Advancing Horticulture: The Role of Marker-Assisted Breeding in Crop Improvement

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Marker-assisted breeding (MAB) is revolutionizing the cultivation of horticultural crops by enabling more accurate genetic selection of desired traits. Unlike traditional breeding methods, which depend heavily on selecting traits based on visible characteristics, a process often hindered by complex inheritance patterns and environmental influences, MAB uses molecular markers tied to specific genes. This allows breeders to more efficiently identify and choose plants with the desired genetic features. MAB has significantly accelerated the breeding process for crops with traits like disease resistance, higher yields, and enhanced nutritional and visual qualities. It also reduces the time needed for breeding cycles; while traditional methods may require multiple generations to achieve desired traits, MAB can confirm the presence of beneficial genes early, speeding up the development of improved varieties. This is particularly crucial in addressing challenges like climate change, where crops must quickly adapt to stressors such as drought, salinity, and temperature fluctuations. In essence, MAB represents a major advancement in horticultural crop development, increasing the precision and efficiency of breeding efforts. It also plays a vital role in creating crops suited to specific environmental and market needs, while helping tackle global issues like food security, climate change resilience, and sustainable farming. MAB positions horticulture to better meet evolving consumer expectations and agricultural demands.

Keywords: Horticultural; genetics; sustainable; chemical; traditional; generations.

1. INTRODUCTION

Horticulture, which encompasses the science and art of producing fruits, vegetables, flowers, and decorative plants, is an essential component of agriculture on a worldwide scale. It makes a substantial contribution to the stability of the economy, as well as to the protection of food supplies and the nourishment of people (Sharma et al., 2008). There has been an increase in the demand for horticulture goods, which has resulted in a multitude of issues for producers. This need has increased as the global population continues to grow and urbanization continues to spread (Koshariya et al., 2024). The production of horticulture must not only adjust to satisfy the requirements of a rising population, but it must also adjust to the mounting dangers that are presented by climate change, environmental degradation, insect invasions, and the introduction of new plant diseases. Plant breeders are tasked with the responsibility of developing horticultural crop varieties that are capable of withstanding these stresses while preserving high yields, exceptional quality, and aesthetic appeal (Tiwari et al., 2022).

Traditional techniques of plant breeding, which have their origins in millennia of experience, placed a significant amount of emphasis on the apparent traits of plants, which are referred to as phenotypes, in order to guide selection (Jannink et al., 2010). The use of this strategy has shown to be quite helpful in improving crop characteristics like as yield, flavour, and resistance to disease. The development of a new variety that has desired features, on the other hand, is a labour-intensive and time-consuming process that often takes several years or even decades to complete (Rehman et al., 2023). Furthermore, many of the characteristics that are essential for modern horticultural production, such as the ability to withstand drought, resistance to disease, and nutritional quality, are controlled by multiple genes and are significantly influenced by environmental conditions. This makes it difficult to select for these characteristics using traditional methods (Thudi et al., 2014). The area of plant breeding has been completely transformed as a result of the introduction of new techniques that have been made possible by developments in molecular biology and genetics in recent decades. The invention of marker-assisted breeding, a method that enables breeders to pick plants based on their genetic composition rather than their outward appearance, has been one of the most important achievements that has occurred in the field of plant breeding (Nadeem et al., 2018). For the purpose of tracking the inheritance of these genes within a population, MAB makes use of molecular markers, which are short sequences of DNA that are tightly related to certain genes of interest. Breeders are able to make more informed choices, which speeds up the breeding process and improves the accuracy of trait selection (Kamboj et al., 2020). This is accomplished by finding plants that have the genetic markers that are linked with desired qualities. It is impossible to overestimate the

