

Identification of Polymorphic SSR Markers and Diversity Analysis in a Set of *Desi* Chickpea Genotypes

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ABSTRACT: The chickpea (*Cicer arietinum* L.) is a staple legume crop grown in India, North Africa and the Middle East and Ethiopia. To maintain continuous production of chickpea, it is important to develop new varieties tolerant/resistant to different abiotic and biotic stresses. Therefore, it is important to identify diverse genotypes to select as parents before planning hybridization programme. So, it is necessary to analyse diversity among and between genotypes. The molecular characterization of chickpea for genetic diversity may be used for detection of diverse chickpea genotype(s). The persistence of this investigation is to examine the genetic diversity present among 57 chickpea genotypes by using SSR molecular markers. Thirteen of 33 SSR molecular markers were found to be polymorphic and produced an average of seven amplicons per primer pair. The genetic relatedness between cultivars ranged between 0.4334 to 0.8926 and the polymorphic information content arrayed between 0.3820 (STMS-11) to 0.8833 (GAA-44). Hierarchical tree data indicated 6 different clusters in a dendrogram and in bootstrapping. Overall, the study confirmed that SSRs are effective marker methods for revealing genetic diversity in chickpeas, which may be proved helpful for breeding programme such as parent selection as well as cultivar identification.

Keywords: Chickpea, Genetic diversity, SSR, PCR, scoring, Dendrogram.

INTRODUCTION

The chickpea (*Cicer arietinum* L.) is a staple legume crop grown in India, North Africa and the Middle East and Ethiopia. It is a plant from the Fabaceae family that grows during the winter season. It is a self-pollinating diploid ($2n=16$) crop with a relatively small genome size of 738 Mbp (Varshney *et al.*, 2013). In most of the world's emerging economies, chickpeas are a significant legume crop. It is also known as "poor man's meat" (Grewal *et al.*, 2020), since it is important for supplying protein sources (Sahu *et al.*, 2020a; Gupta *et al.*, 2021). Nutritionists have also emphasised its significance because of its high nutritional content (Grewal *et al.*, 2020; Sahu *et al.*, 2020b). On an average, chickpea seeds include 358 calories, 22% protein, 4.5% fat, 63% crude fibre and 2.7% ash. Numerous minerals, including calcium, magnesium, potassium, phosphorus, iron, zinc, and manganese are abundant in it (Ibrikci *et al.*, 2003; Asati *et al.*, 2022). In more than 50 countries of the world, it is the most widely cultivated (Gaur *et al.*, 2019). Nearly 70% of the world's total production of chickpeas is produced by India (Korbu *et al.*, 2020). Chickpeas were grown on around 1095 million hectares area worldwide in 2020, with a total yield of 15.1 million tonnes (FAOSTAT, 2023).

Marker-assisted selection (MAS) can significantly increase the precision and efficacy of selection of genotypes in crop breeding (Asati *et al.*, 2022; Rathore *et al.*, 2022; Tripathi *et al.*, 2022; Yadav *et al.*, 2023). Through all the pyramiding of genes from several sources and the combination of resistance to diverse stresses, molecular markers might assist indirect selection for traits that are challenging or inconvenient to evaluate directly (Yadav *et al.*, 2016; Tripathi *et al.*, 2022). The short life cycle of the chickpea makes it a fascinating crop for genetic studies. Numerous examples of microsatellite markers are available for deployment in various crop species for the investigation of molecular diversity and marker trait associations (Adu *et al.*, 2019; Mishra *et al.*, 2020; Mishra *et al.*, 2021). High levels of polymorphism have been reported to be produced using microsatellite markers and because of this characteristic, the markers can be used to study genetic diversity (Bocianowski *et al.*, 2021). In chickpea, numerous molecular markers are reported. Now it is important to analyse the genetic diversity present among the targeted set of chickpea genotypes using these already available markers. So, the present investigation was conducted to analyse microsatellite markers-based diversity among *desi* chickpea genotypes.

