**Role of Molecular Marker in Plant Breeding**

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**Abstract**

Plant breeding is a collection of theories and techniques that alters a plant's genetic makeup to make it better suited for human requirements. Traditional plant breeding has progressed over time from basic seed saving of the best harvest to seed selection based on Mendel's rules. The selection process has been accelerated and the scope of traditional plant breeding has multiplied in recent years with the development of morphological and biochemical markers. The discovery that there is widespread polymorphism in natural populations, the degree of which can be determined by sequencing or creating restriction maps an application of new molecular biology tools was the process that actually revolutionised plant breeding in the 20th century. Breeders can produce new kinds with enhanced valuable qualities by utilising the most recent discoveries in the disciplines of genetics, molecular biology, and biotechnology. The widespread use of molecular markers in a variety of fields of plant science, including germplasm evaluation, genetic mapping, map-based gene discovery, trait characterization, and crop improvement, has shown that agronomically important traits in crop plants can be genetically modified using molecular technology. The advantages of MAB over conventional breeding methods are numerous.

**Keywords:** Molecular markers, Molecular breeding, Polymorphism,Marker assisted selection.

 **I. Introduction**

Plant breeding is a combination of ideas and techniques that alters a plant's genetic makeup to make it better suited to human needs. It is a combination of science and art, based on a breeder's capacity to recognise variations in the economic features of plants and to enhance these variations using scientific knowledge. Although current plant breeding techniques are founded on scientific principles of cytogenetics and genetics, which only began with the rediscovery of Mendel's article that was first published in 1866 (Mendel, 1866), plant breeding has been practised for about 10,000 years. Mendel's law of inheritance served as the cornerstone for the wealth of information that has collected in genetics, establishing that the genetic building blocks (genes), which can be passed down from one generation to the next, are what truly control how traits are passed down. Since then, plant breeders have worked to rearrange these genes in an effort to combine beneficial features into a single variety that is more suited to human needs [1].

This goal has been mostly attained by conventional plant breeding, which entails crossing the best plants with the most desirable features (such as high yield or disease resistance). It can take up to 15 years for wheat, 18 years for potatoes, and even longer for some other crops to establish a variety, thus thousands of individual plants are chosen and examined for this purpose [1]. Even while hybridization and selection techniques are significantly more advanced in modern plant breeding, there are still a number of issues that need to be resolved.

The term "molecular breeding" (MB) can be described broadly as the process of using genetic engineering or gene modification, molecular marker-assisted selection, genomic selection, etc. to modify DNA at the molecular level to enhance desirable traits in plants and animals. However, the term "molecular breeding" is more frequently used to refer to "molecular marker-assisted breeding" (MAB), which is defined as the use of molecular biotechnologies, specifically molecular markers, in conjunction with linkage maps and genomics, to improve and change plant or animal traits based on genotypic assays [2].

Breeders can produce new kinds with enhanced valuable qualities by utilising the most recent discoveries in the disciplines of genetics, molecular biology, and biotechnology. Molecular markers are tags that can be used to identify certain genes and position them in relation to other genes. The introduction of DNA markers, especially those based on the Polymerase Chain Reaction (PCR), has led to a breakthrough in the study of plant genetics. Molecular markers are the focus of interest for cutting-edge research today. These can be divided into two groups: PCR-based techniques and hybridization-based approaches. The PCR-dependent polymorphic markers Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD), Inter-Simple Sequence Repeats (ISSR), and Single Nucleotide Polymorphism (SNP) techniques have been developed, while Restriction Fragment Length Polymorphism (RFLP) is a hybridization-based marker [3].

The use of molecular markers in plant breeding and genetics is now well established as a powerful tool for indirect selection of challenging traits during the seedling stage of plant breeding. These speeds up the process of traditional plant breeding and makes it easier to improve challenging traits that are difficult to improve by the conventional methods of plant breeding. In this direction, many genes and quantitative trait loci (QTLs) regulating agronomic traits and conferring tolerance to both abiotic and biotic stresses have been identified and tagged using molecular markers in several crop species, especially cereals [4 ,5,6].

Beginning with a brief introduction to molecular markers as a potent tool for plant breeding, we will address general principles and methodologies of marker-assisted breeding in plants and discuss some issues related to the procedures and applications of this methodology in practical breeding, including marker-assisted selection, marker-based backcrossing, marker-based pyramiding of multiple genes, etc.

