

ROLE OF MOLECULAR MARKER IN PLANT BREEDING

Abstract

Plant breeding is a field that aims to improve plants to meet human requirements by altering their genetic makeup. Over time, traditional plant breeding has evolved from basic seed saving of the best harvest to seed selection based on Mendel's rules. However, recent developments in molecular biology have revolutionized this process. The discovery of widespread polymorphism in natural plant populations, determined through sequencing or creating restriction maps using molecular biology tools, marked a turning point in plant breeding in the 20th century. This breakthrough accelerated the selection process and expanded the scope of traditional plant breeding. With the aid of genetics, molecular biology, and biotechnology, breeders can now create new plant varieties with enhanced and valuable traits. One significant advancement in this area is the use of molecular markers. These markers have found widespread application in germ plasm evaluation, genetic mapping, map-based gene discovery, trait characterization, and crop improvement. Molecular technology has provided breeders with the ability to genetically modify agronomically important traits in crop plants. As a result, molecular-assisted breeding (MAB) has numerous advantages over conventional breeding methods.

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

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I. INTRODUCTION

Plant breeding is amalgamation of ideas and techniques that alters a plant's genetic makeup to make it better suited to human needs. It is a blend 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. Plant breeding has been practiced for about 10,000 years, although its current techniques are based on scientific principles of cytogenetic and genetics, which only began with the rediscovery of Mendel's article first published in 1866 (Mendel, 1866). 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].

The goal has been largely achieved through conventional plant breeding, involving the crossing of the best plants with the most desirable traits, 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) encompasses various processes such as genetic engineering, gene modification, molecular marker-assisted selection, and genomic selection, all aimed at enhancing desirable traits in plants and animals at the molecular level by modifying DNA. However, "molecular breeding" is commonly used to specifically refer to "molecular marker-assisted breeding" (MAB). MAB involves the utilization of molecular markers in conjunction with linkage maps and genomics to improve and modify plant or animal traits based on genotypic assays [2].

Utilizing the latest discoveries in genetics, molecular biology, and biotechnology, breeders can create new varieties with improved valuable traits. Molecular markers play a significant role in this process by identifying specific genes and their positions relative to other genes. The introduction of DNA markers, particularly those based on the Polymerase Chain Reaction (PCR), has revolutionized the study of plant genetics. Nowadays, molecular markers are a focal point of cutting-edge research. These markers can be categorized into two groups: PCR-based techniques and hybridization-based approaches. PCR-dependent polymorphic markers, such as Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD), Inter-Simple Sequence Repeats (ISSR), and Single Nucleotide Polymorphism (SNP) techniques, have been developed, along with the hybridization-based marker known as Restriction Fragment Length Polymorphism (RFLP) [3].

The use of molecular markers in plant breeding and genetics has become a well-established and powerful tool for indirectly selecting challenging traits during the seedling stage of plant breeding. This accelerates the traditional plant breeding process and simplifies the improvement of difficult traits that are not easily amenable to conventional breeding methods. As a result of these advancements, numerous genes and quantitative trait loci

(QTLs) responsible for agronomic traits and providing tolerance to both abiotic and biotic stresses have been identified and marked using molecular markers in various crop species, particularly cereals [4,5,6].

Starting with a concise introduction to molecular markers as a potent tool for plant breeding, we will cover the fundamental principles and methodologies of marker-assisted breeding in plants. We will then explore various aspects related to the procedures and applications of this approach in practical breeding, which includes marker-assisted selection, marker-based backcrossing, and marker-based pyramiding of multiple genes, among others.

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:

- The analysis must be locus-specific.
- In the population under study, it ought to be polymorphic.
- It ought to be simple to phenotype.

A genetic markers quality is often assessed by:

- Heterozygosity in the relevant population.
- Polymorphism Information Content (PIC).

Polymorphism Information Contents is defined as the likelihood that one parent's homologue, with the other parent's genotype also known, passed along an allele to a particular offspring.

