



Mining Microsatellites: SSR Markers and their Role in Plant Breeding

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Traditional plant breeding has long relied on phenotypic observations and classical methods, often requiring extensive time and resources. In the realm of modern plant breeding, the integration of advanced molecular tools has revolutionized the precision and efficiency with which desirable traits are identified and selected. (Kordrostami and Rahimi, 2015). The emergence of molecular markers has revolutionized the field of plant breeding by providing tools that allow for a more precise and accelerated approach to the selection of desirable traits. Among these markers, Simple Sequence Repeats (SSRs), also known as microsatellites, have gained prominence due to their high polymorphism, reproducibility and wide distribution across plant genomes. (Powell et al., 1996).

SSRs are short, tandemly repeated DNA sequences consisting of 1-6 base pair motifs and their polymorphic nature arises from variations in the number of repeat units. This inherent variability makes SSR markers valuable tools for assessing genetic diversity, population structure and linkage disequilibrium within plant populations (Toth et al., 2000). This article explores the multifaceted role of SSR markers in plant breeding, highlighting their features, classification, applications and challenges.

Key features of SSR markers

- **Tandem Repeat Structure:** SSR markers consist of short sequences of DNA, usually 1-6 base pairs in length, which are repeated in a tandem array. The repetitive nature of these sequences contributes to the high polymorphism observed in SSRs.
- **Polymorphism:** One of the most significant features of SSR markers is their high degree of polymorphism. The number of repeat units in the SSR motif can vary among individuals within a population, making SSRs ideal for assessing genetic diversity and distinguishing between closely related genotypes.
- **Codominant Inheritance:** SSR markers exhibit codominant inheritance, meaning that both alleles at a given locus are independently expressed. This feature allows for the discrimination of heterozygous and homozygous genotypes, providing more detailed information about an individual's genetic makeup.
- **Abundance in Genomes:** SSR motifs are abundant and widely distributed throughout eukaryotic genomes. This ubiquity makes SSR markers applicable to a broad range of species, facilitating comparative genomics, population genetics and evolutionary studies.
- **Ease of Detection:** SSR markers are relatively easy to detect and analyze. Polymerase chain reaction (PCR) is commonly used to amplify the SSR loci and the resulting PCR products can be visualized through gel electrophoresis or more advanced methods, such as capillary electrophoresis.
- **Multiallelic Nature:** SSR markers often exhibit multiple alleles at a single locus due to variations in the number of repeat units. This multiallelic nature enhances the discriminatory power of SSRs in genetic analyses.

- **High Reproducibility:** SSR markers are highly reproducible, allowing for consistent results across different laboratories and experiments. This reliability is crucial for comparing genetic information across studies and population.

Classification of microsatellite markers

Based on the number of SSR motif in locus, it can be divided in to two types:

1. **Simple Sequence Repeats (SSRs):** Simple Sequence Repeats, or SSRs, are tandem repeats of short DNA sequences, typically 1-6 base pairs in length, which make them highly polymorphic and widely distributed across plant genomes (Toth et al., 2000).
2. **Compound Microsatellites:** Compound microsatellites consist of two or more adjacent SSR motifs within the same locus, adding an additional layer of complexity to the genetic structure (Katti et al., 2001).

Based on interruption in repeats it can be divided in two types:

1. **Perfect Microsatellites:** Perfect microsatellites are characterized by uninterrupted tandem repeats of the same motif, offering straightforward analysis and high polymorphism (Li et al., 2002).
2. **Imperfect Microsatellites:** Imperfect microsatellites contain variations, such as point mutations or insertions/deletions, within the repeated motifs, contributing to their diversity (Kashi et al., 1970).

Based on number of nucleotide repeats, it can be further classified in to...

1. Mononucleotide Repeats: ex. (A)_n, (T)_n, (C)_n and (G)_n repeats
2. Dinucleotide Repeats: ex. (CA)_n, (GT)_n, (AG)_n and (TG)_n repeats
3. Trinucleotide Repeats: ex. (CAG)_n, (GAA)_n, (AAT)_n and (TGC)_n repeats
4. Tetranucleotide Repeats: ex. (ACAT)_n, (GATA)_n, (AGCT)_n and (CATG)_n repeats.
5. Pentanucleotide Repeats: ex. (AAAGA)_n, (AGCTT)_n and (CACAC)_n repeats.

