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UTILIZING MOLECULAR MARKERS IN FRUIT PLANTS: A REVIEW

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ABSTRACT

Markers can be defined as heritable items connected with the economically relevant characteristic under polygene control, or they can be defined as any trait of an organism that can be identified with certainty and relative ease, and that can be followed in a mapping population. Molecular markers are widely used in fruit crop development, especially in the fields of disease diagnostics, sex differentiation, hybrid detection, genetic diversity and varietal identification investigations, and marker assisted selection. Plant breeders can focus their efforts in new directions thanks to molecular markers, especially in the areas of taxonomy, phylogenetic analysis, and gene localization. Molecular markers also significantly reduce the time needed to generate new and superior cultivars. The use of molecular markers for marker-assisted selection (MAS) is the most intriguing application. Marker-assisted selection (MAS) is the most fascinating use of molecular markers. DNA markers that are suitable should possess polymorphism and exhibit expression in all organs and tissues at different stages of development. Programs for breeding fruits can be made more effective and efficient by using molecular markers as opposed to traditional breeding methods.

Keywords: Linkage maps, microsatellites, polymorphism, phylogenetic analysis, and polygenes.

Introduction

A phenotypic or morphological test has been the cornerstone of almost all recent advancements in contemporary genetics and breeding. The most effective indirect selection for target genes at the DNA level has been made possible by the development of molecular (DNA) markers, which have made gene selection in plant breeding a viable and strong method. According to Beckman and Soller (1986), markers are any characteristic of an organism that can be consistently and easily identified, and that can be tracked in a mapping population. Alternatively, markers can be described as heritable entities linked to the economically significant attribute that is controlled by polygenes. Observational selection was typically used in traditional plant breeding to identify genetic variability. Molecular biology has advanced to the point where this task is now decided at the molecular level by DNA alterations and their impact on phenotype. Following the extraction of DNA from the plant, modifications in the samples are identified using PCR or hybridization, followed by agarose or acrylamide gel electrophoresis (which identifies distinct molecules according to their size, chemical makeup, or charges). The genetic variants in DNA samples are labeled and tracked using genetic markers. These biological substances may be identified by allelic © 2022 PP House variations. They can be employed as labels or experimental probes to follow a gene, chromosome, organ, or cell.

Marker types

A morphological Marker: Morphological markers are phenotypic traits; they are also referred to as "classical" or "visible" markers. These are the characteristics, such as lower color, seed form, growth patterns, disease response, pigmentation, etc., that are visually scored or that are genetic markers whose inheritance can be observed with the unaided eye. These physical markers

typically indicate easily controlled genetic variations. As a result, they are typically employed in the traditional two-and/or three-point test used to generate linkage maps. In practical breeding, some of these markers might be utilized as indirect selection criteria because of their relationships to other agronomic features.

A molecular identity

All molecules that signify the presence of a chemical or physical process are considered molecular markers. Molecular markers encompass both macromolecules, such as proteins, and biochemical components, such as secondary metabolites in plants, along with deoxyribonucleic acid) (Joshi *et al*., 1999). These macromolecules exhibit readily discernible variations between various species or strains within a species.

The molecular markers were divided into two types by Strauss *et al*. (1992). Both molecular genetics and biochemical molecular markers are obtained through direct study of variation in DNA sequences, or DNA-based markers, while biochemical molecular markers are acquired from the chemical products of gene expression, or protein-based markers. The main drawbacks of morphological and biochemical markers are that their quantities may be restricted and that their results may be affected by the plant's stage of growth or environmental conditions. A comparison between the five commonly used plant DNA markers (Table 1)

Table 1: Characteristics of different molecular markers used in fruit Crops

	RFLP	RAPD	AFLP	SSR	SNP
Genomic coverage	Low copy coding region	Whole genome	Whole genome	Whole genome	Whole genome
Amount of DNA required	10 µg-50	100 ng^{-1}	100 ng^{-1}	120 ng-50	≥50 ng
Quality of DNA Required	High	Low	High	Medium-High	High
Type of Polymorphism	Single base changes, Indels	Single base changes, Indels	Single base changes, Indels	Changes in length of repeats	Single base changes, indels
Level of Polymorphism	Medium	High	High	High	High
Effective multiplex ratio	Low	Medium	High	High	Medium to High
Inheritance	Co-dominant	Dominant	Dominant/ Co dominant	Co-dominant	Co-dominant
Type of probes/primer	Low copy DNA cDNA clone or	Usually, 10 by random nucleotides	Specific sequence	Specific sequence	Allele specific PCR primer
Technically demanding	High	Low	Medium	Low	High
Radioactive detection	Usually yes	No	Usually yes	Usually no	No
Reproducibility	High	Low to medium	High	High	High
Time Demanding	High	Low	Medium	Low	Low
Automation	Low	Medium	High	High	High
Development/ startup cost	High	Low	Medium	High	High
Suitability utility in diversity, genetics and breeding	Genetics	Diversity	Diversity and Genetics	All purpose	All purpose

