (H), Shannon's information index (I), number of polymorphic loci (NPL) and percentage polymorphic loci (PPL) were also analyzed (Zhao et al. 2006). Within species diversity (Hs) and total genetic

diversity (Ht) (Nei, 1978) were calculated within the species and within three major groups (as per response to diseases, that is,ascochyta blight and Fusariumwilt) using POPGENE software. The STMS data was subjected to a hierarchical analysis of molecular variance (AMOVA) (Excoffier et al., 1992) among the genotypes. The non-parametric analysis of molecular variance (AMOVA) was done via Gen Alex (Excoffier et al., 1992), where the variation component was partitioned among individuals within populations, among populations within groups and among groups. The input files for AMOVA were prepared by using AMOVA-PREP version-1.01 (Miller, 1998). The resolving power of the STMS primers was calculated according to Prevost and Wilkinson (1999). The resolving power (*Rp*) of a primer is: $Rp = \Sigma\ IB$ where,*IB* (band informativeness) takes the value of: 1–[2* (0.5–P)], P being the proportion of the 125 genotypes containing the band. The genetic diversity of the samples as a whole was estimated based on the number of alleles per locus (total number of alleles/number of loci), the percentage of polymorphic loci (number of polymorphic loci/total number of loci analyzed) and polymorphism information content (PIC). The polymorphism was determined according to the presence or absence of the STMS loci. The value of PIC was calculated using the formula:

$$PIC = 1 - \sum_{i=1}^{n} p_i^2$$

Where,p_i is the frequency of an individual genotype generated by a given STMS primer and summation extends over *n* alleles.

In order to determine the utility of each of the marker systems, diversity index (*DI*), effective multiplex ratio (*EMR*) and marker index (*MI*) were calculated according to Powell et al. (1996). *DI* for genetic markers was calculated from the sum of the squares of allele frequencies: $DI_n = 1-\Sigma\ pi^2$ (where, 'pi' is the allele frequency of the *ith* allele). The arithmetic mean heterozygosity, Di_{av}, was calculated for each marker class: $Di_{av} = \Sigma Di_n/_n$, (where, 'n' is the number of markers (loci) analyzed). The *DI* for polymorphic markers is: $(Di_{av})p = \Sigma\ Di_n/n_p$ (where, 'n_p' is the number of polymorphic loci and n is the total number of loci). *EMR (E)* is the product of the fraction of polymorphic loci and the number of polymorphic loci for an individual assay. $EMR\ (E) = n_p\ (n_p/n)$. *MI* is defined as the product of the average diversity index for polymorphic bands in any assay and the *EMR* for that assay, $MI = DI_{avp} * E$.

RESULTS AND DISCUSSION

Thirty one STMS primers generated a total of 153 loci (an average of 4.94 loci per primer) out of which 129 loci were found to be polymorphic and 24 loci were monomorphic (Table 3). The value of PIC varied from 0.128 (Cp-14) to 0.783 (Cp-29).In a similar study Castro et al. (2011) characterized 32 commercial chickpea (*C. arietinum* L.) cultivars by using 15 microsatellite markers distributed across all linkage groups (LG) of the chickpea genetic map. All STMS markers analysed in chickpea cultivars exhibited a high degree of polymorphism, producing a total of 154 different alleles with fragment size ranging from 171 to 297 bp. The most polymorphic marker in the present study was TA186 with a PIC value of 0·897 and 21 alleles identified. Jomova et al. (2009) have also reported the genetic diversity in a set of 49 chickpea accessions using microsatellite markers. They used 5 primer pairs which generated 50 different amplified alleles and 4 of them were found to be polymorphic with 11 to 13 alleles per locus. PIC ranged from 0.972 to 0.991, showing high variation at the $(TAA)_n$ containing microsatellite loci in chickpea. However, Bharadwaj et al. (2010) reported different values of PIC that ranged from 0.48 to 1.0. They scrutinized 35 polymorphic STMS primer pairs which generated 87 amplicons, giving on an average of 2.49 amplicons per primer pair. Based on PIC values obtained, most STMS markers were considered as informative markers (PIC>0·7), indicating the potential use of this set of STMS markers for cultivar identification.

