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21. Marker-Assisted Selection for Crop Improvement

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Abstract:

This scholarly book chapter offers a comprehensive overview of Marker-Assisted Selection (MAS) in plant breeding, focusing on its historical development, principles, techniques, and applications. It critically examines the evolution of MAS techniques and compares them with conventional breeding methods, highlighting the transformative power of molecular markers in accelerating genetic improvement. The chapter explores the fundamental principles and techniques of MAS, their classification based on gene action and detection methods, and the desirable attributes of an ideal molecular marker. It also provides a brief description of various marker types, elucidating their relevance and applicability in plant breeding. The chapter emphasizes the discovery of novel molecular markers, which have become a powerful tool for enhancing breeding strategies. It also investigates genotyping methods and the critical process of marker validation, focusing on the role of validation populations and their benefits in ensuring reliable marker performance. The chapter also explores the principles of trait mapping and the seamless integration of MAS into breeding programs, focusing on quantitative trait loci (QTLs) and their involvement in trait inheritance. It discusses various strategies for harnessing the power of MAS in breeding programs, including Marker-Assisted Backcrossing (MABC), markers-assisted recurrent selection, marker-assisted gene pyramiding, genomic prediction, marker-assisted heterosis breeding, targeting induced local lesions in the genome, virus-induced gene silencing, and genome editing using CRISPR technology. It concludes with a forward-looking perspective, highlighting prospects and emphasizing the immense potential for ongoing advancements in this field. By encapsulating the breadth and depth of marker utilization, this scholarly work endeavors to engage readers and inspire them to embrace and exploit the remarkable potential of molecular markers to revolutionize plant breeding practices.

Keywords:

Molecular Markers, Marker-Assisted Selection, Marker Validation, Marker-Assisted Backcrossing, Marker-Assisted Gene Pyramiding, Marker-Assisted Recurrent Selection

21.1 Introduction:

Marker-assisted selection is a molecular breeding technique that utilizes DNA markers linked to genes or traits of interest to select those traits indirectly at an early stage in the

breeding process. MAS relies on the principle that DNA markers located near a gene or genomic region controlling a trait are physically linked and tend to be inherited together with that trait. Plant breeders identify DNA markers that are closely associated with traits of interest, such as disease resistance, drought tolerance, or yield potential.

"MAS utilizes DNA markers linked to genes or traits of interest to indirectly select those traits at an early stage, before the phenotypic expression."(Collard & Mackill, 2008; Varshney et al., 2005)

21.1.1 Historical Development and Evolution of MAS Techniques:

The foundations for MAS were laid in the early MAS were laid in the early 20th century with the rediscovery of Mendel's laws of inheritance and the development of genetic linkage maps, which allowed for the identification of genetic loci associated with traits of interest (Allard, 1999). However, limited tools were available for molecular marker analysis at this time. In the late 1970s and 1980s, restriction fragment length polymorphism (RFLP) markers started being identified and used to construct early genetic maps in crops like tomatoes and maize (Tanksley et al., 1989).

RFLP markers provided the first opportunities to link DNA markers to traits, laying the groundwork for MAS (Collard & Mackill, 2008). However, RFLP analysis was laborious and had a low throughput.

The 1990s saw the development of polymerase chain reaction (PCR)-based markers like random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP), which accelerated genetic mapping and MAS (Mueller & Wolfenbarger, 1999). Simple sequence repeat (SSR) or microsatellite markers also emerged as a popular marker class during this decade (Varshney et al., 2005). From the late 1990s to the early 2000s, marker technologies advanced rapidly. SSRs became widely used, and new marker types like single nucleotide polymorphisms (SNPs) were discovered (Gupta et al., 2008). High-throughput marker platforms further increased efficiency (Ribaut & Hoisington, 1998).

The advent of next-generation sequencing (NGS) technologies in the 2010s has led to the development of new MAS approaches, such as genomic selection. Genomic selection uses a large number of SNPs to predict the genetic merit of individuals for a wide range of traits. This allows breeders to select desirable traits without having to phenotype individual plants (Desta & Ortiz, 2014). At present, MAS has had a significant impact on plant breeding and has helped develop new crop varieties with improved resistance to pests and diseases, increased yields, and better nutritional quality (Song et al., 2023). MAS is also being used to develop crop varieties that are more tolerant to abiotic stresses such as drought and salinity.

21.1.2 Comparing MAS over conventional breeding:

Compared to Marker-Assisted Selection (MAS), conventional breeding methods have several notable drawbacks. Conventional breeding relies on phenotypic observations, which

can be time-consuming and resource-intensive. It often requires growing and evaluating a large number of plants over several generations to select those with the desired traits. This process is slow and may not guarantee the selection of the most desirable genetic traits. Furthermore, conventional breeding can be limited by the need for controlled crosses between plants, which might only sometimes be feasible. The drawbacks of conventional breeding, including its time-consuming nature, resource requirements, and limited precision, highlight the advantages of MAS in modern plant breeding programs. MAS can be achieved with greater efficiency and precision and may be at a lower cost compared to conventional breeding methods while minimizing environmental impact by reducing resource usage and the number of crossings. Here is a brief comparison between them….

