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Assessment of Genetic Diversity in Tomato (*Solanum lycopersicum)* Varieties by Using RAPD Markers

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Assessment of genetic diversity in Tomato (*Solanum lycopersicum)* varieties by using RAPD markers.
Place and Duration of Study: Department of Plant Biotechnology of K. K. Wagh College of Agricultural Biotechnology, Nashik, Maharashtra.
Methodology: Tomato is one of the most important vegetable crops in the world and normally human being daily consumed in diet. The five Varities were collected from Mahalaxmi Hi-Tech Nursery Nashik. The present study was conducted at K K Wagh college of Agricultural Biotechnology Nashik. DNA was isolated by using modified CTAB method Five Varities were analyzed through RAPD markers to determine the molecular characterization at individual level.

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Results: Six arbitrary oligonucleotide primers used in the RAPD-PCR produced a total 38 different marker bands with an average 8 band per primer. Based on the banding pattern 100% polymorphism observed among the tomato varieties. Size range of amplified DNA bands varied from 0.1-10 kb. A total of 14 unique bands were amplified from the genome of the 5 tomato varieties. The dendrogram clustered the tomato varieties into two major clusters. The clusters were subdivided into small groups including the highly related Keshar and 0225, Anisha and Sai-22 varieties. The other Cluster included an isolated variety of Aryaman as a distinctive one. The RAPD data revealed a high genetic polymorphism that can be used to select diverse parents in breeding programs and maintain genetic variation.

Keywords: Tomato; DNA. RNase; gel electrophoresis; RAPD markers and genetic diversity.

1. INTRODUCTION

"Tomato (*Solanum lycopersicum*) is one of the most economically important vegetable crops, which is consumed as fresh and processed products. Tomato is considered a functional and protective food because of its health-beneficial compounds such as vitamins A and C, minerals, and antioxidants mainly lycopene and beta-carotene. Breeding new tomato cultivars with current consumers' demand and processing industrial requirements is very important, which needs wide genetic resources with target traits. Natural genetic variability has led to the development of many varieties with agronomic traits. Initially, induced mutagenesis caused by chemical or physical mutagens has been successfully applied in breeding, but it is quite labor-intensive, cumbersome, and time-consuming. Similarly, conventional breeding also relies upon phenotypic selection and requires a long breeding cycle. Hence, to overcome these issues the availability of genome sequence (900 Mb) allows functional genomics and rapid breeding via dissecting complex traits" (Tomato Genome Consortium, 2012). Furthermore, tomato pan-genomes have been reported for 1000 accessions and 725 cultivated and wild species. Recently, progress in genomics-assisted breeding has been reviewed for accelerated tomato improvement.

"The diploid chromosome number of tomatoes is 2n = 24. However, some varieties have 26 chromosomes, and one commercial hybrid variety has 25. All tomato species are diploid and have similar chromosome number and structure" [1-4]. "There are nine species of tomatoes, which have been grouped into two complexes. There are more than 15,000 known varieties of tomatoes worldwide. These varieties are all of the same species, Solanum lycopersicum. Tomatoes are a member of the nightshade family, which includes more than 3,000 species. Lycopene is a potent antioxidant carotenoid pigment found in tomatoes and other red fruits. In green tissues, lycopene acts as a free-radical scavenger conferring protection during photosynthesis. High-lycopene tomato genotypes containing knockout mutations within the lycopene catabolic CYC-B gene include the spontaneous old-gold (og) or old-gold crimson(ogc) mutations, and the chemically-induced mutation A949G. It has a high level of macro and micro-synteny within this plant family which comprises more than 3000 species among which some are important crops such as the fruit-bearing vegetables tomato, eggplant, and pepper, and the tuber-bearing potato, in addition to several medicinal and ornamental plant. In 2022, India's tomato production was 206.9 lakh tonnes. In 2022-23, production moderated by 0.35% to 206.2 lakh tonnes. India is the third largest producer of tomatoes in the world, contributing 10% of the world's total production. The top five tomato producers are: China, the United States, India, the European Union and Turkey" (APEDA 2022-23). The top tomato producing states in India in 2021-22 were: Madhya Pradesh: 14.63%, Andhra Pradesh: 10.92%, and Karnataka: 10.23% Other major tomato producing states in India include: Odisha, Gujarat, West Bengal, Chhattisgarh, Maharashtra, Bihar, Haryana, Uttar Pradesh, Telangana, Tamil Nadu. Sources: Agricultural and Processed Food Products Export Development Authority (APEDA) 2021-22.

