Journal of
Current Opinion in Crop Science

Journal homepage: www.jcocs.com; ISSN(O):2583-0392

REVIEW ARTICLE

DNA marker development and assay execution: a practice-oriented perspective

V. G. Shobhana*[1] and K. Ashokkumar[2]

[1]Department of Plant Molecular Biology and Biotechnology, CPMBB, Tamil Nadu Agricultural University, Coimbatore, TN, India; [2]Department of Genetics and Plant Breeding, School of Agriculture and Animal Sciences, Gandhigram Rural Institute- Deemed to be University, Gandhigram, Dindigul, TN, India.

Edited by:

M.S. Jeberson, PhD., Agricultural University Jodhpur, Jodhpur, Rajasthan, India.

Reviewed by:

G. Amente, PhD., Addis Ababa University, Addis Ababa, Ethiopia; A. Karthikeyan, PhD., Jeju National University, Jeju, South Korea.

Article history:

Received: June 01, 2025
Accepted: June 27, 2025
Published: June 30, 2025

Citation:

Shobhana, V. G., & Ashokkumar, K. (2025). DNA marker development and assay execution: a practice-oriented perspective. *Journal of Current Opinion in Crop Science*, *6*(2), 146-156.
https://doi.org/10.62773/jcocs.v6i2.329

*Corresponding author e-mail address:
vgshobhana@gmail.com
(V. G. Shobhana)

ABSTRACT

DNA markers serve as indispensable tools in molecular biology, enabling precise identification of genetic traits, assessment of genetic diversity, and implementation of marker-assisted selection across a wide range of organisms. This review presents a comprehensive, practice-oriented framework for DNA marker development and assay execution, encompassing primer design, PCR optimization, validation, and downstream data analysis. Emphasis is placed on the selection of informative genomic regions, evaluation of polymorphism through gel and capillary electrophoresis, and standardization of assay conditions to ensure reproducibility. The workflow integrates troubleshooting protocols and quality control measures to enhance genotyping accuracy and reliability. Applications of DNA markers in crop improvement, functional genomics, and biodiversity conservation are highlighted, demonstrating their versatility in plant breeding, phylogenetic analysis, and conservation of genetic resources.

Keywords: DNA markers; gel electrophoresis; MegaSSR; microsatellites; MISA; PCR; primer designing; SSR analysis tool (SAT)

INTRODUCTION

DNA markers are segments of DNA with known locations on chromosomes that can be used to identify individuals or species and detect genetic variation. They have revolutionized molecular biology by serving as robust tools for genotype fingerprinting, trait selection, evolutionary mapping, and species identification

(Collard et al., 2005; Amiteye, 2021). Microsatellite DNA markers, also known as Simple Sequence Repeats (SSRs), are short tandemly repeated nucleotide motifs (1–6 base pairs) widely distributed throughout eukaryotic genomes. Their high polymorphism, co-dominant inheritance, and reproducibility make them powerful tools for genetic analysis, species authentication, and population studies (Gupta et al., 1996; Tóth et al., 2000; Gupta & Varshney, 2000). Their development typically involves mining repeat motifs from genomic sequences, designing specific primers flanking the repeat regions, and validating them through PCR-based assays. SSR assays are particularly advantageous in plant molecular biology due to their capacity to resolve intra-species variation and establish phylogenetic relationships (Yang et al., 2016; Serrote et al., 2020). Also, the development and assay of microsatellite markers have become integral to molecular biology, enabling researchers to explore genetic diversity, construct linkage maps, and support marker-assisted selection (Kantartzi, 2013; Dubey, 2021; Zargar et al., 2015).

SSR assays typically involve primer design flanking conserved regions, PCR amplification, and allele scoring through gel or capillary electrophoresis. These steps enable researchers to detect polymorphisms, assess genetic diversity, and construct high-resolution genetic maps (Gil et al., 2017; Du et al., 2012). Moreover, SSR markers have been successfully applied in conservation biology to monitor genetic erosion, guide germplasm preservation, and support sustainable breeding programs (Wei et al., 2017; Nadeem et al., 2018; Wang, 2020; Priyanka, 2021).

The broader implications of practicing DNA marker development and assay extend into conservation biology, breeding strategies, and biotechnology. SSR markers enable researchers to monitor genetic erosion (Hu et al., 2023), protect endangered medicinal taxa (Wang, 2020; Su et al., 2025), and design informed breeding programs aimed at enhancing complex yield traits (Senan et al., 2014; Daware et al., 2016). With the advent of Next-Generation Sequencing (NGS) technologies, the discovery of SSR loci has become more efficient and scalable, allowing for high-throughput marker development across diverse taxa (Vignes & Rivallan, 2021). Bioinformatics tools such as MISA and QDD have further streamlined the identification and primer design process, enhancing the accuracy and speed of SSR marker generation (Beier et al., 2017). Moreover, SSR assays facilitate the detection of allelic variation linked to agronomic traits, making them indispensable in crop improvement programs (Daware et al., 2016). In light of this interest, the present study aimed to develop a procedure for DNA markers, specifically focusing on microsatellite marker development and practicing assays for its applications in crop improvement.

