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Molecular Markers: Their Importance, Types, and Applications in Modern Agriculture

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Abstract: To meet the expanding global population's need for food, high-yielding hybrids, varieties, and superior populations of food crops must be developed. This can be accomplished by employing a molecular marker system. Breeders can use molecular markers to choose genotypes directly because they are not affected by the environment. Crop yields must be increased to meet the needs of growing people around the world, as well as the threat of new viruses, if climate change is to be avoided in the next decades. Agriculture is confronted with rising demand from a growing population, as well as dangers of restricted production area as a result of climate change, such as water scarcity, soil salinity, or harvest weather that is unpredictable. Plant breeding's ultimate goal is to create better crops. Crop productivity is something that can be improved. Any region (locus) in an organism's genome where the DNA base sequence differs among different individuals in a population is referred to as a molecular marker. DNA markers have been created in recent years and have shown to be effective tools for cereal breeding. The overall goal of this review paper is to evaluate the role, kind, and utility of markers in in crop improvement program.

Keywords: Molecular Marker, Crop Improvement, Mapping, Genetic

1. Introduction

To improve crop varieties, breeders previously utilized phenotypic selection based on morphological traits [41]. Today's geneticists are frequently educated to extrapolate information from what individual genes operate in a model species in a controlled setting to how similar genes are anticipated to act in complicated genomes in a corresponding field environment [26]. Understanding the genetic arrangement of plant populations and the level of diversity within and across them is critical for optimal plant utilization and conservation [6]. To keep up with the predicted growth in population, food production in tropical developing countries must skyrocket. Plant breeders can use genetic markers to make genetic advances more precisely and quickly than they can with phenotypic selection [9]. DNA markers also open the door to previously impossible aims, such as disease resistance gene pyramiding.

Agricultural and plant breeding achievements have moved as molecular marker technology has advanced [10]. Current molecular marker developments, such as the development of high-throughput genotyping devices, genotyping by sequencing, and the availability of significant agricultural plant genome sequences, have opened up new opportunities for crop improvement [40]. With the progress of molecular biology, a new type of marker based on polymorphisms in the DNA sequence known as molecular markers has arisen, opening up new possibilities for plant breeding difficulties [23]. Molecular markers can be located across the genome, are unaffected by environmental factors, and can be discovered in any tissue or developmental stage [16].

Plant breeders currently use marker-assisted methodologies to aid in the selection of favorable gene combinations that occur naturally within a crop species [33]. Variation or polymorphisms in certain sections of the DNA can be detected using molecular techniques among people in a population [28]. Many crop species have already benefited from the use of genetic markers for genetic characterisation and development. Individual organisms or species have genetic variations, which are represented by genetic markers [18].

Molecular markers are increasingly used to study the genetic basis of agronomic traits and to facilitate the transfer

and accumulation of useful traits between breed lines [42]. Advances in molecular plant breeding, genetics, genomic selection, and genome editing have led to a better understanding of molecular markers, as well as a deeper awareness of the diversity of plants available, and vastly improved breeding tactics [5, 27].

The use of molecular techniques to identify changes in the DNA of individual plants is known as molecular genetics, and it has a wide range of uses in crop production [18]. Marker aided selection is a breeding approach in which genetic markers closely linked to the target trait/gene(s) are utilized in segregating and non-segregating generations for indirect selection. Breeders can generate new kinds with better useful qualities thanks to advances in genetics, molecular biology, and biotechnology [29]. Generally the purpose of this review was to assess the various types and applications of molecular marker systems for crop development.

2. Molecular Markers' Importance in Plant Breeding Programs

Molecular markers were first used in breeding programs in the 1980s. DNA marker technology has the potential to dramatically increase the efficiency of plant breeding, just as molecular biology has transformed research in the life sciences [30]. Molecular markers offer several advantages over traditional phenotypic and biochemical markers in plants [20].

2.1. Cultivar Identification/Purity Assessment/Hybrid Testing

Predicting heterosis in hybrids is another important application of DNA markers. Field testing of hybrids for heterosis or combinability is expensive. Molecular markers have been used to correlate genetic diversity and heterosis in a wide variety of cereal crops (such as rice, oats, and wheat) [13]. Individual plants' genuine identities can be confirmed using markers. The genetic diversity and heterosis of diverse crop plants are investigated using molecular markers. SSR markers have recently been used to explore heterotic groups and trends. In order to take advantage of heterosis in cereal hybrid development, high levels of genetic purity must be maintained [43]. The conventional 'grow-out tests,' which entailed growing the plant to maturity and analyzing morphological and floral features, were replaced with SSR and STS markers to certify purity in hybrid rice [8].