"Tomato is highly susceptible to infection by several viruses transmitted by the *whitefly Bemisia tabaci* (Genn.). A recent survey of tomato-growing regions in SA revealed that infestations of this polyphagous insect pest are widespread and particularly severe in the Limpopo province which is a major tomato-producing region. Tomato yellow leaf curl virus (TYLCV) and an ever-expanding group of related whitefly-transmitted viruses having circular

ssDNA genomes belonging to the genus *Begomovirus* (family *Geminiviridae*) continue to cause devastating losses to the tomato crop worldwide. *Tomato curly stunt virus* is a distinct tomato-infecting monopartite begomovirus species closely related to a group of solanaceous plant host-infecting begomoviruses from sub-Saharan Africa and the Southwest Indian Ocean island" [1].

"Genetic variability is important in plant breeding for crop improvement which should be available from germplasm, the reservoir of variability for different characters The evaluation of genetic variability among and within populations of tomato genotypes can be assessed by using morphological, biochemical and molecular characterization Morphological characterization has been the effective tool used for the improvement of new genotypes over the years where improved plants are developed by solely selecting plants with desirable phenotypes. In crop improvement program evaluation of germplasm is imperative, to the genetic background and the breeding value of the available germplasm However, developing a new improved plant genotype by ways of phenotypic selection can easily exceed 10 years and is highly dependent on the environment for expression, hence, their ability to estimate genetic diversity in plants is reduced. Genetic parameters and character associations provide information about the expected response of various characters to selection and it will help in developing the optimum breeding procedure" [2,3].

"The development of DNA (molecular) markers has enhanced plant genetics and plant breeding. These molecular markers are effective tools for efficient selection of desired agronomic traits because they are based on the plant genotypes and are independent of environmental variation. While there are several applications of DNA markers in breeding, the most promising for cultivar development is marker-assisted selection (MAS)" (Pervaiz *et al*., 2009). "Estimating genetic variation in tomato landrace and cultivar collections using several molecular techniques including Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeats (SSR) is more efficient. Random Amplified Polymorphic DNA (RAPD) is a highly reproducible, locus-specific, high proportion of single-fragment amplification, high sensitivity that allows distinctions even between closely related individuals and cost-effectiveness" (Dongre and Parkhi, 2005).

To achieve sustainable agriculture and obtain high-quality products in terms of food safety, the use of resistant varieties has been implemented as a high-impact tool to reduce the damage caused by pathogens. This can be achieved by genetic improvement through molecular markers, which allow the detection and identification of genes of interest in plants at early developmental stages, taking less time than conventional morphological markers. Moreover, it is not affected by epigenetic changes such as DNA methylation, histone modification and microRNAs. Different types of techniques are used to detect molecular markers and allow the analysis of variation in the DNA molecule; restriction, and different types of Polymerase Chain Reaction (PCR) are some of them, with multiplex PCR being one of the most frequently used to identify molecular markers by using primer sets within a single PCR mix to produce amplicons of different sizes [4-8].

2. MATERIALS AND METHODS

2.1 Plant Material

Five tomato Varieties, namely L1, L2, L3, L4, and L5 obtained from Mahalaxmi Hi- Tech Nursery, Nashik, Maharashtra.

2.2 DNA Isolation and Purification

The DNA was isolated from each plant sample by using the CTAB DNA extraction protocol. [Doyle and Doyle (1990)].

Before extraction, all the required materials viz., CTAB buffer pestle and mortar, spatula, scissors, Eppendorf tubes, and tips were autoclaved. Tomato leaves (1- 2 g) were crushed into a fine mixture with pre-warmed CTAB buffer in a mortar and pestle. The samples were suspended in 1ml pre-warmed extraction buffer (2 % CTAB), 100mM Tris-HCl (pH 8), 20 mM EDTA, and 1.4 M NaCl and incubated at 60℃ for 1 hr. Tubes were cooled to room temperature and centrifuged at 10,000 rpm for 10 min. The supernatant was transferred to fresh tubes and an equal volume of Phenol: Chloroform: Isoamyl alcohol (25:24:1) was added. The mixtures were inverted several times to allow mixing and centrifuged at 10,000 rpm for 10 min at 4℃. To achieve maximum deproteination the upper aqueous phase was

