Molecular Markers Technology for Genetic Improvement in Fruit Crops

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Abstract

The conventional breeding procedures for fruit crop genetic improvement are extremely time-consuming, expensive, and difficult to manage, making them insufficient on their own. The selection and improvement process for these species is hindered by a number of additional limitations, such as high heterozygosity, sterility, sexual incompatibility, polyploidy, etc. Therefore, it is imperative that modern biotechnological tools such as molecular marker approaches be added to traditional breeding procedures in order to facilitate precise and accurate plant selection based on genotypes. Many markers, particularly molecular ones, allow for direct selection of genomic areas controlling the desired characteristic, such as high quality, yield, and resilience to various stresses, reducing time and space and assisting in the screening of fruit quality and other related desired traits at early stages. The DNA-based molecular markers, such as RFLP, RAPD, SSR, AFLP, SNP, etc., have been used to screen germplasms for various types of stress tolerance and the QTL mapping of gene.

Introduction

The 1980s was the beginning year of the use of molecular markers in crop improvement. This was first done with hybridization-based molecular markers, which are no longer as widely used. PCR-based molecular markers gained enormous traction as soon as they were made accessible since they were simple to use and could be used to a wide variety of agricultural plants. The latter two decades of the 20th century, the introduction of molecular markers to help with the numerous tasks associated with fruit crop breeding programmes was initiated. Because phenotypic-based markers are less effective at discriminating phenotypes and are more susceptible to environmental influences, DNA-based markers have been developed in response to these shortcomings. The term "molecular markers" refers to naturally occurring polymorphisms in nucleic acids. A particular fragment of the DNA which shows variations at the genome levels comprises a molecular marker. Molecular markers are the landmarks whose position in the genome is known.

Molecular markers directly reveal the polymorphism at the level of DNA. It has been characterized into three types a) Hybridization based marker (First generation marker) viz., restriction fragment length polymorphism (RFLP) (Botstein *et al.,* 1980); PCR based markers (second generation markers) viz., Simple sequence repeat (SSR), Random amplified polymorphic DNA (RAPD) (Williams *et al.,* 1990), sequence characterized amplified regions (SCARs) (Michelmore *et al.,* 1991); and quasi types viz., amplified fragment length polymorphism (AFLP) (Vos *et al.,* 1995). Prior to the development of PCR, the RFLP marker system was widely employed in DNA fingerprinting; however, because SSR markers are automated and easier to use, they have superseded RFLP markers. Nonetheless, RFLP markers continue to be the preferred marker for identifying unidentified genes and numerous transgene insertions in the genomes of transgenic plants. These days, high-throughput genotyping and deciphering the vast genetic variation down to single nucleotide polymorphisms (SNPs) throughout the entire genome are made feasible by a variety of genome-wide sequencing techniques. Consequently, third-generation markers, or SNP markers, have supplanted SSR markers as the most valuable marker system for creating saturated linkage maps, precise DNA fingerprinting, phylogenetic and evolutionary investigations, gene tagging and marker assisted selection (MAS). The development of high-throughput molecular markers and genetic maps facilitated the location of genes governing the agronomically

important traits and may possibly help in boosting the breeding through MAS (marker-assisted selection).

The nucleotide sequences, or molecular markers, are typically distinguished across various living organisms based on the polymorphism or variability observed in these nucleotide sequences (Nadeem et al., 2018). It is possible to improve many crop species through evolutionary research, marker-assisted breeding, and genetic marker patterns of heredity and genome variants. Singh et al. (2017) reported on the use of molecular markers in the characterization and enhancement of several genotypic and phenotypic features in brassica. Current developments in molecular markers offer several opportunities to evaluate genetic diversity across a wide variety of germplasms. Molecular marker approaches opens up tremendous possibilities to reveal valuable information about genetic diversity (Panthee, 2023) and phylogeny to boost the efficacy of selection through genome sequencing and thus cultivar improvement with respect to traits in horticultural crops.The molecular markers mentioned in Table 1 have been used for characterization, DNA fingerprinting, genome mapping and genome editing of fruit crops.

