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Identification of Highly Polymorphic Molecular Markers and Potential Genotypes for Harnessing Chickpea Breeding Strategies

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ABSTRACT

Background: STMS markers and morphological traits were used to investigate the genetic relationship and allelic diversity in chickpea. In this study, we focused on the selection and more efficient utilization of core germplasm in breeding programs for chickpea crop improvement using STMS and quantitative/morphological traits.

Methods: Seeds of elite accessions of chickpea were obtained from ICRISAT, Patancheru, Andhra Pradesh, India. 50 STMS markers and 11 quantitative traits were used for exploring the genetic variability and relationship in 35 chickpea accessions.

Result: A total of 97 alleles were produced out of the 32 polymorphic STMS loci with an average of 3.03 alleles per locus ranging between 2-6 alleles per primer. The PIC value ranged from 0.029 to 0.768 with an average of 0.502. PIC value showed a highly positive correlation (r = 0.718) with number of alleles at the STMS loci. In both molecular and morphological markers/traits-based clustering, out of 35 chickpea accessions only one accession ICC-13892 was isolated at the end of clustering. The results indicated that highly polymorphic microsatellite markers NCPGR 68, NCPGR 50, NCPGR 81, NCPGR 48 and NCPGR 77 along with the accessions ICC-13892 having distant associations with ICC-13816, ICC-15697, ICC-15610, ICC-15868, ICC-15888, ICC-15996 with novel findings should be useful resources for strategies of allele mining, association genetics, mapping and cloning of gene(s) and in applied breeding to broaden the genetic base of chickpea.

Key words: Allelic diversity, *Cicer arietinum* L., DNA fingerprinting, Genetic diversity, Polymorphic information content, STMS.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an annual, self-pollinating, diploid (2n = 2x = 16) food legume that ranks second in world legume production (Gaur *et al.,* 2012). It is primarily cultivated in arid and semi-arid areas of the world. Chickpea is valued for its nutritious seeds, which contain 20-30% protein, ~ 40% carbohydrate and only 3-6% oil (Gil *et al.*, 1996). Chickpea is often grown as a disease break in rotation with other crops and contributes to the maintenance of soil fertility through the fixation of atmospheric nitrogen. Hence, chickpea does not only serve as a good source of nutrition to the people but also improves the fertility of the soil.

Quantitative traits provide an estimate of genetic diversity and cluster analyses have been successfully used to classify and measure the pattern of genetic diversity in germplasm, as in chickpea (Naghavi and Jahansouz, 2005), black gram (Ghafoor *et al.*, 2001), pea (Amurrio *et al.*, 1995), soybean (Perry and McIntosh, 1991), alfa-alfa (Smith *et al.*, 1995), lentil (Sultana *et al.*, 2006) and cowpea (Nkoana *et al*., 2019). Among numerous techniques available for assessing the genetic variability and relatedness among crop germplasm, DNA-based markers provide very effective and reliable tools for measuring genetic diversity in crop germplasm and studying evolutionary relationships (Iruela *et al.*, 2002). Molecular techniques using DNA polymorphism have been increasingly used to characterize and identify a novel germplasm for uses in the crop breeding process (O'Neill *et al.*, 2003).

Among various types of DNA marker, microsatellite markers [simple sequence repeats (SSRs) or sequence-

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tagged microsatellite site (STMS)] have been used extensively in genetic diversity analysis and DNA typing in recent years (Choumane *et al.*, 2000; Abe *et al.,* 2003; He *et al.,* 2003). Microsatellite markers have also been developed for *C. arietinum* (Hüttel *et al.,* 1999; Muehlbauer and Kahl, 1999; Winter *et al.,* 1999; Sethy *et al.,* 2003; Lichtenzveig *et al.,* 2005; Bhardwaj *et al*., 2014). Microsatellites consist of tandemly repeated units, each between one and 10 base-pairs in length, such as (TG)n or (AAT)n (Bruford and Wayne, 1993). They are widely dispersed through eukaryotic genomes and are often highly polymorphic. The aim of the present study was to assess the genetic diversity and relationship amongst 35 chickpea accessions to facilitate the selection and more efficient utilization of this germplasm in breeding programmes using STMS markers and morphological traits.

MATERIALS AND METHODS

Seed material, field experiment and genomic DNA extraction

Seeds of elite accessions of chickpea were obtained from ICRISAT (International Crop Research Institute for the Semi-Arid Tropics), Patancheru, Andhra Pradesh, India representing different geographical areas of the world (Table 1). The experiments were conceptualized, standardized and conducted at Crop Research Centre (CRC), Sardar Vallabhbhai Patel University of Agriculture and Technology, Modipuram, Meerut, Amity University during the period 2009-15. Healthy seeds of 35 chickpea accessions were sown in the experimental field in augmented block design (Federer, 1956) with three replications under all suitable agronomic practices. Eleven morphological traits including plant height (cm), internode length (cm), days to 50% flowering, number of primary branches per plant, number of secondary branches per plant, number of pods per plant, number of seeds per plant, number of seeds per pod, days to plant maturity, 100-seed weight (g), seed yield per plant (g) were recorded on 10 competitive plants basis in the middle of each row for each accessions. For extraction of DNA young and fresh leaves of each chickpea accessions were collected and total genomic DNA was extracted using CTAB procedure as described by Doyle and Doyle (1990) with minor modifications. The quantity and quality of DNA were determined by biophotometeric analysis and agarose gel electrophoresis, respectively. All DNA samples were stored at -20°C for use in PCR amplification.