The UPGMA generated dendrogram (Figure 1) showed the grouping of all the 125 chickpea cultivars into two major clusters and one small cluster. The cluster I consists of eight genotypes (25, 30, 35, 36, 50, 48, 52 and 85) while cluster III comprised of 34 genotypes, that is, 14, 28, 68, 19, 70, 12, 59, 1, 3, 46, 39, 41, 54, 40, 53, 34, 23, 16, 27, 37, 86, 109, 82, 76, 74, 47, 43, 2, 33, 13, 26, 58, 51 and 83 which is further subdivided into five subgroups. The cluster II consists of the rest of 83 genotypes which is further subdivided into seven subgroups. The 2-D PCA analysis (Figure 2) also confirmed the similar grouping pattern as observed by UPGMA analysis.STRUCTURE analysis can help to identify clusters of genetically similar genotypes. The final population subgroups were determined based on plotting a distribution of$\Delta\kappa$. The value of $\Delta\kappa$ was calculated as mean of absolute values of difference between successive likelihood values of K divided by the standard deviation of L(K) (Figure 3). Based on the graph, we choose K=3 as the optimal grouping pattern. Structure analysis gave three groups including a total of 125 genotypes (Figure 4).Percentage of polymorphic loci using POPGENE analysis was 50.98, 58.82 and 96.73 for susceptible, resistant and miscellaneous genotypes, respectively (Table 4). Nei's genetic diversity (H) values-0.200, 0.188 and 0.237 and Shannon's information index (I) values obtained were 0.299, 0.278 and 0.375 for resistant, susceptible and miscellaneous genotypes, respectively. There occurred a great genetic divergence within each of the three groups. The estimated gene flow was 6.117. AMOVA for among groups (10%) and among genotypes (90%) indicated that there are more variations within the population (Table 5).The overall Nei's gene diversity (0.238) and Shannon's information index (0.372) indicated high degree of genetic polymorphism revealed by the STMS molecular markers (Table 6). Our results are in accordance with previous report ofChoudhary et al. (2006) who analyzed polymorphism in 35 accessions by using 13 STMS markers. Ten primer pairs produced polymorphic amplification patterns and a maximum of 2 to 4 alleles were obtained, generating a total of 30 alleles with an average of 2.3 alleles per locus. The observed heterozygosity ranged from 0.1143 to 0.4571 with an average of 0.2284. Out of 10 polymorphic STMS markers, 5 markers were able to detect polymorphism within the *Cicer reticulatum* accessions while all the 10

Table 3. Sequence of primers used for STMS amplification and their GC content, annealing temperature (T_A), total number of loci (TL), percentage of polymorphic loci (PPL), total fragments amplified (TF), resolving power (Rp), and polymorphic information content (PIC).

Primer	Primer sequence (5'~3')	GC(%)	T_A (^{0}C)	TL	PPL	TF	R_P	PIC
Cp1	F-TTACAGCTTGTGCTCAG R- AGTCAGATTCTTATCCGA	47 39	42.6	5	80	119	1.904	0.480
Cp 2	F-GACCATAATGGTGAACGA R- GGCACAATGTATGTATTG	44 39	48.5	4	75	109	1.744	0.369
Cp 3	F- GACCAAGATTAGTAGAACCT R- TATGTCTACACCTATGCATC	40 40	50.5	6	66.6	263	4.208	0.746
Cp 4	F- GACCAAGATTAGTAGAACCT R- CTTGATAAGGATGAGTCATG	40 40	43.6	6	100	162	2.592	0.671
Cp 5	F- CCTTGTTAGTGTGTATAGGT R- GTAATGACCAAGTGAACA	40 39	48.5	7	85.7	181	2.896	0.682
Cp 6	F- GTTGTTGCCGTGACTT R- TGAATCGGACTGACACT	50 47	44.6	9	77.7	283	4.528	0.725
Cp 7	F- TCCATTGTAGCTTAGCTTAG R- TCTTACTCTTAGCTTACCTCTT	40 36	47.2	2	50	94	1.504	0.238
Cp 8	F- TCAAAGGCAGACGTGTAGAA R- TAGAGGAAGATTTCGGAGGA	45 45	58.7	3	100	78	1.248	0.167
Cp 9	F- GCCTACATTGCTTTCCCTTT R-TCATGTGTGTATGAAGTGGAATGA	45 38	54.6	4	75	138	2.208	0.602
Cp 10	F-AAACCAAAACTGAAGTTAATAGGG R-GAAAGAAGTGAAAAAGTAGTGGAA	33 33	54.7	5	100	77	1.232	0.612
Cp 11	F- GCTCAAGGCTGAAGGAGATA R- ACCCTGCAAGTCAAGTCTTC	50 50	53.2	5	60	238	3.808	0.736
Cp 12	F- TGAGAATCACTTTTGGCTCT R- ATCCTGATGAAGGTCGTATG	40 45	55.8	3	66.6	98	1.568	0.152
Cp 13	F- TCCTATCACTCACCAGAAGG R- TTAGGATTACGGCAAGTAGC	50 45	57.3	2	100	109	1.744	0.460
Cp 14	F- GCTTTTTGGAAGCTGAAGTG R- CCCTTTTCTGTTTCCATTTG	45 40	50.9	5	80	105	1.68	0.128
Cp 15	F- TCTACCTCGTTTTTCGTGCC R- TTGCTCCTTCAACAAAACCC	50 45	53.4	6	100	148	2.368	0.675
Cp 16	F- ACATCTTGAAGTGCCCCAAC R- TGCAAGCAGACGGTTACAAG	50 50	60.3	4	100	173	2.768	0.701
Cp 17	F- AGTCGCATCTCTGCCAAAGT R- CATTCCCTGACCTGCTGC	50 61	54.2	5	80	124	1.984	0.569