2.2. Population Genetics and Genetic Diversity Analysis

The stability and identification of crop varieties have become increasingly important in forecasting plant breeder/farmer success. Recent advances in molecular markers and genome sequencing provide a tremendous excellent chance to assess the genetic diversity of a large germplasm collection [7]. The effectiveness of using genetic markers to determine genetic diversity and to classify genetic material has been demonstrated. Several methods of measuring genetic diversity in germplasm, cultivars, and advanced breeding material have been successful, including restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and microsatellites [15].

According to Nadeem et al. [30], the most often employed markers for determining genetic diversity in various crops are DArT markers and SNPs markers. DNA marker analysis is used to determine the genetic structure of plant populations, species, genera and families in order to maximize the acquisition, maintenance and use of germplasm collections.

2.3. Constructing a Genetic Linkage Map

Genetic Link Maps are a useful tool in genetic and genomic research. DNA markers can be used to create genetic linkage maps. A unified core genomic map for rice has been created [31].

2.4. Quantitative Trait Loci Mapping (QTLs)

Small segments of DNA (loci) that contribute to the phenotypic value of quantitative traits are called quantitative trait loci (QTLs). QTL mapping is the process of using molecular markers to find the genes that affect the traits of interest. Quantitative trait locus analysis (QTL) is a statistical approach that combines two types of data: phenotypic data (trait measurements) and genotypic data (usually molecular markers) to try to explain the genetic basis of variation in complex traits [44]. Molecular markers are of particular importance in QTL research and are considered an optimal tool for this task.

2.5. Simple Trait Mapping/ Major Gene Mapping

One of the most recent applications of modern molecular biology techniques is the rapid improvement of genomic studies [18]. With the help of DNA-based markers, researchers can determine the sequence of genes along the chromosomes and the distances between them. Genetic mapping uses methods to determine the distance between two genes, as well as to identify the location of a gene. Molecular markers are used in molecular biology and biotechnology to identify a specific DNA sequence in a pool of unknown DNA [37]. Genetic mapping is based on chromosomal recombination during meiosis, which leads to gene segregation. Some markers are categorized as linked markers because they are on the same chromosome as the gene of interest [30].

2.6. Genetic Mutation Mapping

Genetic mapping of a mutation-described gene is the first step toward isolating and cloning the corresponding ordinary gene, and eventually discovering its encoded protein. To offer genetic mapping, next-technology sequencing combines unmarried nucleotide polymorphism detection, mutation area, and the capability identification of causal sequence editions [24].

2.7. Selection Aided by a Marker (MAS)

Resistance to diseases, drought, salt, heat tolerance, and quality attributes (basmati rice aroma, vegetable flavor) are among the features that MAS is most beneficial for. When introducing traits from exotic germplasm, DNA markers can be used to select children with the least amount of additional genetic material from the exotic parent. Once tightly connected markers for genes or QTLs have been found, they should be employed for MAS [30]. In marker assisted or marker aided selection, DNA markers are employed. MAS has a number of advantages over traditional selection.

2.8. Evolutionary Classification and Molecular Taxonomic

Advances in molecular biology techniques have provided more information on the genetic structure. In the study of crop evolution, DNA markers are useful. Molecular markers are currently widely utilized in order to obtain complete information on phylogeny and evolution. Extensive investigations of the evolution of a range of crops, including the identification of crop progenitors, have been made possible because to the discovery of increasingly informative molecular markers [2].

2.9. Pyramiding with the Help of Markers

Pyramiding is the technique of simultaneously integrating many genes/QTLs into a single genotype. DNA markers may aid selection in the absence of phenotyping [20]. The most common use of pyramiding is the integration of many disease resistance genes into a plant to achieve "durable" or stable disease resistance. A single stripe rust gene and two QTLs were pyramided using the SSR marker, resulting in quantitative resistance. It's likely that field testing won't be able to tell the difference between different sources of disease resistance [8]. Combining numerous sources of resistance in the same cultivar, on the other hand, can reduce the probability of the pathogen acquiring resistance-breaking mechanisms.

2.10. Backcrossing a Gene of Interest

Introgression is a way of transferring genes of importance from plant genetic resources to crop cultivars. Some desired features are picked from exotic germplasm and backcrossed into crop plants in this procedure [14]. The use of wild genes and their transfer into crop plants has been greatly aided by MAS. Almost all economically relevant cultivated plants have received several genes of relevance from wild plants [30]. SSR markers are commonly employed for this purpose. DNA markers can be used to select children with the least amount of extra genomic material from the exotic parent while also indirectly choosing that trait when introgressing traits from exotic germplasm [34].