Table 21.1: Comparing MAS over Conventional Breeding

Character	Conventional Breeding	Marker-Assisted Selection
Selection Method	Based on visual observation of expressed traits/phenotypes.	Relies on DNA markers linked to traits of interest for indirect selection.
Selection Timing	Selection occurs later, after trait expression.	Allows for early selection before trait expression at the seedling stage.
Selection Accuracy	Accuracy depends on heritability and environment.	Highly accurate due to the direct selection of DNA.
Selection Intensity	Limited by trait heritability and expression.	Enables selection of traits with low heritability or complex inheritance.
Breeding Cycles	Longer breeding cycles.	Shortens breeding cycles through early selection.
Introgression of Wild/Donor Traits	Difficult to introgress traits from unadapted germplasm.	Facilitates introgression of traits from diverse germplasm.
Recessive Trait Selection	Cannot select traits masked in hybrids	Enables selection of recessive traits.
Linkage Drag Reduction	Prone to retaining linked undesirable traits.	It helps minimize linkage drag during backcrossing.
Applicability	Depends on trait heritability and expression	Applicable to both simple and complex inherited traits.
Cost and Labor Requirements	More labor-intensive and time-consuming.	Less labor-intensive and time-consuming.

Marker-assisted selection (MAS) offers several significant advantages over conventional breeding methods for crop improvement. One of the significant benefits is that MAS allows for more efficient and accelerated development of new crop varieties.

Through the use of molecular markers linked to traits of interest, MAS enables direct selection of desired genes or QTLs at the seedling or seed stage before phenotypic expression of the traits. This early-generation selection saves substantial time compared to

conventional breeding, where selection is only possible in late generations after visual observation of trait expression under field conditions. By facilitating selection much before field trials, the entire crop breeding process, from cross-breeding to variety release, can be reduced by 1-3 years through MAS.

Another key advantage is the higher accuracy and precision of selecting the best-performing lines achieved with MAS. Molecular markers provide a more reliable method for identifying genotypes with target genes than visual phenotypic selection alone. This makes MAS an efficient approach for transferring traits governed by recessive genes or pyramiding multiple genes controlling complex traits, tasks that are difficult with conventional breeding methods. MAS also enables year-round breeding as it is not constrained by crop seasons, further expediting variety development. Overall, MAS offers substantially higher efficiency, accuracy, and cost-effectiveness for crop improvement compared to traditional phenotypic selection techniques through early-generation selection.

21.2 Principles and Techniques of Marker-Assisted Selection:

21.2.1 Introduction to markers:

A marker in plant breeding acts like a flag, indicating the location of a particular gene for a plant to grow and look a certain way; specific genetic instructions must be carried out. This means you can use markers to identify the gene responsible for a particular feature, such as disease resistance, if you wish to develop a plant with disease resistance. There are four types of genetic markers:

Biochemical markers are measurable molecules or substances in biological fluids or tissues that can act as indicators of normal or abnormal biological processes, disease states, conditions, or characters. **Morphological markers** refer to visible traits or characteristics that can be observed externally, such as hair or eye color, height, etc. and are used to identify individuals or trace biological relationships. Examples include hair color, eye color, facial features, and fingerprints.

Cytological markers in plant breeding refer to observable banding patterns on chromosomes produced by staining techniques, which reveal the distribution of euchromatin and heterochromatin and can be used to identify chromosomes and genetic loci but are limited for direct use in genetic mapping and breeding due to their physical nature.

Molecular markers are distinct DNA segments that are identifiable across the whole genome. Molecular markers are present in particular genomic regions. They serve to "flag" the location of a particular gene or the inherited trait of a specific character. Molecular indicators do not affect phenotype in any way.

21.2.2 Molecular markers are:

classified into various groups on the basis of:

- Mode of **gene action** (co-dominant or dominant markers);

- Method of **detection** (hybridization-based molecular markers or polymerase chain reaction (PCR)-based markers)
- Mode of **transmission** (paternal organelle inheritance, maternal organelle inheritance, bi-parental nuclear inheritance, or maternal nuclear inheritance) (Nadeem et al., 2018)

A. Markers Based on Mode of Gene Action:

a. Dominant Markers A dominant marker is one where only one form of the trait being studied is associated with the presence of the marker. In contrast, the other form shows no association.

Dominant markers cannot distinguish between an individual who is heterozygous or homozygous for the marker allele. Since only one allele is marked, both the heterozygous and one homozygous genotype will test positive for the marker, while the other homozygous genotype will test negative. These markers are less informative than co-dominant markers.

b. Co-Dominant Markers: The co-dominant marker differs in that both forms of the trait being studied are associated with detectable variants of the marker. Both alleles of a co-dominant marker can be identified independently of each other. This allows the marker to discriminate between heterozygotes, which express both markers and homozygotes, which express only one marker type.

Since both alleles are detected, co-dominant markers provide more information than dominant markers. They allow for the identification of genotypes at a locus and are thus more advantageous for genetic mapping and breeding applications where knowing the exact genotype is essential.