PIC= Probability that the Parent Is Heterozygous X Probability that the Offspring is Informative

$$PIC = 1 - \sum_{i=1}^n p_i^2 - \sum_{i=1}^n \sum_{j=i+1}^n 2p_i^2 p_j^2$$

Where,

p_i and p_j 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:

$$H = 1 - \sum_{i=1}^n p_i^2$$

1. 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.

- 2. Molecular Markers:** Molecular markers are specific DNA fragments dispersed throughout the genome, residing in certain regions. These markers serve the purpose of 'flagging' the presence of particular genes or the inheritance of specific traits. Unlike phenotype-based indicators, molecular markers are unbiased in their assessments.

III. TYPES OF MOLECULAR MAKER

Molecular markers are broadly categorized into three groups:

- Hybridization-based markers, such as RFLPs.
- PCR techniques-based markers, including RAPD, AFLP, microsatellites, or SSR.
- Single Nucleotide Polymorphisms (SNPs).

Additionally, molecular markers can be categorised according to the mechanism of gene action (dominant or co dominant markers) and according to the mode of heredity (cytoplasm 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

The characteristics of an ideal molecular marker for plant breeding include:

- Highly polymorphic in breeding material to differentiate between different genotypes.
- Not influenced by environmental factors, ensuring independence of genotype inference from the environment or developmental stage.
- Ubiquitous distribution throughout the genome, occurring uniformly.
- Requires small amounts of leaves and DNA samples.
- Demonstrates repeatability and reproducibility of results.
- Cost-effective, simple, and quick assay for practical application.
- Co-dominant, enabling differentiation between homozygous and heterozygous individuals.

A breeder must select a suitable molecular marker that meets the majority of the requirements, considering the specific circumstances and available resources for the breeding program. [11].

V. USE OF MOLECULAR MARKERS IN BREEDING PROGRAMMES

Using DNA markers that are intimately linked to the desired feature or gene(s), a breeding method known as marker-aided selection applies indirect selection for that trait in segregating or non-segregating generations. It can be used to replace an assessment of a trait

that is time-consuming or expensive to examine in its most basic form. When a marker is found to co-segregate with a relevant gene for an important trait, it may be easier and less expensive to screen for the presence of the marker allele associated to the gene than to examine the characteristic. 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. When marker assisted, selection is employed to enhance a breeding plan using the current breeding material, the problem of restricted genetic variability that is typically seen in breeding stocks is not remedied. Marker-assisted selection can be employed in a variety of ways to enhance the genetic quality of breeding stock [12].

It may be possible to support a regulated influx of new genetic material by marker assisted selection. The wild form of a species frequently has desired elements that the produced form can be missing. These elements can be added to elite grown material by frequently backcrossing. However, because of the uncertainty of linkage drag, breeders are generally unwilling to adopt this strategy. These are brought on by other genes that mistakenly cross-transfer with the genes that regulate the desired phenotype. It can take a lot of work and screening to get rid of the undesired genes and restore the material's proper agronomic potential. Markers can be used to pinpoint the genetic components in the un adopted material that are responsible for the desired features. A backcross programme can continuously verify the presence of the desired QTL alleles by looking at related markers [12].

Marker Assisted Selection (MAS): The most challenging traits to select, such as disease resistance, salt tolerance, drought tolerance, heat tolerance, and quality attributes (e.g., aroma in basmati rice and vegetable flavor), are the most valuable for Marker-Assisted Selection (MAS). This approach involves conducting a single large-scale marker-assisted selection while retaining significant allelic segregation in the population. It entails screening large populations and early-stage selection of plants with a fixed, favorable genetic background at specific loci to achieve the breeding program's objectives. The selection is confined to the target genomic regions, maintaining ample Mendelian allelic segregation among the selected genotypes. The use of DNA markers for selection allows breeders to preserve genetic diversity at unselected loci, potentially enabling the creation of novel varieties and hybrids through conventional breeding in response to the breeding program's goals [12].