Table 3. Contd.

Cp 18	F- TGTTTTCAAATCAAACAGGC R- GATACACACCAAGGCACAGT	35 50	46.8	1	100	57	0.912	0
Cp 19	F- CCCAAAACTGAAATGGAAAC R- GGCAGTTACTACCAAGGCAT	40 50	52.6	3	66.6	140	2.24	0.453
Cp 20	F- TGGGCTATGAATTAAGATGG R- TAATTGATGAGGGAGAGAGC	40 45	46.8	6	100	91	1.456	0.553
Cp 21	F- ATGATGGATTTTCGGAATGT R- AAAAATGCTGGAAGGAACTG	35 40	51.5	4	100	108	1.728	0.628
Cp 22	F- CATAATGCAAGGGCAATTAG R- CTCTTATCTTCATGTTGCCG	40 45	52.6	3	33.3	189	3.024	0.530
Cp 23	F- CCCTCCTTCTTGCTTACAAA R- TAATGGTGAACGAATCATGG	45 40	54.1	5	60	279	4.464	0.736
Cp 24	F- CGATGATATTCTCAGCGAAC R- TGTATGAAAACACTTTGACTCATT	45 29	54.6	4	100	93	1.488	0.663
Cp 25	F- AGAAATCACAAACCTCTTCG R- GCTTGGATCTTCAAAACTTG	40 40	52.8	5	60	298	4.768	0.756
Cp 26	F- TGGACTAACCCTTCTTTCTTC R- TTATATTATGCAGGACCGCT	43 40	57.1	11	90.9	198	3.168	0.758
Cp 27	F- CCGAATGTCCATAAATCAAT R- TGTTTGACTGGGATAACTCC	35 45	52.4	4	100	59	0.944	0.689
Cp 28	F- CTACTGCAGAAAAATCAGGG R- ATAGTTCTTGACCAGAGGCA	45 45	47.8	5	80	96	1.536	0.212
Cp 29	F- TAGCATACCATTGTCAACCA R- AAGAGCACATACGGTTTTGT	40 40	46.8	14	100	130	2.08	0.783
Cp 30	F- ATTGAATCCTTTCTGAACCG R- CTGTTCTCTTTTCTCCTCCG	40 50	49.2	4	75	148	2.368	0.627
Cp 31	F- GGTTTGATGTGTTCTTGGCT R- CCCTCAATTCCCTCGATTTA	45 45	50.9	3	100	74	1.184	0.482
	Total			153		4459		

primers identified polymorphism between *C. arietinum*and *C. reticulatum*.