Molecular Marker Applications in Fruit Crops

Evaluation of genetic variation

A multitude of papers exists regarding the application of DNA markers to evaluate genetic diversity and confirm genetic relatedness among various horticulture crop species. This has broad applications, particularly for woody perennials that are challenging to breed. Mandarin germplasm found in

the North East Himalayas was found to have significant heterogeneity when RAPD markers were used. Mandarin landraces and wild races of mandarins, sweet oranges, grapefruits, lemons, and citranges had their genetic diversity resolved in China using SSR markers. Table 2 lists a few examples of DNA markers that are used to evaluate genetic diversity.

Identification of variants

DNA fingerprinting is the only method used for varietal identification. Molecular markers can produce patterns specific to each genotype, either individually or in groups.

Genetic finger printings are their patterns, regardless of how they are produced by PCR, hybridization, or single-, multi-, or repeat-containing sequences. Table 3 lists a few instances of DNA markers used for varietal identification.

Disease diagnostics

Molecular markers have made it possible to develop diagnostic techniques to identify pathogen with an unprecedented accuracy and speed and to tap genes from as diverse sources including bacteria, plants, and animals to help the scientists create disease-resistant plants (Table 4).

Linkage map construction and QTL mapping

Among the primary uses of DNA markers in agriculture and Linkage maps for various crop varieties are created through study. Using QTL analysis, linkage maps are utilized to locate chromosomal regions containing single gene characteristics (traits regulated by a single gene) and quantitative traits (37). The combined effect of multiple genes results in a large number of significant heritable traits. These traits are commonly denoted as quantitative or polygenic. Numerous plant species' characteristics, including those with agronomic significance, are inherited quantitatively. Characteristics such as drought tolerance, yield, and maturity date are examples. Quantitative trait loci (QTLs) have been used to describe the genetic loci corresponding to these features. The connection between a QTL and a known marker locus that segregates with Mendelian ratios is the crucial characteristic that allows for the identification and characterization of a QTL. This potential is made possible by DNA markers, which enable the identification, mapping, and measurement of the effects of the genes underpinning quantitative traits. Several publications about DNA markers associated with genes or QTLs have been made available (Table 5).

Table 2: Genetic diversity estimate using DNA markers in fruit crops

Sl. No.	Fruit	Marker Type	References
1	Apple	AFLP and RAPDs	Coart *et al.* (2003)
2	Avocado	Mini satellite DNA	Ashworth *et al.* (2003)
3	Banana	RAPDs	Brown *et al.* (2009)
4	Mango	ISSR and RAPDs	Bora *et al.* (2018)
5	Pistachio	Mini satellite marker	Riaz Ahmad *et al.* (2003)
6	Cashew	RAPD and ISSR	Thimmappaiah *et al.* (2009)
7	Pear	SSRs and AFLP	Sisko *et al.* (2009)
8	Peach	RAPD	Lu Zx *et al.* (1996)
9	Peach	RAPD	Warburton *et al.* (1996)
10	Almond	RAPD	Bartolozzi *et al.* (1998)

Table 3: DNA markers for varietal identification

Sl. No.	Fruit	Marker type	References
1	Raspberry	RAPD	Parent *et al.* (1993)
2	Apple	RAPD	Koller *et al.* (1993)
3	Grape cultivar	SSR	Thomas *et al.* (1993)
4	Lemon	RAPD	Deng *et al.* (1995)
5	Mango	RAPD	Schnell *et al.* (1995)
6	Peach	SSR	Swapnil *et al.* (2019)

Table 4: DNA markers for disease diagnostics

Character	Fruit crops with population	Major gene (symbol)	Markers linked	Reference
Grey mold (Botrytis cinerea)	Strawberry		RAPDs	Rigotti *et al*. (2002)
Brown spot disease (Alternaria alternata)	Clementine ×LB-8-10 (Clementine× Minneola)	Aa M1/ aaM1	P12 (15.3 cM) and AL3 (36.7 cM) (RAPDs)	Dalkilic *et al*. (2005)
Eastern filbert blight (Anisogramma anomala)	Hazelnut OSU 245.098×OSU 408.040		5 AFLP markers B2-125 at 4.1 cm	Chen *et al*. (2005)
Citrus tristeza virus Sharka disease	Different citrus hybrids Apricot (Padre ×54P455)	Ctv-R Y	RAPDs	Cristofani *et al*. (2007)
Peach root knot nematodes resistance	Peach cv. 'Juseitou'	Mj	STS-834b	Yamamoto and Hayashi (2002)

Marker assisted selection (MAS) this is one of the important applications of molecular markers as microbes, plants and animals to enable the researchers to develop plants resistant to diseases (Table 4).