Table:1 Classification of Molecular Markers

Markers	Examples
Non-PCR based	RFLP
PCR based	RAPD, AFLP, ISSR, SSR
Functional markers	SCAR
Genome/Gene editing	CRISPR/Cas, TALEN, ZFN
Sequencing technologies based	GBS, NGS, DArT, SNP

Ideal properties of Molecular Markers

They need to be polymorphic with co-dominant inheritance, affordable able to resolve genetic differences adequately, easily accessible, frequently occurring in the genome, requiring small amounts of DNA sample, related to a variety of phenotypes, and easily interchangeable throughout laboratories. Unfortunately, the use of molecular markers varies depending on the nature of the work and can vary depending on the circumstances. The following characteristics of molecular markers distinguish one another on the basis of genomic richness, polymorphism detection level, locus specificity, assay cost, and repeatability. Finding a molecular approach that can satisfy all of the aforementioned parameters is difficult. Techniques, however, can be adjusted based on the goals of the research that is being done. This study assessed the significance of molecular markers for fruit crop genetic improvement initiatives._A no. of various molecular markers have been developed in different fruit crops as listed below in Table 2.

Table:2 List of Molecular Markers in horticultural crops

Fruit Crops	Molecular Markers
Citrus	RFLPs, RAPDs, AFLPs, SSRs,ISSRs, SNPs and DArTs
Mango	AFLPs, RAPDs, SSRs and ISSRs
Banana	RAPDs, SSRs and ISSRs
Apple	RFLPs, RAPDs, SSRs, ISSRs, SCARs, SNPs and DArTs
Kiwifruit	SNP
Plum	ISSR
Apricot	SNP
Grapes	AFLPs, RAPDs, SSRs, ISSRs,SNPs
Pomegranate	RAPDs, SRAPs, SSRs, ISSRs and SNPs
Pear	AFLPs, RAPDs, SSRs, SNPs, ISSRs,
Guava	RAPDs and SSRs
Date palm	RAPDs, SSRs and ISSRs
Pineapple	RFLPs, RAPDs, SSRs and ISSRs
Peach	RAPDs, AFLPs, SSRs, SRAPs and SSAPs
Strawberry	RAPDs, SNPs, SSRs and SCARs,

(Source: Riaz et al., 2020)

Applications of Molecular Markers

- Genetic Diversity Assessment
- Genetic Linkage Maps
- Tagging of targeted genes
- DNA fingerprinting for varietal identification
- Hybrid identification
- Detection of QTLs
- Marker Assisted Selection

Variations within or among populations or individuals of a species or genus are referred to as

genetic diversity. Evaluating genetic diversity within plant species is a need for high-quality crop production. A great deal of genetic variation can be found in certain plant species, such as fruits. Any attribute, including fruit quality and size, can exhibit diversity. A few studies found that the primary causes of the loss of priceless germplasm are biotic and abiotic stressors. Crop development and breeding programmes rely heavily on the assessment of genetic variability in the available germplasm for their effectiveness. A great method to determine the convolution of the available germplasm, identify hybrid combinations with the highest levels of heterozygosity and heterosis, and determine the relationships between the available germplasm using molecular markers in fruit crops.

Conclusion

Molecular markers added to conventional plant breeding has already shown to be an effective strategy for improving horticulture crops. Next-generation sequencing technology and the creation of new genomic tools will aid in the creation of a saturated linkage map, which will guarantee more accurate QTL location. Additionally, the creation of closely related molecular markers can help with marker-assisted selection, which has a high selection efficiency. These molecular techniques are extremely useful for utilising a variety of genetic resources to create improved crops. Furthermore, the next great green revolution will be fueled by present and future genetic advancements. Deep insights on the genes controlling attractive fruit attributes, their ripening, and scent development mechanisms, including biotic and abiotic stress responses, have been made possible by the present advancements in molecular marker technology.

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