Use of Molecular Marker in Fruit crops for their traits and Genetical Diversity Analysis

Ritik Thakur, Vikram Singh*, Dhrubajyoti Banerjee

Department of Horticulture, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India.144411.

*Corresponding author: vikram.2603@lpu.co.in

Abstract. The introduction of molecular markers has caused a shift in the genetic diversity of fruit crops. They are crucial to a wide range of disciplines, such as taxonomy, gene mapping, phylogenetic analysis, and the assessment of disease resistance. This extensive study looks at various molecular markers, including AFLP, RAPD, SSRs, SCoT, and SNPs, for the purpose of characterizing fruit crop genomes. We examine how they contribute to our understanding of disease resistance, genetic diversity, and evolutionary, dynamics in a wide variety of fruit crops, such as nuts and tropical, subtropical, and temperate fruits. Breeders can now create new cultivars with improved traits, quicker breeding schedules, and better genetic resource conservation. They have made it feasible to perform customized genetic analyses and gain a deeper understanding of genetics and evolution in domains other than agriculture. The sustainable use of genetic resources from fruit crops, conservation initiatives, and the larger scientific and medical fields are all significantly impacted by this historical perspective.

1 Introduction

DNA sequences known as molecular markers serve as stand-ins for alterations at the genome level. Thanks to recent advancements in molecular techniques, it is now possible to accurately identify plant species and genera. Numerous disciplines, such as taxonomy, gene mapping, phylogenetic analysis, genome tagging, genetic relationship assessment, genetic diversity evaluation, and cultivar, clone, or hybrid identification, have employed molecular markers [1]. To characterize various germplasms, one must comprehend genome sequencing, phylogenetic analyses, genetic diversity, and genome barcoding using molecular markers. By employing markers based on sequence, it is feasible to recognize and group desired attributes. When assessing disease-resistant genes in horticultural crops, functional markers are useful tools. There are opportunities to sequence important yield-related genes and agronomic traits through QTL mapping and genome association. Understanding genetic differences within and between populations benefits evolutionary biology as well as conservation efforts. The introduction of new cultivars and the replacement of genotypes that differ from one another are the main causes of low genetic variability. Future breeding programs should take serious note of this lack of diversity [2]. Furthermore, random DNA markers (RDMs) are DNA-based markers that can originate from any genomic region. Recently, there has been a lot of interest in the synthesis of molecular markers from transcribed genome regions. This problem has been approached using both lab-based and computational techniques. Using quantitative polymerase chain reaction in real-time or PCR for short, has increased in environmental science. This technique monitors the amplification of particular DNA molecules in real-time as it happens, in contrast to conventional PCR, which evaluates amplification at the end of the process. Predicting the patterns of gene expression is
possible, and a review of gene expression is becoming more and more important in many biological research domains [15]. Simple sequence-repeats (SSRs), random amplified polymorphic DNA RAPD, microsatellites, and amplified fragment-length polymorphism are some of the more recent and extensively utilized PCR-based DNA marker technologies. Nevertheless, these methods have certain drawbacks. For instance, RAPD is not very repeatable, AFLP is expensive, and developing SSR polymorphism primers for species-specificity necessitates understanding flanking sequences. ISSR-PCR is a solution that deals with a lot of these problems [25]. Numerous (SNP) markers have been found because of extensive research projects and the sequencing of the entire human genome. New technologies that can analyze up to one million SNP markers at once allow for the simultaneous analysis of multiple markers. Using methods like (whole-genome scanning) WGS, Genome-wide association studies or association-genetics, the entire genome can be thoroughly examined to find correlations between particular markers and quantitatively inherited traits [17].

Table 1 Molecular markers and their applications.

<table>
<thead>
<tr>
<th>Marker Type</th>
<th>Application</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microsatellite</td>
<td>Genetic connections between cultivars; Ancestry information</td>
<td>(Ashworth et al., 2003)</td>
</tr>
<tr>
<td>QTL analysis</td>
<td>Identification of genetical areas linked to leprosis resistance</td>
<td>(Bastianel et al., 2009)</td>
</tr>
<tr>
<td>Morphology &amp; Microsatellite</td>
<td>Assessment of genetical diversity and relationships</td>
<td>(Bora et al., 2018)</td>
</tr>
<tr>
<td>EST &amp; In Silico Hybridization</td>
<td>Genes that are particularly expressed in effect to the detection of (CTV) infection.</td>
<td>(Yaly et al., 2007.)</td>
</tr>
<tr>
<td>RAPD</td>
<td>Identification linked to Alternaria resistance.</td>
<td>(Dalkilic et al., 2005)</td>
</tr>
<tr>
<td>Grapevine Repetitive DNA</td>
<td>Identification of repetitive DNA for cultivar identification</td>
<td>Thomas et al., 1993</td>
</tr>
<tr>
<td>Advanced Backcross-QTL</td>
<td>Using sophisticated backcross-QTL analysis to improve crops</td>
<td>Wang et al., 2010</td>
</tr>
<tr>
<td>STS Markers</td>
<td>Identification of new nematode resistance genes</td>
<td>Yamamoto, 2002</td>
</tr>
</tbody>
</table>
2 Sub-tropical and Tropical fruits:

2.1 By using Quantitative trait loci (QTL) analysis:
The genetic relationships between microsatellite markers and domesticated avocado cultivars (*Persea americana Mill*). Genetic relationships amongst various avocado cultivars, providing vital information for breeding initiatives and genetic resource preservation. The history of avocado cultivars, which supports deliberate breeding efforts to preserve genetic diversity [4]. *Citrus reticulata Blanco*, *Citrus sinensis L.*, and *Citrus sinensis Osbeck* as a collective were subjected to quantitative trait loci (QTL) analysis to test for citrus leprosis resistance. This interspecific hybrid citrus family has been shown to contain genetic regions associated with resistance to leprosis. The genetic mechanisms responsible for disease resistance in citrus breeding [6]. Microsatellite markers and morphology in a polymorphic evaluation of mango (*Mangifera indica L.*) genetic diversity. The morphological and microsatellite marker-based methods assess the degree of genetic diversity and relationships amongst mango cultivars. A thorough comprehension of the diversity of mango germplasm was generated through the amalgamation of morphological characteristics and molecular markers [9]. The Citrus tristeza virus (CTV) displayed unique patterns of gene expression using in silico hybridization and EST research. The study used EST analysis and silico hybridization to investigate changes in gene expression, which provided insight into the molecular responses of citrus plants to viral infections, particularly CTV [12].

2.2 Using (RAPD) Randomly Amplified Polymorphic DNA marker:
Mandarin hybrids have RAPD fragments connected to an anti-Alternaria gene. Disease-resistant Mandarin hybrids were found to have RAPD fragments associated with a gene linked to Alternaria resistance. This contributes to the development of resistant cultivars by providing citrus cultivars with the genetic foundation for disease resistance [13]. Lemon used RAPD markers mutations were found both in vivo and in-vitro. Lemon mutants were created both in controlled and open condition using (RAPD) markers. Molecular marker usefulness in identifying genetic changes and mutation among different lemon cultivar [14]. RAPD marker aid in the recognition of *Mangifera indica L.* cultivar and the verification of genetic connections. The usefulness of molecular markers in this situation is demonstrated by their capacity to distinguish between various mango varieties and verify their genetic relationships [30].

2.3 Analysis of fruit crops for genetical diversity by using RFLP marker:
Agricultural species exhibit both restriction fragment length polymorphism (RFLP) and genetic development. The idea of utilizing RFLP analysis for genetic enhancement in agricultural species was first presented by this work. The role of RFLP marker in selection support, genetic diversity,
and plant breeding [7]. Genetic markers' function in improving fruit crops. Molecular markers: their usefulness in improving fruit crops. It illustrated how diverse molecular markers, breeding techniques by enabling effective gene mapping and diversity analysis, such as RAPD and SSR markers, and selection [8]. Utilizing molecular marker technology for plant genome analysis. The role of molecular markers in plant. It includes a number of marker strategies and how they can be used to map genes, identify genes, and comprehend genetic diversity in plant genomes [20]. The prospects and obstacles for molecular breeding and analysis of the challenges and possibilities of molecular breeding. Tools' potential to boost crop yields and support sustainable agriculture in resource-poor areas [28].

2.4 Analysis of fruit crops for Genetical diversity by using (RAPD) and (ISSR) marker:

Both molecular genetics and biochemical markers are used in forest tree biosystematics research. Understanding the evolutionary divergence and adaptation of distinct tree species can be aided by these genetic markers [33]. Using RAPD and ISSR markers, assessment of genetic diversity of cashew germplasm is done. Analysis of genetic diversity of cashew germplasm by the use of RAPD and ISSR markers for better interpretation of the genetic variability found in cashew cultivars [17]. Repetitive DNA sequences found in grapevines can be used to distinguish between cultivars and classes. Cultivar differentiation is made possible by the repetitive DNA of grapevines. By analyzing these sequences, the study improves the accuracy of grapevine cultivar identification, thereby advancing grape breeding and research [35].

