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Characterization of genetic diversity in chickpea (*Cicer arietinum*) genotypes using ISSR markers

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Abstract

To evaluate the genetic diversity of seven chickpea genotypes, ISSR (Inter Simple Sequence Repeat) analysis was employed using seven specific primers. The annealing temperatures for these primers ranged from 46 °C to 53 °C. The seven primer pairs, namely USB 836, USB 820, USB 815, USB 811, USB 846, USB 801, and USB 808 exhibited different levels of polymorphism amongst the seven chickpea varieties under evaluation in the present investigation. A total of twenty seven different loci were assigned to the seven ISSR primers. The average number of amplicons per primer is 4.57 while the average number of polymorphic amplicons per primer is 3.86. The total percent polymorphism by marker is 84.38. The resulting dendrogram categorized the genotypes into two primary clusters. Cluster I represent the largest group, which is further subdivided into two sub-clusters: Sub-cluster A (comprising Phule Vikram, Phule Vikrant, Phule Vijay, and Phule Kripa) and Sub-cluster B (containing Phule Vishal and Phule Virat). Within this group, Phule Vikram and Phule Vikrant exhibited the highest genetic similarity, joining at a coefficient of approximately 0.62. In contrast, Cluster II is a mono-genotypic cluster consisting solely of Phule Vishwaraj. This genotype separated from the others at a lower similarity coefficient of 0.42, identifying it as the most genetically diverse variety among the studied samples. These findings indicate a significant range of genetic diversity, which is essential for future crop improvement programs.

Keywords: ISSR, chickpea, genetic diversity, dendrogram, polymorphism

Introduction

Cultivated chickpea (Scientific name-*Cicer arietinum* L.) is a self-pollinated, diploid ($2n = 16$) annual pulse crop with a genomic size of 740 Mbp. Chickpea is an important agricultural crop and 4th most important grain-legume crop after soybean, bean, and pea (Upadhyaya *et al.* 2008) [7]. Chickpea is a cheap protein source in most developing countries in Asia and Africa. (Saeed *et al.* 2011; Jannatabadi *et al.* 2014) [4, 2].

The development of high-yielding and stress-resistant chickpea varieties requires a thorough understanding of genetic diversity among available genotypes. The conservation and use of diverse collections of plant genetic resources is the backbone in plant breeding programs, so this genetic variability is the raw material for crop breeding industry on which selection acts to evolve superior genotypes (Saeed *et al.* 2011; Upadhyaya *et al.* 2008) [4, 7]. Traditional methods based on morphological characters are often influenced by environmental conditions and may not accurately reflect genetic variation. Therefore, molecular characterization has become an effective and reliable approach for studying genetic diversity in chickpea.

Molecular genetic markers have been widely used in the last decades for both assessment of original material and search for valuable plant phenotypes. Genetic diversity in the chickpea has been explored using a range of molecular markers such as SSR (Saeed *et al.* 2011; Ghaffari *et al.* 2014) [4, 1], RAPD (Talebi *et al.* 2008b) [6] and AFLP (Talebi *et al.* 2008a) [5]. Among various molecular marker techniques, Inter-Simple Sequence Repeat (ISSR) markers are widely used because they are highly reproducible, cost-effective, and capable of detecting high levels of polymorphism without prior sequence information. ISSR markers help in identifying genetic relationships, assessing variability, and supporting marker-assisted breeding programs for the development of improved chickpea cultivars with better yield, quality, and resistance to biotic and abiotic stresses.

Materials and Methods

Plant Materials

The seven different genotypes of chickpea (*Cicer arietinum* L.) were collected from Pulses improvement Project, Mahtma Phule Krishi Vidyapeeth, Rahuri-413722 (MS) (Table 1). Seeds were thoroughly rinsed with sterile water and sown in a sterilized soil mixture to ensure healthy seedling development.

Table 1: Details of chickpea genotypes tested for molecular characterization.

Sr. No.	Name of Genotype
1.	Phule Vishwaraj
2.	Phule Vikram
3.	Phule Vishal
4.	Phule Vikrant
5.	Phule Vijay
6.	Phule Virat
7.	Phule Kripa

Methods

Genomic DNA extraction and Purification

Total genomic DNA was extracted from a chickpea leaves of each genotypes following a CTAB extraction protocol. Isolated DNA was purified using RNase enzyme to remove any possible contamination of RNA. The quality of isolated DNA was evaluated through agarose gel electrophoresis. The DNA sample diluted with appropriate quantity of 1X TE buffer to yield a working concentration of 20 ng/μl, and stored at 4 °C for further work until PCR amplification.

