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19. Marker- Assisted Selection

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Abstract:

The progress made in molecular genetics has greatly enhanced plant breeding by deepening our comprehension of trait genetics. Marker-assisted selection (MAS) is becoming crucial in breeding, particularly for traits with straightforward genetic foundations, expediting the breeding process. The efficacy of MAS is contingent upon the genetic constitution of the trait, the correlation between molecular markers and target genes, and the genetic context for gene transfer. Advancements in genotyping and genomic techniques are currently improving the process of selecting genotypes with superior agricultural features, namely in areas like as disease resistance and crop quality.

Keywords:

Molecular Genetics, Plant Breeding, Marker-Assisted Selection (MAS), Genotyping Technologies, Crop Improvement

19.1 Concept:

Marker-assisted selection (MAS), pioneered by Smith, Simpson, Soller, and Beckmann more than 25 years ago, has transformed plant breeding by replacing traditional phenotypic selection with genetic-based selection. Marker-assisted breeding (MAB) is a technique that utilizes durable molecular markers that are effective during all stages of plant growth. This method can be applied to both major gene characteristics and quantitative trait loci (QTLs). Marker-assisted selection (MAS) allows for early selection in plantlets, which has the potential to decrease the required assessments and associated expenses. However, it does not expedite the breeding cycle as field evaluations are still essential. The success of this relies on the caliber and abundance of markers, which are determined using methods such as RFLP, AFLP, SSR, RAPD, CAPS, SSCP, and SNPs. Linkage analysis is then used to connect DNA markers to certain phenotypes. Although MAS is efficient in determining simple features regulated by a small number of genes, its effectiveness is constrained when dealing with complex traits influenced by several genes.

19.2 History:

In the early 1900s, Sax (1923) determined that a Mendelian-inherited gene could indicate a quantitative trait gene. Almost sixty years after the discovery of RFLPs (Botstein et al., 1980), a tomato DNA marker map with 57 RFLP markers was created. Neimann-Sorensen and Robertson (1961), Smith (1967), Soller (1978), and Stuber et al. (1982) proposed using DNA markers for breeding and selection. The first plant Marker-Assisted Selection (MAS) experiment was by Tanksley et al. in 1981. The proposition argues that tomato isozyme selection precedes morphological selection but does not replace it. Since DNA markers (RFLP) were initially used to explore quantitative features in tomatoes, the number of research initiatives identifying genetic markers connected to major phenotypic traits has expanded (Paterson et al., 1988). Young (1999) was optimistic about MAS but warned that marker-assisted selection is widely used in plant breeding but has led to few successful and viable results. More than 2500 plant QTL mapping research have been published. However, Young's (1999) optimistic vision has not been realized, and few breeding projects use DNA markers to improve plant quantitative traits.

19.3 Key Objectives of Marker-Assisted Selection in Plant Breeding:

The primary objectives of marker-assisted selection (MAS) in plant breeding involve three key applications of DNA-based markers:

- Tracking beneficial alleles, whether dominant or recessive, across multiple generations to enrich the accumulation of favourable genetic traits.
- Selecting the most promising individuals from the offspring, guided by the analysis of their allelic structure, which may involve part or the entirety of the genome.

- Disrupting any potential association between desirable alleles and unfavourable genetic regions.

Utilizing Marker-Assisted Selection in Breeding Programs:

- **Definition**: Marker-assisted selection (MAS) is the process of using genetic markers to select specific alleles affecting desired traits.
- **Indirect Selection Method**: In MAS, selection is based on markers (morphological, biochemical, DNA/RNA-based) rather than the trait itself.
- **Alternate Name**: MAS is also known as marker-aided selection.
- **Trait Focus**: Key traits for MAS include productivity, disease resistance, abiotic stress tolerance, and quality.
- **Application in Breeding**: MAS is employed in both plant and animal breeding.
- **Selection Process**: It's an indirect process where the selection is linked to a marker associated with the trait, not the trait directly.
- **Benefits for Challenging Traits**: MAS is particularly useful for traits that are hard to measure, have low heritability, or appear late in development.
- **Complementary to Traditional Methods**: MAS can enhance traditional phenotypic-pedigree-based selection methods.

When to Apply Marker-Assisted Selection in Plant Breeding:

- When the desired trait manifests late in the plant's development, such as characteristics related to flowers and fruits.
- In cases where the trait is controlled by a recessive gene, as MAS can identify both dominant and recessive genes.
- For screening plants for disease and insect resistance in regions where field inoculation with pathogens is restricted for safety reasons, also helping to negate unwanted environmental influences.
- When the phenotype is influenced by two or more non-linked genes (epistasis), like in the selection of multiple genes for resistance against diseases or insect pests, a process known as gene pyramiding.