MICROSATELLITE MARKERS

Microsatellites (1–10 nucleotide repeats) and minisatellites (>10 nucleotide repeats) are subclasses of tandem repeats (TRs) that, along with dispersed repetitive elements and residual fragments of transposable sequences, contribute to the repetitive DNA architecture found approximately every 6 kilobases within plant genomes (Ellegren, 2004; Kalia et al., 2011).

Microsatellites serve as highly informative molecular markers due to their:

- Dense and widespread genomic distribution
- Elevated mutation rates, resulting in substantial allelic variability
- Co-dominant inheritance patterns
- Simplified detection through PCR-based methods

This hypervariability enables microsatellite profiling to reveal polymorphism across and within species. In plant genomics, microsatellites function as gene-based or functional markers in selective breeding programs by elucidating genetic variation at economically and agronomically significant loci (Gupta & Varshney, 2000).

Terminologically, microsatellites are also referred to as:

- Simple sequence repeats (SSRs)
- Short tandem repeats (STRs)
- Inter-simple sequence repeats (ISSRs)
- Simple sequence tandem repeats (SSTRs)

- Variable number tandem repeats (VNTRs)
- Simple sequence length polymorphisms (SSLPs)
- Sequence-tagged microsatellites (STMSs)

Tandem repeats, from an evolutionary viewpoint, represent genomic instability (Gemayel et al., 2010). SSRs, being naturally co-dominant, are instrumental in kinship analysis, population structure studies, phylogenetic inference, genotype identification, and investigations of gene duplication or deletion. Furthermore, their application extends to sex determination, species lineage analysis, taxonomic classification, assessment of genetic purity, linkage map construction, QTL mapping, and genome-wide association studies. In addition to their utility in crop improvement and marker-assisted selection, STRs have been implicated in human pathologies, including neurodegenerative disorders and various cancers (Mirkin, 2007).

EXAMPLES OF MICROSATELLITE SEQUENCE MOTIFS

Microsatellites, also known as simple sequence repeats (SSRs), are tandemly arranged DNA segments composed of repeating units ranging from one to six nucleotides in length. These sequences exhibit considerable variability due to differences in the number of repeat units and are widely distributed across both coding and non-coding genomic regions (Tautz, 1989; Vieira et al., 2016). The repeated units may be as:

(a) Common Repeat Units: The abundance of microsatellites follows a motif-dependent hierarchy, with dinucleotide repeats being most prevalent, followed by tri- and tetranucleotide repeats (Table 1). Hexanucleotide repeats are comparatively rare in most plant genomes (Kalia et al., 2011).

Table 1. Common repeat units of microsatellite sequence motifs

Repeat Type	Example	Structure	Length
Mononucleotide	TTTTTTTTTT	$(T)_{10}$	10 bp
Dinucleotide	CGCGCGCGCG	$(CG)_5$	10 bp
Trinucleotide	ATGATGATGATGATG	$(ATG)_5$	15 bp
Tetranucleotide	ATCGATCGATCGATCGATCG	$(ATCG)_5$	20 bp

(b) Microsatellite Genotypes: Microsatellite loci can exhibit either homozygous or heterozygous configurations depending on the repeat number uniformity across homologous chromosomes (Ellegren, 2004).

1. Homozygous Microsatellite

…ATGCTGCTAGCTGCTAG—GTGTGTGTGTGTGTGT--GCTACGTCGATCA… (46 bp)

…ATGCTGCTAGCTGCTAG—GTGTGTGTGTGTGTGT--GCTACGTCGATCA… (46 bp)

5’ flanking region ----------------- microsatellite locus -------------- 3’ flanking region

2. Heterozygous Microsatellite

…CGTATGCTACTCGGC--ATATATATATAT--ACGTCACTCATAGC…(46bp)
…CTGCTACTAGCTACTCGATC--ATATATATATATATATAT—ACGTTCCG... (50 bp)

5’ flanking region ----------------- microsatellite locus -------------- 3’ flanking region

These structural polymorphisms form the basis for numerous applications in plant genetics, including cultivar identification, population genetics, and marker-assisted selection (Gupta & Varshney, 2000).

DETECTION OF PCR PRIMERS

PCR primers are designed to amplify specific loci within the genome, flanking unique base pairs adjacent to microsatellite regions. These primers are locus-specific and typically function across individuals of the same species or closely related taxa (Selkoe & Toonen, 2006). Microsatellites, also known as short tandem repeats (STRs), consist of variable-length repeat motifs commonly dinucleotides such as "AG" which result in PCR products of differing sizes depending on the number of repeat units present (Abdurakhmonov, 2016). Following amplification, PCR products are separated using gel or capillary electrophoresis, enabling precise fragment size determination and inference of repeat count per allele (Vieira et al., 2016). Ideally, each microsatellite locus yields two distinct bands corresponding to the diploid alleles. However, PCR slippage during amplification can produce minor stutter bands, which typically differ from the main allele by one or two repeat units (Pompanon et al., 2005). Microsatellite analysis remains a powerful tool in population genetics, biodiversity studies, and marker-assisted selection due to its high polymorphism and reproducibility (Figure 1) (Hodel et al., 2016),