Table 1: List of chickpea accessions used in the present investigation.

Accession	Country of origin
ICC-13816	Russia and CISs
ICC-13863	Ethiopia
ICC-13892	Ethiopia
ICC-14077	Ethiopia
ICC-14098	Ethiopia
ICC-14199	Mexico
ICC-14402	ICRISAT
ICC-14595	India
ICC-14669	India
ICC-14778	India
ICC-14799	India
ICC-14815	India
ICC-14831	India
ICC-15264	Iran
ICC-15294	Iran
ICC-15333	Iran
ICC-15406	Morocco
ICC-15510	Morocco
ICC-15518	Morocco
ICC-15567	India
ICC-15606	India
ICC-15610	India
ICC-15612	Tanzania
ICC-15697	Syria
ICC-15802	Syria
ICC-15868	India
ICC-15888	India
ICC-15996	ICRISAT
ICC-16207	Myanmar
ICC-16261	Malawi
ICC-16269	Malawi
ICC-16487	Pakistan
ICC-16524	Pakistan
ICC-16903	India
ICC-16915	India

PCR amplification and gel electrophoresis

A total of 50 STMS primers were used (Sethy *et al.,* 2006, Table 2) to assess the polymorphism in 35 chickpea accessions. The PCR amplification was carried out in 10 µl reaction volumes that contained 1X PCR buffer, 50 µM $Mgcl_2$, 10 µM of each primer, 2.5 mM of dNTPs and 1 U Red *Taq* DNA polymerase (Bangalore GeNei, India). The PCR amplification was carried out in Master cycler gradient PCR (Eppendorf, Germany) with following profile: 2-minute initial denaturation at 95°C followed by 35 cycles of 95°C for 20 second, 53-62°C (primer specific annealing) for 50 second, 72°C for 50 second and final extension at 72°C for 7 minute. The amplified PCR products were resolved at 1.5% Agarose gel in 1X TBE buffer by horizontal gel electrophoresis for 2 hrs at 50 Voltage.

STMS amplification, data scoring and statistical analysis

The gel was scored both manually and with the help of the gel doc system. Allele size was determined on the basis of bands position. Alleles were numbered as 'a_1', 'a_2', 'a_3' *etc.* sequentially from the largest to the smallest sized band. The allelic band for all the chickpea accessions were scored in a binary matrix on the basis of presence (1) or absence (0) of bands. The binary matrix was used to estimate DNA polymorphisms and genetic relatedness of chickpea accessions.

Table 2: List of chickpea STMS primers used in DNA fingerprinting of chickpea.

Primers	Sequences (Forward and Reverse)	Repeat motif	Length (bp)
NCPGR 37	F: AGTCGCATCTCTGCCAAAGT	$(CT)_2$gc $(CT)_{19}$	191
	R: CATTCCCTGACCTGCTGC		
NCPGR 38	F: CGGTTAGTTGGGGACTTTCA	$(CA)_{11}(CT)_8$	197
	R: CCCGACGTAAAATGGAAGAA		
NCPGR 39	F: GCGCGTGGACTACTCATGT	$(GA)_{17}$taaa $(GA)_2$ta $(GA)_8$	152
	R: GGTGGCTGCCTTTTCTTTC		
NCPGR 40	F: TGAACGAATCATGGCAAGAG	$(GA)_{12}$gt (GA)ca $(GA)_3$gtaagt $(GA)_4$gtgg	193
	R: GCCCTCCTTCTTGCTTACAA	$(GA)_{10}$gt (GA)gt $(GA)_6$	
NCPGR 41	F: GGGAGGAGGATCAAAATTAC		
	R: CAACTATAAAGAGGCATGTTCC	$(CT)_8(CA)_{17}$	262
NCPGR 42	F: CCCCTAGTAGCAAATATTTTGACC		
	R: TTTGAATGCATTTCTTCATAGCA	$(CT)_{27}$	170
NCPGR 43	F: GAAGTCGAGATGCTGAAAAG		
	R: AATTCTAGAAGGGAAGGGTG	$(CT)_{12}$at $(CT)_3$	255
NCPGR 44	F: AAATGTTTTGTATGATCGGG		
	R: CTAAACAAGTGCATACAGCG	$(CT)_{25}$	212
NCPGR 45	F: TGTTTTCAAATCAAACAGGC		
	R: GATACACACCAAGGCACAGT	$(CT)_2$gtcat $(CT)_5$cc $(CT)_2$cc $(CT)_{17}$	223
NCPGR 46	F: CCCAAAACTGAAATGGAAAC		
	R: GGCAGTTACTACCAAGGCAT	$(CT)_6$at $(CT)_6$at (CT)$(CA)_{14}$	217
NCPGR 47	F: AGCGGAACCTTGTTTACATA		
	R: ATTCTTTAGGGATACCAGCC	$(CT)_{19}$t$(CT)_3$	147
NCPGR 48	F: TGGGCTATGAATTAAGATGG		
	R: TAATTGATGAGGGAGAGAGC	$(CT)_{22}N_{13}(CT)_3$cg $(CT)_3$	206
NCPGR 49	F: TGTCTCAGATGTAGTGGCCT		
	R: TTCATGGCAAGAAATTGAAC	$(CT)_{17}$c$(CT)_3$ttcttt $(CT)_6$tt $(CT)_7$	234
NCPGR 50	F: ATGATGGATTTTCGGAATGT		
	R: AAAAATGCTGGAAGGAACTG	$(GA)_{26}$	209
NCPGR 51	F: CATAATGCAAGGGCAATTAG		
	R: CTCTTATCTTCATGTTGCCG	$(GA)_{20}$	203
NCPGR 52	F: CAAGCTCTTTCAGAATTTGC		
	R: TACTGGTGGAAAAATGGATG	$(GA)_2$aa $(GA)_{25}$	245
NCPGR 53	F: CCCTCCTTCTTGCTTACAAA		
	R: TAATGGTGAACGAATCATGG	$(CT)_5$ca (CT)ca $(CT)_{10}$ca $(CT)_4$ ca	194
NCPGR 54	F: GAAGTCGAGATGCTGAAAAG	(CT)ta $(CT)_4$ gtca$(CT)_{12}$	
	R: AATTCTAGAAGGGAAGGGTG	$(CT)_{16}$	255
NCPGR 55	F: TCCATTGGATACATCACAGG		
	R: GGGCAAATTCAGTATTTTGG	$(GA)_{16}$	204
NCPGR 56	F: CATGACAATAATGGTGAACG		
	R: GATCTTGACTTCTGTTTGTGC	$(GA)_{12}$gt (GA)ca $(GA)_3N_6(GA)_4$gt	162
NCPGR 57	F: CGATGATATTCTCAGCGAAC	$(GA)_9$gt (GA)gt $(GA)_6$	
	R: TGTATGAAAACACTTTGACTCATT	$(GT)_{14}(GA)_{24}$ $(GT)_2$	219
NCPGR 58	F: TGAAGATCTCCAACGGTAAC		
	R: TTTCTTTTGATGTGTTCTTGG	$(GT)_{13}(GA)_{27}$	215
NCPGR 59	F: CTTGACCAGAGGCATTTATC		
	R: AACATAATGGTGTCCAAAGC	$(GT)_{12}(GA)_{13}$	267
NCPGR 60	F: AGAAATCACAAACCTCTTCG		
	R: GCTTGGATCTTCAAAACTTG	$(GT)_2$c(GT)cgat $(CT)_5$ca $(CT)_{26}$	259
NCPGR 61	F: AAATGGTTTGAGAGGTGATG		
	R: AAGGGAGAAGGAAGAAAAGA	$(AT)_5$gtat $(GTCT)_2(GT)_{20}$	226