19.4 Steps of MAS:

The Marker-Assisted Selection (MAS) process has two main steps: Gene Mapping and Marker Assisted Selection.

a. Gene Mapping: The primary objective of this initial phase is to determine the specific gene or quantitative trait locus (QTL) that is being targeted. The process comprises the subsequent pivotal phases:

- **Parental Selection**: choice of parents who possess unique alleles or opposing qualities.
- **Population Development:** The process involves the creation of mapping populations, which include recombinant inbred lines (RILs), near isogenic lines (NILs), backcrosses, double haploids (DH), and F2 segregations.
- **DNA Isolation**: The process of extracting DNA for the purpose of analysis.

- **DNA Marker Scoring:** utilization of markers like as RFLPs or AFLPs to create linkage maps. This is done by examining the connection between the phenotypic and markers that have already been mapped, in order to determine the position of the QTL.

b. Marker Assisted Selection (MAS): This process utilizes gene mapping data to carry out selection using molecular markers. The procedure encompasses:

- **Indirect selection:** involves the use of molecular markers to aid in the process of selection.
- **Marker Correlation**: The process of linking DNA markers with morphological markers, usually by using two markers that are closely located (less than 5 recombination units away) to reduce errors caused by recombination. The utilization of two adjacent markers with a genetic distance of approximately 20 centimorgans significantly enhances the probability (99%) of successfully identifying the desired gene.

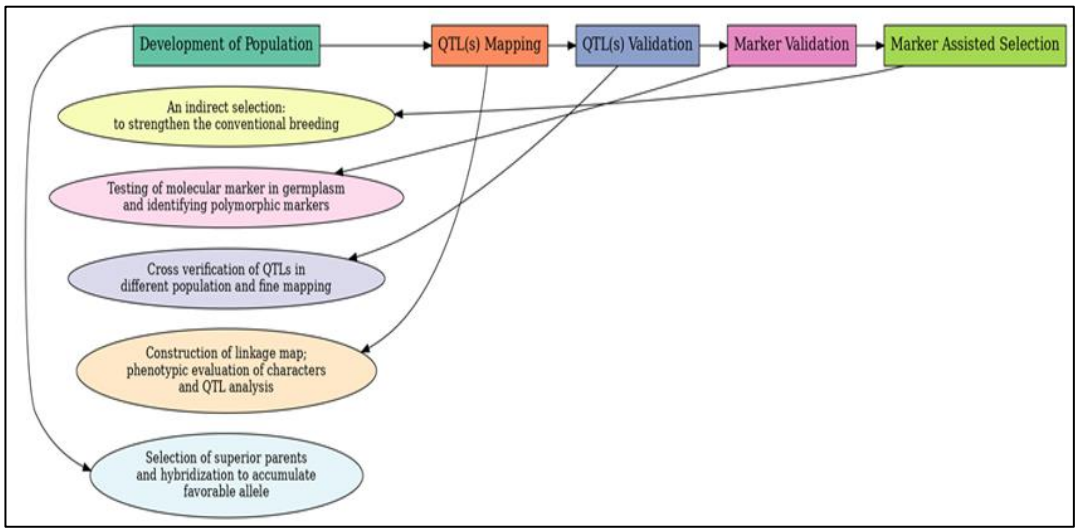


Figure 19.1: Steps in Marker-Assisted Selection: From Population to Selection

19.5 Conventional Backcross V/s MAS Backcross:

Transferring a specific genetic segment from a donor to a recipient might greatly increase a trait whether it involves a single gene or a gene with a large influence. The utilization of Molecular Assisted Selection (MAS) is progressively employed to expedite the reinstatement of the recurrent parent in backcross (BC) programme. Molecular markers enhance BC breeding efficiency in three fundamental ways when compared to traditional backcrossing:

- Enabling the choice of demanding characteristics in situations where direct observation is challenging.
- By minimizing the incorporation of undesired donor genetic material beyond the intended location, the negative effects of genetic linkage drag are reduced.

- Facilitating the transmission of recessive genes without the requirement for subsequent generations of self-pollination after each backcross.