2.5 Fruit crop genetic diversity analysis by RAPD marker and SSR marker:

Utilizing quantitative-trait locus application analysis (QTL) with advanced backcross in crop improvement plans to increase crop yields. These methods facilitate the process of creating improved crop varieties [36]. To interpret the genetic diversity of Prunus persica L. Batsch was RAPD markers were utilized and investigated. It uses RAPD markers to find species-level heterogeneity, with a focus on peach genetic diversity. The peach genetic resources and aids in breeding efforts [37]. Recently discovered genes that shield peaches from nematodes that tangle roots and their STS markers. The peaches were resistant to root-knot nematode, there were novel resistance genes and related STS markers. By creating peach varieties that are resistant to nematodes [40].

2.6 Genetical diversity analysis of fruit crops by use of SCoT Marker:

Using the Start Codon Targeted Technique (SCoT), genic variation was observed in 14 isolates of Ralstonia solanacearum from various locations in Egypt. After the data were analyzed using the allelic distance and UPGMA-method, apart from the two main groups, the isolates were further classified into multiple subgroups [16]. The start codon targeted (SCoT) polymorphism of Cicer arietinum L. for genic diversity interpretation and fingerprinting. The SCoT data revealed that between 84% and 98% of the genotypes showed genetic similarity. Principal Coordinate Analysis (PCA) revealed that the ten genotypes of chickpeas clustered in four quadrants, indicating genetic variation between the samples [3]. According to the clustering analysis, P.mairei, P.polyphylla var.yunnaensis, P.thibetica, P.delavayi var.petiolata, P.axiparis var.axialis, P.fargesii, and P.polyphylla var.delavayi are clustered together. P.polyphylla var. stenophylla is found in a single cluster. The SCOT markers, which can also be used to reliably identify plants in Paris, provide molecular evidence supporting the taxonomic status of species and interspecific relationships [22].
3 Nuts

3.1 Utilizing AFLP and RAPID markers for analysis:
Randomly-amplified polymorphic DNA (RAPD) was used to analyze California almond cultivars along with breeding lines in order to determine genetic and relatedness traits. Using RAPD profiling, California almond cultivars and lineages for breeding have genetically characterized. The usefulness of RAPD markers for assessing genetic relatedness and diversity in almond genetic material, which supports almond breeding techniques [5]. The 408.040 at OSU AFLP markers from hazelnuts are linked to protection against the eastern filbert blight. In this work, marker linked to hazelnut resistance to the blight in the east were identified using AFLP markers. The identification of resistant genotypes, which has implications for the breeding of hazelnuts [10]. Microsatellite marker analysis of Pistacia vera L. nuts. To use micro-satellite markers to identify pistachio nuts. Pistachio cultivar authentication through genetic markers is essential for business and quality control [27].

4 Temperate fruit crops

4.1 Examination using RAPD marker and AFLP marker:
Using the method of AFLP and micro-satellite markers, genic variation analysis was conducted on the critically endangered Belgian wild apple (Malus sylvestris L. Mill.). AFLP and microsatellite markers are used to study genetic diversity in the critically endangered wild apple species. The diversity of genes and population dynamics of wild apples aided conservation efforts. [11]. RAPD markers are used for apple cultivar identification and analysis. RAPD markers were used in this work to identify apple varieties. The molecular markers can be used practically to differentiate between apple types, supporting varietal authentication and quality management [21]. Rootstock cultivars of peaches are identified using the AFLP-DNA marker. The peach rootstock varieties and used RAPD-DNA marker to identify the subjects. The utilization of molecular markers to differentiate and describe several peach rootstock variants [23].

4.2 By use of QTL analysis:
Genetic control of sugar content in an intricate autopolyploid sugarcane crop using QTL analysis. QTL analysis was performed to gain a better understanding of the genic regulation of sugar quantity in sugarcane, an intricate auto-polyploid crop. The potential use of molecular-marker to determine the genic basis of complicated characteristics in poly-ploid plants [24]. The genetic relationships of Slovenian pears were investigated using molecular markers. Slovenian pear varietals and evaluated genetic links using molecular markers. The effective molecular methods are at determining genetic relatedness and diversity among pear varieties [31]. Discovering and recognizing a locus in two apricot enhanced linkage maps that encode resistance to the plum-pox virus. The maps identify and map a genetic locus in apricot, with a focus on plum-pox virus resistance. Plant-virus interactions and resistant variety formation [32]. Botrytis cinerea Pers.: Fr. in strawberries is detected with PCR-based molecular marker characterization, which is sensitive and specific (Fragaria × ananassa Duch.). Molecular markers for the fungal pathogen Botrytis cinerea were created to identify the disease in strawberries. PCR-based markers' application for quick and precise disease diagnosis in horticulture crops [29].