MATERIALS AND METHODS

Plant materials: At the Research Farm of the Department of Genetics and Plant Breeding, College of Agriculture, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior Gwalior, Madhya Pradesh, India during Rabi 2021–2022, total 57 chickpea

genotypes (Table 1) were grown in a Randomized Block Design in two replications with an R × P distance of 30 × 15 cm. These genotypes were gathered from Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh, India and RAK College of Agriculture, Sehore, RVSKVV, Gwalior, Madhya Pradesh, India.

Table 1: List of chickpea genotypes with their parentage/source used in the study.

Sr. No.	Genotype	Parentage/ Source of collection	Sr. No.	Genotype	Parentage/ Source of collection
1.	ICCV-201102	COA, JNKVV, Jabalpur	30.	SAGL-152258	JG 135 × FG 711
2.	ICCV-201104	COA, JNKVV, Jabalpur	31.	SAGL-152265	PUSA 1088 × VIJAY
3.	ICCV-201105	COA, JNKVV, Jabalpur	32.	SAGL-152273	KAK 2 × IPC 9494
4.	ICCV-201109	COA, JNKVV, Jabalpur	33.	SAGL-152278	JSC 37 × JSC 36
5.	ICCV-201111	COA, JNKVV, Jabalpur	34.	SAGL-152318	JSC 19 × JG 16
6.	ICCV-201112	COA, JNKVV, Jabalpur	35.	SAGL-152324	IPC 4958 × IPC 9494
7.	ICCV-201113	COA, JNKVV, Jabalpur	36.	SAGL-152327	KAK 2 × JSC 19
8.	ICCV-201115	COA, JNKVV, Jabalpur	37.	SAGL-152330	ICC 4958 × PHULE G 5
9.	ICCV-201116	COA, JNKVV, Jabalpur	38.	SAGL-152339	JG16 × KAK 2
10.	ICCV-201118	COA, JNKVV, Jabalpur	39.	SAGL-152344	IPC9494 × JG16
11.	ICCV-201205	COA, JNKVV, Jabalpur	40.	SAGL-152347	KAK 2 × JSC 19
12.	ICCV-201206	COA, JNKVV, Jabalpur	41.	SAGL-162299	RAK, Sehore, RVSKVV, Gwalior
13.	ICCV-201209	COA, JNKVV, Jabalpur	42.	SAGL-152349	KAK 2 × PHULE G5
14.	ICCV-201210	COA, JNKVV, Jabalpur	43.	SAGL-152403	RAK, Sehore RVSKVV, Gwalior
15.	ICCV-201211	COA, JNKVV, Jabalpur	44.	SAGL-152404	RAK, Sehore RVSKVV, Gwalior
16.	ICCV-201212	COA, JNKVV, Jabalpur	45.	SAGL-152405	RAK, Sehore RVSKVV, Gwalior
17.	ICCV-201214	COA, JNKVV, Jabalpur	46.	SAGL-162370	PG 9425-9 × BG 2064
18.	ICCV-201217	COA, JNKVV, Jabalpur	47.	SAGL-162376	JSC 52 × RSG 888
19.	SAGL-152210	IPC 9494 × ICC 506	48.	SAGL 22-101	KAK-2 × BG-362
20.	SAGL-152216	JG 16 × VIJAY	49.	SAGL 22-102	JG-6 × RVSSG-2
21.	SAGL-152223	RAK, Sehore, RVSKVV, Gwalior	50.	SAGL 22-103	JG-130 × FG-703
22.	SAGL-152231	KAK2 × JG130	51.	SAGL 22-104	JSC-33 × JG-11
23.	SAGL-152234	JSC 19 × ICC 4958	52.	SAGL 22-105	JAKI-9218 × BGD-112
24.	SAGL-152236	KAK-2 × BG 362	53.	SAGL 22-106	RVG-204 X JSC-37
25.	SAGL-152237	BG 2064 × KAK -2	54.	GCP-101	RAK, Sehore, RVSKVV, Gwalior
26.	SAGL-152238	PG -9425-9 × IPC 9494	55.	RVSSG-64	RAK, Sehore, RVSKVV, Gwalior
27.	SAGL-152250	KAK 2 × BG 2064	56.	JG-36	COA, JNKVV, Jabalpur
28.	SAGL-152252	ICC 4958 × BG 1108	57.	JG-14	COA, JNKVV, Jabalpur
29.	SAGL-152254	BG 362 × ICC 506			

