

Molecular Markers for Better Crops

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In ancient times, the farmers and plant breeders used the technique of selecting the best quality plants to develop improved crops. Later, Charles Darwin and Gregor Mendel described the inheritance of traits, which act as the foundation for plant Breeding and Genetics. Today, recent advancements in biotechnology and the use of molecular markers have led to the development of a new era known as molecular plant breeding.

In agriculture, the fundamental objective of the plant breeder is to improve the quality of crops by crossing them with a genotype that possesses desired traits. In a conventional breeding program, superior recombinant selections are essential to introgress traits of interest. This process is quite challenging because it requires a significant amount of time, demands high labour efficiency, involves multiple crosses and generations and also necessitates careful phenotypic selection. The development of molecular marker technology helped plant breeders to overcome these problems encountered during conventional breeding. By allowing the indirect selection of desired characters at the DNA level, molecular markers enabled breeders to identify superior genotypes more accurately and at earlier stages of plant development, thereby speeding up the breeding process and upgrading the selection efficiency.

Molecular markers: A brief history

Molecular markers have greatly transformed crop improvement over the past few decades. The first molecular markers, known as Restriction Fragment Length Polymorphism (RFLP), were invented in 1980 and is regarded as the beginning of molecular crop breeding. Later, Random Amplified Polymorphic DNA (RAPD) were developed in the late 1980s. RFLP and RAPD together are considered the first-generation molecular markers and are largely used for DNA fingerprinting and partly used for marker-assisted selection, but they have some limitations. In order to overcome these limitations, second-generation markers were developed during the 1990s, which included Simple Sequence Repeats (SSR), Amplified Fragment Length Polymorphism (AFLP), and a variety of their modified forms. Markers were highly polymorphic, easy to use and led to broad acceptance in plant breeding. In the mid to late 1990s, third-generation molecular markers, including Expressed Sequence Tags (ESTs) and Single Nucleotide Polymorphisms (SNPs) were developed. These markers show high

productivity and do not require time-consuming gel-based analysis.

Understanding molecular markers

Molecular markers are DNA sequences located at a known position on chromosomes. Markers show polymorphism, which is due to variation in nucleotides in the genome. These changes enable us to find out the difference between organisms.

Molecular markers offer many advantages over morpho-biochemical markers. Morphological markers are affected by environmental factors and require a lot of time for evaluation. Biochemical markers show very low genetic variation and their use is limited to small-scale studies. So, for studying genetic variation, molecular markers are good, because they are more reliable, faster and highly effective.

What makes a molecular marker perfect?

- A good marker should be highly polymorphic, which means it must show differentiation between different genotypes. High polymorphism gives an idea to find out the genotypic variation among closely related varieties, identify genotypes with superior characters and trace desirable genes effectively during the selection process.
- Another important quality of a molecular marker is that it is stable and not affected by environmental factors. This ensures that the genetic information gained truly indicates the genotype and is not as a result of environmental factors, which helps to identify the genotypes at any developmental stage and in any location correctly.
- An ideal DNA marker must have repeatability and reproducibility. The marker should show the same result everywhere and every time it is tested.
- Another important characteristic of a DNA marker is that it should be uniformly distributed across the genome. It helps the breeder to study genome organisation and construct genetic maps.
- Co-dominant nature is an important property of DNA markers, which allows clear demarcation between homozygous and heterozygous individuals.
- For practical applications, it should be cost-effective, simple and rapid.

In reality, no single system satisfies all these characteristics. Therefore, plant breeders select the DNA markers based on their objectives and available resources. Many times, more than one marker system is used to achieve the desirable results.

From lab to field: Applications of molecular markers

✓ Genetic diversity evaluation

Molecular markers such as ISSR, SSR and RAPD are extensively used to study the genetic variability within and between species. Due to their co-dominant nature, high polymorphism, reproducibility and wide genomic distribution, SSR markers are the most preferred markers and are effectively employed in the diversity study of crops such as cassava, elephant foot yam, sweet potato, taro, etc. RAPD markers are highly valued for their simplicity and ability to generate genetic profiles without prior knowledge and are effectively used to evaluate genetic diversity in many crops such as *Cannabis sativa*, eggplant and Nigerian crop species. However, due to a problem in its reproducibility, it is not much used nowadays.