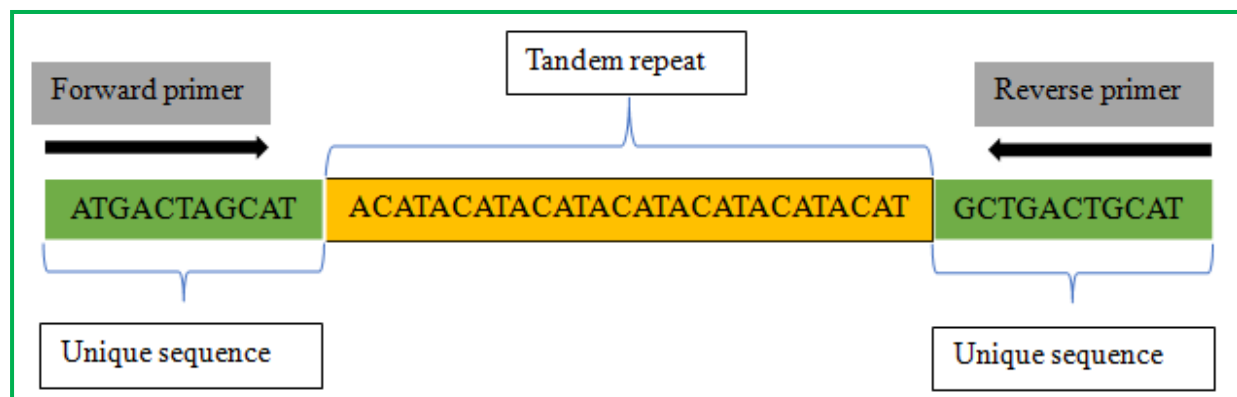


Figure 1: General diagram of microsatellite or SSR marker

Role in plant breeding

Microsatellite markers or Simple Sequence Repeats (SSRs), play a important role in plant breeding, offering valuable applications that contribute to the development of improved crop varieties. These markers are extensively used in genetic mapping, enabling the construction of high-density linkage maps. This facilitates the identification of Quantitative Trait Loci (QTLs) associated with agriculturally important traits, such as disease resistance, yield and quality characteristics. The high level of polymorphism exhibited by SSR markers allows for precise and efficient discrimination between individual genotypes, making them ideal for parentage analysis and assessing genetic diversity within plant populations. Additionally, SSR markers are crucial components of Marker-Assisted Selection (MAS) programs, aiding breeders in the identification and introgression of specific traits into elite cultivars. The scalability and transferability of SSR markers across different plant species further enhance their utility in various breeding programs. Overall, the application of microsatellite markers

in plant breeding contributes to accelerating the breeding process, enhancing the efficiency of trait selection and ultimately fostering the development of crops with improved agronomic traits.

Applications of SSR markers in plant breeding

- Genetic Mapping and QTL Identification
- Marker-Assisted Selection (MAS)
- Genetic Diversity Assessment
- Linkage Mapping for Crop Improvement
- Parentage Analysis
- Population Genetics and Phylogenetic Studies
- Assessment of Gene Flow
- Trait Association Studies
- Varietal Identification and Certification
- Functional Genomics and Gene Tagging

Challenges

One significant challenge is the resource-intensive and time-consuming nature of SSR marker development. Designing primers for specific microsatellite loci requires significant investment in laboratory work and sequencing efforts. Additionally, the cost associated with genotyping using SSR markers, especially when a large number of markers are needed for comprehensive studies, can pose financial constraints for researchers and breeding programs. Another challenge is the limited transferability of SSR markers across different plant species. While some markers show cross-species applicability, many are species-specific, requiring the development of new markers for each species of interest.

Lastly, as SSR markers are often located in non-coding regions of the genome, their direct association with functional genes may be limited. This can pose challenges when trying to link specific markers with traits of interest, especially in the context of marker-assisted selection. Despite these challenges, Advancements in high-throughput sequencing technologies and bioinformatics tools may help mitigate the challenges related to marker development and transferability.

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