DNA extraction: Molecular Analysis work was performed at Plant Molecular Biology Laboratory, Department of Plant Molecular Biology & Biotechnology, College of Agriculture, Rajmata Vijayaraje Scindia Agricultural University Gwalior, Madhya Pradesh, India. High quality genomic DNA was extracted from 8-10 days old young and fresh leaves by employing CTAB method as proposed by Doyle and Doyle (1987) with some modifications as suggested by Tiwari *et al.* (2017). Extracted DNA was quantified through electrophoresis on 0.8% agarose gel and compared after loading a known quantity DNA marker (λ DNA) on the same gel as a standard. Apart from it a Spectrophotometer was also used for quantification of DNA.

SSR markers analysis: The genetic profile of 57 chickpea genotypes was analysed based on difference in allele size produced using 33 SSR markers (Table 2).

The polymerase chain reaction was performed in 10 μ l reaction mixture comprising of 1X PCR buffer, 0.1 μ l *Taq* DNA polymerase, 1 μ l dNTP (1 mM), 0.5 μ l of primers (10 pM) and 20 ng/ μ l of genomic DNA in a thermocycler (Bio-Rad, USA). The PCR protocol comprised of initial denaturation step of 94°C for 3 min followed by 35 cycles of 94°C for 1 min, annealing cycles (from 52°C to 57°C) varied for different markers system for 30 sec, elongation at 72 °C for 1 min with final extension at 72 °C for 7 min. PCR amplified products of SSR markers along with standard markers (100 bp) were separated through electrophoresis on 3% agarose gel respectively at 75 V for two hrs. The agarose gels were stained with Ethidium Bromide (1 μ g/ml). After electrophoresis the agarose gels were visualized under UV light and photographed under Bio-Rad Gel documentation system.

Table 2: List of sequences of SSR markers used in present investigation.