significance of MAB in the process of crop growth in horticulture. Horticultural crops, which include fruits, vegetables, and ornamental plants, are valued for a broad variety of characteristics. These characteristics include flavour, nutritional content, appearance, and shelf life, in addition to productivity and tolerance to environmental challenges (Banoth et al., 2023). As a result of the fact that many of these characteristics are governed by intricate gene networks, it was formerly a difficult task for breeders to concurrently improve all of these characteristics. It is possible to solve this issue with the help of MAB, which enables breeders to choose for numerous characteristics at the same time, so significantly enhancing the effectiveness of the breeding process (Murray and Thompson 1980). In the context of contemporary horticulture crop production, the significant part that marker-assisted breeding really plays. This article digs into the scientific foundations that underpin MAB, then compares it to more conventional techniques of breeding, and last investigates the numerous ways in which this methodology might be used in the field of horticulture. Furthermore, the essay explores the future potential for this technology in the context of a fast-changing agricultural environment, as well as the obstacles and constraints that are associated with MAB (Rafalski 2002, Turan et al., 2012, Kaushal et al., 2020).

2. THE SCIENCE BEHIND MARKER-ASSISTED BREEDING

In the field of plant breeding, the powerful technique known as marker-assisted breeding has brought about a revolution by making it possible to choose plants with desirable characteristics in a more accurate and efficient manner (Lander and Botstein 1989). It combines molecular biology with traditional plant breeding, which enables breeders to make choices that are better informed. This is accomplished by the use of molecular markers, which facilitate the identification of plants that contain certain genes linked with characteristics such as resistance to disease, tolerance to drought, or increased fruit quality (Flint-Garcia et al., 2003). MAB is based on the fundamental premise of using genetic markers, which are segments of DNA that are closely related to target genes of interest, as proxies for the existence of such genes in the genome of a plant. It is necessary to have a solid understanding of a few fundamental ideas in genetics in order to comprehend the scientific principles that underlie MAB (Rohilla et al.,

2022). These topics include the structure and function of DNA, the existence of genes, and the connection between genotypes (the genetic makeup of an organism) and phenotypes (the qualities that can be seen). DNA (deoxyribonucleic acid) is the hereditary material that contains the instructions for building and maintaining an organism (Ribaut and Ragot 2007). In plants, as in all living organisms, DNA is organized into chromosomes within the cell nucleus. Each chromosome carries thousands of genes, which are specific sequences of DNA that encode the instructions for producing proteins. These proteins, in turn, play critical roles in determining an organism's traits—such as plant height, fruit colour, disease resistance, or tolerance to environmental stresses. Not all sections of DNA code for proteins (Oliver et al., 2012). Some regions are non-coding but still serve important regulatory or structural functions, and certain DNA sequences are known to vary between individuals of the same species. These variable DNA sequences are called genetic markers. Genetic markers can take many forms, including simple sequence repeats (SSRs), single nucleotide polymorphisms (SNPs), and restriction fragment length polymorphisms (RFLPs), among others (Liu and Muse 2005). In marker-assisted breeding, the key is to identify markers that are closely linked to specific genes of interest. Once such markers are identified, they can be used to screen plants at the seedling stage to determine whether or not they carry the desired genes. This process greatly accelerates the breeding cycle, as breeders do not have to wait for plants to mature and express the target traits (Basin et al., 2014).

For a genetic marker to be useful in marker-assisted breeding, it must be tightly linked to a gene or group of genes that control the trait of interest. In other words, the marker must be located very close to the target gene on the chromosome, so that the two are inherited together during plant reproduction (Yamamoto 2021). The closer the marker is to the gene, the less likely it is that the two will be separated by recombination, the natural process by which genetic material is exchanged between chromosomes during sexual reproduction. When breeders use markers to select plants, they are essentially using the presence of the marker as a proxy for the presence of the gene (Shekhar et al., 2024). For example, if a breeder is trying to develop a disease-resistant tomato variety, they might identify a marker linked to a gene that confers resistance to a particular pathogen. By

screening plants for that marker, the breeder can quickly and accurately identify those that carry the resistance gene, even before the plants are exposed to the pathogen or show any visible symptoms of resistance. The strength of this linkage between the marker and the trait is critical (Zhang 2024). If the marker is too far away from the gene on the chromosome, recombination may separate the two, leading to inaccurate selection. Therefore, identifying tightly linked markers is a key step in the MAB process, and this is typically achieved through detailed genetic mapping and association studies (De Mori and Cipriani 2023).

2.1 Types of Genetic Markers

Genetic markers are essential tools in marker-assisted breeding, allowing breeders to identify specific genes or regions of the genome that are associated with desirable traits. These markers serve as signposts within the genome, making it easier to track the inheritance of genes linked to traits such as disease resistance, drought tolerance, and improved yield (Sagar et al., 2024). The choice of genetic marker depends on the organism being studied, the complexity of the trait, and the available genomic resources. Here are the main types of genetic markers commonly used in plant breeding:

1. Restriction Fragment Length Polymorphisms (RFLPs)

RFLPs were one of the earliest types of molecular markers used in genetic studies. They are based on variations in DNA fragment lengths produced by restriction enzymes, which cut DNA at specific sequences. These enzymes recognize short DNA sequences and cleave the DNA at or near these sites. In different individuals, the DNA sequences at these sites may vary due to mutations, leading to different fragment lengths after digestion (Liu et al., 2024, Mendoza and Loeb 2022). These differences are detected through gel electrophoresis, creating distinct patterns that can be used as markers.

- **Advantages**:

  - Highly reliable and reproducible.
  - Useful in the creation of genetic maps.
- **Disadvantages**:

  - Labour-intensive and time-consuming.

  - Requires large amounts of high-quality DNA.
  - Limited use with high-throughput genotyping technologies.

Application: Although RFLPs have been largely replaced by more efficient marker systems, they were instrumental in the early development of genetic maps in crops like maize, rice, and wheat (Alemu et al., 2024, Smith et al., 2015).

2. Random Amplified Polymorphic DNA (RAPD)

RAPD markers are generated by amplifying random segments of the genome using short, arbitrary primers in a polymerase chain reaction (PCR). This process leads to the amplification of DNA fragments that vary in size between individuals, creating a unique banding pattern. RAPD is a simple, quick, and inexpensive method that requires no prior knowledge of the genome (Alemu et al., 2024).

- **Advantages**:

  - Rapid and cost-effective.
  - No requirement for sequence information.

- **Disadvantages**:

  - Low reproducibility due to the randomness of primer binding.
  - Sensitivity to experimental conditions.
  - Limited to dominant markers, meaning that heterozygous individuals cannot be distinguished from homozygous ones.

Application: RAPD markers have been widely used in genetic diversity studies and population genetics but are less commonly used in marker-assisted breeding due to their limitations in reproducibility (Singh et al., 2020, Saini et al., 2020, Sharma et al., 2021).

3. Amplified Fragment Length Polymorphisms (AFLPs)

AFLPs combine elements of RFLPs and PCR-based markers, providing a more sensitive and versatile approach. AFLP markers are generated by digesting DNA with restriction enzymes, followed by selective amplification of a subset of

these fragments using PCR (Bidyananda et al., 2024). The resulting amplified fragments are separated by size, typically through gel or capillary electrophoresis.

- **Advantages**:

    - High reproducibility.
    - Can generate a large number of polymorphic markers quickly.
    - No prior genomic information is required.

- **Disadvantages**:

    - Technically complex and requires specialized equipment.
    - Dominant marker system, making it difficult to distinguish heterozygotes from homozygotes.

Application: AFLP markers have been used extensively in the development of genetic maps and in the assessment of genetic diversity in crops such as barley, wheat, and grapes (Tyagi et al., 2024).

4. Simple Sequence Repeats (SSRs) or Microsatellites

SSRs, also known as microsatellites, are short, repeating sequences of DNA (usually 2–6 base pairs in length) that are scattered throughout the genome. The number of repeats can vary between individuals, and these variations can be detected using PCR, followed by gel or capillary electrophoresis (Morris et al., Johnsson 2023). SSRs are codominant markers, meaning they can differentiate between homozygous and heterozygous individuals.

- **Advantages**:

    - High level of polymorphism.
    - Codominant inheritance, providing more detailed genetic information.
    - Highly reproducible.