transferred into a fresh tube and an equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added and centrifuged again at 10,000 rpm for 10 min at 4°C. The upper aqueous phase was transferred to a fresh tube and an equal volume of chilled isopropanol was added to precipitate the DNA. The tubes were gently inverted several times until precipitation occurred and incubated at –20°C overnight. To get a pellet, the DNA tubes were spun at 10000 rpm for 10 min at 4°C. The supernatant was discarded and the pellets were washed with 70 % ethanol. The pellets were dried and dissolved in the required quantity of 1X TE Buffer. Dissolved DNA samples were stored at 4°C for further analysis. The quality of isolated DNA was evaluated through agarose gel electrophoresis. 1X TAE buffer was prepared from the 50X TAE stock solution. Agarose (0.8%) was weighed and dissolved in TAE buffer by boiling. Then Ethidium Bromide was added at a concentration of 2-3µl/50 ml TAE and mixed well. The open end of the gel casting tray was sealed with cello tape and kept on a horizontally leveled surface. The comb was inserted desirably and the dissolved agarose was poured onto the tray. The gel was allowed to sit for 20-25 minutes after which the comb was removed carefully [9-15]. The tray was kept in the electrophoresis unit with the good side directed towards the cathode. 1X TAE buffer was added to the tank. Then DNA sample (3 µl) and the loading dye (2 µl) were loaded into the wells using a micropipette carefully. After closing the tank, the anode and cathode ends were connected to the power pack, and the gel was run at a constant voltage (65 V) and current (50 A). The power pack was turned off when the loading dye reached the 2/3rd length of the gel. Then the gel was taken from the electrophoresis unit, viewed under a UV trans- illuminator, or documented in the Gel Documentation System.

Then quantification of DNA Take 100 µl TE buffer in a cuvette and calibrate the BioSpectrometer at 260 nm and 280 nm. Added 1 µl of each DNA sample to 99 µl TE (Tris – EDTA buffer) and mixed it well. TE buffer was used as a blank in the other cuvette of the spectrophotometer. Take OD260 and OD280 values on a BioSpectrometer. Calculate the OD260 and OD280 values. The ratio between readings at 260 nm and 280 nm (OD260 / OD280) provided an estimate for the purity of nucleic acid. Any sample showing a ratio below 1.8 or above 2.0 was further subjected to purification. The Ratio between 1.8-2.0 denotes that absorption in the UV range is due to nucleic acids. The ratio lower than 1.8 indicates that the presence of protein. A ratio higher than 2.0 indicates that the sample may be contaminated with Chloroform, phenol, or RNA.

2.3 RAPD Amplification

In total, 6 RAPD primers were used for the study. The PCR reaction was carried out using a BIO-RAD Thermal Cycler (T100 Bio-Rad Laboratories, Inc., United States). The PCR reactions were repeated one time for each primer to ensure reproducibility. Amplified products were separated on 1.5% agarose gels in 1x TAE buffer and visualized with ethidium bromide staining [16-20].

2.4 Agarose Gel Electrophoresis

Amplified products of RAPD were analyzed in 2.0 % Agarose in 1X TAE buffer and the bands were visualized on gel a Gel Doc system and documented.

2.5 Data Analysis and Scoring

Only well-separated and intense alleles that were observed in all independent amplifications were selected for scoring. Each RAPD allele was considered a character and the presence or absence of the allele was scored in binary code (present = 1, absent = 0). Based on the allele data, Dendrogram was created using the PAST 4.03 software.

3. RESULTS AND DISCUSSION

3.1 RAPD Analysis

A total of 14 RAPD alleles were scored from the selected 5 RAPD primers, out of which 100% RAPD i.e., All primers (OPA- 01, OPA-02, OPA-05, OPA-09, OPA-10 and OPD-13) are 100%. The amplification profile generated by the RAPD primer for 5 Tomato varieties.

To compare the efficiency of primer, polymorphic information content (PIC) was calculated by using the formula.

$$PIC = 2f (1-f)$$

Where f is the frequency of the present allele for the RAPD marker.

3.2 RAPD-Based Cluster Analysis

A genetic similarity matrix was constructed using the Jaccard coefficient. Based on the proximity

matrix obtained from Jaccard's coefficient PAleontological STatistics (PAST 4.03) clustering was done using Unweighted Pair Group Method with Arithmetic Averages (UPGMA) method. The dendrogram is grouped into two main clusters based on the reference line drawn at a similarity coefficient. Two main clusters are formed in dendrogram cluster A and cluster B. Cluster A consists of four varities namely Keshar, 0225, Anisha and Sai-22. While cluster B consist of one varities namely Aryaman [21-25].