4.3 Using the MAS and SSR techniques: To improve fruit quality, low-chill peach cultivars were crossed within their own species; the crossings were verified using SSR markers. The low chill
peach cultivars employed SSR markers to confirm hybridity and intraspecific hybridization. The work shows how to employ molecular markers to guarantee the genetic integrity and quality of hybrid plants [26]. Testing apple (*Malus domestica*) cultivars for fire-blight resistance using molecular testing and creating clones of them. Apple cultivars and breeding clones were screened for fire blight resistance using molecular methods. The hardier varieties of apples by discovering genetic markers associated with resistance [19]. The process for Marker-assisted selection in fruit crops was shown in Fig. 1. The QTL to day-neutrality in octoploid strawberries was found with a linkage mapping technique. To find QTL linked to day-neutrality in octoploid strawberries. The strawberry flowering traits, which help with breeding initiatives [38]. Plant molecular breeding is becoming more widely used. the growing importance of molecular breeding in attempts to improve plants. Molecular-marker approaches being incorporated into conventional plant breeding to produce more accurate and efficient crop development [39].

![Fig.1 Steps involved in marker assisted selection (MAS) for fruit crops.](image)

**5 Conclusion**

The creation of molecular markers has significantly improved genetic diversity analysis, an essential part of fruit crop conservation and improvement. These markers allow researchers to determine breeding objectives, prioritize conservation strategies, and assess genetic diversity within and between fruit crop populations, ensuring the crops’ capacity to adjust to shifting weather patterns and consumer preferences more accurately. Moreover, molecular markers have provided insight into the ecological adaptation, migration trends, and domestication of fruit crops over the course of their evolutionary histories. Decisions in context to the use and protection of genic resources as well as conservation efforts have been affected by this knowledge. Beyond agriculture, these markers have advanced personalized genetic analysis and encouraged innovation and specialized care in a range of scientific and medical fields. They have also improved our understanding of genetics, evolution, and disease. This historical context guides conservation initiatives and aids in our decision-making about the preservation and use of genetic resources. by
promoting customized care, enabling accurate genetic testing, and assisting in the evolution of agriculture. Their capability to distinguish certain genes and variations has enormous potential to advance our knowledge of illness, genetics, and evolution. This will therefore promote creativity and personalized treatment across a range of scientific and medical domains.

6 Research gap:

Further investigation into functional genomics is needed to pinpoint the exact genetic mechanisms underlying desirable traits in the field of fruit crop genetics and conservation. Furthermore, a more thorough examination of the biodiversity of fruit crops worldwide in a variety of climates and regions is imperative. Research on genetic markers for resistance is necessary given the persistent problems caused by pests and newly emerging diseases. A key component of sustainable agriculture is the adaptation of fruit crops to shifting climatic patterns. There is an urgent need for efficient genetic resource management and conservation strategies, especially in relation to endangered species. Breeding fruit crops can be matched to market demands if the genetic foundation of consumer preferences is recognized. Molecular breeding must be implemented in resource-constrained developing nations. Two potential directions for development are to investigate CRISPR-Cas9 genetic editing and to integrate molecular markers with other omics technologies. Last but not least, evaluating the socioeconomic effects of improved fruit crop varieties created using molecular markers is critical for the advancement of both agriculture and the economy.

7 Future scope and prospective:

The use of sophisticated molecular markers and genetic editing technologies may facilitate precise breeding, leading to fruit varieties that are suited to characteristics and consumer preferences. Improved nutrient profiles and health benefits may result from further research on nutrigenomics in fruit cultivars. Research on disease and pest resistance remains essential because there are always new threats that call for genetic remedies. Global fruit varieties that are rare or endangered can be identified and protected through conservation efforts. It is crucial to assess the social effects of improved varieties and to match breeding to market demand. A future in agriculture that is more resilient and sustainable will also result from research into the function of fruit crops in delivering ecosystem services and from supporting educational initiatives.

References:
