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2. Molecular Breeding and Its Current Scenario in Crop Improvement

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Abstract:

Increasing the global population and change in climatic conditions is a major threat to the food security. Rapid shrink in agricultural lands have been observed due to urbanization which is a matter of concern. Conventional plant breeding strategies will not be able to fulfill the food security for the world population due to its time consuming process and molecular breeding strategies are the only source to produce optimum food in a limited period of time. In the current scenario, molecular breeding has gained widespread acceptance and adoption by plant breeders and researchers worldwide. It has facilitated the development of improved crop varieties with enhanced yield, resistance to diseases and pests, tolerance to abiotic stresses, and improved nutritional content. The application of molecular breeding techniques has contributed to the development of genetically diverse and adapted crop varieties, addressing the challenges of global food security and sustainable agriculture.

Keywords:

Climate Change, Global Population, Food Security, Crop improvement.

2.1 Introduction:

By 2025, the global population will surpass seven billion. In the interim, as biotic and abiotic stresses rise, there will be a yearly decline in per-capita availability of arable land and irrigation water.Food security, which is best described as having access to a healthy diet and safe drinking water on an economic, physical, and social level, will be jeopardized. A holistic approach to nutritional and non nutritional factors needed to achieve success in the eradication of hunger. Science and technology can play a very important role in stimulating and sustaining an Evergreen Revolution leading to long-term increases in productivity without any associated ecological harm.[1][2] The objectives of the plant breeder can be realized through conventional breeding integrated with various biotechnology developments.[3] With the development of molecular tools, plantbreeding is becoming quicker, easier, more effective and more efficient.[4]Plant breeders will be well equipped with innovative approaches to identify and/ or create genetic variation, to define the genetic feature of the genes related to the variation (position, function and relationship with other genes and environments), to understand the structure of breeding populations, to recombine novel alleles or allele combinations into specific cultivars or hybrids, and to select the best

individuals with desirable genetic features which enable them to adapt to a wide range of environments.

The term molecular breeding is used to provide a simple umbrella for the multidisciplinary field of modern plant breeding that combines molecular tools and methodologies with conventional approaches for improvement of crop plants.Molecular breeding (MB) used to describe several modern breeding strategies that incorporates molecular biology tools and techniques to improve the efficiency and effectiveness of traditional plant breeding programs. Molecular breeding, which includes marker-assisted selection; marker-assisted backcrossing (MABC) ; more recently, marker-assisted recurrent selection (MARS); genome-wide selection (GWS), all these strategies involve use of molecular markers, which are specific regions of DNA associated with desirable traits, to select individuals with the desired genetic characteristics.

The main goal of molecular breeding is to accelerate the breeding process by identifying and selecting individuals with desirable traits more accurately and efficiently. Traditional breeding methods rely on phenotypic observations, which can be time-consuming and influenced by environmental factors. Molecular breeding allows breeders to make selections based on the genetic makeup of individuals rather than relying solely on observed traits.

The process of molecular breeding typically involves the following steps:

A. Identification of target traits: Breeders determine the traits they want to improve, such as yield, disease resistance, or nutritional quality.
B. Marker identification: Researchers identify molecular markers that are associated with the target traits. These markers can be DNA sequences, genes, or other genetic variations.
C. Marker development: Specific markers are selected and developed into reliable and cost-effective tools for screening large populations. This may involve techniques such as polymerase chain reaction (PCR), DNA sequencing, or genotyping arrays.
D. Marker screening: Large populations of plants or animals are screened using the developed markers to identify individuals that possess the desired traits.
E. Selection and breeding: Individuals with the desired markers and traits are selected as parents for the next generation. By focusing on the molecular markers associated with the target traits, breeders can increase the likelihood of offspring inheriting those traits.
F. Evaluation and validation: The selected individuals and their offspring are evaluated for the target traits through field trials, laboratory tests, or other relevant methods. This helps validate the effectiveness of the molecular markers in predicting trait performance.
G. Iterative cycles: The breeding process continues through multiple cycles of marker-assisted selection, evaluation, and validation to further refine the breeding population and enhance the desired traits.

Molecular breeding has been successfully applied in various crops, leading to the development of improved varieties with enhanced traits. It offers advantages such as increased breeding efficiency, more precise trait selection, and reduced time and resources required for traditional phenotypic-based breeding programs.