Table 2: Continue...

Table 2: Continue...

NCPGR 62	F: TCTTAGACTCGGACCTGGTA		
	R: TTCGTTTTTCTCTTACGCTC	$(GT)_{14}(GA)_4$ gg $(GA)_2$	295
NCPGR 63	F: CTCTCTTCTCCTCCCAAAAT		
	R: GGGGATTTGTTTTAAGTCCT	$(GA)_{11}$gc $(GA)_{12}$	270
NCPGR 64	F: GGCGCGTGGACTAACTAAT		
	R: CACATGATTTCTACATGGTGTT	$(GT)_{23}$	239
NCPGR 65	F: CGTGGACTAACGTTCACTG		
	R: GGTTCTCCCTCTGTTCTTCT	$(GA)_7$gg $(GA)_{29}$	296
NCPGR 66	F: GAAGCCATTGTTTTGTTGGT		
	R: ATTATAGCACCCCGCAAC	$(CT)_{24}(CA)_2$	281
NCPGR 67	F: GCGTGGACTAACTAGAGGTC		
	R: ATGGAATCCAGGACGTTAAT	$(AC)_{40}(AT)_6$	300
NCPGR 68	F: GCGTGGACTACTCACTAGCA		
	R: GAAAAACGGGGTGTTACATT	$(CA)_{11}(CGAG)_4(CA)_2N_{34}(CA)_3N_{16}(CA)_{10}$	195
NCPGR 69	F: GACCGAATGTCCATAAATCA		
	R: GGAGCTGGAAAAACTACAGC	$(GA)_{36}$	252
NCPGR 70	F: TATCCAAAGCACATCTCACA		
	R: CTTAGTTTTGGTAGGGGGTC	$(GA)_{33}$ta (GA)ta $(GA)_{10}$	270
NCPGR 71	F: CAACGACACTTAAGCAATCA		
	R: AGCAATTGGCCTTACATTAG	$(GA)_{38}$	249
NCPGR 72	F: TTAACCCATTAGCGTGACTT		
	R: GATCAGCTTCTTGCTTTCAT	$(GA)_{21}$	250
NCPGR 73	F: GGATGAACGAGAGTTGGTAT		
	R: TATCTGTCAATTGAGTTGCG	$(CA)_{10}$ga (CA)ta $(CA)_{10}$cg $(CA)_{30}$aa$(GA)_2$	282
NCPGR 74	F: TCCGTCCACACATTTCTACT		
	R: CTTTTAGTTGGTCGAAGCC	$(GA)_{39}$aa $(GA)_2$	231
NCPGR 75	F: AACTGAAATGGAAACACAGG		
	R: GAAAGCTGACTCCTCTACCA	$(CT)_{15}(CA)_{14}$	192
NCPGR 76	F: GAAAGCTGACTCCTCTACCA		
	R: GAAAATGCTCTCAGTCAAGG	$(GT)_{13}(GA)_2$ta $(GA)_7$ta $(GA)_6$	245
NCPGR 77	F: TGGACTAACAAATACGACGA		
	R: AGGCCACCCTAAATTTTATT	$(GA)_{36}$ta (GA)ta $(GA)_{10}$	225
NCPGR 78	F: CTCTGTGAGGAGGAAGATGA		
	R: AGAAGTTAAAGCAATGCACC	$(CT)_{21}$	235
NCPGR 79	F: ATTGGTTTGAGAAGTGATGG		
	R: AGAAAAGATGGAGTTCGTGA	$(GT)_{14}$gc (GT)gc(GT)gc$(GT)_6$	203
NCPGR 80	F: TGGACTAACCCTTCTTTCTTC		
	R: TTATATTATGCAGGACCGCT	$(AT)_4(AC)_2$at $(AC)_{24}$	256
NCPGR 81	F: CCGAATGTCCATAAATCAAT		
	R: TGTTTGACTGGGATAACTCC	$(AG)_{31}$	211
NCPGR 82	F: ATTGGTTTGAGAAGTGATGG		
	R: GGGAAGTTCAGGACTCTTTT	$(GA)_{29}$	280
NCPGR 83	F: GCTTGACCTATTTATGGTCTG		
	R: AGGTGATGTGGAAATGATGA	$(CA)_{45}$cg (CA)cg $(CA)_{15}$	314
NCPGR 84	F: GCATGAGAGATGGGTCATTA		
	R: GAGGCGCGTGGACTAACA	$(AT)_8(GT)_{19}$	216
NCPGR 85	F: CGCGTGGACTAACATAGAGT		
	R: GAAGTGGGTGTTGTGTTTTT	$(CA)_{10}$aag $(CT)_4$tt $(CT)_4$	180
NCPGR 86	F: CTACTGCAGAAAAATCAGGG		
	R: ATAGTTCTTGACCAGAGGCA	$(CT)_{13}(CA)_{11}$	208