**II. Genetic marker**

Any easily measurable phenotype associated with an interesting trait that is intended to be marked. They are employed to 'flag' the location of a certain allele or the inheritance of a specific feature. Phenotypes where a single "Mendelian" factor accounts for all or a portion of the variance seen in the population of interest. A genetic marker has the following three characteristics:

* It should be locus-specific.
* It should be polymorphic in the studied population.
* It should be easily phenotyped.

The quality of a genetic marker is typically measured by its:

* Heterozygosity in the population of interest
* Polymorphism Information Content (PIC)

Polymorphism Information Content is defined as the probability of identifying one homologue of a given parent that transmitted an allele to a given offspring, the other parent being genotyped as well.

PIC= probability that the parent is heterozygous x probability that the offspring is informative

 *i i −1 n*

*PIC = 1 − ∑ pi 2 − ∑ ∑ 2pi 2 p j 2*

 *i=1 i=1 j=i+1*

Where,

*pi* and *pj* represents the population frequency of the *i*th and *j*th allele.

Heterozygosity (H) is a value that measures the genetic variation, calculated according to the formula:

 *i*

*H=1-∑ pi 2*

 *i*

**A. Morphological marker**

A special and distinctive morphological characteristic represents the expression of a morphological marker. Environmental factors may influence morphological markers. It is typically only partially connected to the desired gene. The growth stage may have an impact on how it manifests phenotypically. These markers have a very low amount of polymorphism and are uncommon in a natural population.

**B. Molecular markers**

Molecular markers are particular DNA snippets that can be found throughout the entire genome. Particular parts of the genome contain molecular markers. They are employed to 'flag' the presence of a specific gene or the inheritance of a specific trait. Molecular indicators have no phenotypic bias.

**III. Types of molecular maker**

Molecular markers are broadly divided into three groups

* Hybridization based, e.g., RFLPs
* PCR techniques based, e.g., RAPD, AFLP, microsatellites or SSR
* Single Nucleotide Polymorphisms (SNPs)

Additionally, molecular markers can be categorised according to the mechanism of gene action (dominant or codominant markers) and according to the mode of heredity (cytoplasmic inheritance vs. genomic inheritance). There are currently a wide range of molecular markers accessible, but selecting the right ones to achieve goals is crucial. High levels of polymorphism, co-dominance, and ease of allele detection are required of ideal markers. Numerous species, including cotton, maize, Brassica napus, and other plants have been characterised using the molecular marker technique [ 7, 8, 9, 10].

**IV. Properties of ideal DNA markers:**

* Level of polymorphism. Ideally, the marker should be highly polymorphic in breeding material (i.e., it should discriminate between different genotypes)
* Not subject to environmental influences. The inference of a marker’s genotype should be independent of the environment in which the individual lives or its developmental stage
* Occurrence and uniform distribution throughout the genome (ubiquitous distribution in the genome)
* Required small amounts of leaves and DNA sample
* It should have repeatability/reproducibility of results
* Cheap, simple and quick assay
* Co-dominant (able to differentiate homozygous from heterozygous individual)

According to the circumstances and resources available for the breeding programme, a breeder must choose a good molecular marker that satisfies the majority of the requirements [11].

**V. Use of Molecular Markers in Breeding Programmes**

A breeding technique called marker-aided selection uses genetic markers that are closely connected to the desired trait or gene(s) to indirectly select for that characteristic in segregating or non-segregating generations. In its most basic form, it can be used to take the place of an examination of a trait that is time-consuming or expensive to assess. It may be simpler and less expensive to screen for the existence of the marker allele related to the gene than to analyse the trait when a marker is discovered that co-segregates with a significant gene for an important trait. The relationship between the marker and the gene should then periodically be confirmed. The breeder must figure out how to combine the most advantageous alleles for the QTLs that were discovered when it comes to more complex, polygenic regulated traits. In this situation, it is possible to examine the breeding material for markers connected to QTLs. Based on this study, customised crosses can be designed to combine QTL alleles from various sources to produce the best genotype. The issue of restricted genetic variety that is frequently observed in breeding stocks is not resolved when marker assisted selection is used within the current breeding material to improve a breeding programme. To improve the genetic quality of breeding stock, marker-assisted selection may be used in numerous ways [12].

It may be possible to support a regulated influx of new genetic material by marker assisted selection. The wild species frequently has desired components that the produced material could lack. By repeatedly backcrossing, these components can be introduced to elite cultivated material. Breeders, however, are frequently hesitant to utilise this technique because to uncertain linkage drag. These are brought on by other genes that mistakenly cross-transfer with the genes that regulate the desired phenotype. To remove the undesirable genes and bring the material back to an appropriate agronomic value, it can take a lot of work and screening. The genetic elements causing the desired traits in the unadopted material can be identified using markers. By examining linked markers during a backcross programme, the existence of the targeted QTL alleles can be continually confirmed [12].