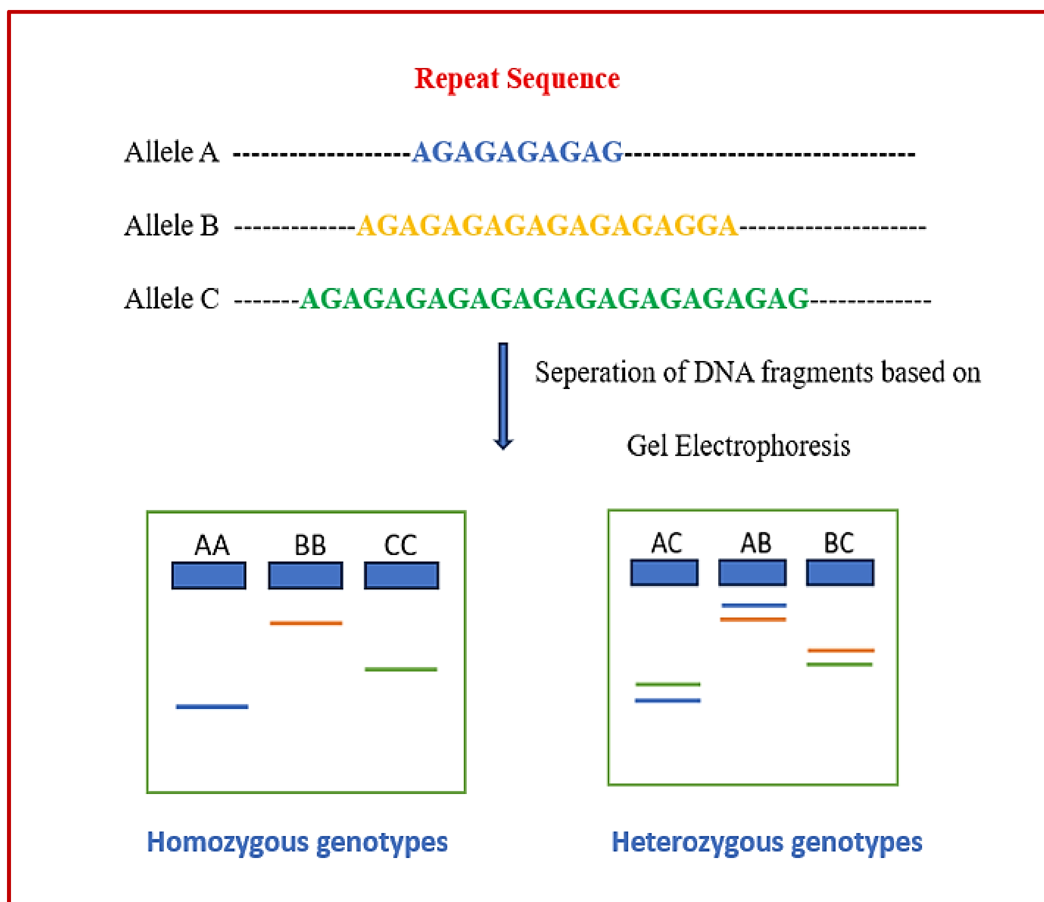

Figure 1. PCR amplification and gel electrophoresis visualization of homozygous and heterozygous genotypes.

Genomic simple sequence repeats (SSRs) are distributed across coding, intronic, and intergenic regions of the genome. These SSRs tend to exhibit high polymorphism and low cross-species transferability due to their location in non-conserved regions (Abdurakhmonov, 2016; Vieira et al., 2016). In contrast, genic SSRs are located within coding regions and are generally more conserved, resulting in lower variability but higher cross-transferability across related taxa (Dang et al., 2020; Singh et al., 2016).

Tandem sequence repeats are primarily formed through DNA polymerase slippage during replication, which leads to insertions or deletions of repeat units (Shinde et al., 2003; Viguera et al., 2001). This replication slippage is facilitated by the misalignment of the nascent strand with the template, especially in regions rich in direct repeats. Additionally, mutations and recombination events contribute to the expansion and diversification of SSRs within genomic sequences, further influencing genome structure and evolution (Tørresen et al., 2019; Levinson & Gutman, 1987).

APPLICATIONS OF MICROSATELLITES

Microsatellites, also known as simple sequence repeats (SSRs), have diverse applications in molecular biology and genetics. Their utility includes:

- Cost-effective analysis and minimal sample requirement, making them suitable for high-throughput genotyping (Pei et al., 2018).
- Efficient PCR amplification and multiplexing, enabling simultaneous detection of multiple loci (Sundaram et al., 2008).
- Forensic DNA profiling, where microsatellites serve as robust markers for individual identification and parentage testing (Chen et al., 2020).
- Hybridity detection in F1 crop hybrids, facilitating confirmation of crossbreeding events (Manohara et al., 2020).
- Assessment of repeat motif abundance and distribution, both in coding and non-coding regions (Meglécz et al., 2012).
- Phylogenetic tree construction using mitochondrial SSRs, offering insights into evolutionary relationships (Takezaki & Nei, 1996).
- Evaluation of motif length dynamics and conservation of flanking regions, which aids in understanding microsatellite evolution (Chatrou et al., 2009).