B. Markers Based on The Method of Detection:

a. Hybridization-based molecular markers -Nucleic acid hybridization is the underlying concept of hybridization-based molecular markers. (Cite 4) In order to identify a specific DNA sequence, they employ a probe, which is a little DNA fragment that is complementary to that sequence. The probe has a radioactive or fluorescent tag on it to make it detectable. Denatured DNA indicates that the two strands of DNA have been split, and this DNA is being evaluated. After the probe is applied to the denatured DNA, it will hybridize to a DNA sequence if it is complementary to that sequence. Following that, the probe's radioactivity or fluorescence can be used to identify its existence.

b. Polymerase chain reaction (PCR)-based markers -Molecular markers that are PCR-based amplify a particular DNA sequence using the polymerase chain reaction (PCR). In PCR, the DNA is heated to denature it, cooled to allow primers to attach to it, and then heated once again to activate the DNA polymerase enzyme and amplify the DNA. (Cite 5) The size or sequence of the amplified DNA can then be used to identify it.

Although hybridization-based molecular markers might take a while to produce, they are comparatively simple to employ. Although PCR-based molecular markers are quicker to produce and often more sensitive, they are more challenging to employ.

21.2.3 Salient features of an ideal molecular marker:

An ideal molecular marker should:

- Exhibit a high degree of variation between individuals in a population. This allows for greater discrimination power.
- Show co-dominant inheritance so that homozygotes and heterozygotes can be distinguished from each other.
- It occurs frequently throughout the genome to provide abundant marker coverage.
- Be selectively neutral so as not to be influenced by environmental factors or natural selection.
- Be easily accessible for detection and analysis.
- Allow simple, straightforward evaluation using standard laboratory techniques and tools.
- Provide highly reproducible, consistent results that are not prone to error.
- Facilitate easy sharing of data between different research laboratories. The data should be portable and comparable between labs.

21.2.4 Brief Description of Different Markers:

Restriction Fragment Length Polymorphisms (RFLPs) were one of the earliest molecular marker techniques developed. RFLPs detect variations in DNA sequences recognized by restriction enzymes. When genomic DNA is digested with these enzymes, individuals may differ in the length of DNA fragments produced. Fragment length polymorphisms can be identified after gel electrophoresis. RFLPs provide locus-specific markers but require large DNA quantities and are labor-intensive.

Random Amplified Polymorphic DNA (RAPD) uses short arbitrary nucleotide primers in PCR to amplify random DNA fragments. This technique is simple and can be performed with small amounts of DNA. However, RAPD markers can lack reproducibility between experiments and are dominant.

Amplified Fragment Length Polymorphisms (AFLPs) employ restriction enzymes and PCR amplification with selective nucleotides to detect multiple loci simultaneously. AFLPs generate highly informative and reproducible co-dominant markers. However, the multi-step process is time-consuming and consumes more significant amounts of DNA.

Microsatellites, also known as Simple Sequence Repeats (SSRs), target short tandemly repeated motifs 1-6 base pairs in length. Variation in repeat number leads to length polymorphisms detectable by PCR. SSRs are highly polymorphic, multi-allelic, and co-dominant. Their transferability between related species also makes them valuable genetic tools.

Single-nucleotide polymorphisms (SNPs) represent single base pair changes in genomic sequences. SNPs are abundant in plant genomes, and high-throughput SNP genotyping platforms now enable efficient simultaneous analysis of hundreds to thousands of loci.

While SNPs exhibit low allelic diversity, their co-dominant nature and distribution across genomes provide opportunities for association mapping. (Rajendra et al., 2023)

Diversity Arrays Technology (DArT) is a microarray-based method for whole genome profiling. Genomic representations are hybridized to microarrays containing probes for polymorphic loci. DArT markers provide dense genome coverage and are highly reproducible. However, they exhibit dominant inheritance.

Restriction-site Associated DNA (RAMP) markers combine PCR amplification with restriction enzyme digestion to detect polymorphisms adjacent to restriction sites. RAMPs generate multi-allelic, co-dominant markers targeted to specific loci. Their development requires prior sequence information for the species.

Inter-Simple Sequence Repeats (ISSRs) utilize PCR with single primers complementary to microsatellite repeat motifs to amplify regions between repeats at different loci. ISSRs are simple and inexpensive but lack reproducibility between experiments.

Cleaved Amplified Polymorphic Sequences (CAPS) involve PCR amplification of specific ... loci followed by restriction enzyme digestion. When digestion patterns differ, a CAPS marker is detected. CAPS markers are locus-specific and co-dominant but require sequence information for marker development.

Insertions and Deletions (Indels) markers detect small insertions and deletions in DNA sequences when compared to a reference genome. These indels provide an abundant source of genetic polymorphisms for plant breeding and genetics research.

Due to their bi-allelic nature, indel markers allow for simple and reliable genotyping, which has made them a valuable tool for applications like genetic mapping and tracking inheritance in crop populations. The continued discovery of novel indel markers through genome sequencing is enhancing their utility for accelerating genetic gains in agriculture.

