

Development and Characterisation of Novel Microsatellite (SSR) Markers in Tomato (*Solanum lycopersicum* L.)

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Abstract: Tomatoes are one of the most significant vegetable crops grown worldwide due to their nutritional worth and economic relevance. Continuous genetic progress necessitates the use of dependable molecular techniques to measure genetic diversity and aid breeding efforts. Microsatellites, also known as simple sequence repeat (SSR) markers, are PCR-based, highly polymorphic, co-dominant markers that are frequently employed in plant genetic studies. The current work produced and characterised new SSR markers in tomato utilising a variety of farmed and wild accessions. Genomic DNA was extracted using a modified CTAB technique, and SSR loci were discovered by genome screening and primer design. The proposed markers generated unambiguous and consistent amplification patterns while revealing considerable allelic diversity between genotypes. These SSR markers are useful tools for genetic diversity studies, cultivar identification, and marker-assisted selection in tomato breeding efforts.

Keywords: SSR Markers, Genetic Diversity, Tomato, *Solanum Lycopersicum*, Molecular Breeding.

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I. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most widely cultivated and economically important vegetable crops worldwide, valued for its adaptability, high yield, and rich nutritional content, including vitamin C, provitamin A, lycopene, and β -carotene [1,2]. Continuous genetic improvement is essential to enhance productivity, fruit quality, and resistance to biotic and abiotic stresses. Traditional evaluation of genetic variability based on morphological traits is often influenced by environmental factors and genotype $\times$ environment interactions, limiting its precision [3,4]. Molecular markers provide a reliable and environment-independent approach for assessing genetic diversity and population structure. Among them, simple sequence repeats (SSRs) are highly polymorphic, co-dominant, reproducible, and widely distributed across the genome, making them effective tools for genetic diversity analysis, cultivar identification, QTL mapping, and marker-assisted selection [5–8]. Although many SSR markers have been developed in tomato, additional genome-wide and

highly informative markers are needed to enhance breeding efficiency and germplasm characterisation [9,10].

Therefore, the present study aims to develop and characterise novel tomato-specific SSR markers to facilitate precise genetic diversity analysis and support advanced molecular breeding programs.

II. PLANT MATERIAL

Twenty accessions of *Solanum lycopersicum* L. representing diverse genetic backgrounds were selected for the present investigation. The accessions were maintained under field or greenhouse conditions, depending on the experimental requirements, and standard agronomic practices were followed to promote healthy and uniform plant growth. Young, fresh, and actively developing leaves were collected from each accession for genomic DNA isolation. Leaves exhibiting visible symptoms of disease, insect infestation, or mechanical injury were excluded to minimize the possibility of contamination and to ensure the suitability of the material for molecular analysis.

The collected leaf samples were thoroughly washed with distilled water to remove adhering soil, dust, and other external contaminants. Excess moisture was removed using sterile blotting paper, and the cleaned samples were either processed immediately or preserved at -80°C until DNA extraction. The low-temperature storage conditions were maintained to minimize degradation and preserve the integrity of the samples prior to molecular analysis.

➤ Genomic DNA Extraction

Genomic DNA was isolated from the collected leaf tissues using a modified cetyltrimethylammonium bromide (CTAB) method. Prior to extraction, the young leaf material was finely ground to a powder under liquid-nitrogen conditions. The powdered tissue was then subjected to extraction using a CTAB-based buffer containing Tris-HCl (pH 8.0), ethylenediaminetetraacetic acid (EDTA), sodium chloride (NaCl), and CTAB.

Following extraction, the lysate was treated with a chloroform alcohol mixture (24:1) to facilitate separation of the aqueous and organic phases. The aqueous phase containing nucleic acids was carefully recovered, and genomic DNA was precipitated using chilled isopropanol. The resulting DNA was collected as a pellet and washed with **70% ethanol** to remove residual salts and other impurities.

The DNA pellet was subsequently air-dried and dissolved in TE buffer for further analysis. The concentration and quality of the extracted genomic DNA were assessed using spectrophotometric measurements, while its integrity was examined by agarose gel electrophoresis. DNA samples showing satisfactory quality and integrity were considered suitable for subsequent molecular characterisation.