3. Types Molecular Markers

Molecular markers systems reveal variations in genomic DNA sequence. Many types of molecular markers have been developed, and developments in sequencing technology have improved crop improvement [30].

3.1. Restriction Fragment Length Polymorphism (RFLP)

RFLP is a molecular marker based on the differential hybridization of cloned DNA to DNA fragments in a sample of restriction enzyme digested DNAs. Before being applied to plants, RFLP technology was developed in the 1980s for use in human genetics [38]. The restriction fragment length polymorphism (RFLP) approach was the first to detect polymorphism at the DNA sequence level.

RFLP was the first approach for detecting polymorphism at the DNA sequence level [37]. The sole marker method based on hybridization was RFLP, which was the first molecular marker technique. RFLP was the first molecular marker technology and the only hybridization-based marking system. Genetic information, which makes up the genes of higher RFLP was the first molecular marker technique and the only hybridization-based marking system. Individuals. Because they relied on DNA-DNA hybridization, early molecular markers were slow and expensive. Restriction fragment length polymorphisms (RFLPs) were the first generation of hybridization-based markers to have a substantial impact on agricultural biotechnology [39].

The sole marker system that represents hybridization-based markers is restriction fragment length polymorphism (RFLP). The initial stage in the RFLP process is to isolate pure DNA. The RFLPs in this study are tiny and co-dominant in nature [25]. A typical technique is used to examine all RFLP markers. However, the analysis necessitates the use of a time-consuming and expensive technique. To visualize the hybridization findings, autoradiography (if the probes are radioactively tagged) or chemiluminesence might be used (if non-radioactive, enzyme-linked methods are used for probe labelling and detection). The same findings can be obtained using any visualization tool. Several visualization approaches will be employed depending on the laboratory settings [37].

The use of gel electrophoresis to separate DNA fragments of various sizes, followed by the transfer of the fragments to a nylon membrane (Southern blot), and visualization of specific DNA sequences using radioactive or chemiluminescent probes exposed to an X-ray film, is required for the identification of RFLPs [37].

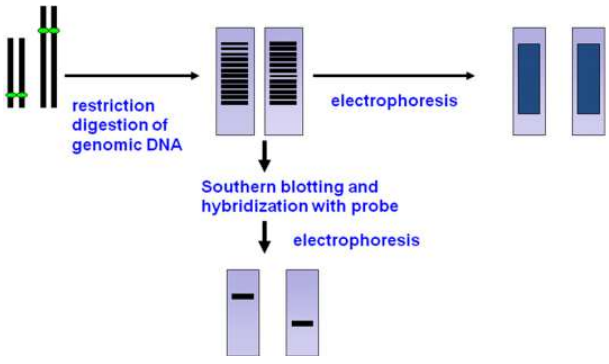

Figure 1. *Illustration of RFLP development.*

3.2. Simple Sequence Repeats (SSRs) or Microsatellite

Simple sequence repeats (SSRs), also known as microsatellites, cover a considerable percentage of the genome. Simple sequence repeats (SSRs), commonly known as microsatellites, are a form of repetitive DNA element. These are useful genetic markers for detecting variations in genes of all eukaryotes between and within species [25]. Microsatellites include mononucleotide (A), dinucleotide (GT), trinucleotide (ATT), tetranucleotide (ATCG), pentanucleotide (TAATC), and hexanucleotide (TAATC) (TGTGCA). Microsatellites can be found throughout the genome, as well as in the chloroplast and mitochondria. Plants have a lot of SSRs, with one every 6-7 kb on average [30].

These markers are notable for being dominant fingerprinting markers as well as codominant sequence tagged microsatellites (STMS) markers. At each locus, microsatellites systems are made up of DNA repeats in tandem. Simple dinucleotides (such as (TG) n) are commonly used as tandem repeats, with each dinucleotide being repeated 10 times. These repeat motifs are bordered by conserved nucleotide sequences that can be used to build forward and reverse primers for PCR-amplifying the DNA portion containing the SSR [17]. SSR alleles, which are variable-length amplified products, can be sorted and seen by silver staining, autoradiography (if primers are radioactively tagged), or automation (if primers are fluorescently labelled). SSR analysis lends itself to automation and multiplexing, allowing genotyping over large numbers of lines and simultaneous study of several loci. SSRs can be discovered by scanning DNA databases (such as EMBL and Genebank) or by enriching genomic DNA libraries with short inserts (200-600bp) for specific repeats [23].