Sr. No.	Marker	Forward sequence 5'-3'	Reverse sequence 5'-3'
1.	TA-1	TGAAATATGGAATGATTACTGAGTGAC	TATTGAAATAGGTCAGGCTTATAAAAA
2.	TA-2	AAATGGAAGAAGAATAAAAAACGAAAC	TTCCATTCTTTATTATCCATATCACTACA
3.	TA-3	AATCTCAAAAATCCCAAAAT	ATCGAGGAGAGAAGAACCAT
4.	TA-18	AAAATAATCTCCACTTCACAAATTTTC	ATAAGTGCCTTATTAGTTGGTCTTGT
5.	TA-27	ACAATTCACCTTAATCTTTGC	AATTTAGCCTACAGACACACACA
6.	TA-28	TAATTGATCATACTCTCACTATCTGCC	TGGGAATGAATATTTTTGAAGTAAA
7.	TA-64	ATATATCGTAACCTCATTAATCATCCGC	AAATTGTTGTCATCAAATGGAAAATA
8.	TA-71	CGATTTAACACAAAAACACAAA	CCTATCCATTGTCATCTCGT
9.	TA-135	TGGTTGGAAATGTAGTGT	GTGGTGTGAGCATAATTCAA
10.	TA-180	CATCGTGAATATTGAAGGGT	CGGTAAATAAGTTTCCCTCC
11.	TA-194	TTTTGGCTTATTAGACTGACTT	TTGCCATAAAAATACAAAATCC
12.	TAA-60	TCATGCTTGTGGTGTACTAGAAA	CAAAGACATAACGAGTTAAAGAAAA
13.	TR-1	CGTATGATTTGCGCTAT	ACCTCAAGTTCCGGAAT
14.	TS-45	TGACACAAAATTGCTCTTGT	TGTTCTAACGTAACCTAACCTAA
15.	TS-82	TCAAGATTGATATTGATTAGATAAAAGC	CTTTATTACAACCTGCACAACACTAA
16.	STMS-2	ATTTTACTTTACTACTTTTTCTTTC	AATAAATGGAGTGAAATTTTCATGTA
17.	STMS-11	GTATCTACTTGTAATATCTCTTCT	ATATCAAAAACCCCCAC
18.	STMS-13	TATGTTAAAAGAGAAAAGCAAGTGAT	TTTTATTAGTTGTGAAAATGTATATCA
19.	STMS-20	CTTNTCGTCATCATCGTTTTG	CACCCTACTTTTTTCCACCAC
20.	STMS-24	AAAGACAGGTTTAAATCCAAAA	CTAATCTTTCTTCTTTTGTGAT
21.	GA-4	TTGCGTGTCAATCTCATTTGG	TCAACACCCTAAGTCCGGAC
22.	GA-11	GTTGAGCAACAAGCCACAA	TTCTTGTCTGGTTGTGTGAGC
23.	GAA-40	TTGACGCAGAGAAGTCTCAA	ATTGGTGTGATGGGTGGATT
24.	GAA-42	CGCTTCAGTGTAGATATTATCAAAACA	TCTCTCTTCTTCAACACGC
25.	GAA-44	AGCAAGCCCATGATTTTCTC	ATGACATTCCAATCGGCTTC
26.	GAA-45	TTGGGATCCATTTCATCCAT	GCCTGGAAGTCACACTTGT
27.	GAA-46	TCTCCTGTGAATGAACCGAA	CTGAGCAACAAAATCAGCCA
28.	TA-22	TCCTCAACCCTTTAGATTGA	TCGTGTTACTGAATGTGGA
29.	TA-46	TTTATTGCAATAAAATCATTCTTATC	TTCTTTTTGTGTGAAAAAAAATATAGTGA
30.	NCPGR-1	TTACAGCTTGTGCTCAG	AGTCAGATTCTTATCCGA
31.	TAA-58	CATTGCTTAAAGAACCAAAATGG	CAATTTTACATCGACGTGTC
32.	TaaSH	GGTAGACGCAAAAGAGTGTGGG	GCCACATTGACCAGGAATG
33.	TR-9	GCCCACTGAAAAATAAAAAAG	ATTTGAACCTCAAGTTCTCG

Band scoring and data analysis: The genetic profiles of genotypes were assessed based on allele size variations. Power Marker v3.25 software (Liu and Muse 2005) was used to examine the major allele frequency, number of alleles per locus, polymorphism information content (PIC) and gene diversity. The dendrogram based on unweighted pair group method for arithmetic average (UPGMA) was also constructed using Marker v3.25 software. Based on the banding pattern data was recorded with allele pattern A/A and B/B homozygous condition and A/B for heterozygous condition and in case of no amplification (-/-) was used.

RESULTS AND DISCUSSION

Genetic diversity analysis of plant genetic resources has played a major role in effective conservation, management, and exploitation. For this purpose, a systematic categorization of targeted plant material and knowledge of the genetic relationships in the germplasm are required (Pramanik *et al.*, 2021; Kumar *et al.*, 2022a; Kumar *et al.*, 2022b; Makwana *et al.*, 2023; Yadav *et al.*, 2022; Tomar *et al.*, 2022). Different genetic diversity parameters like numbers of alleles per locus, major allele frequency, genetic diversity and PIC values are indicators of the efficiency of SSR markers used in molecular diversity analysis, geographical relationship, phylogenetic analysis, and genetic differentiation pattern of the studied genotypes. Numbers of alleles per locus is important parameters to

determine PIC value of a particular marker (Mandloi *et al.*, 2022).