**A. Marker assisted selection (MAS)**

The most helpful qualities for MAS are those that are challenging to select, such as disease resistance, salt tolerance, drought tolerance, heat tolerance, and quality traits (such as the aroma of basmati rice and vegetable flavour). In order to accomplish the goals of the scheme, the approach entails selecting plants with a fixed, favourable genetic background at specific loci at an early stage of development, carrying out a single, large-scale marker-assisted selection while preserving as much allelic segregation in the population, and screening large populations. In order to preserve as much Mendelian allelic segregation among the genotypes that were chosen, no selection is applied beyond the target genomic regions. As a result of selection using DNA markers, the genetic diversity at unselected loci may enable breeders to produce novel varieties and hybrids through traditional breeding in response to breeding programme goals [12].

**VI. Application of DNA markers in crop improvement**

**A. QTL mapping**

Some of the most challenging tasks of plant breeders relate to the improvement of traits that show a continuous range of values. Genetic factors that are responsible for a part of the observed phenotypic variation for a quantitative trait are called quantitative trait loci (QTLs). The term QTL was coined by Gelderman. Conceptually it can be a single gene or may be a cluster of linked genes of the trait. Although similar to a gene, a QTL merely indicates a region on the genome comprised of one or more functional genes. These quantitative characteristics, such as yield, plant length, and days to flowering, among others, are significant ones. Because the relationship between reported trait values in the wild (the phenotype) and the underlying genetic makeup (the genotype) is not clear-cut, selection for quantitative traits is challenging [12].

Through marker-trait association analysis, [13] reported using a broad panel of 96 genotypes of lentil in their study to find QTL for nine agronomic characteristics. For nine agronomic parameters, this study found significant genetic variation among the genotypes of lentils, with estimates of medium to large broad sense heritability (h2 = 0.58–0.95). 534 SSR markers were screened, and 266 polymorphic loci were found that produced 697 alleles, ranging from 2 to 16 per locus across genotypes. In the current study, a small number of EST-SSR markers shown significant relationships with phenotypic variance (7.3-23.8%), days to maturity, pods/plant, secondary branches/plant, 100 seed weight, yield/plant, and reproductive duration. Therefore, these markers can be employed as functional markers in the breeding of lentils to create improved varieties.

[14] created a QTL map from an inter-subspecific population of Lens culinaris ssp culinaris x Lens culinaris ssp orientalis for plant height, pod dehiscence, number of shoots, and seed diameter. Using RAPD, SSR, and AFLP markers, [ 15] found QTLs in lentil for earliness and plant height. In a population of RILs produced from a cross between the lentil accessions WA 8649090 x Precoz,[16] discovered five distinct QTLs for winter hardiness. In MAS, the IISR marker Ubc 808-12 was found to be helpful in predicting winter survival in populations that segregate. Through composite interval mapping, [17] discovered eight QTLs for the ascochyta blight resistance gene in lentils. Five QTLs were identified in F2 population of ILL 5588/ILL 7537 whereas three QTLs were detected in F2 of the cross ILL 7537/ ILL 6002.

**B. Tagging of disease and insect resistance genes**

Plant breeding techniques have showed considerable potential when using DNA-based markers. Finding molecular markers that are strongly associated with resistance genes helps speed up the pyramiding of important genes into the elite background, which reduces costs. The selection of resistant plants in segregating generations is made simple once the resistance genes are marked with molecular markers [12].

From a population of 131 recombinant inbred lines resulting from a wide cross between Cicer arietinum and Cicer reticulatum, DNA markers associated with two closely connected genes for resistance to fusarium wilt races 4 and 5 in chickpea were found [18]. Nineteen novel markers were discovered using bulk segregant analysis around the fusarium wilt resistance genes on linkage group 2 (4.1–9.0 cM). R-2609-1 displayed the strongest linkage (2 cM) to the race 4 resistance locus. In a population of recombinant inbred lines, [19] discovered flanking markers for chickpea fusarium wilt resistance genes. Foc 1 resistance gene was flanked by H3A12 and TA101 SSR, while Foc 2 was located between TA96 and H3A12. The TA194 and H1B06y markers surrounded the Foc3 locus.

Through the use of bulk segregant studies, [20]. discovered two RAPD markers, OPF04700 and OPA091375, that were connected to the open and tall plant type gene in the pigeon pea F2 population of the cross between TT44- 4 and TDI2004-1. In 15 genotypes of the open-tall plant type, these markers were validated. In a pigeonpea F2 population descended from GS1 x ICPL87119, [21] employed bulk segregant analysis using 39 RAPD primers to identify two markers (OPM03704 and OPAC11500) that were linked to Fusarium wilt susceptibility alleles.