MAJOR APPLICATIONS OF MICROSATELLITE MARKERS

Determination of parentage: The breeding value of an organism is generally estimated using the information available from its related species. The knowledge of exact parentage is hence inevitable. Parentage testing using molecular markers has a higher exclusion probability of more than 90%. Highly reproducible polymorphic DNA markers are used for this kind of fingerprinting. Due to this advantage, these markers are used in forensic genetics in the determination of parenthood or criminals through tissue samples like blood, hair etc., ((Flanagan & Jones, 2019).

Determination of twin zygosity: Individual-specific microsatellite profiles help distinguish monozygotic from dizygotic twins in animal studies (Chen et al., 2020).

Identifying the disease carrier: Microsatellite polymorphisms within or near disease-associated genes can identify heterozygous carriers that are phenotypically indistinguishable (Anmarkrud et al., 2008).

ADVANTAGES OF MICROSATELLITES

- Require low quantities of template DNA (10–100 ng).
- Abundant and sequence-specific across the genome.
- Evenly distributed in coding and non-coding regions.
- Exhibit high polymorphism and co-dominant inheritance.
- Multi-allelic with mean heterozygosity often exceeding 70%.
- Amenable to automation and multiplexing.
- Unaffected by environmental factors.
- Not pleiotropic on QTLs.
- Highly reproducible and robust (Schlötterer, 2000; Sundaram et al., 2008).

CHALLENGES OF MICROSATELLITES

- Development is time-intensive and costly for certain crops.
- Null alleles may cause misclassification of heterozygotes as homozygotes due to primer site mutations (Estoup et al., 2002).

- Mutation models are complex and not fully understood.
- Stutter bands can interfere with allele scoring accuracy.
- Homoplasy may lead to underestimation of genetic divergence (Navascués & Emerson, 2009).
- Greater marker density and sequence variability are needed to resolve complex trait inheritance (Goldstein & Schlotterer, 1999).

DEVELOPMENT OF SSR MARKERS USING SSR IDENTIFICATION TOOLS

1. MISA (MIcroSAtellite Identification Tool): Simple sequence repeats (SSRs), also known as microsatellites, were identified from previously annotated unigene sequences of various crops using the MISA tool (https://webblast.ipk-gatersleben.de/misa/), a freely accessible web-based application for SSR mining (Beier et al., 2017). The default search parameters were applied, which include a minimum of six repeats for dinucleotide motifs and five repeats for tri-, tetra-, penta-, and hexanucleotide motifs. Primer pairs flanking the identified SSRs were designed using Primer 3 software (Untergasser et al., 2012). To evaluate the utility of the developed SSR markers, primer pairs specific to the experimental objectives were selected for assessing genetic diversity and population structure in crop germplasm panels. Marker informativeness was quantified using parameters such as expected heterozygosity (He), Nei's gene diversity (H), and polymorphism information content (PIC) (Botstein et al., 1980).

2. SAT (SSR Analysis Tool): SSR loci were also identified through sequencing of genomic DNA libraries enriched for SSR motifs. The raw sequencing output, typically in the form of chromatogram or trace files, was processed using SAT-a flexible and optimized web-based pipeline for SSR marker development (Dereeper et al., 2007). SAT integrates multiple bioinformatics tools, including Phred for base calling, Lucy for sequence cleaning, d2-cluster for clustering, Phrap and CAP3 for sequence assembly, and ePrimer3 for primer design. The tool facilitates automated SSR detection, primer design, and virtual PCR validation, and is available both as a web application and a downloadable command-line version (http://sat.cirad.fr/sat)

The SAT workflow: Eight steps

1. **Base calling:** Fed with Chromatograms (SCF or ABI format) → Base calling with Phred and assigned with corresponding quality scores resulting in a multi-FASTA sequence file and a corresponding quality file (Ewing & Green, 1998a, 1998b).
2. **Sequence Cleansing:** Raw DNA sequences are cleaned by removing the cloning vector and adaptor removal with Lucy. Quality threshold, vector sequence and cloning site are required. Regions with a higher quality value are retained (Chou & Holmes, 2001).
3. ***In silico* restriction digestion:** SAT performs a virtual restriction by detecting chimaeras or partially digested sequences formed during SSR-enriched library construction. Cleaned DNA sequences fed to BioPERL module Bio::Tools:: Restriction Enzyme. The restriction enzyme used has to be specified. All the resulting fragments will contain an SSR motif with no chimeric sequences (Stajich et al., 2002).
4. **SSR detection:** Restriction fragments: SSR detection by SAT with SSRIT (searches for the minimal number of repeats for each pattern of di, tri or tetranucleotide) or Sputnik (reporting imperfect SSRs), (Temnykh et al., 2001).
5. **Clustering:** Fragments containing SSR are masked and are clustered using d2_cluster (Burke et al., 1999).
6. **Assembly:** Clusters of similar consensus sequences are assembled with Phrap or CAP3. This is followed by additional alignment by Craw (Huang & Madan, 1999; Green, 1999).
7. **Primer designing:** Consensus sequences and singletons – PCR primer designing with ePrimer3 to analyze the flanking DNA sequences for forward and reverse primers to assay each SSR loci. Melting temperature of the oligonucleotide, length of the primer, GC content, size of the PCR product, and positional constraints around the SSR are factors to consider during primer designing (Untergasser et al., 2012).