SSR markers are regarded as the preferred molecular markers among the available markers created and employed in breeding efforts. SSR markers are particularly desirable for characterization of germplasm because of their widespread use, high density in many genomes, and other benefits (Tiwari *et al.*, 2019). They have been widely utilised to identify variation in chickpea germplasm lines. During the current investigation, initially at screening stage, total 33 SSR markers were tested for their polymorphic nature with DNA template of *Desi* chickpea genotypes. Out of 33 SSR markers, 13 including GAA-44, STMS-11, STMS-24, GA-4, NCPGR-1, TA-71, TA-135, TA-180, STMS-2, TR-9, TA-18, GAA-40 and TS-45 markers were found reproducible and polymorphic in all 57 lines of *desi* chickpea (Table3). Ninety-one alleles were detected as polymorphic and homozygous. Therefore, 91 alleles were considered effective alleles for 13 markers varied from three to 14 alleles per marker. The aim of the present experiment was to analyse the genetic dissimilarity among 57 lines of *desi* chickpea through SSR markers. The allele size range varied from 150 to 300bp. Similar results are reported by Yadav *et al.* (2016) while using SSR markers for genetic diversity analysis among chickpea cultivars. The genetic divergence among SSR markers ranged from 0.4334 to 0.8926 with an average value of 0.7376. The highest gene diversity was found in GAA-44 (0.8926)

followed by TR-9 (0.8870), NCPGR-1 (0.8655), TA-18 (0.8495), TS-45 (0.8433) and GA-40 (0.7639) (Fig.1). The results are in agreement with findings of earlier studies including Bakshi *et al.* (2016); Aggarwal *et al.* (2018); Amina *et al.* (2020). The polymorphic information content value of the entire polymorphic marker during the present investigation ranged between 0.3820 (STMS-11) to 0.8833 (GAA-44) with a mean value of 0.6955 (Fig. 2). The highest PIC value was

recorded with the primer GAA-44 however, lowest value with STMS-11. In accordance with the present findings, Safera *et al.* (2011); Naghvi *et al.* (2012); Ghaffari *et al.* (2014); Samyuktha *et al.* (2018); Seyedimoradi *et al.* (2019); Sachdeva *et al.* (2019); Shanmugam and Kalaimagal (2019) reported almost similar ranges of PIC values in their studies on use of SSR markers for genetic diversity analysis in chickpea genotypes.

Table 3: Major allele frequency, polymorphic information content, number of alleles per locus, and Gene Diversity of polymorphic SSR markers.

Marker	Major Allele Frequency	Number of Genotype	Number of Allele	Gene Diversity	PIC value
GAA-44	0.1930	14.0000	14.0000	0.8926	0.8833
STMS-11	0.7193	3.0000	3.0000	0.4334	0.3820
STMS-24	0.4211	6.0000	6.0000	0.6931	0.6429
GA-4	0.3684	4.0000	4.0000	0.6716	0.6021
NCPGR-1	0.2632	12.0000	12.0000	0.8655	0.8529
TA-71	0.3860	4.0000	4.0000	0.6808	0.6159
TA-135	0.3684	4.0000	4.0000	0.6845	0.6196
TA-180	0.4035	4.0000	4.0000	0.6630	0.5957
STMS-2	0.3860	3.0000	3.0000	0.6611	0.5870
TR-9	0.2105	13.0000	13.0000	0.8870	0.8774
TA-18	0.2456	10.0000	10.0000	0.8495	0.8324
GAA-40	0.3158	5.0000	5.0000	0.7639	0.7250
TS-45	0.2632	9.0000	9.0000	0.8433	0.8252
Mean	0.3495	7.0000	7.0000	0.7376	0.6955