Therefore, it is obvious from the previous studies that STMS markers are inherited in a co-dominant fashion. This allows one to discriminate between homo- and heterozygous states and increases the efficiency of mapping and population genetic studies. There was reasonably high rate of polymorphism for 31 out of 32 STMS markers loci in the present study. Among these Cp-4, Cp-8, Cp-10, Cp-13, Cp-15, Cp-16, Cp-20, Cp-21, Cp-24, Cp-27, Cp-29 and Cp-31 exhibited higher polymorphism pointing towards the scope for further utilization of these markers for chickpea germplasm characterization. The occurrence of unique alleles or rare STMS alleles provides an immense opportunity for gene-ration of comprehensive fingerprint database. The present

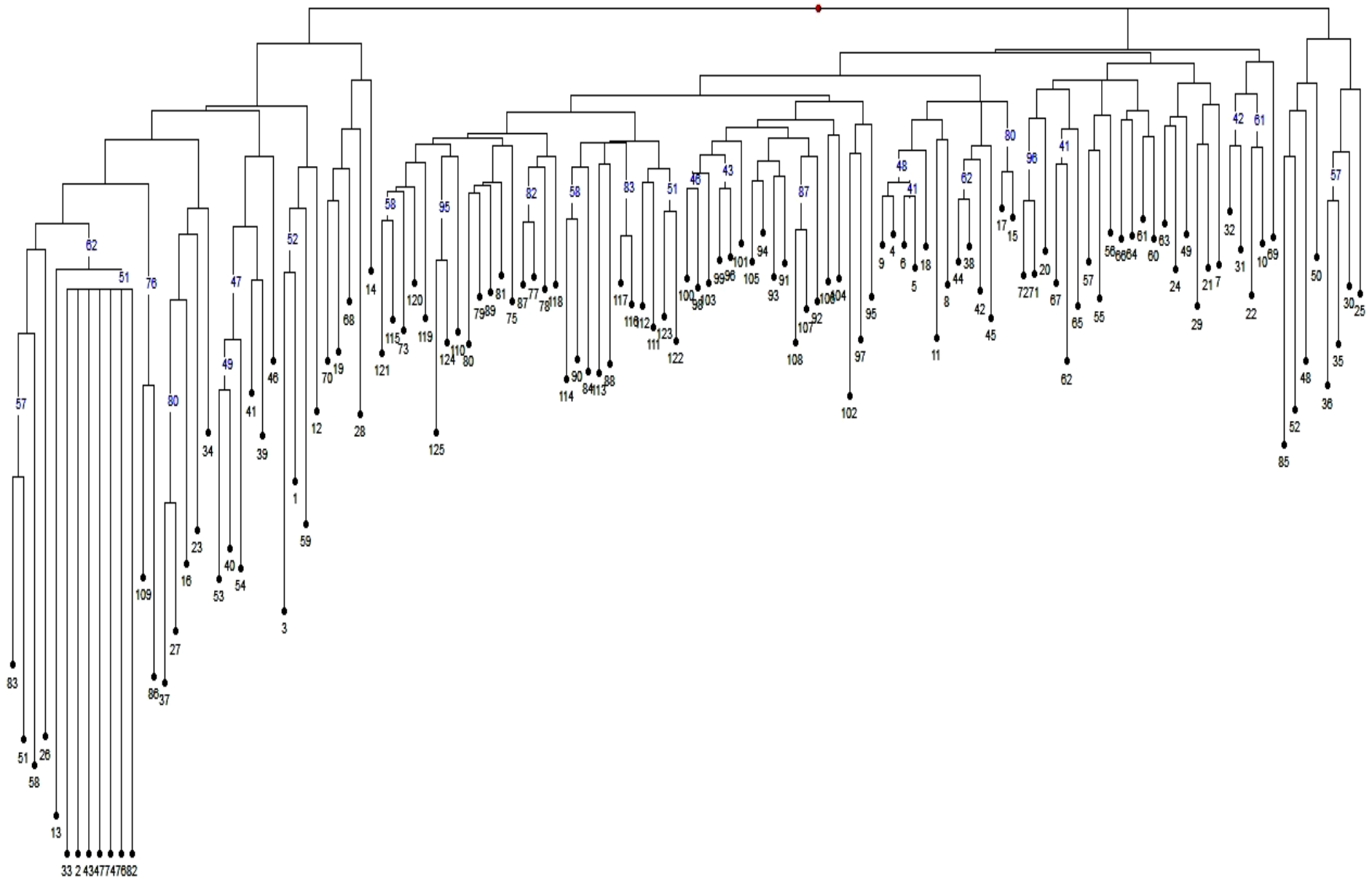

Figure 1. NJ tree representing clustering of cultivars along with supported bootstrap values based on STMS profiling.