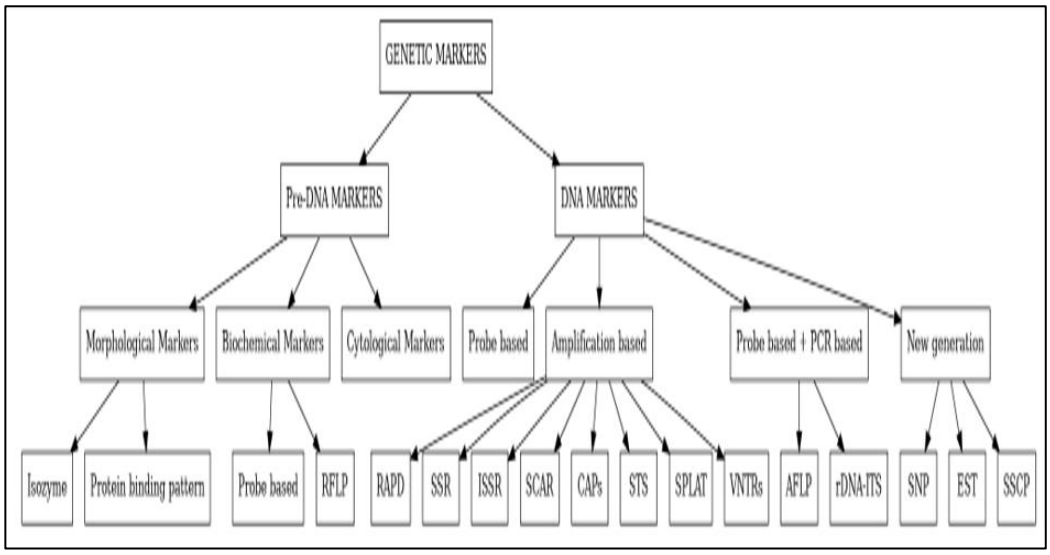


Figure 19.2: Genetic Markers

(source: https://jeas.agropublishers.com/2022/11/molecular-marker-breeding/)

19.6 DNA Markers:

Genetic variation, especially phenotypic variation, has long been studied. RFLP, discovered by Botstein et al. (1980), enables quantitative, accurate, genome-wide DNA polymorphism testing, revolutionizing this discipline. Plants, animals, humans, and other species use DNA markers for MAS. Interestingly, many of these DNA markers were discovered and created in humans then applied to plants based on their traits.

A. The Ideal DNA Markers Should be:

- Highly polymorphic (as assessed by the number of alleles in the population) to have the potential to identify the analyzed phenotypes
- Highly abundant and consistently distributed in the genome, enabling identification of tight linkages between markers and genes and creating saturated genetic maps.
- Co-dominant (distinguishing heterozygote) links marker allele to associated gene allele for phenotype regulation.
- Easy genotyping allows for cost-effective genotyping of large numbers of individuals in several marker loci.

B. Features:

Several factors determine the success of molecular markers in breeding.

- Genetic map with markers linked to agronomic interest genes or QTLs
- Tight association between markers and genes
- Adequate recombinations between markers and genome
- Rapid and cost-effective analysis of many individuals MAS success depends on marker location relative to target gene.

19.6.1 Types of DNA Markers:

Table 19.1: DNA Markers

Sr. No.	Name of Marker	Genotyping Technology	Genotyping Description	Source of Polymorphism	Characteristics
1	RFLP	Enzyme digestion, probing	Allele differentiation by size	Restriction site presence/absence	Biallelic, lower diversity, low polymorphism
2	RAPD	Short primer PCR	Band pattern analysis	DNA sequence homology	Simple, biallelic, moderate polymorphism
3	AFLP	Restriction ligation, selective PCR	Band pattern scoring	Restriction site polymorphism	High resolution, biallelic, high polymorphism
4	SSR	Primer PCR, gel/sequencer	Repeat number determination	Tandem repeat variation	Multiallelic, high diversity, high polymorphism
5	SNP	Sequencing, automated genotyping	Single nucleotide discrimination	Point mutations	Biallelic, specific, low polymorphism

A. Restriction Fragment Length Polymorphism (RFLP):

The development of RFLP was attributed to Botstein et al. in 1980. The number of accessible Restriction Fragment Length Polymorphism (RFLP) loci in plants ranges from tens to several thousands. RFLPs are genetic markers that are specific to a single location on a chromosome, and their inheritance follows a co-dominant pattern. The discovery of RFLP, the first DNA marker, was the impetus for the development of the MAS idea. It is important to mention that RFLP is still used in studies of synteny since it can accurately determine the genetic makeup of different species that are closely related.

B. Random Amplified Polymorphic DNA (RAPD):

The development and application of Random Amplified Polymorphic DNA (RAPD) markers in plants were documented by Welsh and McClelland (1990) and Williams et al. (1990). The number of RAPD loci utilized for the analysis of plant genomes ranges from tens to hundreds. Random Amplified Polymorphic DNA (RAPD) markers are a type of genetic markers that involve several loci, and their inheritance follows a dominant pattern.

The genotyping technology is characterized by its simplicity, which is the primary advantage of this system. As a result, RAPD has gained significant popularity in numerous laboratories. The primary drawbacks of this system include a limited degree of polymorphism, a prevailing method of inheritance that is not well-suited for MAS, and a diminished level of reliability.

C. Amplified Fragment Length Polymorphism (AFLP):

The development of amplified fragment length polymorphism (AFLP) markers was carried out by Keygene, as described by Vos et al. in 1995. A typical run of AFLP often yields several tens of bands. AFLP markers are characterized by several loci and exhibit a dominant pattern of inheritance. The primary benefits of this method include the relatively simple genotyping process, the detection of a large number of loci in each response, and the system's high level of dependability. The primary drawbacks include the limited degree of polymorphism and the prevailing form of inheritance, which is not well-suited for MAS.

D. Simple Sequence Repeats (Also Referred to As Microsatellites) (SSR):

Simple sequence repeats (SSRs) were initially identified and pioneered by Litt and Luty (1989) and Edwards et al. (1991) in the context of human genetics. The application of SSRs to plants was first introduced by Akkaya et al. (1992). SSRs are markers that exhibit co-dominance, allowing them to differentiate between individuals who are heterozygous (possessing two different alleles) and those who are homozygous (possessing two identical alleles). SSRs offer significant benefits in terms of their extensive polymorphism and exceptional dependability.

E. Single nucleotide polymorphism (SNP):

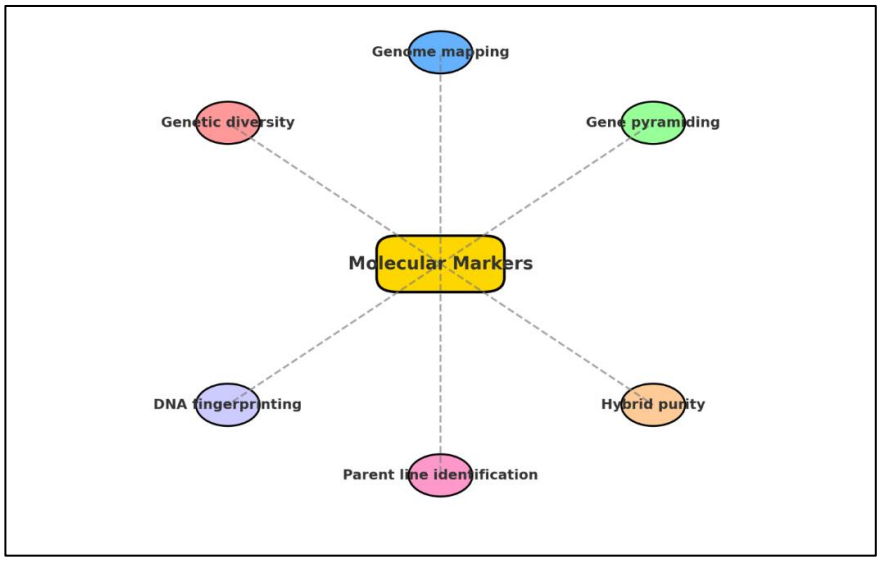
A single nucleotide polymorphism (SNP) refers to a specific type of genetic mutation when there is a change in a single nucleotide. This mutation is characterized by the presence of a short sequence of nucleotides surrounding it. The system is employed in people, livestock breeding, and to a lesser degree in plants. Their inheritance pattern is characterized by co-dominance. SNPs are extremely prevalent, with over 10 million of them present in the human genome. Additionally, there are advanced genotyping technology that can efficiently analyze hundreds of thousands of SNPs in each individual. These benefits are offset by their limited degree of polymorphism.

Table 19.2: Marker-Assisted Selection in Plant Breeding

DNA marker	Laboratory technique	Number of detected loci	Source of polymorphism	Level of polymorphism	Dominance	Abundance	References
RFLP	Southern blot, agarose gel	Single locus	Point mutation	Low	Co-dominant	Medium	Botstein et al. (1980)
RAPD	PCR, agarose gel	Multiloci	Point mutation	Low	Dominant	Low	Welsh and McClelland (1990)
AFLP	PCR, acrylamide gel	Multiloci	Point mutation	Low but one reaction detects many loci	Dominant	Medium	Vos et al. (1995)
SSR	PCR, acrylamide gel	Single locus	Variation in the no. of the repeats	Very high	Co-dominant	Medium	Litt ant Luty (1989)
SNP	Primer extensiom, chips	Single locus	Point mutation	Low for each locus, but high for high-throughput genotyping	Co-dominant	Very high	Rafalski (2002)

(Source: Ben-Ari, G., & Lavi, U. 2012)

19.6.2 Application of Molecular Marker:



(Source: https://www.researchgate.net/Figure/application-of-molecular-markers-molecular-markers-are-used-in-the-determination-of_Figure5_257940735)

Figure 19.3: Application of Molecular Marker

19.7 Technologies Used In MAS:

Some of the high throughput technologies that have been applied to plants for genotyping SNPs are described in the following sections.

a. Mass spectrometry: Kwon et al (2001) pioneered the use of mass spectrometry (MS) for DNA sequencing.
b. Diversity arrays technology: The diversity arrays technology (DArT) was developed and first demonstrated in barley (having a large genome) by Wenzl et al. (2004). It is based on microarray hybridizations.
c. SNP arrays:

Modern sequencing technologies

- Solexa-Illumina
- 454 (now Roche)
- Pacific biosciences

A. MAS For Improvement of Quantitative Traits:

The challenges of identifying and characterizing quantitative trait loci (QTLs) in complex traits are multifaceted. Due to the low heritability and the involvement of numerous QTLs with minor individual effects, the identification of specific genomic regions influencing traits is challenging. This complexity necessitates multiple genomic manipulations for significant impact and requires replicated field tests to accurately assess QTL effects and their environmental stability.

