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Applications of Molecular Markers in Fruit Crops: A Review

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Abstract

Markers are any trait of an organism that can be identified with confidence and relative ease, and can be followed in a mapping population or they can be defined as heritable entities associated with the economically important trait under the control of polygenes. Molecular markers have diverse applications in fruit crop improvement, particularly in the areas of genetic diversity and varietal identification studies, disease diagnostics, hybrid detection, sex differentiation and marker assisted selection. Molecular markers provide new directions to the efforts of plant breeders particularly in gene localization, taxonomy, phylogenetic analysis and also play an important role to decrease the time required for development of new and excellent cultivars. The most interesting application of molecular markers is marker-assisted selection (MAS). Suitable DNA markers should be polymorphic in the nature and should be expressed in all tissues, organs, at various developmental stages. Compared with traditional breeding programs, molecular markers can increase the efficiency and effectiveness of fruit breeding programs.

Keywords: Polygenes, phylogenetic analysis, microsatellites, polymorphism, linkage maps

1. Introduction

Until recently virtually all progress in both breeding and modern genetics have relied on the phenotypic or morphological assay. Development of molecular (DNA) markers has created a powerful and practicable tool to perform gene selection in plant breeding, although it is not a real gene selection but the best indirect selection for target genes at the DNA level. Markers are any trait of an organism that can be identified with confidence and relative ease, and can be followed in a mapping population or they can be defined as heritable entities associated with the economically important trait under the control of polygenes (Beckman and Soller, 1986).

In traditional plant breeding, genetic diversity was usually diagnosed through observational selection. But now, with the development of molecular biology this work is determined at molecular level based on DNA changes and their effects on the phenotype. Once DNA was extracted from plant, changes in the samples are determined using PCR or hybridization and subsequent agarose or acrylamide gel electrophoresis (to recognize different molecules based on their size, chemical composition or charges). Genetic markers are used for labeling and tracking the genetic variations in DNA samples. These are biological compounds which can be determined by allelic variations and can be used as experimental probes or labels to track an individual, tissue, cell, nucleus, chromosomes or genes.

2. Types of Markers

2.1. Morphological marker

Morphological markers (also called "classical" or "visible" markers) are phenotypic traits. These are those traits that are scored visually, or they are those genetic markers whose inheritance can be followed with the naked eye such as flower color, seed shape, growth habits, disease response, pigmentation etc. These morphological markers generally represent genetic polymorphisms which are easily identified and manipulated. Therefore, they are usually used in the construction of linkage maps by classical two- and/or three-point tests. Some of these markers are linked with other agronomic traits and thus can be used as indirect selection criteria in practical breeding.

2.2. Molecular marker

Molecular markers are any kind of molecule indicating the existence of a chemical or a physical process. Molecular markers include biochemical constituents (e.g., secondary metabolites in plants) and macromolecules (e.g., proteins

and deoxyribonucleic acid) (Joshi et al., 1999). These macromolecules show easily detectable differences among different strains of a species or among different species. Strauss et al. (1992) distinguished the molecular markers into two classes. Biochemical molecular markers derived from the chemical products of gene expression i.e., protein-based markers and molecular genetic markers derived from direct analysis of polymorphism in DNA sequences i.e., DNA based markers. The major disadvantages of morphological and biochemical markers are that they may be limited in number and are influenced by environmental factors or the developmental stage of the plant.

2.2.1. Comparison of the five widely used DNA markers in plants (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

3. Applications of Molecular Markers in Fruit Crops

3.1. Assessment of genetic diversity

A number of reports are available on the use for DNA markers to assess genetic diversity among species of several horticultural crops, as well as validation of genetic relatedness among them. This has significant application, especially for difficult to breed woody perennials. Using RAPD markers, the wide variability was observed in the mandarin germplasm present in N. E. Himalayas. In China using SSR markers, genetic diversity in mandarin landraces and wild races of mandarins, sweet orange, mandarins, grapefruit, lemon and citranges was resolved. Few examples of DNA markers used for assessment of genetic diversity are mentioned in Table 2.

3.2. Varietal identification

Varietal identification is nothing but DNA fingerprinting. Singly or in groups, molecular markers are capable of producing patterns that are unique for each individual genotype. Their patterns, whether they are generated by PCR or by hybridization with single copy, multi copy, or repeated sequences are referred to as genetic finger printings. Few examples of DNA markers used for varietal identification are mentioned in Table 3.

3.3. 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

as microbes, plants and animals to enable the researchers to develop plants resistant to diseases (Table 4).