Table 1. RAPD amplification 6 RAPD primers were used for the study

Sr. No	RAPD Primer	Sequence	Temperature
1.	OPA- 01	5'-CAGGCCCTTC-3'	34 ^{o}C
2.	OPA- 02	5'-TGCCGAGCTC-3'	34 ^{o}C
3.	OPA- 05	5'-AGGGGTCTTG-3'	36 ^{o}C
4.	OPA- 09	5'-GGGTAACGCC-3'	34 ^{o}C
5.	OPA-10	5'-GTCATCGCAG-3'	28 ^{o}C
6.	OPD-13	5'-GGGGTGACGA-3'	34 ^{o}C

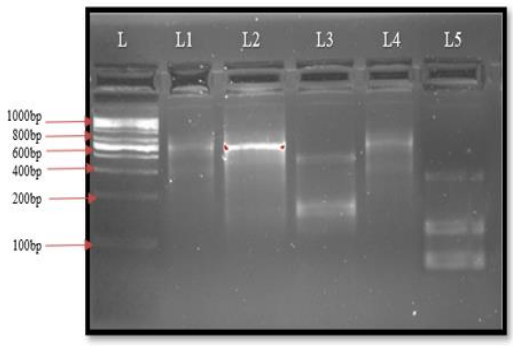
Table 2. The primer names, amplified DNA bands, and polymorphism percentages generated by Random Amplified Polymorphic DNA (RAPD)

Marker	Primer	MB	PB	TAB	PIC	P (%)
	OPA- 01	0	3	3	0.32	100%
	OPA- 02	0	1	1	0.21	100%
	OPA- 05	0	3	3	0.46	100%
RAPD	OPA- 09	0	3	3	0.22	100%
	OPA-10	0	2	2	0.38	100%
	OPD-13	0	2	2	0.46	100%
		0	14	14		100%

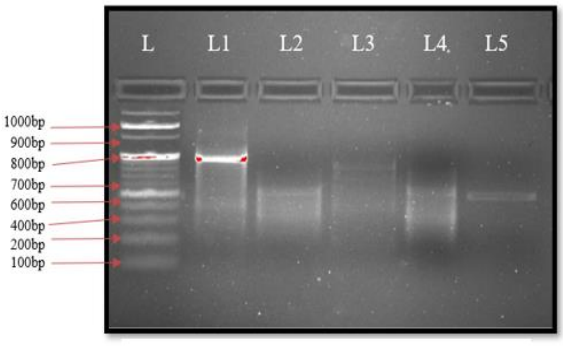
MB: Monomorphic band, PB: Polymorphic band, TAB: Total amplified bands, PIC: Polymorphic information content, %P: Percentage of polymorphism.

Table 3. Results of cluster analysis

Variety	Aryaman	Keshar	Anisha	0225	Sai-22
OPA-01	1.000	0.066	0.055	0.066	0
OPA-02	0.666	1.00	0.307	0.4	0.083
OPA-05	0.055	0.307	1.00	0.307	0.454
OPA-09	0.066	0.4	0.307	1.00	0181
OPA-10	0	0.833	0.454	0.181	1.00
OPD-13	0.66	0.38	0.48	0.48	0.48



