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Variability analysis among advance breeding lines of chickpea using microsatellite markers

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Abstract
Cicer arietinum L. is the third greatest widely planted imperative pulse crop worldwide, belongs to the Leguminosae family. Modern plant breeding and agricultural systems have narrowed the base for the genetic variability of cultivated chickpea and to explore new sources of variation that might be used in plant breeding programmes. The main aim of this study was to quantify the genetic variability present among the selected 20 advance breeding lines of chickpea using molecular markers. MAB, which is part of genomics-assisted breeding (GAB), is a tool that can significantly improve the precision and efficiency of breeding in chickpeas. This comprehensive approach combining traditional breeding with advanced genomic tools could lead to the development of chickpea varieties better suited to selected environments. The analysis revealed that set of 24 SSR primers were able to produce variability present within the advance breeding lines. UPGMA dendrogram were constructed by the genetic variability present within the selected lines. This analysis will be useful in the selection of advance breeding lines for mapping populations and further breeding programmes attempting to broaden the genetic base of future chickpea cultivars.

Keywords: Marker assisted breeding (MAB), Simple sequence repeat (SSR)

Introduction
Chickpea (*Cicer arietinum*) is a diploid, annual, self-pollinated crop. It ranks as the third most important pulse crop globally, following faba bean and field pea. It has a relatively large genome size of 738 megabytes. India is the largest producer of chickpeas, with an annual production of 10.13 million tonnes from 9.44 million hectares, yielding 1073 kg per hectare. Chickpeas are cultivated in 52 countries.

The breeding approaches such as hybridization, mutation, and marker-assisted breeding (MAB) can be used to improve the chickpeas. Furthermore, genome sequencing and omics technologies, which provide a deeper understanding of plant genetics and physiology, also play a significant role in improving drought resilience. Root traits, such as root depth and biomass, are particularly important for managing biotic and abiotic stresses.

Marker-assisted selection (MAS), a form of genomics-assisted breeding (GAB), can enhance breeding accuracy and efficiency, making it a valuable tool in developing high yielding multiple disease resistant, biotic and abiotic stress tolerant chickpea cultivars. The combination of breeding technologies, omics approaches, and an understanding of plant physiology is essential for advancing chickpea breeding and creating more resilient crops to withstand future breeding strategies.

At the local level, it offers essential insights for farmers, breeders, and researchers to identify well-suited varieties for specific agro-climatic conditions, improve crop management practices, and boost overall productivity. On a global scale, morphological and molecular characterization plays a critical role in conserving germplasm, assessing genetic diversity, and advancing the development of enhanced crop varieties, thereby contributing to food security, sustainability, and adaptation to evolving environmental conditions (Karishma *et al.* 2023) [1].

In context of climate change, yield potential of chickpea is hampered by heat stress. Increasing area of rice fallows, shifting in cropping system and global warming, needs to identify the chickpea genotypes tolerant to high temperature (Katkani *et al.* 2022) [3].

Modern genomics has enabled the identification and tagging of genes associated with important agronomic traits, including stress tolerance and disease resistance. These advancements facilitate the transfer of beneficial genes through molecular breeding techniques (Salahvarzi *et al.* 2021) [4].

Through molecular breeding and genomic tools, it is now possible to more efficiently identify and transfer genes for important traits like drought tolerance and disease resistance in chickpeas. These techniques, including QTL mapping, marker-assisted backcrossing, and association mapping, are revolutionizing chickpea breeding, enabling faster and more precise improvements in crop resilience (Verma *et al.* 2015) [5]

The objectives of this study were to employ SSR markers for investigation of genetic relationship and genetic variability among chickpea breeding lines.

Material and Methods

Plant materials

The experimental material consisted of 20 advanced breeding lines of chickpea including three check varieties *viz*. JG24, JG36 and JG18 during *Rabi* 2023-24 and 2024-25, obtained from AICRP on Chickpea, JNKVV, Jabalpur in 3 replications in a Randomized Complete Block Design.