III. METHODS

➤ Sample Preparation and Surface Cleaning

Young, actively developing leaves were collected from each selected accession of *Solanum lycopersicum* L. The harvested leaves were initially washed thoroughly under running tap water to remove adhering soil, dust, and other visible contaminants. This was followed by rinsing with double-distilled water to eliminate residual surface impurities. Prior to genomic DNA extraction, the washed leaf samples were gently blotted with sterile blotting paper to remove excess moisture.

➤ Genomic DNA Extraction

Approximately 200 mg of fresh leaf tissue from each accession was finely ground to a powder in liquid nitrogen using a sterile mortar and pestle. The powdered tissue was immediately transferred into a sterile microcentrifuge tube and mixed thoroughly with pre-warmed CTAB extraction buffer. The homogenized samples were incubated at 65°C for 60–90 min to facilitate cell disruption and release of genomic DNA.

After incubation, an equal volume of chloroform alcohol (24:1) was added to the extracts and mixed carefully to facilitate separation of cellular components. Following

phase separation, the aqueous phase was recovered and used for DNA precipitation. Genomic DNA was precipitated using chilled ethanol in the presence of sodium acetate. The resulting DNA pellet was collected and washed with 70% ethanol to remove residual salts and impurities. The pellet was subsequently dried and dissolved in TE buffer for further molecular analysis.

The concentration and quality of the extracted DNA were evaluated by spectrophotometric analysis, while DNA integrity was examined through agarose gel electrophoresis. DNA preparations showing satisfactory concentration, purity, and integrity were used for subsequent SSR-based molecular analysis.

➤ Identification of SSR Loci

The tomato genome was examined using appropriate bioinformatics approaches to identify regions containing simple sequence repeat (SSR) motifs. Candidate SSR loci were selected based on the presence of suitable repeat sequences, and primers flanking the identified regions were designed for amplification. During primer development, parameters including GC content, melting temperature, expected amplicon size, and primer specificity were considered to obtain reliable and reproducible amplification.

➤ PCR Amplification

SSR amplification was carried out in a total reaction volume of **25 μL** containing template genomic DNA, forward and reverse primers, dNTPs, MgCl_2 , PCR buffer, and Taq DNA polymerase. The amplification program consisted of an initial denaturation step followed by 30–35 cycles of denaturation, primer annealing, and extension under optimized thermal conditions.

The amplified PCR products were separated by electrophoresis on 3% agarose gel prepared in $1\times$ TBE buffer. Fragment sizes were estimated by comparison with an appropriate molecular-weight DNA ladder. Distinct and reproducible amplification bands were recorded as allelic variants at the corresponding SSR loci. The number of alleles detected at each locus was determined to evaluate the extent of marker polymorphism. SSR loci producing consistent amplification and clear polymorphic profiles were retained for subsequent genetic analysis [14,6].

The informativeness of the SSR markers was assessed by calculating the Polymorphism Information Content (PIC) according to established procedures [15]. Expected heterozygosity (H_e) was also estimated to quantify genetic diversity among the studied accessions [16]. Standard population-genetic equations and procedures were followed for the calculation of genetic parameters [17,18].

Based on the observed SSR allele-frequency data, a genetic similarity matrix was generated. Cluster analysis was subsequently performed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) to assess genetic relationships among the tomato accessions. The resulting relationships were represented graphically as a dendrogram [19,6,4].

➤ Analysis of Genetic Diversity and Polymorphism Using SSR Markers

The SSR markers evaluated in the present study exhibited different levels of polymorphism among the tomato accessions (Table 1; Fig. 1). The number of alleles detected per locus varied from 2 to 5, with a mean of 3.6 alleles per marker, demonstrating appreciable genetic variation within the studied material [6,5]. Among the loci examined, SSR-03 displayed the greatest allelic richness, with five alleles, whereas SSR-04 produced two alleles. The higher number of alleles observed at individual loci indicates greater discriminatory potential and genetic variation among the accessions [6,5].