B. Key Issues in This Field Include:

- Identifying major QTLs that control the trait of interest.
- Addressing the uncertainty in QTL positioning, especially for those with minor effects, where current analysis techniques can yield a broad confidence interval (up to 30 cM in small populations).
- Overcoming deficiencies in QTL analysis that lead to misestimation of the number and impact of QTLs.
- Challenges in identifying QTL-marker associations that are effective across different breeding materials.
- Risk of losing target QTLs in marker-assisted selection (MAS) due to double crossovers, a risk that increases with the length of the marker interval.
- Difficulty in precisely assessing epistatic effects and Q×E (quantitative trait loci by environment) interactions.

An innovative strategy to tackle these challenges is linkage disequilibrium (LD) mapping or association mapping. Unlike linkage mapping, which is limited to alleles from bi-parental crosses and samples only a fraction of potential alleles, LD mapping evaluates associations between genotypes (haplotypes) and phenotypic variation by examining genetic

polymorphisms across diverse genetic backgrounds. This approach takes into account the historical recombination events and factors like population history and recombination frequency in the genome segment under study, offering a more comprehensive analysis of genetic influences on complex traits.

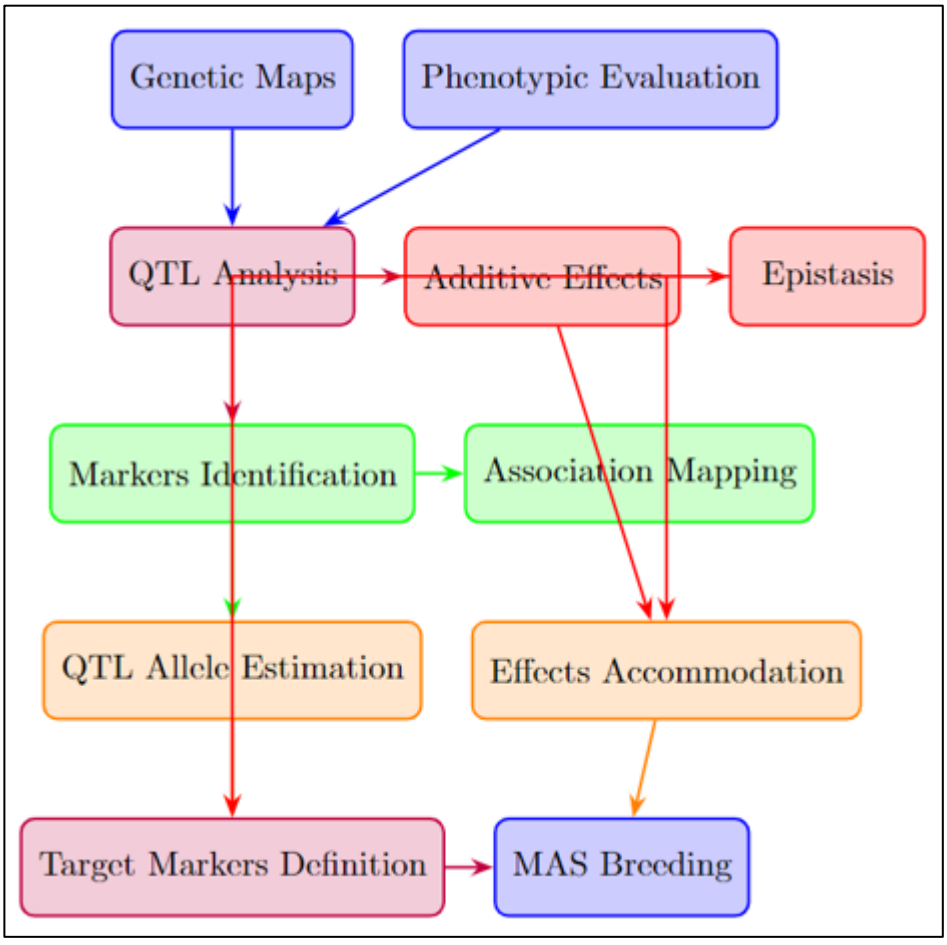


Figure 19.4: Deciphering Quantitative Trait Loci: Advancements in Marker-Assisted Selection for Crop Improvement (Source: Francia, E. et al. 2005)

19.8 MAS for Biotic Stress:

Pathogens, including viruses, bacteria, nematodes, and fungus, present substantial risks to the health of plants. Therefore, it is essential to do research on the mechanisms by which these pathogens cause disease and to identify genes in plants that may resist them. The regulation of these resistance mechanisms is determined by either prominent dominant/recessive genes or quantitative trait loci (QTLs). DNA markers are essential for identifying these resistance genes, which are then incorporated and combined into new cultivars. An essential component in this process is the presence of Plant disease resistance genes (R-genes), which play a crucial role in identifying specific proteins produced by avirulence (Avr) genes in pathogens.