Polymorphic information content (PIC) was determined as per Senior *et al.* (1998) for each STMS primer pair. The PIC, as a measure of the allele diversity at a locus, was determined as equal to $1-\sum P_{ij}^2$, where P_{ij} is the frequency of the j^{th} allele for i^{th} locus summed across all alleles in the locus. The 0-1 data matrix was further used to calculate genetic similarity (GS) between pairs of accessions using SIMQUAL module of NTSYS-pc software ver.2.2 (Exeter software, New York) (Rohlf, 1993). The similarity matrix was then used to generate a dendrogram depicting clustering pattern of accessions using unweighted pair group method with arithmetic averages (UPGMA) methods under sequential agglomerative hierarchical nested clustering (SAHN) module of NTSYS.

RESULTS AND DISCUSSION

Allelic analysis and polymorphism of STMS markers

Out of 50 STMS primer pairs were used for the fingerprinting of 35 chickpea accessions, 18 primer pairs namely NCPGR 40, NCPGR 41, NCPGR 42, NCPGR 43, NCPGR 44, NCPGR 51, NCPGR 52, NCPGR 53, NCPGR 54, NCPGR 55, NCPGR 56, NCPGR 58, NCPGR 60, NCPGR 62, NCPGR 63, NCPGR 64, NCPGR 79 and NCPGR 84 were found to be monomorphic and the rest 32 primer pairs as polymorphic. The detailed statistical parameters of these 32 polymorphic STMS primers are presented in Table 3. The STMS markers used in the present investigation exhibited a high degree of polymorphism producing a total of 97 alleles with an average of 3.03 alleles per locus. Most of the 15 marker loci produced 2 alleles, followed by 9 primers produced 3 alleles, 4 primers produced 5 alleles, 2 primers produced 4 alleles and 2 primers produced 6 alleles. The range of alleles observed in our study is comparatively less than the previous reports (Sethy *et al.,* 2006; Chaudhary *et al.,* 2012) where they have reported allele range 2 to 11 with an average of 6.4 alleles / loci in chickpea. However, our report is comparable to several other reports (2011; Ghaffari *et al.*, 2014; Katoch *et al.*, 2016; Kumar *et al.*, 2017; Rashmi *et al.*, 2012; Rizvi *et al.,* 2014; Singh *et al.*, 2011, 2012, 2013; Soi *et al.*, 2014).

Out of 97 alleles detected, 8 were considered as rare alleles due to their low frequency (<0.03), 23 as common (0.04-0.20) and rest 66 as frequent alleles (>0.21) (Table 3). The average polymorphic information content (PIC) value was found to be 0.502 with a range of 0.029 (NCPGR 37) and 0.768 (NCPGR 68) (Table 3). The PIC value reveals the informativeness level and accordingly is defined into high (PIC>0.5), moderate (0.5>PIC>0.25) and low (PIC<0.25) categories (Botstein *et al.,* 1980). In the present investigation, the STMS markers exhibited moderate to high level of informativeness with average PIC value of 0.502 and most of the STMS (53.0%) had PIC value more than 0.50.

A positive correlation was obtained (r = 0.718) between PIC and number of alleles at the STMS locus, which confirms that STMS markers used in this study were highly informative. The positive association of PIC value and allele number was also reported earlier by Saini *et al.* (2004). The PIC value obtained in the present study was lower than reported by several others (Singh *et al.,* 2008; Ghaffari *et al.,* 2014) but it was comparable to as reported by Chaudhary *et al.* (2012). In reference to PIC value the STMS markers namely NCPGR 68, NCPGR 50, NCPGR 81, NCPGR 48 and NCPGR 77 were considered as very good markers. However, NCPGR 37 and NCPGR 49 were considered as very poor markers and the rest of the markers as moderate.

Diversity analysis on the basis of STMS markers

The genotypic data of the polymorphic STMS primers was used to study the genetic relationship among the chickpea accessions. The pairwise genetic similarity coefficients ranged from 0.22 to 0.91. The highest similarity occurred between ICC-15868 *vs* ICC-15888 and ICC-16269 *vs* ICC-16487 with a coefficient value of 91% and the lowest similarity occurred between ICC-13892 *vs* ICC-15610 with a coefficient value of 22%.