3.4. Construction of linkage maps and QTL mapping

One of the main applications of DNA markers in agricultural research is the construction of linkage maps for different types of crops. Linkage maps are used to identify chromosomal regions that contain single gene traits (controlled by a single gene) and quantitative traits using QTL analysis (37). Many important heritable characters are a consequence of the joint action of several genes. Such characters are often referred to as polygenic or quantitative. Several characters of plant species, among which are traits of agronomic importance, are inherited quantitatively. Yield, maturity date and drought tolerance are examples of such characters. The genetic loci for such characters have been referred to as quantitative trait loci (QTLs). The essential feature which makes feasible the finding and characterization of a QTL is its linkage with a known marker locus segregating with Mendelian ratios. DNA markers provide this opportunity by making it feasible to identify, map and measure the effects of genes underlying quantitative trait. Numerous such reports have been provided about DNA markers linked to the genes or QTLs (Table 5).

3.5. Marker assisted selection (MAS)

This is one of the important applications of molecular markers.

Table 2: DNA markers for genetic diversity assessment 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)
6.	Mango	ISSR and RAPDs	Bora et al. (2018)
7.	Pistachio	Mini satellite marker	Riaz Ahmad et al. (2003)
8.	Cashew	RAPD and ISSR	Thimmappaiah et al. (2009)
9.	Pear	SSRs and AFLP	Sisko et al. (2009)
10.	Peach	RAPD	Lu Zx et al. (1996)
11.	Peach	RAPD	Warburton et al. (1996)
12.	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 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)

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)

Molecular markers can potentially increase the importance and usefulness of indirect selection in plant breeding. MAS permits the breeder to make earlier decisions about the further selections while examining fewer plants. An added advantage in breeding for disease resistance behaviour is that this could be done in the absence of pathogen once marker information is available. Earlier markers were being developed for monogenic traits but present markers are developed for traits governed by multigenes or polygenes (Tab. 9). It was previously thought that markers which were tightly linked to the genes or QTLs in primary QTL mapping, can be used directly in MAS.

Molecular marker -assisted breeding (MAB), also called molecular-assisted breeding, is the application of molecular biotechnologies, specifically DNA markers, in combination with linkage maps and genomics, to alter and improve plant or animal traits on the basis of genotypic assays (Jiang, 2013). This term is used to describe several novel breeding strategies, including marker -assisted selection (MAS), marker-assisted backcrossing (MABC), marker-assisted recurrent selection (MARS), and genome wide selection (GWS) or genomic selection (GS) (Ribaut et al., 2010). MAB is regarded as a novel strategy and a powerful methodology for genetic improvement of crop plants, and up to now it has been extensively used in multiple crop species (Jiang, 2013; Xu, 2010). In terms of the resources invested and the expectations presented, however, MAB has not yet been very successful.

3.6. Marker assisted pyramiding

The main advantage of molecular markers in gene pyramiding is their ability to search and discover multiple genes in plants whose phenotypic effects are difficult to be separated. The most widely application of pyramiding is the integration of several genes for disease resistance (i.e., integration of qualitative resistance genes) into a single genotype. The motivation of this work is to develop “durable” or stable resistance to a disease, because pathogens usually overcome single-gene resistance over time due to the emergence of new strains of plant pathogens. Some evidence suggests that the combination of multiple genes (effective against certain strains of the pathogen) can provide durable resistance (broad spectrum resistance). In the past, pyramiding of multiple resistance genes was difficult because they generally had a similar phenotype. Using linked DNA markers, the number of resistance genes per plant can be easily determined. Inserting the quantitative resistance (which is controlled by QTLs) offers another promising strategy for durable disease resistance.

3.7. Markers to detect somaclonal variation in tissue cultured fruit plants

In micropropagation programme, true to type are required. Somaclonal variations in these cases are undesirable. In banana, somaclonal variants were reported. Variants can be detected by RAPD, AFLP and cytological studies.

3.8. Marker for gender identification (*Sex-linked markers in dioecious plants*)

Papaya sex can be identified at an early stage using RAPD, SCAR, ISSR (a single gene is responsible for the sex determination mechanism). In India ICAR has been supporting projects on DNA fingerprinting in a number of institutes. Some of which are shown in Table 6.

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

4. Conclusion

In terms of scientific progress, the old disciplines of quantitative genetics and plant taxonomy have been revived by the molecular marker approach. The markers have immediate applications in supportive research for advanced breeding programmes. The major application of markers lies in the strategic research for rapid understanding of basic genetic mechanisms and genome organization at molecular level. The success of DNA marker technology for bringing genetic improvement in fruit crops would depend on close interaction between plant breeders and biotechnologists, availability of skilled man power and substantial financial investment on research.

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