- **Disadvantages**:

    - Development of SSR markers is labour-intensive and expensive, requiring knowledge of the genome sequence.

    - Limited scalability compared to more modern high-throughput techniques.

Application: SSRs have been widely used in genetic mapping, diversity studies, and marker-assisted selection. They have been employed in crops such as rice, maize, and tomatoes to select for traits like disease resistance and fruit quality (Hasanuzzaman 2023).

5. Single Nucleotide Polymorphisms (SNPs)

SNPs are the most abundant type of genetic variation in genomes. They involve single base-pair changes in the DNA sequence between individuals and are distributed throughout the genome. SNPs are codominant markers and can be easily detected using high-throughput genotyping platforms (Kushanov et al., 2021). These markers are particularly suited for large-scale genetic studies and breeding programs due to their abundance and ease of detection.

- **Advantages**:

    - High abundance throughout the genome.
    - High throughput and scalability, enabling rapid genotyping of thousands of SNPs in a single experiment.
    - Codominant, providing detailed genetic information.

- **Disadvantages**:

    - Requires extensive sequence data for marker development.
    - Expensive to set up initially, though costs decrease with high-throughput genotyping.

Application: SNP markers are widely used in modern plant breeding programs, including for genomic selection, genome-wide association studies (GWAS), and marker-assisted selection. In horticultural crops like grapes, apples, and strawberries, SNPs have been used to select for traits such as fruit flavour, disease resistance, and environmental stress tolerance (Sheoran et al., 2022, Sandhu and Kumar 2017, De Mori and Cipriani 2023).

6. Sequence-Related Amplified Polymorphisms (SRAPs)

SRAPs are based on the amplification of open reading frames (ORFs), the coding regions of the

genome. The technique uses primers that target both exons and introns, leading to the amplification of both coding and non-coding regions (Subedi et al., 2023). SRAP markers are particularly useful for detecting polymorphisms in gene-rich regions of the genome, making them highly informative for trait selection.

- **Advantages**:
  - Targets gene-rich regions, making it useful for identifying markers linked to traits of interest.
  - Simple and cost-effective.

- **Disadvantages**:
  - Lower polymorphism rates compared to SSRs and SNPs.
  - Dominant marker system, which limits its ability to distinguish between homozygotes and heterozygotes.

Application: SRAP markers have been successfully used in genetic diversity studies, mapping, and marker-assisted breeding in various horticultural crops, including lettuce, peas, and cucumber (Sahin et al., 2024).

7. Diversity Arrays Technology (DArT)

DArT is a high-throughput marker system that detects polymorphisms at hundreds to thousands of loci in the genome without the need for sequence information. DArT markers are based on hybridization differences between individuals. Genomic DNA is first digested, and a subset of the fragments is amplified (De Mori and Cipriani 2023). These amplified fragments are then hybridized onto a microarray platform, and differences between individuals are detected based on the presence or absence of specific DNA fragments.

- **Advantages**:
  - High-throughput and cost-effective, allowing for genome-wide analysis.
  - Does not require prior genomic knowledge.

- **Disadvantages**:
  - The technology requires specialized equipment and expertise.

  - Resolution may be lower compared to SNP-based technologies.

Application: DArT has been used in various crops, including wheat, barley, and banana, to assess genetic diversity and assist in marker-assisted breeding (Kumar et al., 2024).

8. Cleaved Amplified Polymorphic Sequences (CAPS)

CAPS markers combine PCR amplification with restriction enzyme digestion. Specific regions of the genome are amplified using PCR, and the resulting products are digested with restriction enzymes. Polymorphisms in the restriction enzyme recognition sites lead to differences in fragment sizes, which can be detected by gel electrophoresis (Tyagi et al., 2024, Oyebamiji et al., 2024).

- **Advantages**:
  - Simple and cost-effective.
  - Codominant, providing detailed genetic information.

- **Disadvantages**:
  - Requires prior sequence information to design primers and select appropriate restriction enzymes.
  - Labour-intensive compared to SNP genotyping.