PCR Amplification

The ISSR markers amplified by polymerase chain reaction (PCR) in a volume of 20μl, containing 20 ng genomic DNA, 1U of Taq DNA polymerase (DSS Takara Biolab) 2 μM of primer (Eurofins Laboratories) (Table 2), 100 μM of each dNTP, 2μl (10×) PCR buffer (DSS Takara Biolab), 2.5 mM MgCl₂ (DSS Takara Biolab) and ddH₂O, using a BIO-RAD Thermal Cycler (T100 Bio-Rad Laboratories, Inc., United States). Amplification was carried for 38 cycles, each consisting of a denaturation step at 95 °C for 1 min, annealing at 46-53 °C for 1 min and extension step at 72 °C for 2 min. PCR products were analyzed using 2.5% agarose electrophoresis gels stained with ethidium bromide.

Table 2: Description of polymorphic ISSR markers used in the genetic diversity analysis.

Sr. No.	Primer Name	Sequence	Annealing Temperature
1.	USB 836	AGAGAGAGAGAGAGAGYA	51 °C
2.	USB 820	GTGTGTGTGTGTGTGTC	53 °C
3.	USB 815	CTCTCTCTCTCTCTCTC	51 °C
4.	USB 811	GAGAGAGAGAGAGAGAC	51 °C
5.	USB 846	CACACACACACACACAT	49 °C
6.	USB 801	ATATATATATATATATT	49 °C
7.	USB 808	AGAGAGAGAGAGAGAGC	46 °C

Scoring of amplified fragments

Frequencies of incidence of all polymorphic alleles for each ISSR markers were calculated and used for determining statistical parameters. The amplified profiles for all the primers was compared with each other and bands of DNA fragments was scored as 1 for presence and 0 for absence, generating 0 and 1 matrix. Percent polymorphism was calculated by using the formula-

$$\text{Percent polymorphism} = \frac{\text{Number of polymorphic bands}}{\text{Total number of bands}} \times 100$$

Analysis of the Amplified Fragment

Pair-wise genetic similarities between chickpea genotypes and polymorphic band was estimated by Jaccard's similarity coefficient. Clustering was done using the symmetric matrix of similarity coefficient and cluster obtained was based on unweighted pair group method for arithmetic mean (UPGMA) using sequential agglomerative hierarchical nested (SAHN) cluster analysis of NTSYS-PC version 2.02 (Rohlf, 1998) [3].

Results and Discussion

Extraction of Genomic DNA and Quantification

Genomic DNA was extracted using CTAB-DNA extraction method. The quantity of DNA was checked by using 1 μl of DNA of each 7 genotype by using Bio-Spectrometer. The quality of the DNA isolated from all the seven varieties were obtained by calculating the ratio of absorbance at 260 and 280 nm. The value varied from 1.763 to 1.964; however most of the value fell in the range of 1.8-2.0, indicating good quality of the isolated DNA. Quantity of all samples was found to be in the range of 150ng/μl to 490 ng/μl. The results of 0.8% agarose gel electrophoresis showed good quality of DNA (Fig. 1).

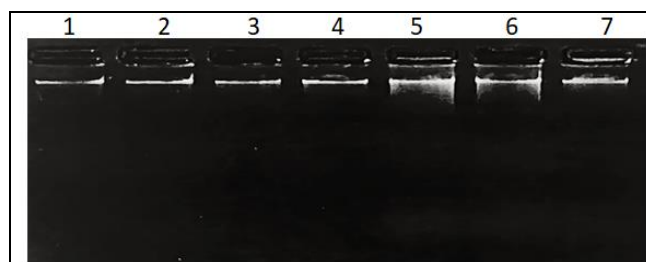


Fig 1: Genomic DNA Isolated from seven different genotypes of chickpea.

1: Phule Vishwaraj, 2: Phule Vikram 3: Phule Vishal 4: Phule Vikrant 5: Phule Vijay 6: Phule Virat 7: Phule Kripa

Detection of SSR polymorphism

DNA fingerprinting database was produced using PCR-based molecular marker (ISSR) system. The seven primer pairs, namely USB 836, USB 820, USB 815, USB 811, USB 846, USB 801, and USB 808 exhibited different levels of polymorphism amongst the seven chickpea varieties under evaluation in the present investigation. A total of twenty seven different loci were assigned to the seven ISSR primers.