All eukaryotes have microsatellites in their genomes. These DNA markers are important for genetic mapping and population studies since they are eukaryotic. These DNA markers are useful for genetic mapping and population research due to their abundance [12]. Because of their abundance, SSR length polymorphisms are used in mapping and population studies. DNA finger printing and genetic diversity analysis have both proven successful with SSR markers. SSR markers are widely used in crop enhancement tactics for genetic analysis. They are widely employed in plants because to their abundance, hyper-variability, and suitability for high-throughput study [18].

3.3. Inter-simple Sequence Repeat (ISSR)

ISSRs are semi-random markers amplified by PCR in the presence of one complementary primer to a target microsatellite [36]. ISSR amplification is a type of polymerase chain reaction that uses simple sequence repeat primers to amplify areas between target sequences. It works by amplification of DNA segments separated by a distance that allows for amplification between two identical but oppositely oriented microsatellite repeat regions [23]. One sort of PCR-based fingerprinting technology is Inter SSR (ISSR) amplification. The method takes advantage of the abundant and random distribution of SSRs in plant genomes by amplifying DNA sequences between tightly related SSRs [18].

Amplification of areas between two SSRs with compatible priming sites using this approach. Utilizing SSR specific primers in combination with an arbitrary primer or in combination with primers that target other abundant DNA sequences such as retrotransposons are two more ways for fingerprinting using primers complementary to SSR motifs [37]. Because of the significant degree of variety in the number of repeats, it can be utilized as a location marker in genomic mapping. PCR is used to determine the length of each allele, with specific oligonucleotide primers flanking the repeating region. After electrophoresis, the DNA products are seen [23].

3.4. Amplified Fragment Length Polymorphisms (AFLPs)

Amplified fragment length polymorphisms (AFLPs) are PCR-based markers that can be used to quickly test for genetic variation. AFLPs are DNA fragments with diverse nucleotide sequences that have been amplified in huge numbers using PCR. The RFLP and PCR techniques are combined in this procedure [1]. The AFLP (Amplified Fragment Length Polymorphism) method of fingerprinting genomic DNA in any organism is extremely sensitive. AFLPS are DNA fragments that have been amplified from genomic DNA restriction using specific primers. AFLP markers combine RFLP and PCR technology, which involves DNA digestion followed by PCR [37].

The discovery of AFLP markers helped to overcome the limitations of the RAPD and RFLP techniques. AFLP definition: Any difference found by the amplified restriction length polymorphism approach between matching DNA fragments from two organisms A and B [23]. RFLP and PCR technologies are used in the amplified fragment length polymorphism (AFLP) procedure. Whole genomic DNA is digested using a pair of restriction enzymes, usually a frequent and a rare cutter. Following that, the DNA segments are ligated with adaptors of known sequence [25]. Primers that are complementary to the adaptors are used to amplify the restriction fragments. Gel electrophoresis can then be used to separate the PCR-amplified fragments, and banding patterns can be seen. To alter the complexity of AFLP fingerprints to fit application, a variety of enzymes and primers are available. Selecting primers for selective bases requires caution [37].

3.5. Randomly-Amplified Polymorphic DNA Marker (RAPD)

RAPD is a PCR-based approach that uses single primers of any nucleotide sequence with 10 nucleotides to amplify anonymous PCR fragments from genomic template DNA. The randomly-amplified polymorphic DNA marker (RAPD) identifies nucleotide sequence polymorphism in DNA using a

single primer of any nucleotide sequence [3, 4]. A single, short (10 nucleotide) and random primer is used to amplify genomic DNA in PCR. RAPD definition: Any DNA segment amplified utilizing arbitrary nucleotide sequence short oligo deoxynucleotide primers (amplifiers) and polymerase chain reaction techniques [11].

PCR is used to create random amplified polymorphic DNAs (RAPDs) using genomic DNA and arbitrary primers. Taq polymerase is an enzyme that works in conjunction with short random oligomers to amplify DNA segments between closely spaced sequences (2 kb) (typically 10-mers). RAPD polymorphism is caused by changes in the primer-binding site in the DNA sequence. Gel electrophoresis can be used to separate PCR results. RAPDs are a common type of marker [22]. The Random Amplified Polymorphic DNA (RAPD) technique, which is based on the polymerase chain reaction, is one of the most extensively used molecular techniques for creating DNA markers [21].