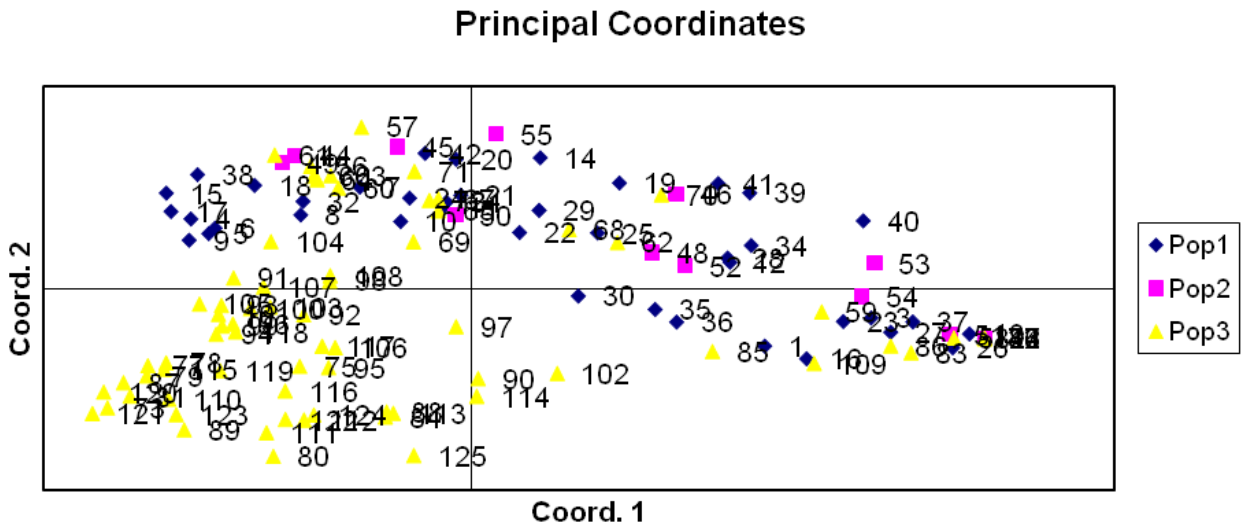

Figure 2. PCA analysis shows distribution of 3 groups as per their genetic relatedness.

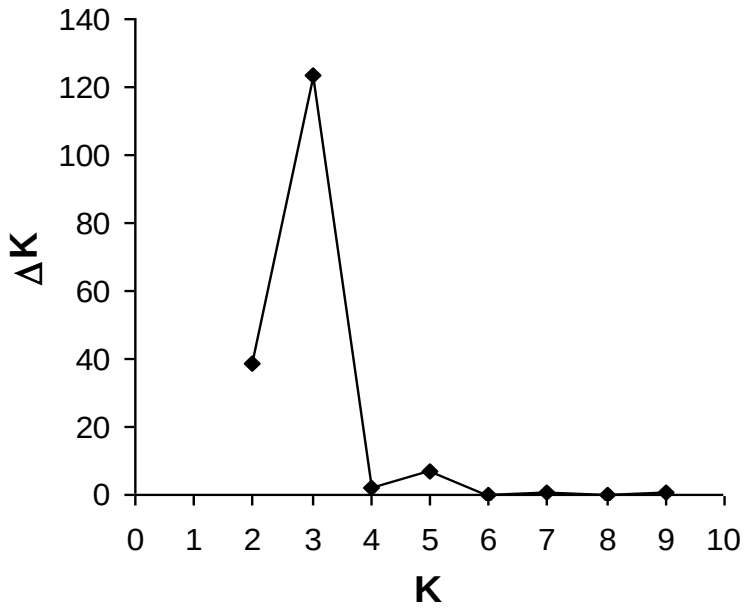

Figure 3. Optimization of the number of populations (K value) varying from 1 to 10 to verify the most appropriate population number for 125 chickpea genotypes using STRUCTURE.

analysis also gives an insight of the interrelationship among the genotypes and highlights the urgency for effective supplementation of pedigree data with the data-base generated by STMS marker to efficiently reveal the genetic inter-relationship among the genotypes as well as finger print the varieties for their protection.