21.2.5 Discovery of New Molecular Markers: A Powerful Tool for Plant Breeding:

Plant breeders rely on molecular markers to accelerate crop improvement through selection. However, existing marker sets often need more coverage or are not transferable between related species. Continuous discovery of novel markers remains vital.

a. Marker discovery begins by selecting candidate genes likely influencing traits of interest. These may control stress tolerance, yield components, or quality parameters. Researchers then sequence candidate regions in diverse germplasm to detect sequence variations like SNPs.
b. Polymorphism detection techniques have advanced rapidly. Early methods like RFLP and RAPD gave way to high-throughput next-generation sequencing. This enables comparison of whole genomes and transcriptomes to uncover an abundance of sequence variants across the species.
c. Candidate markers are evaluated on a test population for their potential usefulness. Markers showing high variability between individuals, known as polymorphic markers,

are selected for further study. Their linkage to trait loci is then examined through genetic mapping.

d. d)Markers tightly linked to genes or QTLs of interest are validated on a larger population. Only markers reliably associating with traits are considered validated and ready for deployment in breeding programs.

The continual discovery of novel molecular markers empowers breeders with new tools for selection. By tracking the inheritance of agronomically essential genes and alleles, markers help deliver crop varieties with higher and more stable yields adapted to changing conditions. This supports global food and nutritional security for the future.

21.2.6 Genotyping and its Methods:

Genotyping of markers refers to the process of determining the genetic makeup of an individual or population based on the presence or absence of specific molecular markers.

Genotyping Methods:

a. Gel Electrophoresis - Separates DNA fragments by size to detect length polymorphisms in RFLPs, SSRs, etc. Low throughput.
b. DNA Microarrays is a hybridization-based method to genotype many loci simultaneously. Early arrays targeted candidate genes or markers.
c. c)SNP Chips - High-density microarrays for genotyping hundreds to millions of pre-selected SNPs simultaneously. Commonly used in association studies.
d. Kompetitive Allele Specific PCR (KASP) - Fluorescence-based genotyping of SNPs and other markers. Medium-throughput, cost-effective.
e. Next-Generation Sequencing - Used for both marker discovery and genotyping. Whole genome resequencing provides the highest marker density but at a higher cost than array-based methods.

A. Phenotyping vs Genotyping vs Sequencing:

Genotyping is typically done using DNA markers, which are short sequences of DNA that vary between individuals. DNA markers can be used to identify specific genes or to track the inheritance of traits.

Phenotyping can be done by observing the plant's physical traits, such as its height, leaf shape, and flower color. Phenotyping can also be done by measuring the plant's performance in different environments. Genotyping and phenotyping are complementary techniques that can be used together to improve plant breeding. Genotyping can be used to identify plants with desirable genes, while phenotyping can be used to confirm that these plants actually have the desired traits. Genotyping and sequencing are both methods of determining the genetic makeup of an individual plant. Genotyping is typically faster and cheaper than sequencing, but it only provides information about a small portion of the genome. Sequencing provides information about the entire genome, but it is more expensive and time-consuming.

21.2.7 Marker Validation:

Marker validation is a crucial step in plant molecular breeding, ensuring the reliability and accuracy of DNA markers for selecting plants with desirable traits.

It involves confirming that the identified markers are indeed linked to the desired traits and that this association holds across different genetic backgrounds and environments.

A. Importance of Marker Validation:

- Enhanced Breeding Efficiency: Marker validation ensures that breeders focus their efforts on selecting plants with the desired traits, saving time and resources.
- Improved Genetic Gain: Validated markers lead to more effective selection of superior genotypes, accelerating genetic progress and improving crop productivity.
- Reduced Risk of False Positives: Validation eliminates the possibility of selecting plants based on spurious marker associations, preventing the spread of undesirable traits.
- Broader Applicability: Validation ensures that markers are adequate across different genetic backgrounds and environments, expanding their applicability in breeding programs.
- Confidence in Marker-Assisted Selection (MAS): Validation instills confidence in MAS, a powerful tool for selecting plants with desired traits based on their genetic makeup.

B. Marker Validation Using Validation Populations:

Validation populations are distinct from the mapping populations used to identify marker-trait associations initially.

They represent a diverse set of genotypes, often from different germplasm sources or breeding lines, that are not included in the mapping population. The process of validation typically involves the following:

a. Selection of Validation Population: Choose a validation population that represents the genetic diversity of the breeding material intended for MAS.
b. Genotyping: Validation populations are genotyped using the same markers identified from the mapping population.
c. Phenotyping: Validation populations are phenotyped for the trait of interest, assessing their performance under relevant environmental conditions.
d. Marker-Trait Association Analysis: The marker-trait association is analyzed in the validation population by statistical analysis, determining the strength and consistency of the association across different genetic backgrounds.
e. Validation Confirmation: Validated markers are those that consistently show a significant association with the trait of interest across different genetic backgrounds and environments.

C. Benefits of Using Validation Populations:

- Robustness of Marker-Trait Association: Validation populations ensure that the marker-trait association is not specific to the mapping population, providing confidence in its broader applicability.
- Genetic Background Independence: Validation across diverse genetic backgrounds demonstrates that the marker-trait association is not influenced by unique genetic interactions within the mapping population.
- Environmental Stability: Validation under different environmental conditions confirms that the marker-trait association is consistent and reliable across varying environments.
- Increased Reliability of MAS: Validation populations enhance the reliability of MAS, ensuring that breeders can confidently select plants based on their marker profiles.

Marker validation using validation populations is an essential step in plant molecular breeding. It ensures the accuracy and reliability of DNA markers, enabling breeders to make informed decisions in selecting superior genotypes for crop improvement.