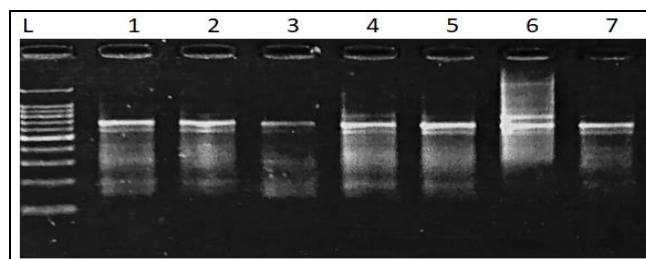


Fig 2: Amplification profile obtained with USB 820 primers detected in chickpea genotypes.

L: 100 bp ladder 1: Phule Vishwaraj, 2: Phule Vikram 3: Phule Vishal 4: Phule Vikrant 5: Phule Vijay 6: Phule Virat 7: Phule Kripa

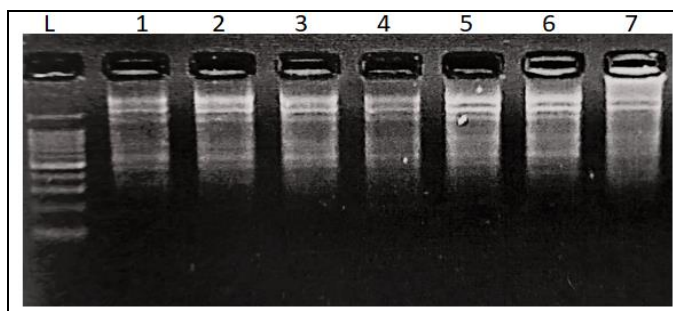


Fig 3: Amplification profile obtained with USB 815 primers detected in chickpea genotypes.

L: 100 bp ladder 1: Phule Vishwaraj, 2: Phule Vikram 3: Phule Vishal 4: Phule Vikrant 5: Phule Vijay 6: Phule Virat 7: Phule Kripa

Table 3: Polymorphism values in chickpea genotypes as revealed by ISSR markers.

Sr. No.	Primer	Total no. of amplicons	Total no. of polymorphic amplicons	Percent polymorphism
1.	USB 836	5	5	100.00%
2.	USB 820	3	1	33.33%
3.	USB 815	5	3	60.00%
4.	USB 811	3	3	100.00%
5.	USB 846	4	4	100.00%
6.	USB 801	5	4	80.00%
7.	USB 808	7	7	100.00%
Total	7	32	27	84.38%

Average number of amplicons per primer = 4.57

Average number of polymorphic amplicons per primer = 3.86

Total percent polymorphism by marker = 84.38%

Analysis of genetic divergence

Allelic diversity data was used to produce a dendrogram in order to elucidate the relationship among seven chickpea genotypes. Presence or absence of each band on the gel was scored and similarity between every pair of entries was

estimated. Genetic Similarity estimate (Jaccard's coefficient) based on ISSR banding pattern used for cluster analysis to present genetic relationship in the form of dendrogram (Fig. 4). Jaccard's coefficient value for 7 chickpea genotypes are presented in following table 4.

Table 4: Jaccard's similarity matrix of seven chickpea genotypes revealed by ISSR analysis.

	Phule Vishwaraj	Phule Vikram	Phule Vishal	Phule Vikrant	Phule Vijay	Phule Virat	Phule Kripa
Phule Vishwaraj	1						
Phule Vikram	0.42	1					
Phule Vishal	0.48	0.40	1				
Phule Vikrant	0.46	0.62	0.56	1			
Phule Vijay	0.53	0.57	0.57	0.61	1		
Phule Virat	0.26	0.47	0.54	0.59	0.48	1	
Phule Kripa	0.42	0.46	0.51	0.55	0.57	0.42	1

The large range of similarity coefficient revealed by ISSR markers provided greater confidence for the assessment of genetic divergence and interrelationship. A perusal of similarity coefficients clearly reflected that a very high degree of similarity exists between chickpea varieties Phule Vikram and Phule Vikrant (0.62). The analysis further revealed that Phule Vishwaraj is the most genetically distinct genotype, as it formed a standalone cluster (Cluster II) and joined the rest of the group at the minimum similarity coefficient of 0.42. This early separation highlights Phule Vishwaraj as a significant source of genetic diversity among the tested samples. The study demonstrates that ISSR markers are highly effective for detecting polymorphism and estimating genetic distance among the selected chickpea genotypes. The large range of similarity coefficient revealed by ISSR markers provided greater confidence for the assessment of genetic divergence and interrelationship. Such characterization is essential for future breeding strategies, as it helps in selecting diverse parents to enhance genetic variability in chickpea improvement programs.