The dendrogram based on UPGMA clustering clearly revealed 7 distinct clusters namely A, B, C, D, E, F and G (Fig 1). Cluster A comprised 6 accessions, which were further divided into two sub-clusters *viz*. A-1 and A-2. Sub-cluster A-1 consisted of three accessions ICC-13816, ICC-15697 and ICC-15610. Sub-cluster A-2 consisted of three accessions ICC-15868, ICC-15888 and ICC-15996. Cluster B consisted of only two accessions ICC-15333 and ICC-15406. Cluster C comprised 7 accessions, which were further divided into two sub-clusters *viz*. C-1 and C-2. Sub-cluster C-1 consisted of three accessions ICC-13863, ICC-14098 and ICC-14199. Sub-cluster C-2 consisted of 4 accessions ICC-14077, ICC-15264, ICC-14595 and ICC-14778. Cluster D consisted of 4 accessions ICC-15510, ICC-15518, ICC-16903 and ICC-15294. Cluster E comprised 8 accessions, which were further subdivided into two sub-clusters *viz*. E-1 and E-2. Sub-cluster E-1 consisted of three accessions ICC-14831, ICC-16207 and ICC-16261. Sub-cluster E-2 consisted of 4 accessions ICC-16269, ICC-16487, ICC-16524 and ICC-16915. Accession ICC-14799 was isolated at the end of cluster E. Cluster F consisted of only two accessions ICC-14815 and ICC-15606. Cluster G consisted of 6 accessions namely ICC-14402, ICC-14669, ICC-15567, ICC-15802, ICC-15612 and ICC-13892. At the end of clustering, out of 35 chickpea accessions only one accession ICC-13892 was isolated.

However, the accessions, ICC-13892, ICC-15612, ICC-15802, ICC-15567 expressed distant association with ICC-13816, ICC-15697, ICC-15610, ICC-15868, ICC-15888 and ICC-15996 revealing very high diversity in their genotypic structure. Similar results have also been reported in chickpea by Monika *et al.*, 2018 and Vishnu *et al.*, 2020. These accessions could be potentially utilized in various hybridization programmes for further genetic improvement of chickpea.

Diversity analysis based on quantitative traits

The pooled quantitative trait data (Table 4) partially published

Table 3: List of 32 polymorphic STMS markers with number of alleles and PIC value.

Primers	Alleles	No. of accessions sharing alleles	Frequency of STMS alleles	No. of accessions showing amplification	No. of alleles	PIC
NCPGR 37	a_1	1	0.016	33	2	0.029
	a_2	33	0.985			
NCPGR 38	a_1	22	0.227	33	3	0.583
	a_2	22	0.227			
	a_3	33	0.560			
NCPGR 39	a_1	12	0.118	28	6	0.724
	a_2	2	0.016			
	a_3	1	0.018			
	a_4	27	0.373			
	a_5	25	0.302			
	a_6	16	0.174			
NCPGR 45	a_1	33	0.500	34	2	0.500
	a_2	33	0.500			
NCPGR 46	a_1	9	0.141	32	2	0.242
	a_2	32	0.859			
NCPGR 47	a_1	20	0.523	22	2	0.499
	a_2	19	0.477			
NCPGR 48	a_1	20	0.250	29	4	0.727
	a_2	24	0.353			
	a_3	18	0.256			
	a_4	6	0.141			
NCPGR 49	a_1	6	0.103	29	2	0.186
	a_2	29	0.896			
NCPGR 50	a_1	10	0.086	29	5	0.761
	a_2	23	0.236			
	a_3	23	0.236			
	a_4	25	0.327			
	a_5	4	0.115			
NCPGR 57	a_1	7	0.125	32	3	0.584
	a_2	28	0.531			
	a_3	23	0.343			
NCPGR 59	a_1	17	0.132	33	4	0.699
	a_2	23	0.192			
	a_3	33	0.434			
	a_4	26	0.242			
NCPGR 61	a_1	28	0.696	28	2	0.424
	a_2	17	0.303			
NCPGR 65	a_1	1	0.018	19	3	0.349
	a_2	17	0.781			
	a_3	6	0.202			
NCPGR 66	a_1	9	0.323	17	3	0.476
	a_2	1	0.029			
	a_3	15	0.647			
NCPGR 67	a_1	8	0.097	31	3	0.549
	a_2	31	0.581			
	a_3	22	0.322			
NCPGR 68	a_1	19	0.159	29	6	0.768
	a_2	28	0.383			
	a_3	15	0.111			
	a_4	15	0.111			

Table 3: Continue...