8. **Virtual PCR:** PCR primer pairs for Virtual PCR was performed with Blast to verify the specificity of the designed primers. Validated/Non validated PCR primer pairs. Results of every step is stored in a MySQL database (Altschul et al., 1990).

3. MegaSSR: A Web-Based and Standalone Pipeline for SSR Marker Development

MegaSSR is a versatile web server and standalone pipeline designed for the identification and development of simple sequence repeat (SSR) markers across any target genome or transcriptome (Mokhtar et al., 2023). The platform integrates multiple computational modules to facilitate SSR mining, motif classification, comparative analysis, PCR primer design, in silico validation, and statistical visualization. Users can generate customized PCR-based primers for selected SSR motifs, enabling high-throughput marker development for genetic diversity studies and breeding applications.

The web interface of MegaSSR (https://bioinformatics.um6p.ma/MegaSSR) provides intuitive tools for accessing, searching, downloading, and visualizing SSR data. Outputs include interactive tables and graphical summaries that detail SSR motif characteristics and associated primer pairs. These features support efficient marker selection and downstream analysis, particularly in plant genomics and molecular breeding programs.For offline use, MegaSSR is available as a standalone version via GitHub (https://github.com/MoradMMokhtar/MegaSSR), compatible with Conda-based environments. Installation is streamlined using the command "conda env create -f MegaSSR.yml", which resolves all dependencies. The pipeline has been validated on Ubuntu 18.04 and 20.04 systems, ensuring broad accessibility for bioinformatics workflows (Figure 2) (Morad et al., 2023).

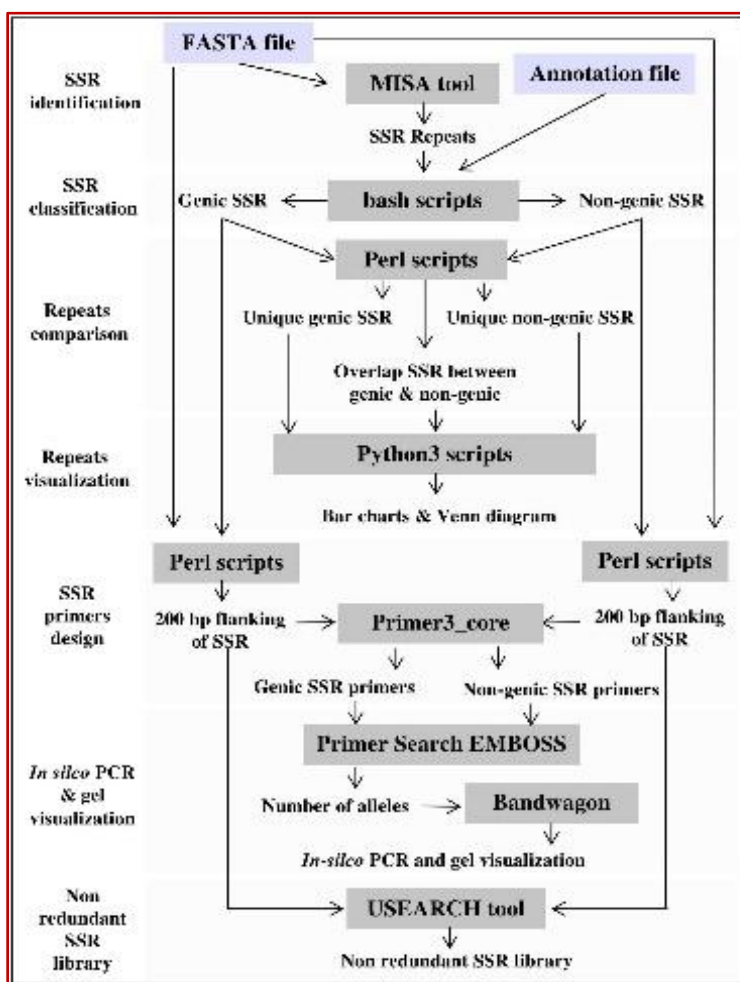

Figure 2. Workflow of MegaSSR (Morad et al., 2023)

CONCLUSION

DNA markers revolutionize molecular biology by enabling precise genetic trait identification, diversity assessment, and marker-assisted selection. This paper substantially reviews and describes a reproducible DNA marker development approach, from primer design and PCR optimization to validation and data interpretation. The method ensures robust genotyping across multiple biological systems by focusing on polymorphism identification and assay standardization. Quality control and troubleshooting improve molecular assay reliability. DNA markers' extensive use in crop improvement, functional genomics, and biodiversity conservation emphasizes their importance in plant breeding and genetic resource management. As molecular technologies advance, standardized, high-throughput marker systems will accelerate genetic research and sustainable agriculture innovation.