In the present study, STMS markers detected sufficient polymorphism among 125 chickpea genotypes. Out of 32 primers, 31 primers generated reproducible DNA fragments while only one primer did not show any amplification. The possible reason could be either it did not find any complementary region in the genomic DNA or some specific requirements for its amplification need to be addressed. Additionally, 82.6% polymorphism is observed in case of STMS markers, which may be due to the higher average annealing temperature used for STMS markers. In a similar study, Bharadwaj et al. (2010) have also reported high level of polymorphism (85.2%) indicating considerable variability in 14 chickpea varieties. The mean diversity index (DI) and mean effective

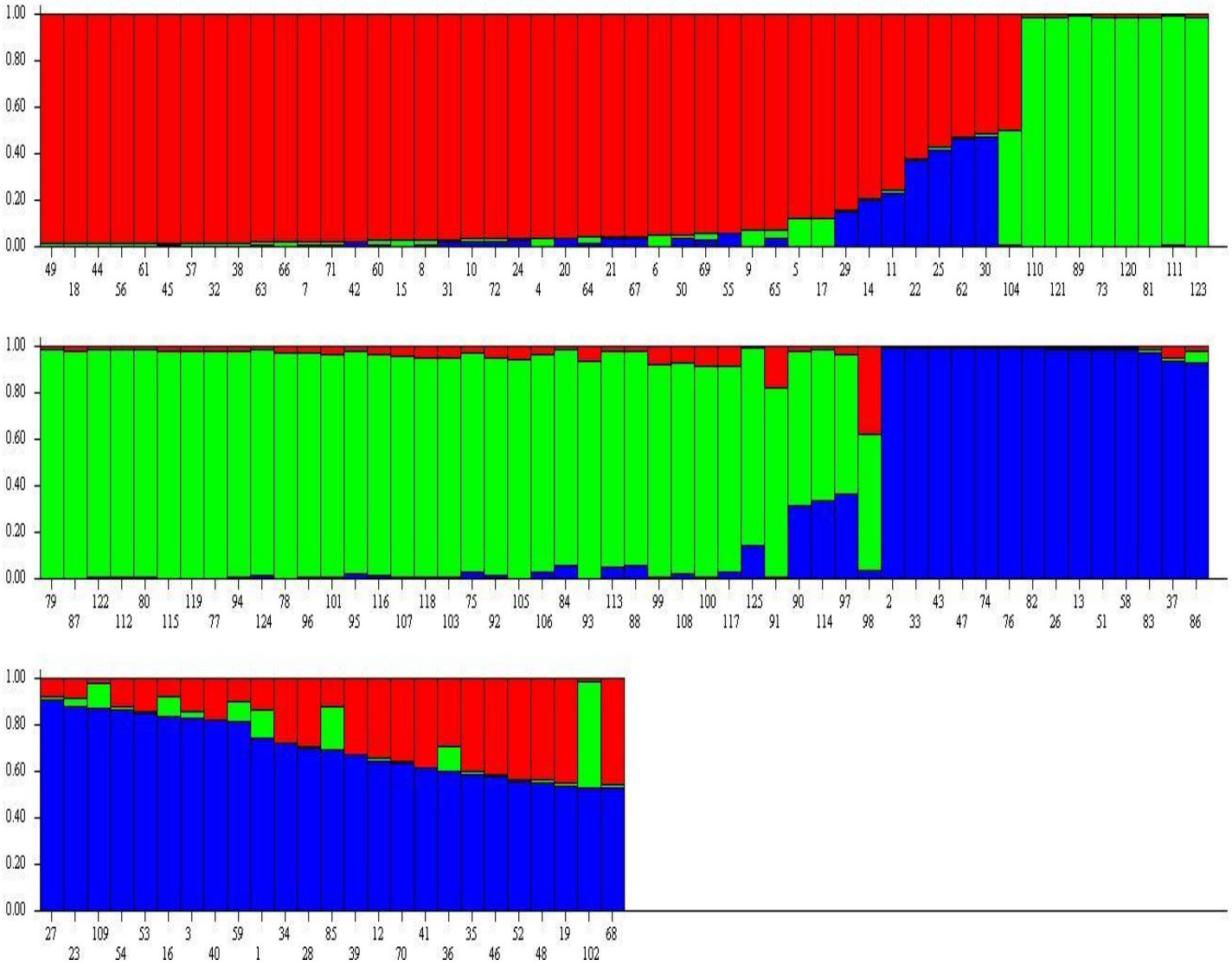

Figure 4. Q-plot showing clustering of 125 chickpea genotypes based on 153 loci produced by STMS primers using STRUCTURE. Each genotype is represented by a vertical bar. The coloured subsections within each vertical bar shows membership coefficient (Q) of the genotype to different clusters.

Table 4. Summary of genetic variation statistics for all loci of STMS among the Chickpea cultivars with respect to their resistance/susceptibility towards diseases Fusarium wilt and Ascochyta blight.

Response towards diseases	Sample size	Na	Ne	H	I	Ht	No. of polymorphic loci	Percentage of polymorphic loci (%)
Resistant	42	1.588 (0.494)	1.344 (0.379)	0.200 (0.202)	0.299 (0.287)	0.200 (0.041)	90	58.82
Susceptible	13	1.510 (0.502)	1.329 (0.391)	0.188 (0.207)	0.278 (0.295)	0.188 (0.043)	78	50.98
Miscellaneous	70	1.967 (0.178)	1.379 (0.323)	0.237 (0.165)	0.375 (0.217)	0.237 (0.027)	148	96.73

Na = Observed number of alleles; Ne = Effective number of alleles; H = Nei's gene diversity; I = Shannon's Information index; Ht = Heterogeneity.

Table 5. Summary of nested analysis of molecular variance (AMOVA) based on STMS analysis. Levels of significance are based on 1000 iteration steps.

Source of variation	d.f.	S.S.D.	M.S	Variance component	Percentage	P-value
Among groups	2.00	171.62	85.81	1.95	10	-