Table 3: Continue...

	a_5	20	0.176			
	a_6	9	0.061			
NCPGR 69	a_1	33	0.636	33	2	0.464
	a_2	24	0.363			
NCPGR 70	a_1	23	0.356	27	5	0.698
	a_2	24	0.372			
	a_3	15	0.180			
	a_4	4	0.038			
	a_5	5	0.054			
NCPGR 71	a_1	29	0.879	29	2	0.213
	a_2	7	0.120			
NCPGR 72	a_1	30	0.441	34	2	0.493
	a_2	34	0.559			
NCPGR 73	a_1	5	0.500	10	2	0.500
	a_2	5	0.500			
NCPGR 74	a_1	30	0.884	30	2	0.204
	a_2	7	0.117			
NCPGR 75	a_1	34	0.500	34	2	0.500
	a_2	34	0.500			
NCPGR 76	a_1	34	0.333	34	3	0.667
	a_3	34	0.333			
	a_2	34	0.333			
NCPGR 77	a_1	2	0.018	25	5	0.715
	a_2	16	0.271			
	a_3	17	0.311			
	a_4	16	0.331			
	a_5	5	0.068			
NCPGR 78	a_1	63	0.098	34	3	0.274
	a_2	2	0.627			
	a_3	30	0.568			
NCPGR 80	a_1	26	0.607	28	2	0.478
	a_2	20	0.392			
NCPGR 81	a_1	31	0.252	33	5	0.754
	a_2	31	0.252			
	a_3	26	0.202			
	a_4	29	0.278			
	a_5	2	0.015			
NCPGR 82	a_1	30	0.441	34	2	0.493
	a_2	34	0.559			
NCPGR 83	a_1	12	0.206	29	3	0.357
	a_2	29	0.775			
	a_3	1	0.017			
NCPGR 85	a_1	23	0.357	28	3	0.665
	a_2	25	0.339			
	a_3	23	0.303			
NCPGR 86	a_1	33	0.485	34	2	0.501
	a_2	34	0.514			
Total					97	-
Average (Mean)					3.03	0.502

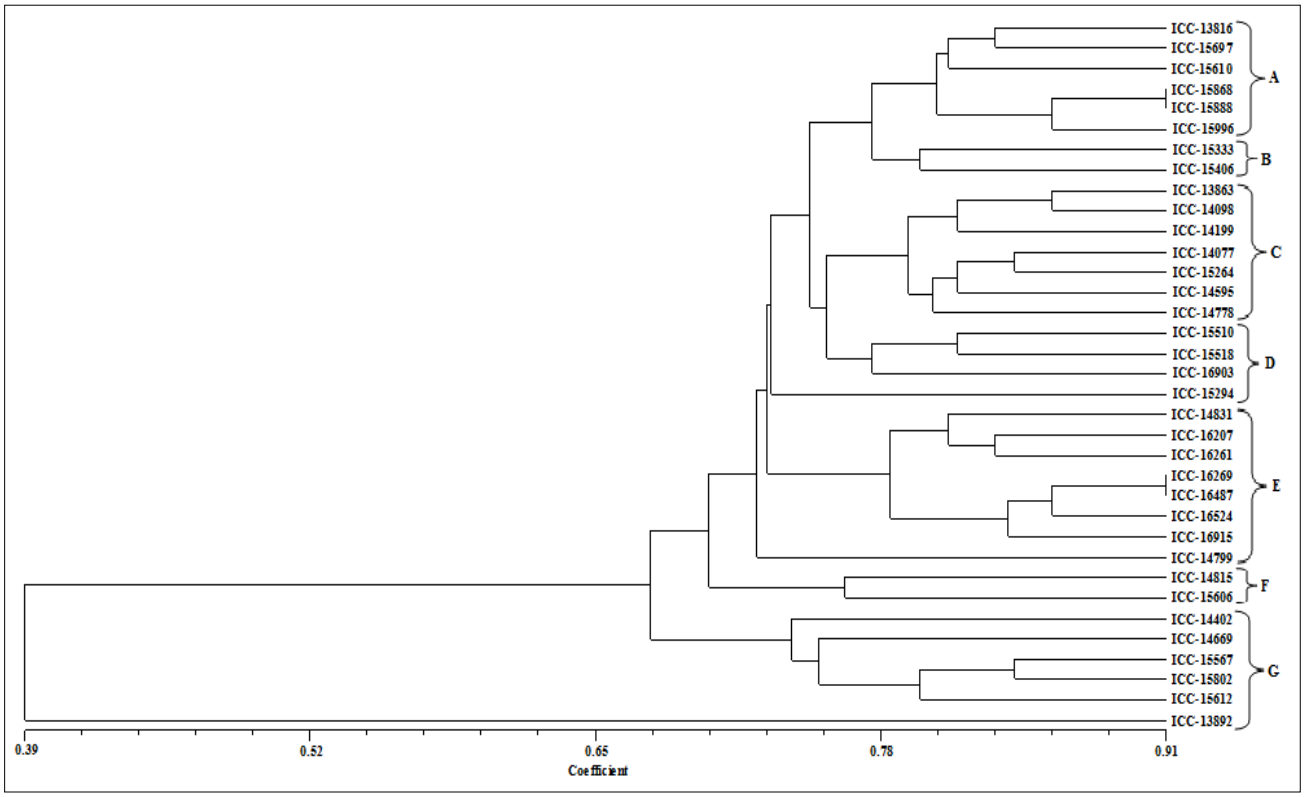

Fig 1: Dendrogram based on STMS markers.