Application: CAPS markers are often used in the fine mapping of genes and in marker-assisted selection for traits such as disease resistance and yield in crops like maize and soybean (Kumar et al., 2024).

3. MARKER-ASSISTED SELECTION (MAS) VS. TRADITIONAL BREEDING

Marker-Assisted Selection (MAS) and traditional breeding are two methodologies employed in plant breeding to develop new crop varieties with desirable traits. While both aim to improve crop performance, they differ significantly in their approach, efficiency, and outcomes (Dormatey et al., 2020). Here's a detailed comparison between MAS and traditional breeding:

3.1 Methodology

Traditional Breeding: Traditional plant breeding relies on selecting plants based on their

observable traits (phenotypes). This process involves crossing plants with desirable traits and then evaluating their offspring to identify those that exhibit the desired characteristics. Selection is typically based on visual assessments or performance in field trials (Zandberg et al., 2022).

- **Process:** Breeders perform controlled crosses between plants, grow the progeny, and evaluate them for traits such as yield, disease resistance, and quality. This process can take several generations and years to achieve desired results (Joshi et al., 2024).
- **Examples:** Traditional breeding methods include selection for higher yield, disease resistance, or improved fruit quality through repeated crossing and selection of the best-performing plants (Mangal et al., 2024).

Marker-Assisted Selection (MAS): MAS involves using molecular markers—specific DNA sequences associated with particular traits—to select plants with the desired genetic traits. Instead of relying solely on observable traits, MAS uses these markers to track the inheritance of genes linked to the traits of interest (Sharma et al., 2024).

- **Process**: Breeders identify genetic markers associated with desired traits, then screen plants at various stages (including seedling stages) to determine whether they carry these markers. This allows for more precise selection and faster breeding cycles.
- **Examples**: MAS is used to select for traits such as disease resistance, drought tolerance, or improved nutritional content based on the presence of specific genetic markers.

3.2 Efficiency and Speed

Traditional Breeding: The traditional breeding process is often time-consuming and labour-intensive. It involves several generations of plant growth and evaluation to identify and select the best performing individuals. Breeding cycles can take many years, especially when dealing with traits influenced by multiple genes or when requiring multiple trait improvements (Anand et al., 2023).

- **Time Frame:** Developing a new variety through traditional breeding can take 10-15 years or more, depending on the crop and trait complexity.
- **Labour and Resources:** Extensive field trials and phenotypic evaluations are required, demanding significant labour and resources (Norman et al., 2009).

Marker-Assisted Selection (MAS): MAS significantly accelerates the breeding process by allowing breeders to identify desirable traits at the molecular level early in the breeding cycle. This can reduce the time required to develop new varieties and increase the efficiency of the breeding program (Bhat et al., 2020).

- **Time Frame:** With MAS, the development of new varieties can be shortened to a few years, as selection is based on genetic information rather than waiting for plants to reach maturity.
- **Labour and Resources:** MAS reduces the need for extensive field evaluations, focusing instead on genotyping and marker analysis (Tiwari et al., 2022).

3.3 Precision and Accuracy

Traditional Breeding: Traditional breeding relies on phenotypic expression, which can be influenced by environmental factors. This makes it challenging to accurately select for traits that are not readily observable or that require multiple genetic factors (Eibeck et al., 2024).

- **Accuracy:** Traditional breeding methods may be less precise when selecting for complex traits that are influenced by multiple genes or environmental conditions.
- **Challenges**: Identifying and selecting for traits like drought tolerance or disease resistance can be difficult if the traits are not easily visible or require specific environmental conditions to express (Sun et al., 2024).

Marker-Assisted Selection (MAS): MAS offers higher precision and accuracy by directly targeting the genes associated with desirable traits. Markers linked to specific genes allow breeders to select plants with the exact genetic makeup needed for the desired traits (Baloch et al., 2023).

- **Accuracy**: MAS provides a more reliable method for selecting traits, as it is based on genetic information rather than observable phenotypes.
- **Advantages**: MAS is particularly useful for selecting traits that are difficult to assess visually, such as resistance to diseases that may not manifest in all environments (Tyagi et al., 2024).