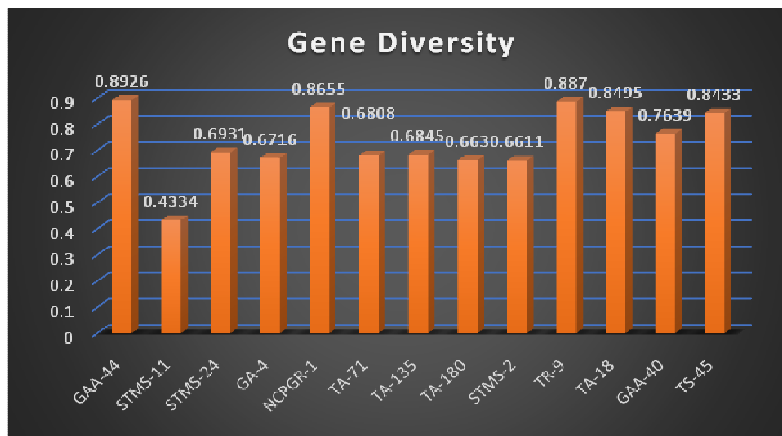


Fig. 1. Graphical representation of gene diversity.

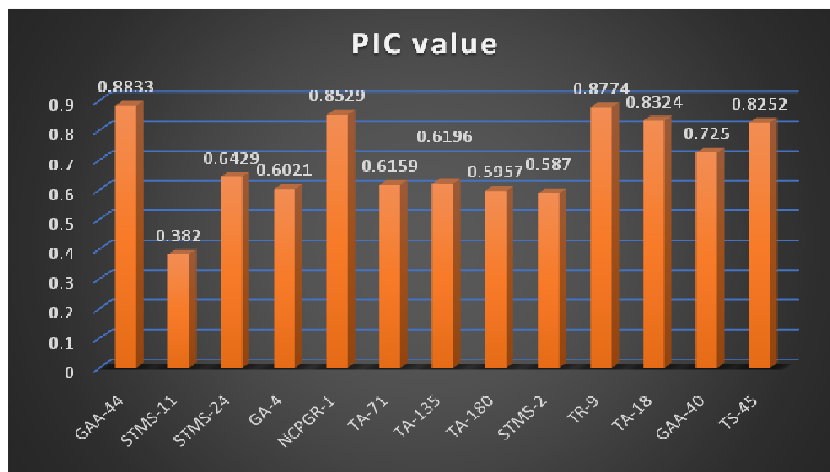


Fig. 2. Graphical representation of Polymorphic Information Content (PIC) Value.