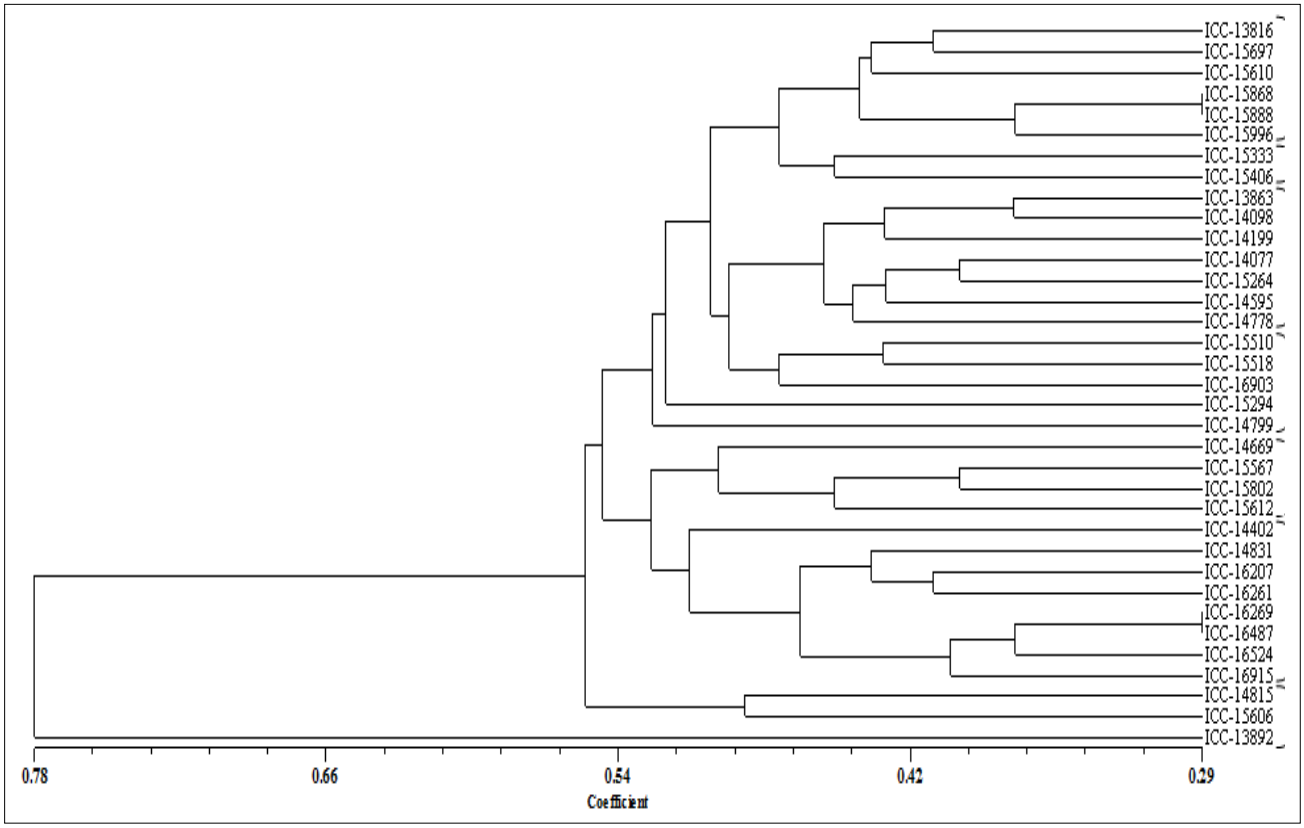

Fig 2: Dendrogram based on morphological data.

(Kumar *et al*., 2013, 2014) across the seasons were used to generate a dendrogram with the help of computer software NTSYS-pc. The genetic dissimilarity coefficients for the 35 chickpea accessions based on 11 quantitative traits ranged from 0.01 to 0.578. The highest dissimilarity coefficient value (57.8%) occurred between two chickpea accessions, ICC-7272 *vs* ICC-15697 and the lowest dissimilarity coefficient values (1.0%.) occurred between ICC-11944 *vs* ICC-14799 and ICC-14595 *vs* ICC-14669.

The resulting dendrogram classified the 35 chickpea accessions into 7 distinct clusters namely A, B, C, D, E, F and G (Fig 2). Cluster A comprised 6 accessions, which were further subdivided into two sub-clusters *viz*. A-1 and A-2. Sub-cluster A-1 consisted of three accessions ICC-13816, ICC-15697 and ICC-15610. Sub-cluster A-2 consisted of three accessions ICC-15868, ICC-15888 and ICC-15996. Cluster B consisted of only two accessions ICC-15333 and ICC-15406. Cluster C comprised 7 accessions, which were further subdivided into two sub-clusters *viz*. C-1 and C-2. Sub-cluster C-1 consisted of three accessions ICC-13863, ICC-14098 and ICC-14199. Sub-cluster C-2 consisted of 4 accessions ICC-14077, ICC-15264, ICC-14595 and ICC-14778. Cluster D consisted of 5 accessions ICC-15510, ICC-15518, ICC-16903, ICC-15294 and ICC-14799. Cluster E consisted of 4 accessions ICC-14669, ICC-15567, ICC-15802 and ICC-15612. Cluster F consisted of 8 accessions, which were further subdivided into two sub-clusters *viz*. F-1 and F-2. Sub-cluster F-1 consisted of three

Table 4: Two years pooled morphological data for 35 chickpea accessions.