✓ Marker assisted selection (MAS)

Marker assisted selection, or MAS, is an advanced breeding method that combines molecular biology with conventional plant breeding, facilitating accurate and early selection of desirable traits in crops. It helps to improve the overall efficiency in improving crops by identifying and selecting plants with desired traits before trait expression. This greatly helps reduce the time and manpower for phenotypic evaluation of a large number of progenies/clones. Cassava mosaic disease (CMD) resistance in cassava, Sweet potato virus disease (SPVD) resistance in sweet potato, Pyramid nematode resistance and high oleic acid traits in peanut, enhanced disease resistance and better yield potential in maize are some successful examples of MAS programs.

✓ Quantitative trait locus (QTL) mapping

Molecular markers play an important role in QTL mapping, which helps plant breeders to identify the specific regions in the genome that control important traits. During QTL mapping, breeders study a group of related plants, evaluate the field performance and examine their DNA using molecular markers like SSRs and SNPs, which helps the breeders to understand which region of the genome is responsible for specific traits. This tool is successfully used in Potato for identifying loci responsible for resistance against late blight disease, cyst nematode, tuber shape, flesh colour and early maturity. SPVD resistance in sweet potato, carotenoid profiling and starch yield are some effective examples of QTL mapping in sweet potato.

✓ Genotyping of cultivars

Identification and analysis of the genetic makeup of the plants are crucial for the assessment of genetic diversity. The repetitive and arbitrary DNA markers are widely used for genotyping of cultivars. SSRs such as (CT)₁₀, (GAA)₅, (AAT)₆, (CAC)₅ are mainly used for DNA fingerprinting to figure out genetic variation and to understand the differentiation among cultivars. These markers are useful for breeders to analyse the genetic diversity, characterise accessions in germplasm collection and taxonomic studies. SSR markers are successfully used in crops like cassava, taro, sweet potato, elephant foot yam, rice and soybean to distinguish cultivars.

✓ Phylogeny and evolution

In earlier days, phylogeny and evolution were mostly studied based on morphological traits and the geographical distribution of organisms. The traits such as shape, colour and other anatomical features were compared to study the relatedness among the species. Although these methods gave valuable insights, it was proven later that morphological characters of unrelated species can develop similar traits due to similar ecological pressures. However, with modern genomic technologies, the focus has been shifted to the genetic structure of organisms. RNA, DNA and protein sequences have been proven more precise and reliable for scientists to trace the ancestry and understand the evolutionary process than using morphology alone.

For phylogenetic studies, RFLP, DNA sequencing and PCR-based markers are widely used. Markers such as STMS (Sequence-tagged microsatellite markers), STS (Sequence-tagged sequence) and ALPs (Amplicon Length Polymorphism) aid the detection of gene pool variation during the development of cultivar and germplasm classification. STS markers act as organelle-specific markers for chloroplast and mitochondrial DNA, which give details about seed and pollen inheritance. RAPD was used for detecting genetic variability within taro and between related species. Transposable elements and RFLPs are also powerful tools to track the origins of crops like rice and wheat, which show a relationship between species and their wild ancestors.

Conclusion

In short, molecular markers have revolutionised modern genetics and biotechnology. They have become fundamental tools in biological research by providing precise, reliable and rapid methods for the identification and selection of crops with desirable traits, avoiding the lengthy phenotype evaluations, molecular assisted selection, DNA fingerprinting, etc. It is crucial to maintain, assess and evaluate genetic diversity for studying genetic variation and

for the construction of genetic and physical maps. They have proved to be instrumental in tracing the ancestry, to assess evolutionary process and in the reconstruction of phylogenetic trees with high efficiency and precision. Furthermore, their applications in plant breeding, genetic diversity analysis, disease diagnostics and conservation biology highlight its broad utility in both basic and applied biological research. As genomic technologies continue to advance, the scope and impact of molecular markers can further expand, making them indispensable for sustainable crop improvement.

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