Table 5: Markers associated to main polygenic traits in fruit crops

Fruit	Trait	Marker Type	References
Apple	Fire blight resistance	SCAR, SSR	Sylwia *et al*. (2009)
Citrus	Citrus leprosies virus resistance	AFLP and RAPD	Bastianel *et al*. (2009)
Banana	Sugar content Seedlessness,	RFLP AFLP, SSR	Ming *et al*. (2001)
Strawberry	Day-neutrality	AFLP	Weebadde *et al*. (2008)
Apricot	Plum Pox Virus	SSR	Soriano *et al*. (2007)
Sour Cherry	QTL analysis of flower and fruits	RFLP	Wang *et al*. (2010)

Molecular markers may make indirect selection in plant breeding more significant and beneficial. With MAS, the breeder can examine fewer plants and decide on subsequent selections earlier. Breeding for disease resistant behavior has the extra benefit of being possible in the absence of a pathogen once marker data is available. Current markers are created for qualities controlled by several genes or polygenes, as opposed to the monogenic features for which earlier markers were created (Tab. 9). It was once believed that markers that showed a strong correlation with the genes or QTLs in primary QTL mapping may be applied straight to multiple association studies (MAS). Molecular biotechnologies, particularly DNA markers, in conjunction with linkage maps and genomics are applied in molecular marker-assisted breeding (MAB), also known as molecular-assisted breeding, to modify and enhance plant or animal traits based on genotypic assays (Jiang, 2013). This phrase refers to a number of novel breeding procedures, such as genomic selection (GS), genome wide selection (GWS), marker-assisted backcrossing (MABC), marker-assisted recurrent selection (MARS), and marker-assisted recurrent selection (MAS) (Ribaut *et al*., 2010). Up till now, MAB has been widely applied in a number of crop species and is thought to be a novel technique and potent technology for genetic improvement of crop plants (Jiang, 2013; Xu, 2010).

Pyramiding with marker assistance

Molecular markers' primary benefit in gene pyramiding is their capacity to look for and identify several genes in plants whose phenotypic impacts are hard to distinguish. The integration of many disease resistance genes (also known as qualitative resistance genes) into a single genotype is the most common use of pyramiding. Since pathogens typically overcome single-gene resistance over time due to the introduction of new strains of plant pathogens, the goal of this work is to establish "durable" or stable resistance to a disease. There is evidence that a broad range of resistance, or persistent resistance, can be produced by combining several genes that are effective against specific strains of the pathogen. There is evidence that a broad range of resistance, or persistent resistance, can be produced by combining several genes that are effective against specific strains of the pathogen. Because they typically exhibited comparable phenotypes, pyramiding several resistance genes

proved challenging in the past. It is simple to ascertain how many resistance genes each plant has by using linked DNA markers. Adding the quantitative resistance, which is regulated by QTLs, presents yet another viable approach to long-lasting disease resistance.

In tissue-cultured fruit plants, markers can identify Somaclonal variation

It is necessary to be faithful to type in micro-propagation programs. In these situations, somaclonal fluctuations are undesirable. There have been reports of Somaclonal variations in banana. Cytological investigations, AFLP, and RAPD can all identify variants.

Gender identification marker (dioecious plants with sex-linked markers)

RAPD, SCAR, and ISSR can be used to detect papaya sex early on (the sex determination mechanism is attributed to a single gene). ICAR has been funding DNA fingerprinting initiatives at several institutions in India. Table 6 displays a few of these.

Table 6: Supporting institutes on DNA projects (Bhat *et al*., 2010).

Institute	Crop	Work
IIHR	Mango, Citrus, Pomegranate	i) Identification of Mango varieties and genetic relatedness through RAPDS ii) Identification of markers linked to bacterial canker resistance in Lemon iii) Development of markers to test clonal fidelity of pomegranate plants raised through tissue culture.
CPCRI-Kasargod	Coconut	i) DNA fingerprinting of all major coconut accessions, hybrids and high yielding palms using RFLP, RAPD markers ii) Development of molecular markers linked with important traits especially root wilt disease resistance/tolerance and drought tolerance.
NRC-Trichy	Banana	i) Marker aided selection for important traits ii) DNA finger printing of new Musa clones
CISH-Lucknow	Mango	i) DNA finger printing for identification and analysis of existing genotypes, promising new hybrids and clones of mango

Summary

In terms of scientific advancement, the molecular marker technique has brought back the ancient fields of plant taxonomy and quantitative genetics. The markers are immediately useful in research that supports advanced breeding initiatives. The strategic study for quick comprehension of fundamental genetic mechanisms and molecular genome structure is where markers are most commonly used. DNA marker technology's ability to improve fruit crops genetically would require close collaboration between plant breeders and biotechnologists, the availability of highly qualified labor, and a significant financial investment in research.

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