Accession	Plant height (cm)	Internode length (cm)	Days to 50% flowering	No of primary branches	No of secondary branches	No of pods/ plant	No of seeds/ plant	No of seeds/ pod	Days to plant maturity	100-seed weight (g)	Seed yield/ plant (g)
ICC-13816	34.30	1.50	117.0	2.3	7.5	13.85	7.95	0.45	158.0	17.60	1.39
ICC-13863	27.35	1.91	117.5	2.5	6.5	9.40	9.80	1.03	159.0	8.22	0.97
ICC-13892	23.00	1.42	114.0	1.9	3.9	10.60	5.20	0.51	155.5	11.25	0.54
ICC-14077	33.90	1.75	116.5	2.4	8.4	20.20	21.00	0.85	154.0	7.31	1.96
ICC-14098	37.90	1.48	114.5	1.8	6.5	26.80	18.00	0.65	153.0	17.04	3.09
ICC-14199	50.10	1.43	116.0	2.7	14.1	40.40	63.70	1.52	153.5	15.86	11.55
ICC-14402	26.60	1.78	115.0	2.0	5.6	13.60	8.10	0.71	152.5	15.34	1.25
ICC-14595	26.90	1.75	116.5	2.1	7.0	26.70	33.90	1.02	154.0	13.70	4.98
ICC-14669	27.90	1.61	117.0	2.2	7.0	29.46	34.75	0.86	153.0	13.20	6.14
ICC-14778	33.00	1.47	115.5	2.2	9.9	79.50	94.38	1.03	151.5	13.68	12.90
ICC-14799	23.40	1.40	119.0	1.8	6.5	25.50	34.20	0.10	155.0	11.98	6.19
ICC-14815	29.20	1.90	110.0	2.4	7.9	37.60	53.95	1.35	152.0	13.81	7.75
ICC-14831	29.60	1.50	111.0	2.0	8.7	31.20	54.95	1.68	153.0	15.04	8.65
ICC-15264	28.40	1.79	112.0	2.0	13.5	23.85	51.00	1.29	154.0	13.14	6.36
ICC-15294	35.60	1.54	110.5	2.6	8.2	13.80	8.55	0.60	156.5	22.87	1.87
ICC-15333	35.60	1.78	112.5	2.1	6.8	16.20	9.00	0.52	155.0	24.58	2.17
ICC-15406	29.00	1.63	114.5	2.1	7.9	17.30	10.60	0.65	155.5	22.75	2.63
ICC-15510	33.10	2.11	120.0	1.9	5.9	14.80	6.59	0.47	156.0	18.89	1.22
ICC-15518	32.00	1.79	117.0	2.2	6.6	17.00	11.45	0.58	155.0	15.59	1.75
ICC-15567	21.30	1.62	116.5	1.7	3.6	5.80	5.90	1.05	154.5	14.80	0.94
ICC-15606	30.90	1.59	118.0	2.5	7.1	42.10	58.20	1.28	157.0	14.90	9.55
ICC-15610	25.30	1.06	119.0	2.7	8.6	27.60	38.83	1.37	156.5	17.35	7.89
ICC-15612	33.90	2.43	120.0	2.2	7.7	48.60	46.20	0.94	157.5	15.04	6.79
ICC-15697	32.60	1.57	117.0	1.9	4.7	4.40	4.00	2.05	152.0	15.00	1.00
ICC-15802	28.00	1.89	118.5	1.9	5.5	22.20	30.70	1.19	153.5	15.19	4.99
ICC-15868	33.30	1.47	115.0	2.1	5.9	68.25	85.30	0.92	153.5	12.43	11.35
ICC-15888	30.90	1.90	117.5	1.9	4.3	24.90	30.75	0.94	156.0	10.58	4.39
ICC-15996	33.30	1.70	114.5	2.3	7.9	61.70	66.75	0.70	153.0	17.83	14.25
ICC-16207	34.30	1.30	116.0	2.2	6.5	84.20	98.35	0.62	154.5	9.96	11.02
ICC-16261	36.90	2.70	114.0	2.9	8.1	63.89	79.06	0.87	151.5	12.50	11.39
ICC-16269	36.30	1.66	116.5	1.9	6.8	78.00	94.30	0.87	157.0	11.05	12.43
ICC-16487	33.10	1.27	116.0	3.1	9.4	116.60	128.95	0.77	155.0	12.50	16.38
ICC-16524	33.10	1.26	111.5	1.9	5.6	85.35	98.25	1.10	156.5	13.51	15.91
ICC-16903	27.70	1.75	117.5	2.1	5.7	16.20	19.20	1.21	157.5	24.07	5.72
ICC-16915	26.90	1.95	114.5	2.1	7.1	60.80	78.94	1.12	154.5	12.86	11.21

accessions ICC-14831, ICC-16207 and ICC-16261. Sub-cluster F-2 consisted of 4 accessions ICC-16269, ICC-16487, ICC-16524 and ICC-16915. Accession ICC-14402 was isolated from the cluster F. Cluster G consisted of three accessions ICC-14815, ICC-15606 and ICC-13892. At the end of clustering, out of 35 chickpea accessions only one accession ICC-13892 was isolated.

The maximum similarity coefficient value occurred between chickpea accessions, ICC-15868 *vs* ICC-15888 and ICC-16269 *vs* ICC-16487 in the tune of molecular data-based similarity index values. However, the accessions ICC-13892, ICC-14815, ICC-15606, ICC-16915 expressed distant association with ICC-13816, ICC-15697, ICC-15610, ICC-15868, ICC-15888, ICC-15996 revealing high degree of diversity in phenotypic expressions.

CONCLUSION

Crop improvement depends on the existence of genetic diversity. We report that STMS markers NCPGR 68, NCPGR 50, NCPGR 81, NCPGR 48 and NCPGR 77 are highly efficient polymorphic markers and should be utilized to assess chickpea genetic and allelic diversity. The morphological as well as molecular data-based dissimilarity values confirm the distant association of accession ICC-13892 with accessions ICC-13816, ICC-15697, ICC-15610, ICC-15868, ICC-15888, ICC-15996 as a novel finding and should be utilized in various hybridization programmes for further genetic broadening and chickpea improvement. Thus, identified polymorphic STMS markers along with distantly related accessions will be useful resources for future strategies of allele mining, association genetics, mapping and cloning of gene(s) and in applied breeding to broaden the genetic base of chickpeas.

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