VI. UTILISING DNA MARKERS TO ENHANCE CROPS

1. QTL Mapping: The enhancement of characteristics with a continuous range of values is one of plant breeders' most difficult challenges. Quantitative trait loci (QTLs) are genetic elements that contribute to a portion of the observed phenotypic variance for a quantitative trait. Gelderland is credited with creating the QTL. Conceptually, it could be a single gene or a group of related genes that contribute to the trait. A QTL simply denotes a section of the genome that contains one or more functional genes, despite being comparable to a gene. These quantitative traits include important ones, including yield, plant height, and days before flowering, among others. Selection for quantitative traits is

problematic functional genes because the relationship between reported trait values in the wild (the phenotypic) and the fundamental genetic structure (the genotype) is ambiguous. [12].

In their study, [13] conducted marker-trait association analysis using a diverse panel of 96 lentil genotypes to identify QTLs for nine agronomic traits. The research revealed significant genetic variation among the lentil genotypes for the nine agronomic parameters, with moderate to large broad sense heritability estimates ($h^2 = 0.58-0.95$). To conduct the analysis, the researchers screened 534 SSR markers, identifying 266 polymorphic loci that generated 697 alleles, ranging from 2 to 16 per locus across the genotypes. Among the EST-SSR markers studied, a limited number exhibited significant associations with phenotypic variance (7.3-23.8%) for traits such as days to maturity, pods per plant, secondary branches per plant, 100-seed weight, yield per plant, and reproductive duration. These findings suggest that these markers can serve as functional markers in lentil breeding to develop improved varieties.

[14] For plant height, pod dehiscence, number of shoots, and seed diameter, a QTL map was made from an inter-subspecific population of *Lens culinaris* ssp *culinaris* x *Lens culinaris* ssp *orientalis*. RAPD, SSR, and AFLP markers are used. [15] discovered QTLs in lentil for plant height and earliness. RILs developed from a cross between the lentil accessions WA 8649090 and Precoz were present in the population. [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.

- 2. 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 broad cross between *Cicer arietinum* and *Cicer reticulatum*, DNA markers associated with two closely related genes conferring resistance to fusarium wilt races 4 and 5 in chickpea were identified [18]. A total of 19 novel markers were discovered using bulk segregant analysis, spanning the region of 4.1–9.0 cM around the fusarium wilt resistance genes on linkage group 2. Among these markers, R-2609-1 exhibited the strongest linkage (2 cM) to the race 4 resistance locus. In another population of recombinant inbred lines, researchers [19] identified flanking markers for chickpea fusarium wilt resistance genes. Specifically, the Foc 1 resistance gene was flanked by H3A12 and TA101 SSR markers, while Foc 2 was situated between TA96 and H3A12. The Foc3 locus, on the other hand, was flanked by the TA194 and H1B06y markers.

Through the use of bulk segregant studies, [20]. Discovered two RAPD markers, OPF04700 and OPA 091375, 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.

[24] evaluated the DNA polymorphism by testing 32 pigeon pea lines using 30 SSR markers. They based their assessment on the polymorphism of marker alleles, genetic dissimilarity coefficient, and phenotypic variation for resistance to Fusarium wilt and sterility mosaic disease. As a result, they identified five parental combinations that are genetically distinct, making them suitable for developing closely linked markers for Fusarium wilt and sterility mosaic disease resistance.

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].

- 3. 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]. In contrast to the developers and presumptive R lines (TRR 5 and TRR 6), male sterile (A) lines 288A (derived from *C. scarabaeoides*) and 67A (derived from *C. sericeus*) produced a unique amplicon of 600 bp in response to primer OPC-11. Genetic distance based on similarity index among male sterile lines, two prospective R lines, and suppliers of male sterility genes demonstrated significant genetic heterogeneity.

- 4. 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.