21.3 Marker-Assisted Selection (MAS):

When Tanksley and Rick (1980) employed isozymes as markers for the introgression of a foreign trait into acclimated tomato cultivars, marker-assisted selection (MAS) was first used. The idea underlying the use of markers is that selection based on genotype, as opposed to phenotype, may accelerate and improve selection.

21.3.1 Principles of Trait Mapping:

A. Quantitative Trait Loci (QTLs)**: -**Quantitative trait loci (QTLs) are regions of DNA that contribute to the variation of a quantitative trait, such as plant height, yield, or disease resistance. Unlike qualitative traits, which are controlled by a single gene and exhibit distinct phenotypes, quantitative traits are controlled by multiple genes and exhibit a continuous range of phenotypes.

B. Role of QTLs in Trait Inheritance: QTLs influence the expression of quantitative traits by affecting the activity of nearby genes involved in trait development. The effect of a single QTL on a trait is typically small, but the combined effects of multiple QTLs can have a significant impact on the trait's phenotype.

C. Process of QTL Mapping Using DNA Markers: -QTL mapping involves identifying associations between DNA markers and quantitative traits. This process typically involves the following steps:

- ***Create a mapping population*****:** A mapping population is a group of individuals with diverse genetic backgrounds, typically created by crossing two parental lines with contrasting traits.
- ***Phenotype the mapping population*****:** The individuals in the mapping population are evaluated for the trait of interest, measuring the trait's phenotype.

- ***Genotype the mapping population*:** The individuals in the mapping population are genotyped for a large number of DNA markers, which are known locations along the genome.
- ***Statistical analysis*:** Statistical methods are used to analyze the association between DNA markers and the trait phenotype.
- ***QTL detection*:** Regions of the genome that show significant association with the trait phenotype are identified as QTLs.

D. Factors Affecting QTL Detection and Interpretation

a. Genetic architecture: The number of QTLs controlling a trait and their individual effects influence the ease of QTL detection.
b. Environmental factors: Environmental conditions can influence the expression of quantitative traits, making QTL detection more challenging.
c. C)Marker density: The number and distribution of DNA markers used in the mapping population affect the precision of QTL detection.
d. D)Statistical methods: The choice of statistical methods and their appropriate application influence the accuracy of QTL detection.
***e.* E) Epistasis:** Interactions between QTLs can complicate QTL detection and interpretation.

21.3.2 Integration of MAS in Breeding Programs:

Here are the steps of marker-assisted selection paraphrased:

a. Identify parent plants that contain desirable traits/genes of interest, preferably those carrying different alleles based on genetic studies. At least one parent must possess the DNA marker for the trait.
b. Perform a cross between the selected parents to generate a hybrid offspring population. Use molecular markers linked to the trait to screen the hybrids and eliminate any non-hybrid plants.
c. Grow the hybrid offspring to generate a segregating F2 generation. Screen F2 plants for the markers associated with the trait and select/harvest only those containing the desired marker alleles.
d. Grow the harvested F2 plants individually to generate F3 rows/populations. Continue marker screening and selection at both the individual plant and row level, retaining only plants/rows with the target markers.
e. In subsequent generations like F4 and F5, marker screening and selection are conducted similarly while focusing on superior lines/individuals that are homozygous for the markers.
f. In late generations such as F6, bulk the best-performing lines together based on marker data and evaluation of traits of interest.
g. Test the selected bulked lines in yield trials and evaluate their performance comprehensively for both the target and other essential traits.

The overall goal is to enrich populations for the desirable trait-linked markers and alleles at each generation through repeated selection.

21.4 Different strategies for utilizing MAS in breeding programs:

21.4.1 Marker-Assisted Backcrossing (MABC):

MABC is a precise method for introducing a single locus controlling a trait while retaining essential characteristics of the RP. It is effective for genes or quantitative trait loci with significant phenotype variations. MABC uses markers to select target loci, minimize donor segment length, and accelerate RP genome recovery during backcrossing. The main objective is to integrate a targeted gene from agronomical substandard sources into an exclusive breeding line. Molecular markers in plants can accelerate backcrossing by reducing the number of backcrosses needed to recover the RP phenotype. Whereas conventional breeding takes more time, more crosses, more resources, and efforts It is possible to recover the RP genotype with only two or three backcrosses when employing high population numbers (400 plants or more) for the backcross F1 generations. The following are the three steps in the selection process.

Foreground selection: - Foreground selection is a breeding method where plants with the donor parent's marker allele at the target locus are selected. The goal is to maintain the target locus in a heterozygous state until the final backcross is completed. The selected plants are self-pollinated, and progeny plants are identified as homozygous for the donor allele. Markers closely linked to the target gene or QTL are used for early progeny selection. This method is helpful for traits with laborious screening procedures and reproductive-stage traits in the seedling stage.

Recombinant selection: - Recombinant selection is a method of selecting BC progeny with the target gene and recombination events between the target locus and linked flanking markers. It aims to reduce the size of the donor chromosome segment, which can be considerable due to linkage drag. Conventional breeding methods can leave the donor segment large, so using markers that flank the target gene can minimize linkage drag. Recombinant selection is typically performed using at least two BC generations, but only for genes or QTLs with well-defined map positions. Fine or high-resolution mapping is usually required before recombinant selection.