ACKNOWLEDGEMENTS

Not applicable

AUTHORS CONTRIBUTIONS

Both authors contributed equally to this work.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

ETHICAL APPROVAL

Not applicable

FUNDING

No funds were obtained for this study.

AVAILABILITY OF DATA AND MATERIALS

All datasets analyzed and described during the present study are available from the corresponding author upon reasonable request.

REFERENCES

Abdurakhmonov, I. Y. (2016). *Introduction to microsatellites: Basics, trends and highlights*. In *Microsatellite Markers* (pp. 1–36). IntechOpen. https://doi.org/10.5772/66446

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Amiteye, S. (2021). Basic concepts and methodologies of DNA marker systems in plant molecular breeding. *Heliyon*, *7*, e08093. https://doi.org/10.1016/j.heliyon.2021.e08093

Anmarkrud, J. A., Kleven, O., Bachmann, L., & Lifjeld, J. T. (2008). Microsatellite evolution: Mutations, sequence variation, and homoplasy in the hypervariable avian microsatellite locus HrU10. *BMC Evolutionary Biology*, *8*(138). https://doi.org/10.1186/1471-2148-8-138

Beier, S., Thiel, T., Münch, T., Scholz, U., & Mascher, M. (2017). MISA-web: A web server for microsatellite prediction. *Bioinformatics*, *33*(16), 2583–2585. https://doi.org/10.1093/bioinformatics/btx198

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*(3), 314–331.

Burke, J., Davison, D., & Hide, W. (1999). d2_cluster: A validated method for clustering EST and full-length cDNA sequences. *Genome Research*, *9*(11), 1135–1142. https://doi.org/10.1101/gr.9.11.1135

Chatrou, L. W., Escribano, M. P., Viruel, M. A., Maas, J. W., Richardson, J. E., & Hormaza, J. I. (2009). Flanking regions of monomorphic microsatellite loci provide a new source of data for plant species-level phylogenetics. *Molecular Phylogenetics and Evolution*, *53*(3), 726–733. https://doi.org/10.1016/j.ympev.2009.07.024

Chen, L., Wu, L., Hou, R., Shen, F., Zhang, W., Tang, Y., Yuan, Y., Zhao, B., & Zhang, L. (2020). Comparative analysis of microsatellite and SNP markers for parentage testing in the golden snub-nosed monkey. *Conservation Genetics Resources*, *12*, 611–620. https://doi.org/10.1007/s12686-020-01147-7

Chou, H. H., & Holmes, M. H. (2001). DNA sequence quality trimming and vector removal. *Bioinformatics*, *17*(12), 1093–1104. https://doi.org/10.1093/bioinformatics/17.12.1093

Collard, B. C. Y., Jahufer, M. Z. Z., Brouwer, J. B., & Pang, E. C. K. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica*, *142*(1–2), 169–196. https://doi.org/10.1007/s10681-005-1681-5

Dang, Z., Huang, L., Jia, Y., Lockhart, P. J., & Fong, Y. (2020). Identification of genic SSRs provide a perspective for studying environmental adaptation in the endemic shrub *Tetraena mongolica*. *Genes*, *11*(3), 322. https://doi.org/10.3390/genes11030322

Daware, A., Das, S., Srivastava, R., et al. (2016). An efficient strategy combining SSR markers and QTL-seq mapping unravels candidate genes regulating grain weight in rice. *Frontiers in Plant Science*, *7*, 1535. https://doi.org/10.3389/fpls.2016.01535

Dereeper, A., Argout, X., Billot, C., Rami, J. F., & Ruiz, M. (2007). SAT: A flexible and optimized Web application for SSR marker development. *BMC Bioinformatics*, *8*, 465. https://doi.org/10.1186/1471-2105-8-465

Du, Q., Wang, B., Wei, Z., Zhang, D., & Li, B. (2012). Genetic diversity and population structure of Chinese white poplar (*Populus tomentosa*) revealed by SSR markers. *Journal of Heredity*, *103*(6), 853–862. https://doi.org/10.1093/jhered/ess056

Ellegren, H. (2004). Microsatellites: Simple sequences with complex evolution. *Nature Reviews Genetics*, *5*(6), 435–445. https://doi.org/10.1038/nrg1348

Estoup, A., Jarne, P., & Cornuet, J. M. (2002). Homoplasy and mutation model at microsatellite loci and their consequences for population genetics analysis. *Molecular Ecology*, *11*(9), 1591–1604. https://doi.org/10.1046/j.1365-294X.2002.01576.x

Ewing, B., & Green, P. (1998a). Base-calling of automated sequencer traces using Phred. I. Accuracy assessment. *Genome Research*, *8*(3), 175–185. https://doi.org/10.1101/gr.8.3.175

Ewing, B., & Green, P. (1998b). Base-calling of automated sequencer traces using Phred. II. Error probabilities. *Genome Research*, *8*(3), 186–194. https://doi.org/10.1101/gr.8.3.186