The process of incorporating a R gene into a high-quality breeding line using conventional means often takes 10-15 years. This technique is additionally hampered by the requirement for comprehensive and labor-intensive artificial inoculation studies to assess resistance. These tests require the careful management of infections or pests, particularly if they are obligate parasites. Some noteworthy instances of characterized R-genes include the ASC1 gene, which confers resistance to Alternaria alternate, the pathogen responsible for Alternaria stem canker, and the RPW8 gene found in Arabidopsis thaliana, which provides resistance against a wide range of mildew infections. Nevertheless, it is important to note that resistance to illnesses is not always determined by a single gene (monogenic). In certain cases, such as those caused by Fusarium species fungi, resistance is influenced by multiple genes (polygenic) and exhibits quantitative characteristics.

Marker-Assisted Selection (MAS) offers distinct advantages in the field of resistance breeding. It allows for faster reactions to resistance failures, promotes the swift incorporation of many genes from diverse genetic sources, assists in the stacking of genes, and facilitates the identification of uncommon recombinants among closely linked resistance genes.

19.9 MAS for Abiotic Stress:

Abiotic stress is defined as the negative impact of non-living factors on living organisms in a specific environment. The stresses include drought, salinity, low or high temperatures, and other environmental extremes. In contrast to plant resistance to biotic stresses, which mostly depends on monogenic traits, the genetically complex responses to abiotic stresses are multigenic and thus more difficult to identify, control, and manipulate.

A. Low-Temperature Stress Tolerance in Plants:

Regarding the ability to withstand low temperatures, overwintering crops necessitate not just vernalization but also the capacity to endure frost and cold. The inheritance of this type of tolerance is quantitatively complex, which makes the application of Marker-Assisted Selection (MAS) for improving cold resistance traits more challenging. For example, in barley, chromosome 5H contains two closely associated quantitative trait loci (QTLs) that are crucial for low-temperature tolerance. These QTLs can be identified using RAPD and RFLP markers. These markers accurately differentiate between genotypes that are tolerant to frost and genotypes that are sensitive to frost. Rice, primarily cultivated in tropical and subtropical regions, is especially vulnerable to low-temperature stress in colder climes and high-altitude regions. Cold stress during the booting stage has a negative impact on the anthers, resulting in delayed heading or maturation and decreased production as a result of spikelet sterility.

B. Drought Stress Tolerance:

Drought stress is a substantial agricultural concern on a global scale. Plants face different types of environmental stresses, but osmotic stress, caused by factors such as drought, salinity, or low temperature, is a major limitation on their growth, productivity, and spread. Crucial mechanisms for withstanding drought conditions consist of drought escape (short

life cycle, sensitivity to photoperiod, ability to adapt developmentally), drought avoidance (improved water absorption, decreased water loss), drought tolerance (adjustment of osmotic pressure, capacity for antioxidants), and drought recovery (ability to withstand desiccation). Other crucial factors for drought tolerance include the depth of the root system for efficient moisture absorption, the regulation of water usage through the reduction of leaf area and growth duration, and the restriction of non-stomatal water loss.

C. Salinity and Aluminum Stress Tolerance in Plants:

Irrigation-induced soil deterioration is a serious issue due to the accumulation of salts from irrigation water after evapotranspiration, which affects salinity and aluminium stress tolerance. Rice has garnered attention for its irrigation requirements, susceptibility to salinity, and compact genome, making it a subject of extensive research. In the context of aluminium toxicity, which is a significant problem in tropical and acidic soils, researchers have discovered a specific genetic location (locus) called AltBH on chromosome 4D of bread wheat (T. aestivum) that is associated with tolerance to aluminium. The Al tolerance gene (Alp) on chromosome 4H in barley has been precisely located, and microsatellite markers linked to it have been found.

19.10 MAS for Agronomic Traits:

Approximately 100 genes and functional polymorphisms that affect plant growth and physiology have been discovered. These genetic factors have a significant impact on important characteristics related to domestication and quality in crop plants. Extensive research has been dedicated to studying the genetic factors behind heterosis, which is a key objective in the field of plant breeding. This phenomenon, which is marked by dominance, overdominance, and epistatic effects, has been thoroughly examined and quantitative trait loci (QTLs) have been identified in several crops such as maize (Frascaroli et al., 2007), rice (Li et al., 2001), tomato (Semel et al., 2006), and rapeseed (B. napus; Radoev et al., 2008).

19.10.1 MAS for Yield:

The subsequent sections present a few chosen instances of successful applications of MAS in enhancing the yield of significant crop plants such as maize, rice, barley, and soybean.

A. Maize:

Marker-mediated backcrossing is a useful technique in maize breeding that allows for the efficient transmission of desirable alleles at quantitative trait loci (QTLs), while also speeding up the restoration of the recipient genotype in other parts of the genome. By employing a mix of backcrossing and selfing, this method effectively produces near-isogenic lines (NILs) that possess diverse introgressed genomic areas. When examined, these NILs exhibit diverse yield performances, which contribute to both the improvement of specific lines and the identification of quantitative trait loci (QTL) associated with yield. Nevertheless, this approach might fail to consider possible advantageous epistatic interactions across quantitative trait loci (QTLs).

Simultaneously, research endeavors to forecast the performance of hybrids by utilising marker and phenotypic data, thereby eliminating the necessity for intensive single-cross testing.