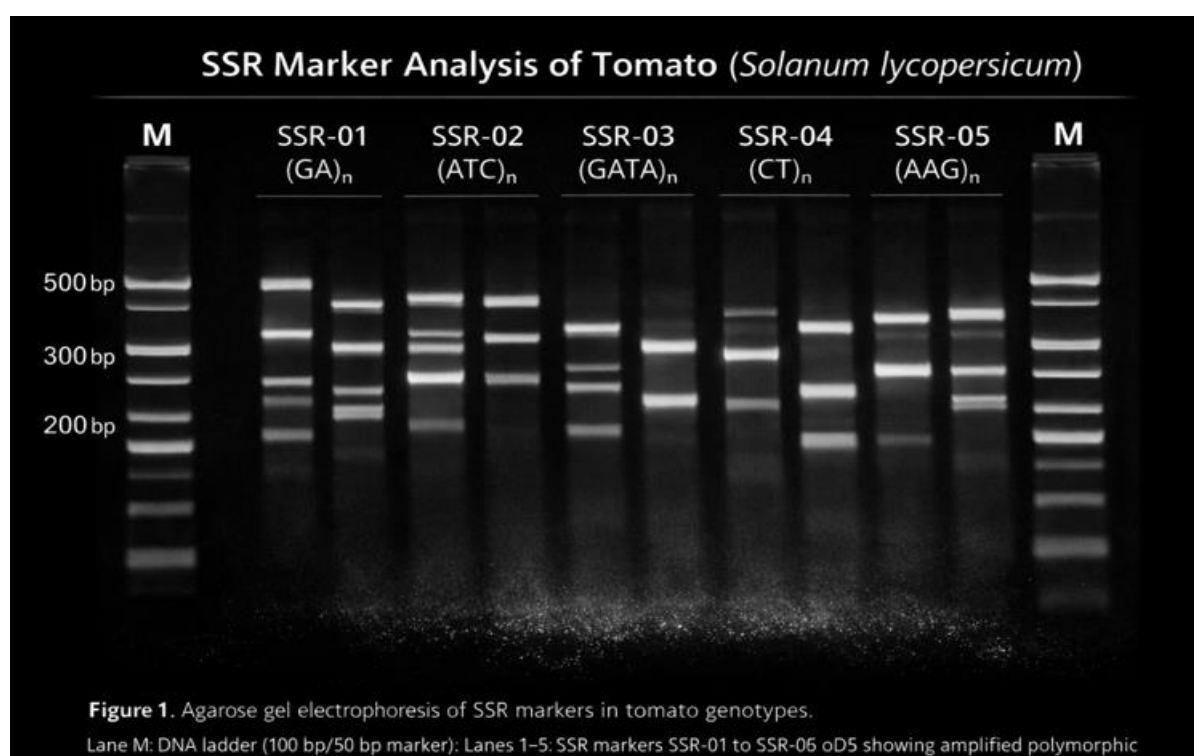
The PIC values ranged from 0.42 to 0.74 across the evaluated SSR loci. Among the markers, SSR-03 exhibited the highest PIC value (0.74), followed by SSR-01 (0.68) and SSR-05 (0.66), indicating relatively high discriminatory ability. In contrast, SSR-04 recorded a lower PIC value of 0.42 [15]. Since SSR markers with PIC values greater than 0.50 are generally regarded as highly informative, most of the loci evaluated in the present study demonstrated considerable usefulness for distinguishing genetically variable accessions [15].

Expected heterozygosity (H_e) varied from 0.48 to 0.79, reflecting differences in gene diversity among the accessions. The maximum H_e value was recorded for SSR-03 (0.79), whereas SSR-04 showed the minimum value (0.48) [16]. The observed relationship between allelic richness, PIC, and heterozygosity was broadly consistent with findings reported in previous studies involving SSR-based characterization of tomato germplasm [9,20].

The amplified SSR fragments ranged in size from approximately 100 to 210 bp, providing suitable fragment sizes for PCR amplification and electrophoretic resolution [14]. Overall, the SSR markers evaluated in this study demonstrated moderate to high levels of polymorphism and successfully discriminated among the cultivated and wild tomato accessions. Their observed allelic diversity and PIC values were comparable with, and in some cases higher than, those reported for previously developed tomato SSR markers. These findings support the potential application of the selected SSR markers in genetic diversity assessment, germplasm characterisation, and marker-assisted breeding of tomato [1,5].