2.2 Molecular Breeding Tool:

Molecular markers: Molecular markers are nucleotide sequencesthat are specific regions of DNA that can be easily identified and associated with certain traits of interest. These specific DNA regions can be investigated through the polymorphism present between the nucleotide sequences of different individuals.All molecular markers occupy specific genomic positions within the chromosome known as 'loci' and used to 'flag' the position of a particular gene or the inheritance of a particular or desired characteristics.Basis of these polymorphisms are insertion, deletion, point mutations duplication and translocation; however, they do not necessarily affect the activity of genes. With the advent of DNA-based molecular markers, the extensive genetic mapping of chromosomes became readily possible for a variety of species. An ideal DNA marker should be co-dominant, highly reproducible, evenly distributed throughout genome, and having abilitytodetecthigherlevelof polymorphism.

Molecular markers are widely used in crop breeding to assist in the selection and improvement of desirable traits in plants.Knowing the complete DNA sequence of a model or reference genome allows genes/traits from this model to be tracked to other genomes. Here are some key types and applications of molecular markers in crop breeding:

A. **Single Nucleotide Polymorphisms (SNPs):** SNPs are the most common type of molecular marker used in crop breeding. They are single base pair differences in DNA sequences and can be easily identified using various techniques, such as DNA sequencing or genotyping arrays. SNPs are used for various applications, including marker-assisted selection (MAS), gene mapping, diversity analysis, and genomic selection.**SNP detection**: SNPs are detected by Oligonucleotide hybridization, DNA chip technology and Solution hybridization technique.
B. **Simple Sequence Repeats (SSRs)**: SSRs, also known as microsatellites, are short tandemlyrepetitive DNA sequences found throughout the genome. One of the most important attributes of microsatellite loci is their high level of allelic variation, making them valuable as genetic markers. They are highly polymorphic and often used for genetic mapping, cultivar identification, and assessing genetic diversity in crop populations.
C. **Insertion/Deletion Polymorphisms (Indels)**: Indels are small insertions or deletions of DNA segments ranging from a few base pairs to several hundred base pairs. They can be easily detected using PCR-based techniques and are used for genetic mapping, linkage analysis, and marker-assisted selection.
D. **Amplified Fragment Length Polymorphisms (AFLPs)**: AFLPs are PCR-based markers that detect variation in DNA fragment lengths. They provide high levels of polymorphism and have been extensively used for genetic diversity analysis, genetic mapping, and phylogenetic studies.
E. **Restriction Fragment Length Polymorphisms (RFLPs)**: RFLPs are markers based on variations in DNA fragment sizes resulting from differences in restriction enzyme recognition sites. They were widely used in the past but have been largely replaced by other more efficient marker systems, such as SNPs and SSRs.
F. **Sequence-Characterized Amplified Regions (SCARs)**: SCARs are PCR-based markers derived from specific DNA sequences associated with a particular trait or gene

of interest. They are used to identify and select plants carrying desired traits in breeding programs.

The use of molecular markers in crop breeding allows breeders to make informed decisions during the selection process, leading to more efficient and targeted breeding programs. They help in identifying and selecting plants with desirable traits, tracking the inheritance of genes of interest, assessing genetic diversity, and accelerating the development of new improved varieties.

2.2.1 Strategies of Molecular Breeding:

A. **Marker-Assisted Selection (MAS):** MAS involves the use of molecular markers, which are specific DNA sequences associated with a particular trait of interest. By analyzing these markers, breeders can identify and select individuals with desired traits more efficiently. MAS can accelerate the breeding process by eliminating the need for time-consuming phenotypic evaluations.Marker-assisted selection enables selection at early stages of plant, even before the traits are fully expressed, accelerating the breeding process. Furthermore, MAS can be particularly useful for traits that are difficult to measure directly or influenced by multiple genes.

B. **Marker-Assisted Backcrossing (MABC)**: Marker-assisted backcrossing (MAB) is a breeding technique used in plant breeding to transfer specific traits from one genotype to another while retaining the genetic background of the recipient genotype. It combines traditional backcrossing methods with the use of molecular markers to accelerate the breeding process.In traditional backcrossing, a desired trait from a donor genotype is introduced into a recurrent or recipient genotype through several rounds of crossing followed by selection of offspring with the desired trait. However, this process can be time-consuming and labor-intensive, especially when the desired trait is controlled by multiple genes or is difficult to select visually.MABC is a variant of MAS that is commonly used in breeding programs for the transfer of a specific trait from one variety or species into another. It involves multiple rounds of backcrossing to introduce the desired trait while simultaneously selecting for the molecular marker associated with that trait.