Yellow mosaic virus (YMV) resistance gene-linked molecular markers were created by [22] in Vigna sp. from a population that was segregating for YMV disease resistance. In order to search for MYMIV resistance genes, [23] found the molecular markers CYR1 and YR4 in an F2 population. In F2 plants and F3 offspring, the MYMV resistance gene co-segregated with CYR1. A multiplex PCR process can be performed to employ these two markers simultaneously.

Using 30 SSR markers to test 32 pigeon pea lines, [24] evaluated the DNA polymorphism. Five parental combinations were identified for developing genetically diverse mapping populations suitable for the development of tightly linked markers for Fusarium wilt and sterility mosaic disease resistance based on polymorphism of marker alleles, higher genetic dissimilarity coefficient, and phenotypic diversity for resistance data to Fusarium wilt and sterility mosaic disease.

An interesting case study of marker-assisted breeding in *Allium* is the introduction of downy mildew resistance from A. roylei into *A. cepa*. Due to the interaction of two dominant genes that condition resistance, the evolution of resistant cultivars has been accelerated [25]. Three A. roylei-specific RAPD markers were discovered on chromosome 3 by [26, 27] using a bulked segregant analysis method [28]. After being transformed to SCAR, these markers were no longer able to distinguish between plants that were susceptible and those that were resistant. Alternative AFLP markers that are closely associated to downy mildew resistance have now been found, and they should be helpful for marker-assisted selection [25].

In cultivars that are tolerant or resistant to sucking insect pests, epicuticular waxes play a crucial influence [29]. In recent years, it has been noted that the amount and types of epicuticular waxes vary naturally in onions [30,31]. Wax concentration is reduced in onion accessions with glossy or semi-glossy foliage, which is related to a lack of affinity for onion thrips. The most prevalent wax is hentriacontanone-16, which is found in higher concentrations in accessions with waxy leaves, followed by semi-glossy and glossy varieties [31]. Through acyl reduction and decarbonylation processes, two loci on chromosomes 2 and 5 regulate the quantity of wax, respectively. In order to change the types and amounts of epicuticular waxes and create cultivars resistant to onion thrips, SNP markers associated with these locations are being found for marker-assisted breeding [30, 31].

**C. Tagging of male sterility genes**

The absence of the necessity for hand emasculation makes a cytoplasmic male sterile system ideal for use in the creation of hybrid seeds. A maternally inherited condition called CMS, which is frequently linked to mitochondrial DNA rearrangements, mutations, and editing, is characterised by the inability to produce viable pollen without impacting the fertility of females. The molecular analysis of the CMS system is made possible by DNA markers connected to several restorer loci that have been discovered using RAPD and STS in various crops. After backcrossing to create restorer lines, these co-dominant markers can be used to determine the homozygous restorer genotypes. The restorer lines could be created in less time this way than by using conventional techniques. RAPD marker was connected to a male sterility gene by [32]. Male sterile (A) lines 288A (derived from C. scarabaeoides) and 67A (derived from C. sericeus) produced a distinctive amplicon of 600 bp in response to primer OPC-11, whereas this amplicon was lacking in the maintainers and putative R lines (TRR 5 and TRR 6), respectively. Between male sterile lines, two potential R lines, and suppliers of male sterility genes, genetic distance based on similarity index revealed significant genetic heterogeneity.

**D. Diversity evaluation**

For the purpose of determining plant breeders' and farmers' rights, crop variety stability and identification have become very important. Comparative anatomy, morphology, embryology, physiology, and other fields with poor genetic resolution are traditionally used as the foundation for evaluation and conservation of genetic variety and biodiversity. A quick and thorough genetic resolution is now possible thanks to recent developments in molecular biology that have produced strong genetic tools.

The application of RFLP of mtDNA for the investigation of pigeonpea diversity was demonstrated by [33]. They used maize mtDNA probes to test restriction enzyme digested segments of 28 accessions, comprising 12 species of the genus Cajanus organised into 6 sections, including 5 accessions of the farmed species C. cajan and 4 species of the genus Rhyncosia. The accessions of wild species (C. scarabaeoides, C. platycarpus, C. acutifolius) and farmed species of C. cajan showed intra-specific diversity in addition to inter-specific variation.