3.4 Application to Complex Traits

Traditional Breeding: Traditional methods can be effective for simple traits controlled by a few genes, but they struggle with complex traits that are influenced by multiple genes or require specific environmental conditions (Hasanuzzaman 2023).

- **Complexity**: For traits like stress tolerance or quality attributes that involve multiple genes, traditional breeding methods can be less effective and slower.

Marker-Assisted Selection (MAS): MAS is well-suited for complex traits, as it allows breeders to target specific genes associated with these traits. By using molecular markers, MAS can efficiently handle traits controlled by multiple genes (Koshariya et al., 2024).

- **Complexity:** MAS can effectively address complex traits such as multi-gene disease resistance or yield improvements by focusing on the underlying genetic factors (Baloch et al., 2023).

3.5 Genetic Diversity and Risk Management

Traditional Breeding: Traditional breeding methods often rely on a relatively small pool of genetic material, which can lead to reduced genetic diversity and potential risks such as susceptibility to new pests or diseases.

- **Genetic Diversity:** Maintaining genetic diversity can be challenging, especially if breeding programs focus on a narrow set of traits or genetic backgrounds (Jannink et al., 2010).

Marker-Assisted Selection (MAS): MAS can help maintain genetic diversity by allowing breeders to select for specific traits without necessarily narrowing the genetic base. It

enables more informed decisions about which plants to cross, thereby preserving broader genetic diversity (Thudi et al., 2014).

- **Genetic Diversity**: MAS helps maintain genetic diversity by allowing for more targeted selections and reducing the risk of genetic bottlenecks (Kamboj et al., 2020).

3.6 Cost and Resource Requirements

Traditional Breeding: Traditional breeding can be resource-intensive, requiring extensive field trials, phenotypic evaluations, and multiple generations of plant growth (Banoth et al., 2023).

- **Costs:** The costs associated with traditional breeding include field trials, labour, and long breeding cycles.

Marker-Assisted Selection (MAS): While MAS requires an initial investment in genetic marker development and genotyping technology, it can ultimately be more cost-effective due to the reduced need for extensive field trials and faster development of new varieties (Nadeem et al., 2018).

- **Costs:** MAS can reduce overall costs by streamlining the selection process and focusing resources on genotyping rather than extensive phenotypic evaluation (Sharma et al., 2008).

4. APPLICATIONS OF MARKER-ASSISTED BREEDING IN HORTICULTURE

Marker-Assisted Breeding (MAB) has made significant strides in horticulture, enabling the development of improved varieties of fruits, vegetables, and ornamental plants. By leveraging genetic markers linked to desirable traits, breeders can accelerate the selection process, enhance trait precision, and address challenges such as disease resistance, yield improvement, and environmental stress tolerance (Koshariya et al., 2024). Here's a detailed overview of how MAB is applied in various horticultural sectors:

4.1 Disease Resistance

Disease resistance is a critical trait in horticulture, as it helps reduce the reliance on

chemical controls and improves crop yield and quality. MAB has been instrumental in developing disease-resistant varieties across a range of horticultural crops (Tiwari et al., 2022, Jannink et al., 2010, Rehman et al., 2023).

- **Tomatoes**: MAB has been used to develop tomato varieties resistant to diseases such as late blight, *Fusarium wilt*, and tomato mosaic virus. Markers linked to resistance genes enable breeders to select plants that are less susceptible to these diseases, resulting in varieties that perform better under field conditions.
- **Cucumbers**: In cucumbers, MAB has been applied to develop varieties resistant to downy mildew and powdery mildew. By using markers associated with resistance genes, breeders can quickly identify and select resistant plants, leading to more durable and high-yielding cucumber varieties.
- **Apples**: Apple breeding programs have utilized MAB to develop varieties resistant to apple scab, a major fungal disease. Markers linked to resistance genes allow for the rapid selection of apple cultivars that can withstand scab infection, reducing the need for fungicide applications.