Background selection: - Background selection is a method used in backcrossing to select genomic regions with the most significant proportion of the RP genome using RP marker alleles. This process is crucial to reduce unnecessary genes introduced from the donor. Background selection is also known as 'negative selection' and is used to select against the donor genome. This method is helpful in accelerating RP recovery, as conventional backcrossing takes at least six BC generations to recover the RP. The use of background selection during MABC is referred to as 'variety development or enhancement' and 'complete line conversion.' This method is essential for reducing linkage drag and ensuring efficient backcrossing.

Scheme of Work in Backcross Breeding:

a. Cross the recurrent parent with the first donor parent to produce F1 hybrids.
b. Backcross F1s to the recurrent parent to generate BC1F1 progeny.

c. Screen BC1F1s for markers linked to the target gene from the first donor and select positive individuals.
d. Backcross selected BC1F1s to the recurrent parent and repeat marker screening in BC2F1s.
e. In Progress segments around the second target gene by crossing BC2F1s with the second donor parent.
f. Repeat backcrossing and screening with the recurrent parent to recover its genetic background.
g. In later generations like BC3F1, select lines possessing all target gene markers. Evaluate top lines through yield trials and identify varieties with pyramided genes.

Activities of Marker-assisted Backcross Breeding:

Marker-assisted breeding involves following steps

a. Planting the breeding populations.,
b. Identification of markers for polymorphism survey.,
c. Sampling plant tissues, usually at early stages of growth.
d. Extract DNA from the tissue sample of each individual and prepare DNA samples for PCR and marker screening.
e. Running PCR with appropriate markers to identify the target plants.,
f. Selecting the best individuals/families with both desired marker alleles for target traits and desirable performance/phenotypes of other traits by jointly using marker results and other selection criteria.
g. Repeating the above activities for several generations

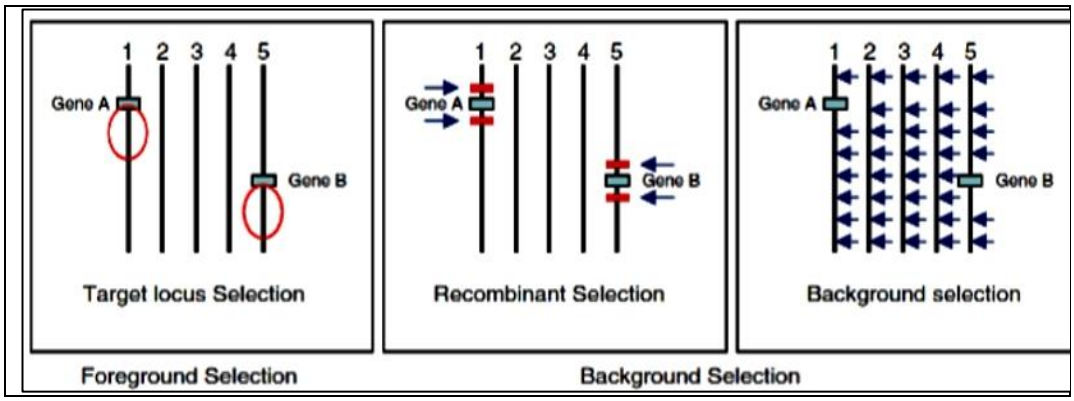


Figure 21.1: Figure depicting the forward, recombinant, and background selection by Jain et al. 2010

21.4.2 Markers assisted Recurrent Selection:

MARS is an advanced plant breeding technique that utilizes molecular markers to speed up the process of accumulating beneficial genes controlling complex traits. It does this by identifying and selecting desirable alleles at multiple locations across the genome from

different parental sources or breeding populations. MARS then facilitates the recombining of these favorable variants through recurrent cycles of crossing within a single growing season. This allows the integration of small-effect favorable variants through recurrent cycles of crossing within a single growing season.

This allows for the integration of small-effect quantitative trait loci more efficiently than traditional recurrent selection methods.

The process involves genotyping early-generation progeny, phenotyping later generations, and performing two to three rounds of selection and recombination based on marker and phenotype data. Using continuous nurseries makes it possible to complete multiple selection cycles in one year.

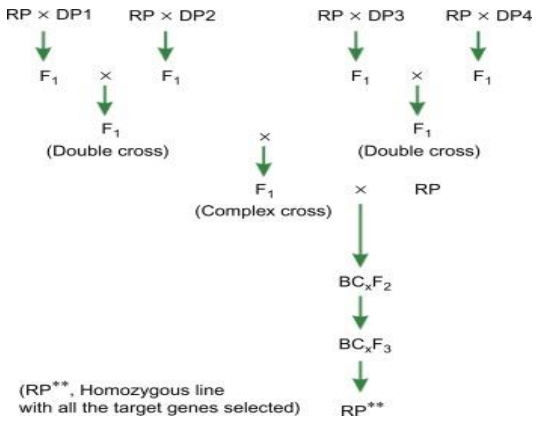
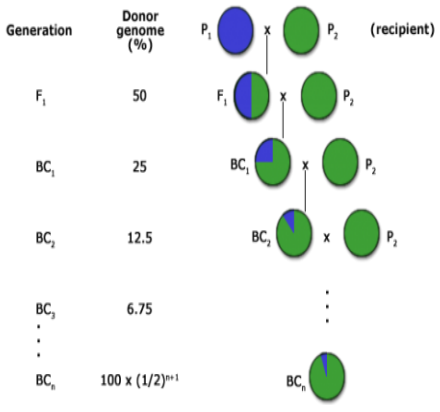
This accelerated approach helps plant breeders more rapidly develop lines optimized for critical agronomic traits like yield, disease resistance, and stress tolerance.