C. **Genomic Selection (GS)**: GS is a breeding strategy that uses high-throughput genotyping technologies to capture and analyze a large number of genetic markers throughout the genome.Genomic selection utilizes genomic information, specifically the analysis of an individual's DNA sequence or genetic markers spread throughout the genome. It allows breeders to identify and select individuals with desirable genetic variations associated with specific traits. The genetic markers used in genomic selection can be single nucleotide polymorphisms (SNPs), microsatellites, or other genetic variations. By correlating these markers with phenotypic data from a training population, breeders can predict the performance of individuals for various traits, even before they are phenotypically expressed. GS is particularly useful for complex traits controlled by multiple genes.

D. **Genetic Engineering**: Genetic engineering involves the direct manipulation of an organism's genetic material by introducing or modifying specific genes. This technique allows breeders to introduce novel traits or modify existing ones by inserting genes from unrelated species. The process of genetic engineering typically involves isolating and manipulating specific genes or DNA sequences from one organism and inserting them

into another organism. This can be done using various techniques, such as recombinant DNA technology, gene editing tools like CRISPR-Cas9, or other methods that facilitate the transfer of genetic material. Genetic engineering has been widely used in crop improvement, such as introducing disease or pest resistance or enhancing nutritional content.

E. **Genome Editing**: Genome editing techniques, such as CRISPR-Cas9, enable precise modifications of specific genes within an organism's genome. Unlike genetic engineering, genome editing does not necessarily involve the introduction of foreign DNA. It allows for targeted changes in the DNA sequence, including gene knockout, gene insertion, or gene replacement, providing breeders with precise control over trait modification.

2.2.2 Importance of Molecular Breeding:

Molecular breeding has a numerous advantages over the classical plant breeding technologies. Using DNA tags for characteristics whose phenotypes emerge in the subsequent phases of crop growth and development, reliable selection becomes feasible at the stage of seedling development. This reduces the expense of growing all the plants until the expression of phenotypic characteristics by allowing undesirable plants to be quickly identified and safely dismissed at the beginning of the seeding stage. It uses a unique sequence of DNA to identify crops with ideal qualities, thus it has an equivalent level of precision and efficiency whether the population is raised in the primary crop's growing season, off-season, outdoor environment, or protected green structures. Retaining a desirable trait dictated by a gene that is recessive in a backcross breeding programme takes a longer approach because each backcross requires an additional intermediate generation (self/test cross) for the recognition of cultivars having the appropriate allelic form. Co dominant DNA markers in MAB aid in detecting and selection of the desirable alleles that are recessive for a specific characteristic even in the heterozygous condition, allowing for continual backcrossing without self or test cross generation interruptions. As a result, the MB saves time and speeds up the breeding process. Genomic marker-based genome assays may be faster, cheaper, and more accurate/precise than traditional phenotypic assays.[5]

2.2.3 Current Scenario of Molecular Breeding in Crop Improvement:

Marker aided selection is a breeding strategy that uses genetic markers that are firmly related to the target trait/gene(s) for the purpose of indirect selection for that trait in segregating/non-segregating generations. When a marker is discovered which co-segregates with a significant gene for a significant behavior, screening for evidence of an associated allele related to the gene may be easier and less expensive than evaluating the characteristic. When it comes to more complex, polygenic regulated traits, the breeder must figure out how to combine as many beneficial alleles as feasible for the QTLs that were discovered. In this situation, the breeding material can be tested for QTL-linked markers. Based on this study, customized crosses can be designed to provide an ideal genotype through the integration of QTL alleles from various sources.[6] Marker assisted selection (MAS) is most beneficial for qualities that are difficult to select, such as disease resistance, salt tolerance, drought tolerance, heat tolerance, and quality traits. The approach entails screening crops at early generation with a fixed, favourable genetic background at associated loci, carrying out a