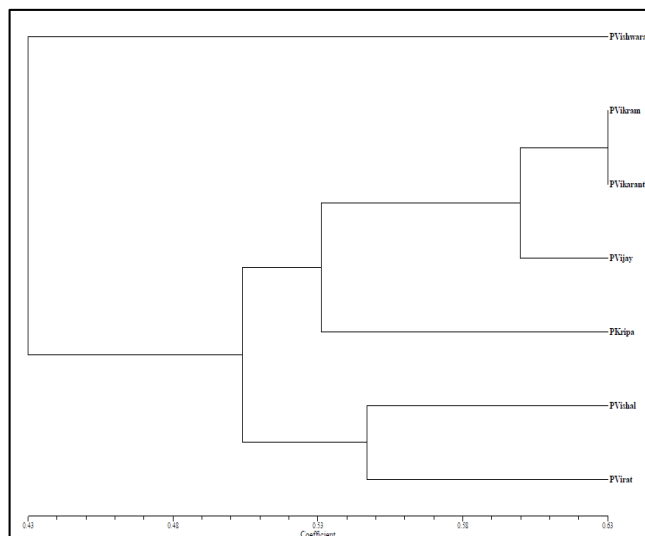


Fig 4: Dendrogram of the seven chickpea genotypes based on the similarity matrix developed using ISSR markers

Conclusion

The molecular analysis and dendrogram interpretation of the seven chickpea genotypes lead to several significant conclusions regarding their genetic architecture. Based on the ISSR marker analysis, it is concluded that Phule Vikram and Phule Vikrant share the highest degree of genetic relationship, exhibiting a maximum similarity coefficient of 0.62. Conversely, Phule Vishwaraj was identified as the most genetically distinct and diverse genotype among the tested samples. It stood alone in Cluster II, separating from the rest of the group at a minimum similarity coefficient of 0.42, which highlights its unique genetic profile.

Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Competing Interests

Authors have declared that no competing interests exist.

References

1. Ghaffari P, Talebi R, Keshavarz F. Genetic diversity and geographical differentiation of Iranian landrace, cultivars and exotic chickpea lines as revealed by morphological and microsatellite markers. *Physiol Mol Biol Plants*. 2014;20(2):225-233.
2. Jannatabadi AA, Talebi R, Armin M, Jamalabadi J, Baghebani N. Genetic diversity of Iranian landrace chickpea (*Cicer arietinum* L.) accessions from different geographical origins as revealed by morphological and sequence tagged microsatellite markers. *J Plant Biochem Biotechnol*. 2014;23(2):225-229.
3. Rohlf FJ. NTSYSpc: Numerical taxonomy and multivariate analysis system. Version 2.0. Setauket (NY): Exeter Software Publishers Ltd.; 1998.
4. Saeed A, Hovsepyan H, Darvishzadeh R, Imtiaz M, Panguluri SK, Nazaryan R. Genetic diversity of Iranian accessions, improved lines of chickpea (*Cicer arietinum* L.) and their wild relatives by using simple sequence repeats. *Plant Mol Biol Rep*. 2011;29:848-858.
5. Talebi R, Fayaz R, Mardi M, Pirsyedi SM, Naji AM. Genetic relationships among chickpea (*Cicer arietinum*) elite lines based on RAPD and agronomic markers. *Int J Agric Biol*. 2008;8:301-305.
6. Talebi R, Naji AM, Fayaz F. Geographical patterns of genetic diversity in cultivated chickpea (*Cicer arietinum* L.) characterized by amplified fragment length polymorphism. *Plant Soil Environ*. 2008;54:447-452.
7. Upadhyaya HD, Dwivedi SL, Baum M, Varshney RK, Udupa SM, Gowda CLL, *et al*. Genetic structure, diversity, and allelic richness in composite collection and reference set in chickpea (*Cicer arietinum* L.). *BMC Plant Biol*. 2008;8:106. doi:10.1186/1471-2229-8-106.