B. Rice:

The examination of QTL alleles derived from the wild rice species Oryza rufipogon has played a crucial role in the research of rice. A notable quantitative trait locus (QTL) for thousand grain weight (TGW) was discovered on chromosome 6 through the utilisation of backcross inbred lines derived from crossings between the high-yielding Nipponbare and the low-yielding Kasalath. Curiously, the gene responsible for the rise in TGW originates from the low-yield variety. When this allele is introduced into the Nipponbare variety, it results in a notable 10-15% enhancement in both TGW and yield per plant. This Quantitative Trait Locus (QTL), identified by many molecular markers, has the potential to improve the production of other high-yielding rice varieties.

C. Barley:

Marker-assisted selection has facilitated the transfer of high grain yield QTL alleles from the adaptive cultivar Baronesse to the high malting quality cultivar Harrington in barley breeding. The BC3 Harrington isolines demonstrated a consistent correlation with Baronesse's yields across several locations and years, frequently exhibiting enhanced malting quality.

D. Soybean:

The lack of variety in the elite germplasm of soybean has sparked interest in G. soja, the wild progenitor, as a potential reservoir of novel genetic diversity. Four quantitative trait loci (QTLs) were identified and mapped in different contexts using backcross populations. These populations were created by crossing G. soja, a wild soybean species, with a soybean cultivar as the parent that is repeatedly used. These quantitative trait loci (QTLs) indicate that the marker alleles from the G. max cultivar have a greater impact on yield compared to those from G. soja.

19.10.2 MAS for Quality Traits:

Many characteristics of high-quality exhibit a constant range of change and are impacted by environmental factors. MAS, which is a dependable method for choosing desirable traits in plants. It is currently applicable to various significant crops such as tomato, barley, wheat, cotton, and rice.

A. Tomato:

The concentration of soluble solids, particularly sugars, plays a crucial role in tomato processing, as higher sugar varietals necessitate less energy for the concentration process, hence enhancing efficiency. The Brix9-2-5 QTL, derived from *Lycopersicon pennellii*,

substantially enhances glucose and fructose concentrations, resulting in a threefold rise in soluble solid content across different genetic backgrounds and environmental conditions. This characteristic exhibit partial dominance and is unrelated to both fruit weight and yield. The ovate gene, found in the early 20th-century, is a major factor that influences the pear-shaped traits in tomatoes and eggplants, thereby determining their fruit form. The ovate locus, which is located on chromosome 2, contains the genetic information for a regulatory protein. Mutations in this gene result in a change from round to pear-shaped fruits. Furthermore, the size of fruits is determined by many Quantitative Trait Loci (QTLs) that have been identified in various species of the Lycopersicon genus. These QTLs have been confirmed in 28 separate investigations. Marker-assisted backcrossing has been successful in improving these desirable characteristics in specific genetic backgrounds.

B. Malting Barley:

Barley suitability for malting is evaluated by examinations of grain characteristics, micromalting, and laboratory assessments of malt quality attributes. The characteristics of barley grain and malt quality often display quantitative variation, which is impacted by genetic and environmental factors as well as G×E interactions.

C. Wheat:

For breadmaking, the most important quality factors in wheat are related to the physical characteristics of the dough, specifically its ability to stretch and resist stretching. These features are mainly influenced by the gluten proteins found in the endosperm of the wheat. The proteins can be categorised into two groups: gliadins and glutenins. Among these, the high molecular weight (HMW) glutenins have a more significant impact on the rheological properties of dough compared to gliadins and low molecular weight (LMW) glutenins. The composition of HMW glutenin subunits has a considerable impact on the breadmaking quality, namely the strength of the dough. The alleles Glu-A1b and Glu-D1d are particularly crucial in this regard.

D. Cotton:

Cotton, which is of utmost importance to the textile industry, possesses yield components and fibre qualities that have a heritability estimated to range between 40% and 80%. The genetic control of fibre quality is modified by interactions between genotype and environment, especially in the context of various water management strategies. A recent study has identified specific molecular markers that are associated with quantitative trait loci (QTLs) responsible for fibre strength. This was achieved by crossing a *Gossypium anomalum* line with superior fibre quality with a standard cotton variety. As a result, three SSRs and six RAPDs markers were discovered, which are linked to two QTLs related to fibre strength.

E. Rice:

In China, the quality of the 'Zhenshan 97' variety of indica hybrid rice has been enhanced by including the Waxy gene region from Minghui 63 through the use of Marker-Assisted

Selection (MAS). This statement pertains to the concerns raised over the high amylose level, hard gel consistency, and chalky endosperm in Zhenshan 97, which are features regulated by the Waxy locus. The MAS approach utilised an SSR marker that specifically targeted the Waxy gene, as well as two RFLP markers that were positioned on either side of the gene. In addition, 118 AFLP fragments were employed for background selection, ensuring the preservation of Zhenshan's genetic background at other genetic loci.