single large-scale marker assisted selection while maintaining as much allelic segregation in the population as possible, and screening large populations to achieve the scheme's objectives. To retain as much Mendelian allelic segregation among the selected genotypes as possible, no selection is applied beyond the target genomic areas. Following selection with DNA markers, genetic variation at un-selected loci may allow breeders to develop novel varieties and hybrids in response to breeding aims. The field of marker-assisted selection for polygenic trait enhancement is in a crucial transitional stage and is about to present impressive outcomes. This method has now been widely utilized in pulse breeding programmes. [7][8] Marker assisted backcross breeding aims to introduce genes from a "donor" line through the chromosomal background of a "recipient" line. When direct phenotypic evaluation is not possible, is too expensive, or is only possible late in development, markers can be employed to evaluate the existence of the introgressed gene ("foreground selection"). Markers can also be employed to speed up the return to the recipient parent genotype at other loci (this is known as "background selection"). The theoretical analysis was limited to background selection because it is assumed that the introgressed gene can be detected without ambiguity. Experiments have shown that using molecular markers for background selection in backcross procedures is quite effective. One of the most promising approaches of MABC is the use of molecular markers to identify and select genes controlling resistance in rice. 'Maudamani', a variety of rice has been improved utilizing this breeding method.[9][10]

Quantitative trait loci (QTLs) are genetic elements that contribute to a portion of the observed phenotypic variance for a quantitative trait. QTL was first used by Gelderman. Conceptually, it could be a single gene or a group of related genes that contribute to the trait. The use of biological markers makes it feasible to distinguish between the effects of quantitative trait loci (QTL) on the variation of a complex trait and makes it easier to transfer these QTL into desired cultivars or lines. In a recent study, QTL information was successfully connected with crop models to substitute the measured parameters, demonstrating that QTL analysis reduces some of the random mistakes of observed model input parameters.[11] The creation of distinct mapping populations for QTL studies involves employing at least two parental genotypes that are different for the desired trait. Sometimes, however, mapping populations are created primarily for linkage map building but are also used for QTL research. In these cases, the parents of the mapping population are diversified but were not chosen for any particular trait of interest. One such example is the International Triticeae Mapping Population (ITMI pop) in wheat, which was created to create genetic maps but later used for numerous QTL studies because the parental genotypes of ITMI were chosen to be diverse and as a result, the recombinant inbred lines (RILs) segregated for a number of traits of economic importance, allowing QTL analysis for these traits.[12][13][14][15][16]

To produce hybrid seeds, a cytoplasmic male sterility technique is used because it does not require hand emasculation. The inability to produce viable pollen but without impacting female fertility characterizes the maternally transmissible trait known as CMS. It is frequently linked to mitochondrial DNA rearrangements, mutations, and editing. Utilizing RAPD and STS, several restorer loci have been discovered in several crops, and DNA markers connected to these loci allow for the molecular analysis of the CMS system. Following backcrossing to create restoration lines, these co-dominant markers are helpful in locating the homozygous restorer genotypes. The restorer lines might be made in less

time this way compared with traditional procedures. Pi2 gene-based TGMS rice lines have been successfully developed which provide resistance to Blast disease is one of the outstanding examples.[17]

Comparative anatomy, morphology, embryology, physiology, and other fields that provide useful information but have low genetic resolution are traditionally used to evaluate and conserve genetic variability and biodiversity. Recent developments in molecular biology have produced strong genetic tools that can quickly and precisely resolve genetic issues. To do genotyping using molecular markers, a person's specific marker profile must be created. It is known as "DNA Fingerprinting" to identify crop varieties in an unmistakable pattern using a DNA identifier. Alec Jeffery created the method in 1985 for humans, and Dallas utilized it for the first time in a crop (rice) in 1988 for cultivar identification. For the investigation of pigeon pea diversity, mtDNA RFLP can be used. RAPD markers were used to create DNA fingerprints for cultivated and wild pigeon pea accessions where low levels of polymorphism were found in crops while high levels of polymorphism were found in wild species. New microsatellite markers were created in lentils to distinguish the molecular diversity. RAPD and IISR markers were employed to determine the genetic diversity among 18 black gram cultivars.[18][19][20][21]

The cost of evaluating hybrids for heterosis or combining ability in the field is high. In some cereal crops including rice, oat, and wheat, genetic diversity and heterosis have been linked using molecular markers. It has been suggested that pedigree information and RFLP-based metrics of similarity can be utilized to forecast the best hybrid pairings. However, both low and large associations between heterosis and DNA-based genetic distance have been found. In three maturity groups of soybean, the relationship between heterosis and molecular (isozyme and RFLP) diversity between parents was studied. Parental RFLP diversity was not substantially correlated with mid-parent and better-parent heterosis, implying that yield heterosis may not be associated with genetic variation at the molecular level as assessed by RFLPs. Although isozyme diversity in parents was associated with yield heterosis, it is of minimal consequence in soybean due to the restricted number of assayable isozyme loci.[22]