Molecular Diversity

The quantity of plant DNA was checked from all ABLs and it was of a high purity ratio DNA was found to be good quality as it was checked by separating the samples on 0.8% agarose gel for presence of any RNA contamination. The DNA bands observed under UV lights were without RNA contamination. Molecular analysis of germplasm diversity would provide information to plant improvement programs regarding the level of genetic variation within and between ABLs.

DNA extraction

Total genomic DNA was extracted using a modified CTAB method based on the protocol of Doyle and Doyle (1990). Young leaves were used for DNA extraction from each plant of every accession. Quality of DNA was tested by submerged horizontal agarose gel (0.8%) electrophoresis and visualized with UV light.

PCR analysis

The SSR markers amplified by polymerase chain reaction (PCR) in a volume of 10 μl, containing 15 ng genomic DNA, 1 U of *Taq* DNA polymerase, 1 µM of each primer, 100 µM of each dNTP, 1 µl (10×) PCR buffer, 1.4 mM $MgCl_2$ and ddH2O, using a Eppendorf PCR System. Amplification was carried for 35 cycles, each consisting of an initial denaturation step at 95 °C for 5 min and 35 cycles of denaturation step at 95 °C for 30 s, annealing at 50-60 °C for 45 s and an extension step at 72 °C for 30 s. The final extension was performed at 72 °C for 7 minutes. PCR products were analyzed using 3% agarose electrophoresis gels stained with ethidium bromide. Each band identified as an allele and scored as '1' for presence and '0' for absence from largest to smallest sized band. Cluster analysis was conducted on the basis of Jaccard's similarity matrix through NTSys PC version 2.2.

Table 2: List of microsatellite markers used for genetic diversity analysis in chickpea

S. No.	MARKER	FORWARDPRIMER(5'-3')	REVERSEPRIMER(5'-3')	Annealing Tm (ºC)
1.	TA76	TCCTCTTCTTCGATATCATCA	CCATTCTATCTTTGGTGCTT	57
2.	NCPGR 200	TTCACACAACAACCTTTTCA	GGTGAGTTTCTTTTTCCCTT	54
3.	NCPGR 196	TTGGGTCATTACCTTCATCT	CTCATCCTTGAGAGAAATCG	56
4.	TR19	TCAGTATCACGTGTAATTCG T	CATGAACATCAAGTTCTCCA	57
5.	NCPGR 206	AACAACACTGGGTGAGAGA T	GATCCACATGCTACCATACC	59
6.	GAA46	TCTCCTGTGAATGAACCGAA	CTGAGCAACAAAATCAGCCA	58
7.	GA2	TGCATTGGAAATACAGCATG A	AATTTTGGTTCGCCACAAAC	57
8.	TA 142	TGATAACATTCCCTAATATCA ATAACTT	TTCCACAATGTTGTATGTTTTG TAAG	69
9.	TA 59	ATCTAAAGAGAAATCAAAATT GTCGAA	GCAAATGTGAAGCATGTATAG ATAAAG	58.9
10.	H4G11	ATCTAAGTGAGCGGCTACTA AATCA	GTAGTCATGCAGCCTATAAAA ACAA	59.7
11.	H4E09	TGCTATTTGTACTAGGACTTA AGGAAA	TGTTTAAAGTACCCATTAAAAA CGTAA	58.9
12.	H3A12	TCAATCTTTTGTTGTTACTAT GAATCTG	AACCTTAGACTGTGTTCGCTG A	58.4
13.	H5A08	AGGAGAGAAAATGTAACATC CTAAATC	CAAATTGGTTATTGATTACAAT TAGGT	58.9
14.	CaSTMS15	CTTGTGAATTCATATTTACTT ATAGAT	ATCCGTAATTTAAGGTAGGTTA AAATA	55.5
15.	TA130	TCTTTCTTTGCTTCCAATGT	GTAAATCCCACGAGAAATCAA	54.2
16.	TA72	GAAAGATTTAAAAGATTTTC CACGTTA	TTAGAAGCATATTGTTGGGATA AGAGT	58.9
17.	TA1	TGAAATATGGAATGATTACTG AGTGAC	TATTGAAATAGGTCAGGCTTAT AAAAA	58.9
18.	TA3	AATCTCAAAATTCCCCAAAT	ATCGAGGAGAGAAGAACCAT	55.5
19.	TA71	CGATTTAACACAAAACACAA A	CCTATCCATTGTCATCTCGT	55.3
20.	TA194	TTTTTGGCTTATTAGACTGA CTT	TTGCCATAAAATACAAAATCC	53.5
21.	SSRG9	CTGTACTCGGACGCAAACT G	CGCGAACTAATAGGCATGGT	59.3
22.	SSR14	ACCTCCGTCCACACATTTCT AC	GTCGAAGCCATTGTTTTGTTG	60.4
23.	TA 110	ACACTATAGGTATAGGCATTT AGGCAA	TTCTTTATAAATATCAGACCGG AAAGA	60.4
24.	SSRG4	CGCATTTTCGCTTTCTTGAT	AGTGGCGGATATTACCGAGA	57.3