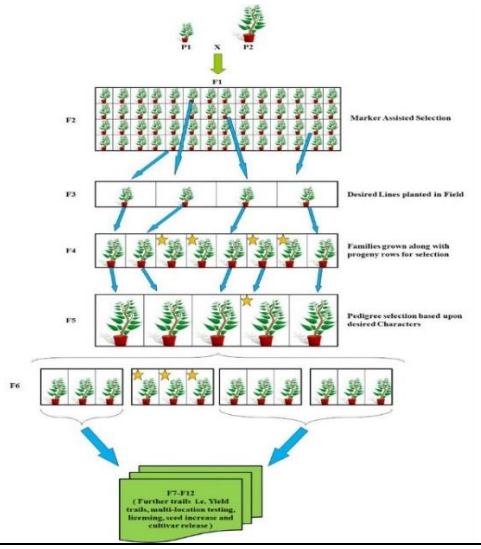
A. Advantages of MARS over other Breeding Methods:

MARS is a faster and more efficient method for selecting plants, combining phenotypic and genotypic information. It offers greater accuracy and precision than MAS, reducing linkage drag and requiring less training populations.

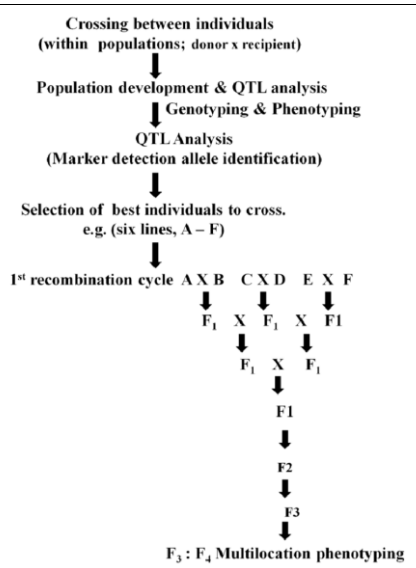
MARS is also more efficient than GS in some instances, as it can be used with fewer markers and does not require large numbers of markers for accurate prediction models.

Table 21.1: Advantages of MARS over other Breeding Methods

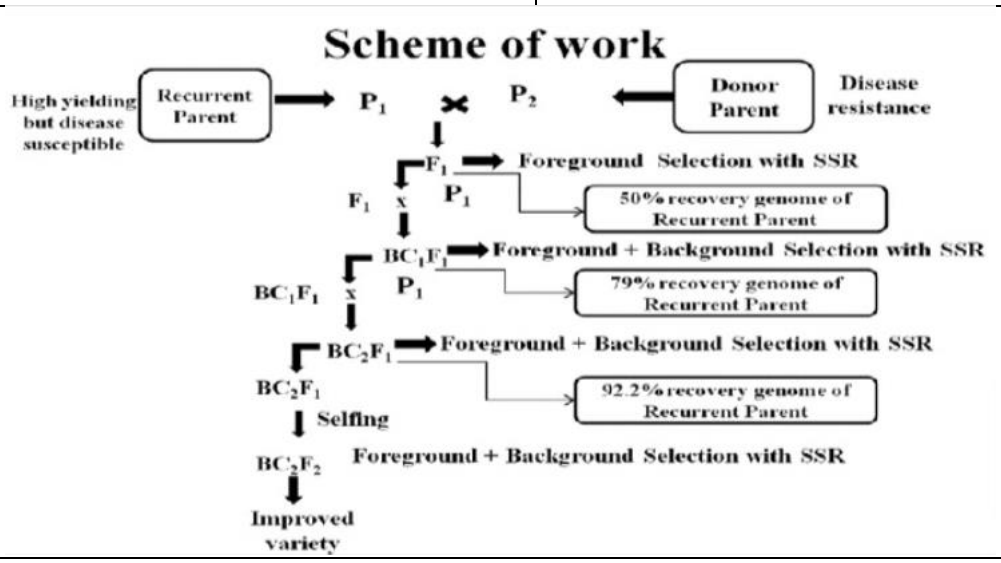
	
Schematic representation of gene pyramiding given by Rana et al. 2019	Schematic representation of % recovery of recipient genome for every Back cross by Lübberstedt et al. 2023



Schematic representation of marker-assisted selection in plants given by Islam et al., 2017



Schematic representation of marker-assisted recurrent selection in crops by Dormatey et al.,2020



Depicting scheme of work in a Backcross Breeding with Foreground and Background selection along with % recovery of the recurrent genome. Given by Hasan et al 2015

21.4.3 Marker-Assisted Gene Pyramiding:

This, also known as gene stacking, is a technique used in plant breeding to incorporate multiple desired genes or quantitative trait loci (QTLs) into a single genotype. It allows for the accumulation of several resistance genes or QTLs with known effects on specific traits,

resulting in durable resistance against various biotic and abiotic stresses. The objective of gene pyramiding is to combine all desirable alleles into a single genotype. The process involves two main parts - first, a crossing scheme is used to accumulate one copy of each target gene from multiple parent lines into a single genotype. The second part focuses on fixing these genes into a homozygous target genotype through continued selfing and selection from the single genotype. Together, these steps are aimed at efficiently integrating all favorable variants into a single high-performing line.