19.10.3 MAS Advantages:

- **Rapid Selection Method**: Marker-assisted selection (MAS) enables fast isolation of DNA from the initial leaves or cotyledons of a plant, thereby expediting the early detection of desirable characteristics. This expedites the selection process, allowing for more knowledgeable breeding choices before pollination.
- **Consistent Results**: The utilisation of markers in MAS ensures consistent outcomes by removing the impact of environmental fluctuations that frequently affect phenotypic evaluations, resulting in more dependable assessments.
- **Enhanced Bio-safety in Breeding**: Particularly important in animal breeding, MAS is a safer screening method as it eliminates the need to introduce infections into breeding populations, therefore guaranteeing a greater level of bio-safety.
- **High Efficiency**: By employing Marker-Assisted Selection (MAS), breeders are able to promptly eliminate unwanted genotypes by screening progeny at early stages. This improves the overall genetic excellence of breeding programmes, even if the number of plants assessed in the field remains the same.
- **Mapping Polygenic Traits or QTLs**: MAS offers the distinct capacity to identify the genetic locations of polygenic characteristics or quantitative trait loci (QTLs), a task that cannot be accomplished using traditional plant breeding methods. This capability is especially advantageous for comprehending and controlling intricate characteristics that are influenced by several genes.

19.10.4 Disadvantages of MAS:

- **High Cost:** The implementation of MAS involves substantial expenses as a result of the requirement for specialized, expensive equipment, glassware, chemicals, and advanced laboratories. The resource requirements of MAS make it a costly technology to implement.
- **Skilled Manpower Requirement:** The approach necessitates proficient workers who are skilled in operating laboratory equipment, extracting DNA molecules, and analyzing DNA markers. This requires highly skilled personnel, contributing to the intricacies and expenses of operations.
- **Labor-Intensive and Time-Consuming:** The procedure of identifying different DNA markers, such as RFLP, AFLP, RAPD, SSR, and SNP, requires a significant amount of labour and time. Conducting screenings on extensive breeding populations necessitates substantial exertion and allocation of resources.
- **Health Hazards from Radioactive Isotopes:** Previous methods of DNA labelling utilised radioactive isotopes, which presented potential hazards to health. Despite the availability of non-radioactive labelling agents, the process of transitioning and handling them still necessitates meticulous control to guarantee safety.

19.11 MAS in Breeding Programs:

Over two decades ago, molecular marker technology was projected to revolutionise breeding programmes and expedite selection advances. Its performance has varied. Concibido et al. (1996) used it to breed soybeans for Heterodera glycines cyst nematode resistance. MAS is efficient in breeding monogenic characteristics but less so in polygenic traits, especially when numerous alleles with minor effects contribute to a phenotype. MAS is widely utilised in gene introgression and pyramiding for disease resistance in major crops such wheat, rice, corn, soybean, tomato, and pepper, as well as less economically important crops.

MAS has targeted many monogenic traits and important QTLs for wheat improvement, including disease and environmental stress resistance, grain protein content, and bread-making quality. Traditional and molecular methods have helped generate drought- and salinity-tolerant cultivars. The International Rice Research Institute (IRRI) has created drought-tolerant introgression lines in elite rice varieties and discovered many drought-tolerant QTLs in barley.

Despite considerable trials, no commercial tomato cultivars are salt or drought tolerant, and fruit recognition markers are currently being developed. Fruit colour is greatly affected by the environment, making marker identification difficult. Few marker loci connected to genes of interest are employed in breeding initiatives, despite numerous reports. However, MAS is increasingly used in breeding, demonstrating its changing function in agricultural development.

19.12 Future Aspects:

The future of agricultural plant genomics is focused on the thorough sequencing of many plant phenotypes, enabled by the increased accessibility of genome sequences. Anticipated are the advancements in plant transcriptome analysis, propelled by novel technologies. Marker-assisted selection (MAS) is advancing, expected to gain advantages from the growing number of quantitative trait loci (QTL) investigations, improved identification of gene functions, and reverse genetic analyses. These advancements will make gene modification easier and encourage the creation of new genomic ideas. Comparative genetic analysis involves the identification of crucial gene sequences among different species, which helps in finding better versions of genes for more effective screening using DNA markers. The implementation of technological advancements like as automation and DNA chips is anticipated to improve the efficiency of MAS. Although there are certain existing difficulties, the prospects for enhancing polygenic traits in plant breeding through the use of DNA markers are positive.

19.13 Conclusion:

Molecular markers have been linked to quantitative trait loci (QTLs), but their use in plant breeding programmes is limited. Marker-Assisted Selection (MAS) is justified when it replaces more expensive or laborious procedures or improves genotype identification. MAS efficiency depends on three factors: the ability to detect DNA variations using DNA markers

or directly identifying genes (as discussed in the DNA Markers section), the composition of the population under study, and the statistical methods used to link a DNA marker to a gene of interest. MAS requires modern genotyping technology and a broad array of DNA markers to cover the genome. These technologies are necessary for genotyping many people with multiple markers.

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