[33] demonstrated the application of RFLP of mtDNA to investigate pigeonpea diversity. They conducted tests on restriction enzyme digested portions of 28 accessions, which comprised 5 accessions of the cultivated species *C. cajan* and 4 species of the genus *Rhynchosia*. Among the 28 accessions, there were 12 species of the genus *Cajanus*, further divided into 6 groups. The study revealed not only inter-specific diversity but also intra-specific diversity within the accessions of *C. cajan*, including both farmed species and wild species such as *C. scarabaeoides*, *C. platycarpus*, and *C. acutifolius*.

[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 black gram cultivars, [37] used RAPD and IISR markers.

- 5. 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].

In their investigation [38], three age groups of soybeans were studied to explore the relationship between heterosis and molecular (isozyme and RFLP) variation among the parents. The results indicated that parental RFLP diversity did not show a significant connection with mid parent and superior parent heterosis, suggesting that heterosis in yield may not be related to genetic variation at the molecular level as revealed by RFLPs. Regarding isozyme diversity in the parents, the soybean species had a modest number of assayable isozyme loci. Despite this, it was found that isozyme diversity in the parents was linked to yield heterosis, implying its importance in contributing to the observed heterosis in yield.

6. Hybrid Seed Purity Testing: In order to evaluate the quality of hybrid seeds, it is essential to make sure that the planned cross has occurred, the amount of self-pollination between the female parents satisfies the required purity, and the product is of appropriate quality. For a long time, the grow out test was the sole way to confirm the integrity of hybrid seeds. The RAPD and RFLP markers are currently used to assess the purity of F1 hybrids. [39] used SSR 218 and SSR 306 gene markers as well as Ty2 gene CAPs gene markers to evaluate the F1 purity of the tomato hybrids Pbc EC 538408, Pbc EC 520061, and H 86 EC 520061.

7. 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. If the pyramided genes were never used as single genes, it is thought that the longevity of resistance may be extended by 50 years. How many resistance genes have, however, been successfully pyramided during production is difficult to verify. Even though the resistance of the latter is likely more durable, plants with three resistance genes are just as resilient as those with just one.

Two phytophthora-tolerant soybean cultivars, Conrad and Hefeng 25, were crossed, and using 161 SSR markers, seven environmentally stable QTLs (QPRR-1 and QRR2, from Conrad, and QRR3 through QRR7, from Hefeng 25) were found. The level of tolerance increases with the number of QTLs. QTLs for soybean phytophthora tolerance that are pyramided.

Table 1: Acceleration in varietal development

Details	References
The US barley variety Tango has been released, which possesses two QTL (Quantitative Trait Loci) providing adult resistance to stripe rust.	[41]
A 'Sloop type' variety with resistance to cereal cyst nematode (CCN) has been developed and is ready for commercial release.	[42]
In 2004, Australia released the 'Flagship' variety after employing a whole genome breeding approach.	[42]
Two Indonesian rice cultivars, 'Angke' and 'Conde', were released using marker-assisted selection (MAS) to introduce xa5 into a background containing xa4.	[43]
Quality Protein Maize (QPM) was developed by transferring the opaque2 gene using marker-aided techniques in backcross programs.	[44]

VII. FUTURE PERSPECTIVES

Plant breeding plays a crucial role in enhancing crop yield, productivity, and food security. However, plant breeders face significant challenges due to factors such as global warming leading to increased production, the emergence of novel disease and insect biotypes, and various abiotic stressors that often reduce crop yield. Nevertheless, recent advancements in biotechnology and genomics offer potential solutions to these challenges, with DNA markers being utilized to identify new genes that provide resistance to important biotic and abiotic stresses. The integration of desired genes from diverse backgrounds of elite cultivars has led to the expansion of the crop gene pool and the development of improved crop varieties suitable for diverse agro-climatic conditions, addressing the impacts of global warming, novel diseases, insect biotypes, and abiotic factors that frequently affect agricultural yield.

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