Gene pyramiding is crucial for developing durable disease resistance, improving elite cultivars, eliminating extensive phenotyping, controlling linkage drag, and reducing breeding duration. Gene pyramiding is influenced by target traits/genes, reproductive characteristics, a breeder's ability to identify desired genotypes, and operating capital.

Other applications and uses, such as

21.4.4 Genomic Prediction:

As a kind of Marker Assisted Selection (MAS), Genomic Selection (GS) estimates the breeding values of lines within a population by assessing the phenotypes and scores of markers throughout the complete genome. The GS prediction models' integration of all marker data helps prevent skewed estimations of marker effects, enabling the capture of variation given by small-effect QTL.

21.4.5 Marker-Assisted Heterosis Breeding:

Molecular markers enhance heterosis breeding by improving the selection of parental lines by identifying haplotype blocks associated with hybrid performance, transferring yield-enhancing and disease-resistant genes, and facilitating the development of new hybrid crop varieties with critical traits, such as resistant rice and pearl millet combinations resistant to bacterial blight and downy mildew.

Overall, markers help enhance genetic gain in heterosis breeding by enabling both the selection of optimal parents and the improvement of parental line genotypes for traits affecting hybrid yield and quality.

21.4.6 Targeting induced local lesions in the genome*:*

Targeting Induced Local Lesions in the Genome (TILLING) is a technique that complements marker-assisted selection (MAS) by creating genetic diversity through induced mutations. TILLING enables the identification of specific mutations within the genome, which can serve as markers for selecting desired traits.

By integrating TILLING with MAS, breeders can screen a large number of individuals for mutations and select those with the desired genetic variations. This synergistic approach enhances the efficiency of MAS by providing a wider pool of genetic variation to choose from, leading to improved breeding outcomes.

21.4.7 Virus-Induced Gene Silencing:

By incorporating genetic markers, Marker-Assisted Selection (MAS) improves Virus-Induced Gene Silencing (VIGS) and speeds up plant breeding. Through momentary gene suppression, VIGS validation is made possible by MAS, which finds candidate genes associated with desirable traits. This collaborative method reduces the need for field trials, speeds up cultivar development, and simplifies the selection process. Applications include improving quality traits, tolerating stress, and developing disease resistance. In spite of obstacles such as transitory impacts, VIGS-MAS shows promise as a focused, quick, and economical approach to improve plant breeding and tackle worldwide farming issues.

21.4.8 Genome Editing (CRISPR):

In the field of genome editing, marker-assisted selection (MAS) is essential, mainly when using CRISPR technology. It makes use of molecular markers linked to the desired gene or locus to make it easier to identify and carefully choose modified plants. By accurately selecting genetically modified plants for the detection of off-target impacts, this integration speeds up the early discovery of altered plants. MAS is especially useful for improving crop quality, as it speeds up the development of superior cultivars with increased yield, quality of nutrition, and resistance to disease pests

21.5 Challenges and Limitations of MAS:

A. Requirement of expert Trained personnel in usage of Molecular Marker for the proficient handling of sophisticated equipment, DNA isolation, and the analysis of DNA markers in molecular marker-assisted selection. (Henkrar and udupa 2020)
B. Identifying various linked DNA markers (e.g., RFLP, RAPD, AFLP, SNP, SRP) is a time-consuming and challenging process, demanding considerable labor.
C. The application of MAS in QTL studies faces difficulties due to cumulative effects influenced by environmental factors and genetic backgrounds, limiting its effectiveness.
D. The implementation of marker-assisted selection methods necessitates a well-equipped laboratory with expensive chemicals and specialized glassware, contributing to elevated costs.
E. In some instances, the use of radioisotopes in marker-assisted selection, especially with RFLP markers, poses serious health risks. Conversely, markers based on PCR are considered safer in this context.

21.6 Future Prospects and Conclusion:

Marker-assisted selection has shown tremendous potential for accelerating genetic gains in plant breeding programs. As genomic resources continue expanding and marker technologies become more high-throughput and cost-effective, MAS approaches will increasingly be integrated into mainstream breeding efforts. For complex polygenic traits, the focus will shift from individual QTL to genome-wide selection based on dense SNP maps. Advanced phenotyping methods combined with predictive models will facilitate indirect selection. Automation and robotics will allow the processing of vast populations with precision.

Cloud computing will help analyze big data and deploy solutions tailored for each cropping system and target environment. International collaborations will optimize germplasm exchange and coordinated breeding efforts. Suppose these scientific and technological advances are matched with adequate investments in both public and private sectors. In that case, the future appears bright for MAS to significantly boost our efforts in feeding the billions and adapting agriculture to climate change.

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