Results and Discussion

For the purpose of creating a breeding plan under particular agro-ecological circumstances, the genetic diversity found in chickpea germplasm collections is crucial. SSR markers were previously discovered to have a high resolving capacity for evaluating the genetic variability and connections among the chickpea accessions (Solanki *et al.*, 2022) [7]. We used score quality, genome coverage, amplified product quality, and polymorphism information content as criteria when choosing the markers. The set of SSR markers that have been presented has several uses, particularly in the study of chickpea genetic variation. DNA fingerprinting database was produced using microsatellite marker system for 20 advanced breeding lines of chickpea developed at Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur. Our results indicated that primers successfully amplified genotype DNAs. All of the markers were able to produce amplicons with chickpea genotypes. Salient features of fingerprinting database obtained using these markers are given in the table 3.

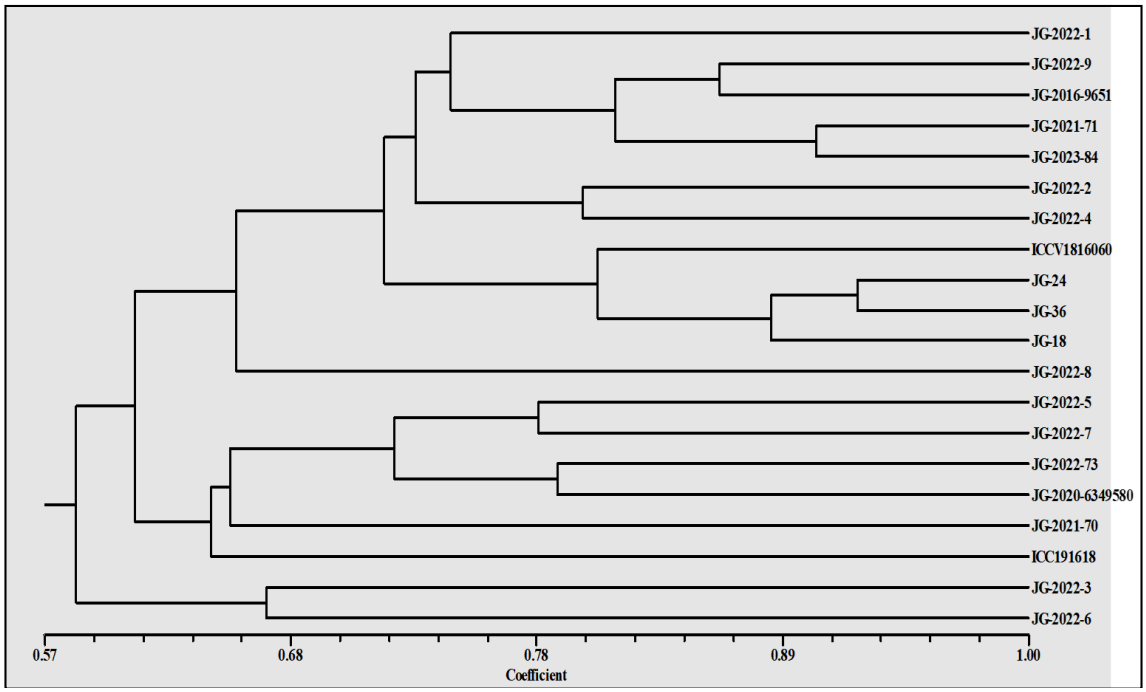
Table 3: Microsatellite markers based parameters obtained during profiling of chickpea genotypes