Among cultivars	122.00	2099.66	17.21	17.21	90	< 0.001

d.f.: degree of freedom; S.S.D.: sum of square deviation; M.S: mean square deviation; P-value: probability of null distribution.

Table 6. Overall genetic variability across all the 125 genotypes of chickpea based on STMS markers.

Sample size	Na	Ne	H	I	Ht	Hs	Gst	Nm	NPL	PPL (%)
125	2.000 -	1.390 (0.345)	0.238 (0.176)	0.372 (0.233)	0.238 (0.031)	0.220 (0.026)	0.076	6.117	153	100

Na = Observed number of alleles; Ne = Effective number of alleles; H = Nei's gene diversity; I = Shannon's Information index; Ht = Heterogeneity; Hs = Homogeneity; Gst = Gene differentiation; Nm = Gene flow (Nm = 0.5(1 - Gst)/Gst); NPL= number of polymorphic loci; PPL = percentage of Polymorphic loci.

multiplex ratio (EMR) is 0.534 and 3.638, respectively. However, Jomova et al. (2009) have reported that the diversity index ranged from 0.885 to 0.904. High values of PIC and DI proved the reliability of microsatellites in deter-mining genetic diversity. Marker index of STMS was significant (0.444) in comparison to other markers like RAPD and ISSR, indicating STMS as the powerful molecular markers for genetic characterization of *C. arietinum* genotypes, which is further supported by grouping pattern in the UPGMA generated dendrogram and PCA analysis, where the intensive and discrete grouping has been monitored.

ABBREVIATIONS

STMS, Sequence-tagged microsatellite; **MAS,** maker-assisted selection; **RAPD,** random amplified polymorphic DNA; **ISSR,** inter simple sequence repeats; **RFLP,** restriction fragment length polymorphism; **AFLP,** amplified fragment length polymorphism; **ITS,** internal transcribed spacer; **SSR,** simple sequence repeats; **CTAB,** cetyltrimethyl ammonium bromide; **NPL,** number of polymorphic loci; **PPL,** percentage polymorphic loci; **PIC,** polymorphism information content; **DI,** diversity index; **EMR,** effective multiplex ratio; **MI,** marker index.

Conflict of interests

The author(s) did not declare any conflict of interest.

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