Table:1 Genetic diversity parameters of SSR markers in tomato accessions

SSR Marker	Motif Type	No. of Alleles	Allele Size Range (bp)	PIC Value	Expected Heterozygosity (H_e)
SSR-01	(GA) _n	4	120–150	0.68	0.72
SSR-02	(ATC) _n	3	180–210	0.55	0.61
SSR-03	(GATA) _n	5	140–190	0.74	0.79
SSR-04	(CT) _n	2	100–120	0.42	0.48
SSR-05	(AAG) _n	4	160–200	0.66	0.7



IV. EXPERIMENTAL METHODOLOGY

Young and healthy leaves were collected from twenty accessions of *Solanum lycopersicum* L. for molecular analysis. The collected leaves were washed thoroughly first with running tap water and subsequently with distilled water to remove dust and other adhering contaminants. The washed material was gently dried using sterile blotting paper. The samples were either processed immediately for genomic DNA extraction or preserved at -80°C until further use.

For genomic DNA extraction, approximately 200 mg of leaf tissue was finely ground in liquid nitrogen using a sterile mortar and pestle. The powdered tissue was transferred into a sterile microcentrifuge tube, and pre-warmed CTAB extraction buffer was added. The samples were incubated at 65°C for 60–90 min to facilitate cell lysis and release of genomic DNA. The CTAB extraction buffer consisted of Tris-HCl (pH 8.0), NaCl, EDTA, CTAB, PVP, and β -mercaptoethanol, prepared using molecular-grade water. The buffer was maintained at 65°C before use.

Following incubation, the homogenized lysate was mixed with chloroform alcohol (24:1) to separate proteins and other cellular components from the nucleic-acid-containing aqueous phase. The mixture was centrifuged at 12,000 rpm for 8 min, after which the upper aqueous phase was carefully transferred to a fresh microcentrifuge tube. The extraction step was repeated where necessary to improve DNA purity and remove residual contaminants.

Genomic DNA was precipitated by adding sodium acetate followed by chilled 100% ethanol. The samples were maintained at -20°C for 2–3 h to facilitate DNA precipitation and were subsequently centrifuged to recover the DNA pellet. The pellet was washed with 70% ethanol to remove residual salts and impurities, followed by gentle air-drying. The dried DNA pellet was finally dissolved in TE buffer and stored appropriately for subsequent molecular analysis.

For preparation of $1\times$ TE buffer, the required quantities of Tris-HCl and EDTA were dissolved in distilled water, and the final volume was adjusted to 100 mL. The extracted genomic DNA was evaluated for integrity and quality using agarose gel electrophoresis, while its concentration and purity were determined spectrophotometrically. DNA samples suitable for PCR analysis were adjusted to an approximate concentration of 20 ng/ μL .

SSR loci were identified by screening the tomato genome using suitable bioinformatics tools. The analysis focused on the detection of simple sequence repeat motifs, including (GA) $_n$, (ATC) $_n$, (GATA) $_n$, (CT) $_n$, and (AAG) $_n$. Suitable regions containing SSR motifs were selected for primer development. Primers flanking the identified SSR regions were designed according to appropriate criteria, including primer length, GC content, melting temperature, specificity, and expected amplicon size. The synthesized primers were dissolved in sterile water or TE buffer and diluted to a working concentration of 10 pmol/ μL .

PCR amplification was performed in a final reaction volume of 25 μL . The reaction mixture contained template genomic DNA, forward and reverse SSR primers, dNTPs, MgCl_2 , PCR buffer, and Taq DNA polymerase in appropriate proportions. Amplification was carried out using a thermal cycler with an initial denaturation step, followed by 30–35 cycles consisting of denaturation, primer annealing, and extension. A final extension step was included at the end of the amplification program.

The amplified PCR products were separated by electrophoresis using a 3% agarose gel prepared in $1\times$ TBE buffer. Following electrophoresis, the DNA fragments were visualized after staining with ethidium bromide under UV illumination. Fragment sizes were determined by comparison with an appropriate DNA molecular-weight marker, and clear, reproducible bands were recorded for subsequent SSR analysis.

The $10\times$ TBE stock solution was prepared using Tris base, boric acid, and Na $_2$ EDTA dissolved in distilled water. The resulting stock solution was diluted tenfold with distilled water to obtain the required $1\times$ TBE working solution for agarose gel electrophoresis.