To establish the quality of hybrid seed, it must be verified that the designated cross happened, the number of self-pollination between the female parents meets the required purity, and the product is of appropriate quality. 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. There is a successful event of SSR 218, SSR 306, and Ty2 gene CAPs gene markers to test the F1 purity of the tomato hybrids Pbc EC 538408, Pbc EC 520061, and H 86 EC 520061.[23]

Gene pyramiding is a method of identifying and introducing numerous genes that confer resistance to an independent insect/microbial pest or confer resistance to a single pest via distinct host pathways. Incorporating numerous resistance genes into a single variety is one technique for enhancing resistance persistence. It has been estimated that if the pyramided genes were never used as single genes, the resistance's durability may be increased by 50 years. It is difficult to verify the number of resistance genes that have been effectively pyramided during the cultivar development process. Even though the resistance of the latter one is likely more permanent, plants with three resistance genes are equally as resistant as

those with only one. Phytophthora tolerance QTLs in soybean were pyramided. By crossing two phytopthora-resistant soybean cultivars, Conrad and Hefeng 25, a recombination inbred line population was created, and was found that the more the number of QTLs the higher was the level of tolerance.[24] Pyramiding of Bt genes is another major aspect of study. Insecticidal cry (crystal) genes from Bacillus thuringiensis (Bt) have been employed as bio pesticides and in transgenic plants for insect control. The discovery of new insecticidal genes is critical for delaying resistance development in target insects. The variety of Bt strains makes it easier to isolate novel types of cry and vip (vegetative insecticidal protein) genes. For the identification of new insecticidal proteins, PCR was used in conjunction with other methods of investigation such as RFLP, gene sequence determination, electrophoretic, immunological, and chromatographic analysis of Cry proteins, and insect bioassays for toxicity evaluation.

In map based cloning of genes, the first step is to locate a molecular marker near the gene of interest. Initially, a small mapping population is used to search for genes, and then a highly saturated genetic map is required to clone a gene. In the case of an initial marker, the region surrounding that initial molecular marker is densely packed with high density markers. A vast number of people are screened in order to locate a marker that seldom recombines with the gene of interest. The next step is to screen a large insert genomic library (BAC or YAC) for clones that hybridize with previously identified closely connected molecular markers. After identifying two flanking markers that show linkage to the target gene, the target gene is found using chromosomal walking. The goal is to identify clones that have a set of flanking markers that co-segregate with the gene of interest. Individuals lacking the target gene are given these hypothetical clones. If a transgenic is shown to rescue the mutant phenotype, the newly cloned gene is subjected to thorough molecular and biochemical study to characterize the gene. To clone the Pto (resistance to bacterial speck disease of tomato) gene, a map-based cloning technique was employed.[25]

2.3 Future Prospects:

Crop improvement has adopted a rapid gain with the onset of molecular breeding as compared to the conventional plant breeding strategies. There is a tremendous growth in world population and issues related to climate change is increasing day by day. Also the urbanization resulted in declining of the cultivable lands which is a major area of concern. To overcome these issues and food security aspects, molecular breeding technique is the only option to increase rapid yield growth and nutritional properties of crops in the upcoming days as it requires very less time compared to the conventional plant breeding.

2.4 Conclusion:

By increasing the genetic gain per crop cycle, or by reducing the number of selection cycles, molecular breeding is unquestionably an effective approach wherever the bare minimum of human and operational resources are available.It is worth noting that the field of molecular breeding is dynamic and rapidly evolving. New technologies, methodologies, and research findings continually shape the landscape of crop improvement. Molecular breeding has transformed crop improvement by providing breeders with powerful tools to select for desired traits, enhance genetic diversity, improve disease and stress resistance, increase

yield potential, and enhance quality characteristics. It has expedited the breeding process, leading to the development of improved crop varieties that contribute to sustainable agriculture and address the challenges of food security and climate change.The ever-decreasing cost of marker technologies and the emergence of platforms for accessing MB tools and support services, plus needs-driven demand for improved varieties to address the global food crisis are all grounds to predict that MB will have a significant impact on crop improvement.

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