3.6. REMAP & IRAP

REMAP stands for retrotransposon microsatellite amplification polymophisms, and it's a popular retrotransposon-based marker for analyzing genetic variation [35]. REMAP definition: Any difference in DNA sequence detected by PCR-mediated amplification of the area between a retrotransposon's long terminal repeat and an adjacent microsatellite between two genomes [39].

REMAP and IRAP are species-specific indicators. The dispersion, ubiquity, and prevalence of retrotransposon-like elements in plant genomes can be exploited for DNA fingerprinting [19]. The IRAP (Inter-Retrotransposon Amplified Polymorphism) markers are made by annealing outward facing primers to two retrotransposons' long terminal repeats (LTRs). In REMAP (RE trotransposon-Microsatellite Amplified Polymorphism), the DNA sequences between the LTRs and surrounding microsatellites (SSRs) are amplified using appropriate primers [23].

3.7. SNPs

Single base-pair differences in a person's genetic sequence are known as SNPs. An SNP is any difference between two genomes caused by a single nucleotide exchange, small deletion, or insertion [25]. SNPs rely mostly on sequence data in their analysis. Automated fluorescence sequencing, denaturing high-performance liquid chromatography, and other techniques can be used to detect SNPs [32].

SNP markers are third-generation markers that are now widely utilized for individual genotyping in a variety of genomic studies. SNPs are more likely to be valuable for defining haplotypes than for their individual information, hence linkage disequilibrium studies employing the haplotype rather than single SNPs as individual molecular markers are more likely to be used [26].

Table 1. *Comparisons of Some molecular marker with their advantages and disadvantages.*

Marker	Advantages	Disadvantages
RFLP	Unlimited number of loci	Labour intensive
	Co-dominant	Large quantity of DNA needed
	Many detection systems	Fairly expensive
	Can be converted to SCARs	very low levels of polymorphism
	Robust in usage	Can be slow (long exposure times)
	Good use of probes from other species	Needs considerable degree of skill
	Detects in related genomes	
	No sequence information required	
RAPD	Results obtained quickly	Highly sensitive to laboratory changes
	Fairly cheap	Low reproducibility within and between laboratories
	No sequence information required	Cannot be used across populations nor across species
	Relatively small DNA quantities required	Often see multiple loci
	High genomic abundance	Dominant
	Good polymorphism	
	Can be automated	
SSR	Fast	High developmental and startup costs
	Highly polymorphic	Species-specific
	Robust - Can be automated	Sometimes difficult interpretation because of stuttering
	Only very small DNA	Usually single loci even in polyploids
	Co-dominant	
	Multi-allelic	
	Multiplexing possible	
	Does not require radioactivity	
ISSR	Highly polymorphic	Usually dominant
	Robust in usage	Species-specific
	Can be automated	
AFLP	Small DNA quantities required	Evaluation of up to 100 loci
	No sequence information required	Marker clustering
	Can be automated	Dominant
	Can be adapted for different uses, e.g. cDNA-AFLP	Technique is patented
		Can be technically challenging

Marker	Advantages	Disadvantages
	Highly polymorphic depends on the transposon	Alleles cannot be detected
	Robust in usage - Can be automated	Can be technically challenging
	Species-specific	
IRAP/REMAP	Small DNA quantities required	
	Highly reliable	
	Usually single-specific	
	Can be automated	
	Robust in usage	Very high development costs
	Polymorphism are identifiable	Requires sequence information
SNP	Different detection methods available	Can be technically challenging
	Suitable for high throughput	
	Can be automated	

4. Conclusion

The art and science of plant breeding is aimed at one of humanity's most pressing problems: feeding, clothing, and nourishing a growing population, water scarcity, renewable energy demands, and the need for environmental stewardship. Molecular markers have proven to be effective techniques for measuring genetic diversity in a wide range of plant species. Because of their simplicity, reproducibility, and precise positioning, molecular markers are widely utilized in crop development. Molecular markers have become an indispensable tool for genetic analysis, as they are critical for deciphering the inheritance of complex traits, identifying genes responsible for these features, and detecting novel variation.

Because high-density linkage maps of traits and markers may be constructed and used in as many genetic backgrounds as needed in a breeding program, molecular markers aid in the plant breeding process. Assessing the level of genetic diversity, parental selection, cultivar identity and assessment of cultivar purity, study of heterosis and identification of genomic selection, marker assisted backcrossing, and marker assisted pyramiding are just a few of the applications of DNA markers in plant breeding. In order to feed the world's rising population, molecular markers are crucial in crop improvement.

Conflict of Interest

The authors declare that they have no competing interests.

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