Flanagan, S. P., & Jones, A. G. (2019). The future of parentage analysis: From microsatellites to SNPs and beyond. *Molecular Ecology*, *28*(3), 486–504. https://doi.org/10.1111/mec.14988

Gemayel, R., Vinces, M. D., Legendre, M., & Verstrepen, K. J. (2010). Variable tandem repeats accelerate evolution of coding and regulatory sequences. *Annual Review of Genetics*, *44*, 445–477. https://doi.org/10.1146/annurev-genet-072610-155046

Gil, J., Um, Y., Kim, S., et al. (2017). Development of genome-wide SSR markers from *Angelica gigas* using next-generation sequencing. *Genes*, *8*(9), 238. https://doi.org/10.3390/genes8090238

Goldstein, D. B., & Schlotterer, C. (1999). *Microsatellites: Evolution and Applications*. Oxford University Press.

Green, P. (1999). Phrap. [Software]. http://www.phrap.org

Gupta, P. K., & Varshney, R. K. (2000). The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica*, *113*, 163–185. https://doi.org/10.1023/A:1003910819967

Gupta, P. K., Varshney, R. K., Sharma, P. C., & Ramesh, B. (1996). Molecular markers and their applications in wheat breeding. *Plant Breeding*, *115*(5), 369–391. https://doi.org/10.1111/j.1439-0523.1996.tb00941.x

Hodel, R. G., Segovia-Salcedo, M. C., Landis, J. B., Crowl, A. A., Sun, M., Liu, X., ... & Soltis, P. S. (2016). The report of my death was an exaggeration: a review for researchers using microsatellites in the 21st century. *Applications in Plant Sciences*, *4*(6), 1600025. https://doi.org/10.3732/apps.1600025

Hu, L., Wang, X., Wang, J., & Shi, W. (2023). Development of SSR markers and evaluation of genetic diversity of endangered plant *Saussurea involucrata*. *Biomolecules*, *14*(8), 1010. https://doi.org/10.3390/biom14081010

Huang, X., & Madan, A. (1999). CAP3: A DNA sequence assembly program. *Genome Research*, *9*(9), 868–877. https://doi.org/10.1101/gr.9.9.868

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*(3), 309–334. https://doi.org/10.1007/s10681-010-0286-9

Kantartzi, S. K. (Ed.). (2013). *Microsatellites: Methods and Protocols*. Humana Press. https://doi.org/10.1007/978-1-62703-389-3

Levinson, G., & Gutman, G. A. (1987). Slipped-strand mispairing: A major mechanism for DNA sequence evolution. *Molecular Biology and Evolution*, *4*(3), 203–221. https://doi.org/10.1093/oxfordjournals.molbev.a040442

Manohara, K. K., Morajkar, S., Shanbagh, Y., Phadte, P., & Patil, K. (2020). Hybridity assessment in experimental F1s of rice using microsatellite markers. *National Academy Science Letters*, *43*, 133–136. https://doi.org/10.1007/s40009-019-00832-y

Meglécz, E., Nève, G., Biffin, E., & Gardner, M. G. (2012). Breakdown of phylogenetic signal: A survey of microsatellite densities in 454 shotgun sequences from 154 non-model eukaryote species. *PLoS ONE*, *7*(7), e40861. https://doi.org/10.1371/journal.pone.0040861

Mirkin, S. M. (2007). Expandable DNA repeats and human disease. *Nature*, *447*(7147), 932–940. https://doi.org/10.1038/nature05977

Mokhtar, M. M., Alsamman, M. M., & El Allali, A. (2023). MegaSSR: A web server for large-scale microsatellite identification, classification, and marker development. *Frontiers in Plant Science*, *14*, Article 1219055. https://doi.org/10.3389/fpls.2023.1219055

Navascués, M., & Emerson, B. C. (2009). Elevated substitution rate estimates from ancient DNA: Model violation and bias of Bayesian methods. *Molecular Ecology*, *18*(3), 439–445. https://doi.org/10.1111/j.1365-294X.2008.04041.x

Pei, J., Bao, P., Chu, M., Liang, C., Ding, X., Wang, H., Wu, X., Guo, X., & Yan, P. (2018). Evaluation of 17 microsatellite markers for parentage testing and individual identification of domestic yak. *PeerJ*, *6*, e5946. https://doi.org/10.7717/peerj.5946

Pompanon, F., Bonin, A., Bellemain, E., & Taberlet, P. (2005). Genotyping errors: Causes, consequences and solutions. *Nature Reviews Genetics*, *6*(11), 847–859. https://doi.org/10.1038/nrg1707

Schlötterer, C. (2000). Evolutionary dynamics of microsatellite DNA. *Chromosoma*, *109*(6), 365–371. https://doi.org/10.1007/s004120000089

Selkoe, K. A., & Toonen, R. J. (2006). Microsatellites for ecologists: A practical guide to using and evaluating microsatellite markers. *Ecology Letters*, *9*(5), 615–629. https://doi.org/10.1111/j.1461-0248.2006.00889.x

Senan, S., Kizhakayil, D., Sasikumar, B., & Sheeja, T. E. (2014). Methods for development of microsatellite markers: An overview. *Notulae Scientia Biologicae*, *6*(1), 1–13. https://doi.org/10.15835/nsb.6.1.9199