V. CONCLUSION

The present study established a systematic workflow for the molecular characterization of twenty *Solanum lycopersicum* L. accessions using SSR markers. High-quality genomic DNA was obtained from young leaf tissues through a modified CTAB-based extraction procedure, followed by quality and concentration assessment using agarose gel electrophoresis and spectrophotometry. SSR loci were identified through bioinformatics analysis of the tomato genome, and suitable primers were designed for their amplification. PCR-based SSR profiling enabled the detection of allelic variation among the selected accessions. The resulting molecular profiles provide useful information for assessing genetic diversity and relationships within the studied tomato germplasm. Overall, the approach demonstrates the usefulness of SSR markers as reliable molecular tools for germplasm characterization, genetic diversity assessment, accession identification, and supporting future tomato breeding and conservation programmes.

REFERENCES

- [1]. Foolad, M. R. (2007). Genome mapping and molecular breeding of tomato. *International Journal of Plant Genomics*, 2007, 1–52.
- [2]. Willcox, J. K., Catignani, G. L., & Lazarus, S. (2003). Tomatoes and cardiovascular health. *Critical Reviews in Food Science and Nutrition*, 43, 1–18.
- [3]. Semagn, K., Bjørnstad, Å., & Ndjiondjop, M. N. (2006). An overview of molecular marker methods for plants. *African Journal of Biotechnology*, 5, 2540–2568.

- [4]. Mohammadi, S. A., & Prasanna, B. M. (2003). Analysis of genetic diversity in crop plants—salient statistical tools and considerations. *Crop Science*, 43, 1235–1248.
- [5]. Kalia, R. K., Rai, M. K., Kalia, S., Singh, R., & Dhawan, A. K. (2011). Microsatellite markers: An overview of the recent progress in plants. *Euphytica*, 177, 309–334.
- [6]. Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J., Tingey, S., & Rafalski, A. (1996). The utility of RFLP, RAPD, AFLP and SSR markers for germplasm analysis. *Molecular Breeding*, 2, 225–238.
- [7]. Collard, B. C. Y., & Mackill, D. J. (2008). Marker-assisted selection: An approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society B*, 363, 557–572.
- [8]. Gupta, P. K., & Varshney, R. K. (2000). The development and use of microsatellite markers for genetic analysis and plant breeding. *Euphytica*, 113, 163–185.
- [9]. Muzzalupo, I., & Perri, E. (2014). Molecular markers and their applications in crop breeding. *Plant Breeding*, 133, 1–12.
- [10]. Varshney, R. K., Graner, A., & Sorrells, M. E. (2005). Genomic-assisted breeding for crop improvement. *Trends in Plant Science*, 10, 621–630.
- [11]. Thiel, T., Michalek, W., Varshney, R. K., & Graner, A. (2003). Exploiting EST databases for the development and characterisation of gene-derived SSR markers in barley. *Theoretical and Applied Genetics*, 106, 411–422.
- [12]. Beier, S., Thiel, T., Münch, T., Scholz, U., & Mascher, M. (2017). MISA-web: A web server for microsatellite prediction. *Bioinformatics*, 33, 2583–2585.
- [13]. Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., & Rozen, S. G. (2012). Primer3—new capabilities and interfaces. *Nucleic Acids Research*, 40, e115.
- [14]. Gupta, P. K., Balyan, H. S., Sharma, P. C., & Ramesh, B. (1996). Microsatellites in plants: A new class of molecular markers. *Current Science*, 70, 45–54.
- [15]. Botstein, D., White, R. L., Skolnick, M., & Davis, R. W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics*, 32, 314–331.
- [16]. Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences USA*, 70, 3321–3323.
- [17]. Weir, B. S. (1996). *Genetic Data Analysis II*. Sinauer Associates.
- [18]. Peakall, R., & Smouse, P. E. (2012). GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research. *Bioinformatics*, 28, 2537–2539.
- [19]. Sneath, P. H. A., & Sokal, R. R. (1973). *Numerical Taxonomy: The Principles and Practice of Numerical Classification*. W.H. Freeman.
- [20]. Shirasawa, K., Hirakawa, H., Nunome, T., Tabata, S., & Isobe, S. (2016). Genome-wide survey of simple sequence repeats in tomato and development of SSR markers for genetic analysis. *BMC Plant Biology*, 16, 1–13. <https://doi.org/10.1186/s12870-016-0709-6>