[34] used RAPD markers to create DNA fingerprints for both domesticated and wild pigeon pea accessions. Low levels of polymorphism were found in the species that were cultivated, whereas high levels of polymorphism were found in the species that were wild. The ability to identify between all pigeonpea accessions, including the cultivars under study, showed the value of RAPD in pigeon pea genetic fingerprinting. For the purpose of grouping domesticated and wild pigeon pea accessions, [35] created 561 amplified fragment length polymorphism (AFLP) loci. According to Jaccard's similarity index, there was more diversity among the various groupings of wild species.

In order to identify the molecular diversity in lentil, [36] generated a novel set of microsatellite markers. In order to determine the genetic diversity among 18 blackgram cultivars, [37] used RAPD and IISR markers.

**E. Heterosis breeding**

DNA markers can also be used to predict heterosis in hybrids, which is a significant application. It costs money to test hybrids for heterosis or combining potential in the field. In a number of cereal crops, including rice, oats, and wheat, molecular markers have been employed to correlate genetic diversity with heterosis. It has been suggested that pedigree information and similarity metrics based on RFLP could be utilised to forecast the best hybrid pairings. However, heterosis and DNA-based genetic distance have been found to have both low and significant correlations [12].

Three age groups of soybeans were examined to determine the relationship between heterosis and molecular (isozyme and RFLP) variation between the parents [38]. Indicating that heterosis in yield may not be related to genetic variety at the molecular level as indicated by RFLPs, parental RFLP diversity was not substantially correlated with mid parent and better parent heterosis. Despite the fact that isozyme diversity in the parents was linked to yield heterosis, the small number of assayable isozyme loci in soybean makes it of little significance.

**F. Hybrid seed purity testing**

It is vital to confirm that the intended cross has taken place, the amount of self-pollination between the female parents meets the required purity, and the product is of sufficient quality in order to assess the quality of hybrid seeds. The grow out test has long been the only way to verify the purity of hybrid seeds. The purity of F1 hybrids is now checked using the RAPD and RFLP markers. SSR 218, SSR 306, and Ty2 gene CAPs gene markers were utilised by [39] to assess the F1 purity of tomato hybrids Pbc EC 538408, Pbc EC 520061, and H 86 EC 520061.

**G. Gene pyramiding**

It basically involves finding and introducing a variety of genes that confer resistance to distinct insect or microbial pests, or that confer resistance to a single pest via distinct host pathways. Including multiple resistance genes in a single variety is one way to increase the resistance's longevity. It is believed that the durability of resistance could be increased by 50 years if the pyramided genes were never used as single genes. However, it is challenging to confirm how many resistance genes have been successfully pyramided during the cultivation process. Plants with just one resistance gene are just as resilient as those with three resistance genes, though the latter's resistance is probably more long-lasting.

A recombination inbred line population was created by crossing two phytopthora tolerant soybean cultivars, Conrad and Hefeng 25, and using 161 SSR markers, 7 environmentally stable QTLs (QPRR-1 and QRR2 from Conrad and QRR3 to QRR7 from Hefeng 25) were identified. The more QTLs, the higher the level of tolerance. [40] pyramided phytopthora tolerance QTLs in soybean.

**Table 1**. **Acceleration in varietal development**

|  |  |
| --- | --- |
| Details | References |
| Release of US barley variety Tango that contains two QTL for adult resistance to stripe rust | [41]  |
| Advancement of a ‘Sloop type’ variety with cereal cyst nematode (CCN) resistance for commercial release | [42] |
| Release of ‘Flagship’ variety in Australia in 2004 after following whole genome breeding approach | [42]  |
| Release of two Indonesian rice cultivars ‘Angke’ and ‘Conde’, in which marker assisted selection (MAS) was used to introduce xa5 into a background containing xa4 | [43]  |
| Development of quality protein maize (QPM) through marker-aided transfer of opaque2 gene in backcross programmes | [44]  |

**VII. Future perspectives**

A major factor in raising crop yield, productivity, and food security is plant breeding. Plant breeders, however, encounter significant challenges. However, plant breeders confront significant obstacles due to increased production as a result of global warming, the emergence of novel disease and insect biotypes, and a number of abiotic stressors that frequently lower crop yield. Recent developments in biotechnology and genomics are paving the way for overcoming obstacles, and DNA markers are also being used to identify new genes that confer resistance to important biotic and abiotic stresses. The growth of the crop gene pool and the creation of enhanced crop varieties suitable for various agro-climatic situations resulted from the integration of desired genes from various backgrounds of elite cultivars. Global warming, the emergence of novel disease and insect biotypes, and a number of abiotic factors, all of which frequently lower agricultural yield.

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