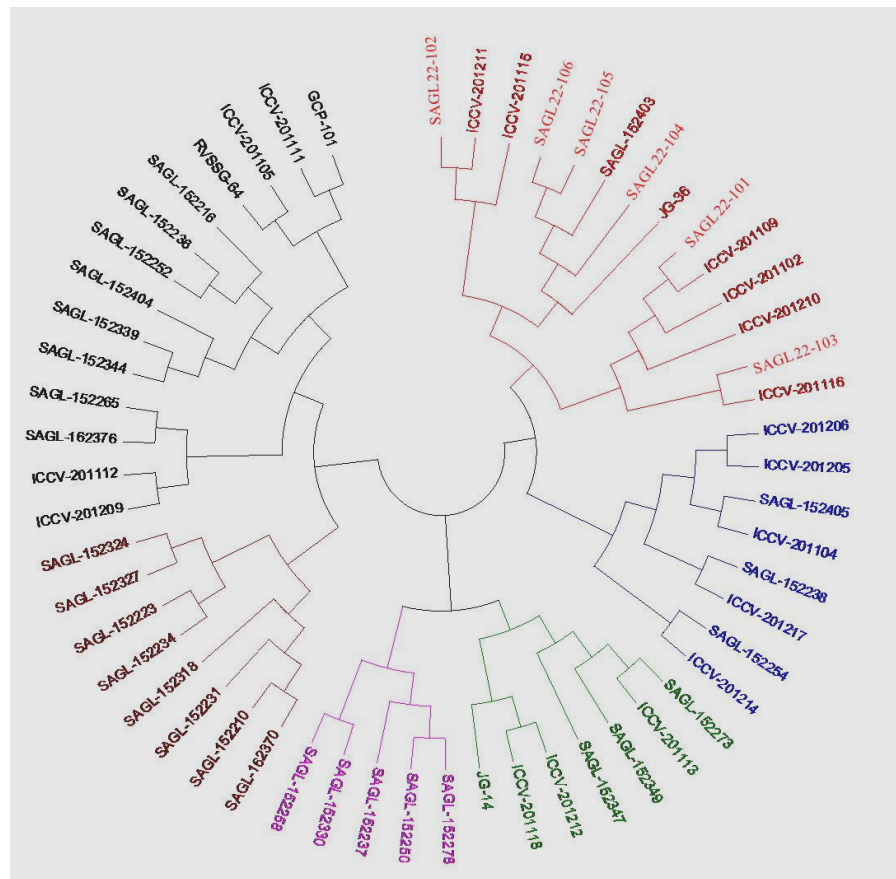


Fig. 3. Dendrogram formed based on UPGMA (Unweighted Pair Group Method with Arithmetic Mean).

Molecular markers based genetic dissimilarity among a set of chickpea genotypes helped to construct an UPGMA tree (Fig. 3). The studied chickpea genotypes were grouped into 6 clusters according to genetic distance among and between them. Cluster 1: Included 14 genotypes *i.e.*, SAGL 22-102, ICCV-201211, ICCV-201115, SAGL 22-106, SAGL 22-105, SAGL-152403, SAGL 22-104, JG-36, SAGL 22-101, ICCV-201109, ICCV-201102, ICCV-201210, SAGL 22-103 and ICCV-201116. Cluster 2 contained 8 genotypes including ICCV-201206, ICCV-201205, SAGL-152405, ICCV-201104, SAGL-152238, ICCV-201217, SAGL-152254 and ICCV-201214 while cluster 3 contains 7 genotypes *viz.*, SAGL-152273, ICCV - 201113, SAGL-152349, SAGL-152347, ICCV-201212, ICCV-201118 and JG-14. Cluster 4 had 5 genotypes *i.e.*, SAGL-152278, SAGL-152250, SAGL-152237, SAGL-152330 and SAGL-152258. Cluster 5 included 8 genotypes *namely* SAGL-162370, SAGL-152210, SAGL-152231, SAGL-152318, SAGL-152234, SAGL-152223, SAGL-152327 and SAGL-152324. Whilst cluster 6 contains 14 genotypes including ICCV-201209, ICCV-201112, SAGL-162376, SAGL-152265, SAGL-152344, SAGL-152339, SAGL-152404, SAGL-152252, SAGL-152236, SAGL-152216, RVSSG-64, ICCV-201105, ICCV-201111 and GCP-101. Grouping of some of the SAGL genotypes collected from the same centre (RAK College, Sehore) confirms their close relationship with each other. Similar results were found by Rizvi *et al.* (2014); Datta *et al.* (2015).

Similarly, most of the ICCV genotypes showed resemblance and grouped together. Similar results have been reported by various research groups (Solanki *et al.*, 2022).

CONCLUSIONS

High genetic diversity within a chickpea population provides an opportunity to the breeders to plant hybridization strategies for improvement of chickpea. Thirteen SSR primers were found to be polymorphic out of the 32 SSR markers used in the current study. Through the crossing of genetically diverse genotypes, traits with a wide range of allele sizes, a large number of genotype-specific alleles per locus, high polymorphic information content, and expected heterozygosity made it possible to improve yield and specific traits like heat, cold, and drought.

Conflict of Interest. None.

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