S. No.	Marker	TA	PA	PP	PIC
1.	TA76	2	2	100	0.34
2.	NCPGR 200	4	4	100	0.37
3.	NCPGR 196	2	2	100	0.24
4.	TR19	2	2	100	0.36
5.	NCPGR 206	2	2	100	0.31
6.	GAA46	2	2	100	0.29
7.	GA2	2	2	100	0.33
8.	TA 142	2	2	100	0.39
9.	TA 59	2	2	100	0.22
10.	H4G11	2	2	100	0.24
11.	H4E09	2	2	100	0.31
12.	H3A12	2	2	100	0.25
13.	H5A08	2	2	100	0.37
14.	CaSTMS15	3	3	100	0.39
15.	TA130	3	3	100	0.34
16.	TA72	2	2	100	0.21
17.	TA1	2	2	100	0.32
18.	TA3	3	3	100	0.41
19.	TA71	4	4	100	0.46
20.	TA194	2	2	100	0.22
21.	SSRG9	2	2	100	0.24
22.	SSR14	4	4	100	0.33
23.	TA 110	4	4	100	0.48
24.	SSRG4	2	2	100	0.19
Total		58	54	-	-
Average		2.42	2.42	100	0.32

TA-Total alleles, PA-Polymorphic alles, PP-Percentage polymorphism, PIC-Polymorphism information content

A total of 58 alleles were produced during the investigation with an average of 2.42. All 54 were alleles produced in the investigation were found to be polymorphic with an average of 2.42. Numbers of alleles were ranged from 2 to 4. The highest number of alleles i.e 4 was produced by TA110, SSR14, TA71 and NCPGR 200 markers. Polymorphism information content was ranged from 0.19 to 0.48 and the markers TA110, SSR14, TA71 and NCPGR 200 were able to discriminate chickpea genotypes used under the investigation. Marker SSRG4 had lowest PIC=0.19 while TA110 had highest PIC=0.48. One of the most crucial indices for determining primer efficiency is polymorphism information content, or PIC (Mazkirat *et al.*, 2023) [6]. The allele diversity among the genotypes under study was reflected in the PIC value. Wide diversity was demonstrated by the high polymorphism in microsatellites, indicating that numerous mutations took place in the genomic areas of microsatellites.

In dendrogram, all genotypes were grouped into two main groups. The major group contained 18 genotypes while minor group had only two genotypes namely JG-2022-3 and JG-2022-6. The major group was further divided into two sub groups. The first subgroup has 12 genotypes namely JG-2022-1, JG-2022-9, JG-2016-9651, JG-2021-71, JG-2023-84, JG-2022-2, JG-2022-4, ICCV1816060, JG-24, JG-36, JG-18 and JG-2022-8. Among these twelve genotypes, JG-2022-8 has shown highest genetic distance and grouped distantly. Second sub group has six genotypes namely JG-2022-5, JG-2022-7, JG-2022-73, JG-2020-6349588, JG-2021-70 and ICC191618. Among these six genotypes, ICC191618 has shown the highest genetic distance and grouped distantly.



Conclusion

Chickpea genotypes used under the current study has considerable amount of genetic variability. In order to pick parents for a hybridisation program efficiently, genetic distance is undoubtedly important. Diverse genotypes may be utilized for the further improvement of chickpea crop.

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