Result of OPA-01 Primer



Result of OPA-02 Primer

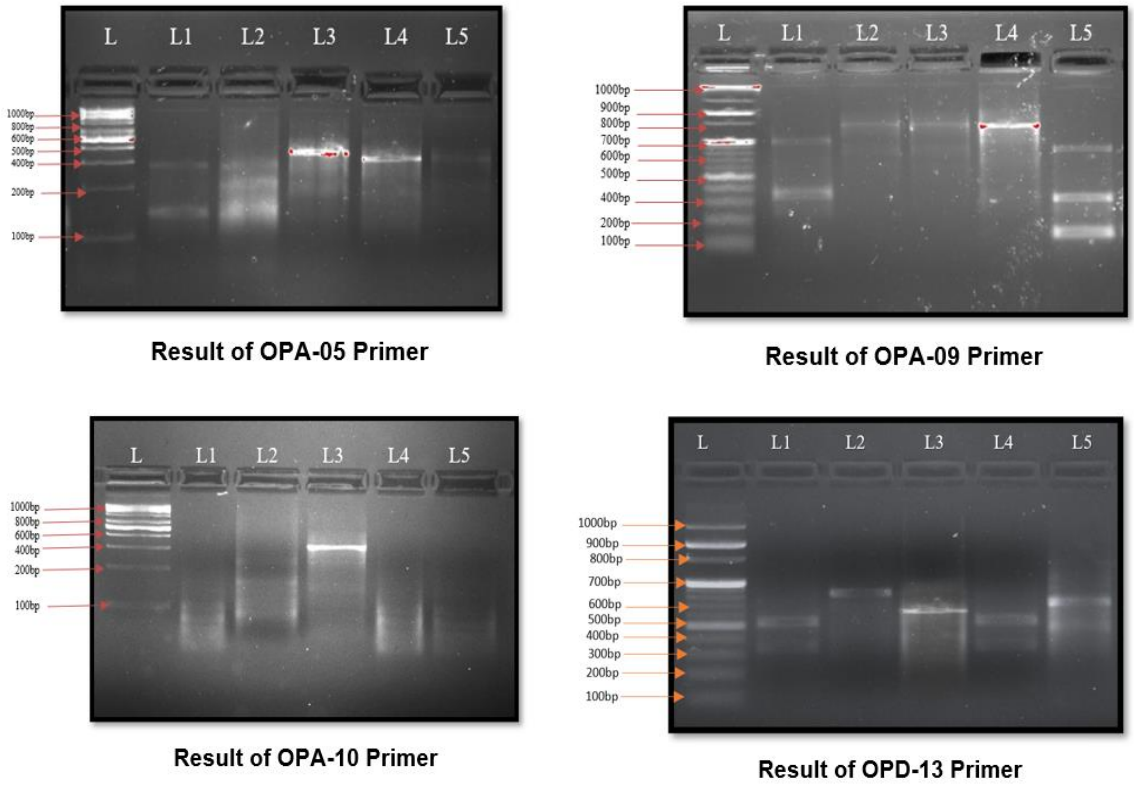


Plate 1. Gel Photograph showing RAPD allelic profile of Tomato Varities

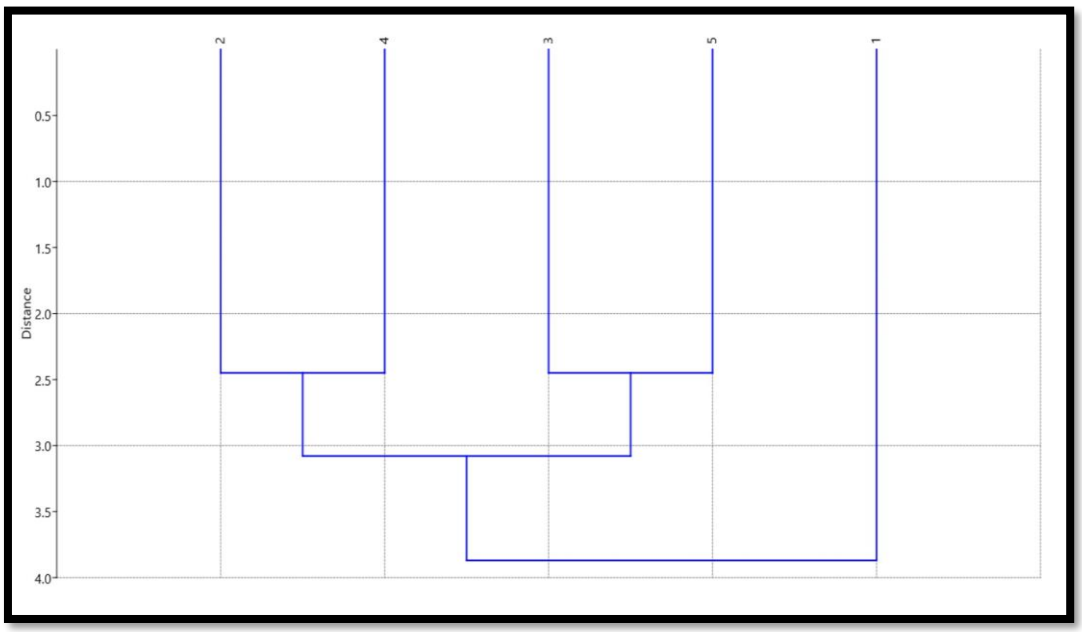


Fig. 1. Dendrogram of RAPD marker

4. CONCLUSION

Six RAPD primers were screened 6 of them showed good amplification with Five tomato varieties. A total of 46 bands were generated and 14 are polymorphic. The Polymorphic percentage was 100%. PCR condition is important for the amplification of specific loci with specific primers,

especially annealing temperature. A total of six RAPD primers were used for the study of 6 RAPD primers (OPA-01, OPA-02, OPA-05, OPA-09, OPA-10 and OPD-13) showed significant amplification. The tomato cultivars were grouped into two main groups by the dendrogram. The closely related Keshar and 0225, Anisha, and Sai-22 types were among the smaller groups that emerged from the division of the clusters. One unique Aryaman type was separated and included in the other Cluster. The current study concludes that the PCR-based fingerprinting method known as RAPD is useful for identifying the pattern of genetic links between tomato cultivars and for measuring the degree of genetic diversity.

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 writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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