Serrote, C. M. L., Reiniger, L. R. S., Silva, K. B., et al. (2020). Determining the polymorphism information content of a molecular marker. *Gene*, *726*, 144175. https://doi.org/10.1016/j.gene.2020.144175

Shinde, D., Lai, Y., Sun, F., & Arnheim, N. (2003). Taq DNA polymerase slippage mutation rates measured by PCR and quasi-likelihood analysis: (CA/GT)n and (A/T)n microsatellites. *Nucleic Acids Research*, *31*(3), 974–980. https://doi.org/10.1093/nar/gkg178

Singh, B. K., Mishra, D. C., Yadav, S., Ambawat, S., Vaidya, E., & Kumar, S. (2016). Identification, characterization, validation and cross-species amplification of genic-SSRs in Indian mustard (*Brassica juncea*). *Journal of Plant Biochemistry and Biotechnology*, *25*(4), 410–420. https://doi.org/10.1007/s13562-016-0353-y

Stajich, J. E., Block, D., Boulez, K., et al. (2002). The Bioperl toolkit: Perl modules for the life sciences. *Genome Research*, *12*(10), 1611–1618. https://doi.org/10.1101/gr.361602

Su, Y., Fu, J., Xie, H., et al. (2025). SSR markers development and their application in genetic diversity of burdock (*Arctium lappa L.*) germplasm. *BMC Plant Biology*, *25*, 196. https://doi.org/10.1186/s12870-025-06203-8

Sundaram, R. M., Naveenkumar, B., Biradar, S. K., Balachandran, S. M., Mishra, B., Ilyasahmed, M., Viraktamath, B. C., & Ramesha, M. S. (2008). Identification of informative SSR markers capable of distinguishing hybrid rice parental lines and their utilization in seed purity assessment. *Euphytica*, *163*, 215–224. https://doi.org/10.1007/s10681-007-9630-0

Takezaki, N., & Nei, M. (1996). Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. *Genetics*, *144*(1), 389–399. https://doi.org/10.1093/genetics/144.1.389

Tautz, D. (1989). Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Research*, *17*(16), 6463–6471. https://doi.org/10.1093/nar/17.16.6463

Temnykh, S., DeClerck, G., Lukashova, A., Lipovich, L., Cartinhour, S., & McCouch, S. (2001). Computational and experimental analysis of microsatellites in rice (Oryza sativa L.): Frequency, length variation, transposon associations, and genetic marker potential. *Genome Research*, *11*(8), 1441–1452. https://doi.org/10.1101/gr.184001

Tørresen, O. K., Star, B., Mier, P., Andrade-Navarro, M. A., Bateman, A., Jarnot, P., ... & Promponas, V. J. (2019). Tandem repeats lead to sequence assembly errors and impose multi-level challenges for genome and protein databases. *Nucleic Acids Research*, *47*(21), 10994–11006. https://doi.org/10.1093/nar/gkz841

Tóth, G., Gáspári, Z., & Jurka, J. (2000). Microsatellites in different eukaryotic genomes: Survey and analysis. *Genome Research*, *10*(7), 967–981. https://doi.org/10.1101/gr.10.7.967

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*(15), e115. https://doi.org/10.1093/nar/gks596

Vieira, M. L. C., Santini, L., Diniz, A. L., & Munhoz, C. de F. (2016). Microsatellite markers: What they mean and why they are so useful. *Genetics and Molecular Biology*, *39*(3), 312–328. https://doi.org/10.1590/1678-4685-GMB-2016-0027

Vignes, H., & Rivallan, R. (2021). Development of microsatellite markers using next-generation sequencing. In *Methods in Molecular Biology* (Vol. 2250, pp. 161–174). Springer. https://doi.org/10.1007/978-1-0716-0997-2_11

Viguera, E., Canceill, D., & Ehrlich, S. D. (2001). Replication slippage involves DNA polymerase pausing and dissociation. *The EMBO Journal*, *20*(10), 2587–2595. https://doi.org/10.1093/emboj/20.10.2587

Wang, S. Q. (2020). Genetic diversity and population structure of the endangered species *Paeonia decomposita* endemic to China and implications for its conservation. *BMC Plant Biology*, *20*, 510. https://doi.org/10.1186/s12870-020-02654-6

Wei, S., Yang, W., Wang, X., & Hou, Y. (2017). High genetic diversity in an endangered medicinal plant, *Saussurea involucrata*, in western Tianshan Mountains, China. *Conservation Genetics*, *18*, 1435–1447. https://doi.org/10.1007/s10592-017-0977-7

Yang, H., Li, X., Liu, D., Chen, X., Li, F., Qi, X., Luo, Z., & Wang, C. (2016). Genetic diversity and population structure of the endangered medicinal plant *Phellodendron amurense* in China revealed by SSR markers. *Biochemical Systematics and Ecology, 66*, 286–292. https://doi.org/10.1016/j.bse.2016.04.003

Copyright: © 2025 by the authors. This work is licensed under a Creative Commons Attribution 4.0 International License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.