4.2 Drought and Stress Tolerance

Drought and environmental stress tolerance are increasingly important in horticulture due to changing climate conditions. MAB helps in developing varieties that can withstand water scarcity and other environmental challenges (Thudi et al., 2014, Nadeem et al., 2018, Kamboj et al., 2020).

- **Tomatoes**: MAB has been used to identify and select for drought tolerance in tomatoes. By using markers associated with genes involved in water-use efficiency and stress response, breeders can develop tomato varieties that maintain yield and quality under limited water conditions.
- **Grapevines**: In grapevines, MAB has been applied to improve drought tolerance and cold hardiness. Markers linked to stress tolerance traits allow breeders to select for vines that can thrive in arid regions or withstand

freezing temperatures, ensuring better performance and yield.
- **Peppers**: MAB has been used to enhance heat tolerance in peppers. Markers associated with heat stress tolerance help breeders develop pepper varieties that can maintain fruit set and quality even under high temperatures.

4.3 Yield Improvement

Yield improvement is a primary objective in horticultural breeding, and MAB can accelerate this process by targeting genes that influence yield components (Nadeem et al., 2018, Kamboj et al., 2020).

- **Corn**: Although not a traditional horticultural crop, MAB in corn can serve as a model for similar applications in vegetables. Markers linked to yield-related traits, such as kernel number and size, are used to develop high-yielding corn varieties. This approach can be translated to vegetable crops like sweet corn.
- **Broccoli**: MAB has been used to increase yield in broccoli by selecting for traits such as head size and uniformity. Markers linked to these traits enable breeders to develop varieties with improved productivity.

4.4 Fruit Quality and Ripening

Fruit quality and ripening are crucial factors for consumer acceptance and marketability. MAB can help enhance these traits by selecting for specific genes involved in fruit development and quality (Koshariya et al., 2024, Tiwari et al., 2022, Jannink et al., 2010).

- **Apples**: MAB has been used to improve fruit quality traits such as firmness, flavour, and colour in apples. Markers associated with these traits allow breeders to develop apple varieties with superior taste and appearance.
- **Strawberries**: In strawberries, MAB has been applied to enhance fruit size, sweetness, and shelf life. By selecting for markers linked to these quality traits, breeders can produce strawberries that are more appealing to consumers and have a longer post-harvest life.

- **Citrus**: MAB has been utilized to improve fruit quality in citrus crops by selecting for traits such as juice content and peel thickness. Markers associated with these traits enable the development of citrus varieties with better flavour and processing qualities.

4.5 Resistance to Abiotic Stresses

Abiotic stresses such as salinity, high temperatures, and poor soil conditions can impact crop performance. MAB helps in developing varieties that are resilient to these environmental challenges (Rehman et al., 2023, Thudi et al., 2014, Nadeem et al., 2018).

- **Tomatoes**: MAB has been used to select for salt tolerance in tomatoes. Markers linked to salt tolerance genes enable the development of tomato varieties that can grow and produce fruit in saline soils, expanding their cultivation potential.
- **Onions**: In onions, MAB has been applied to improve tolerance to soil-borne stresses such as low fertility and high pH. By using markers associated with stress tolerance traits, breeders can develop onion varieties that perform better in challenging soil conditions.
- **Lettuce**: MAB has been used to enhance lettuce tolerance to high temperatures. Markers linked to heat tolerance traits help breeders develop lettuce varieties that can withstand heat stress and maintain quality.

4.6 Ornamentals and Aesthetic Traits

In the ornamental sector, MAB helps improve aesthetic traits and other qualities that enhance the value of ornamental plants (Kamboj et al., 2020, Banoth et al., 2023).

- **Roses**: MAB has been applied to develop rose varieties with improved flower colour, fragrance, and disease resistance. Markers associated with these traits enable breeders to create roses with more vibrant colours and better resistance to common diseases like powdery mildew.
- **Orchids**: In orchids, MAB has been used to select for traits such as flower shape, size, and colour. By using markers linked

to these traits, breeders can develop orchids with enhanced visual appeal and market value.
- **Tulips**: MAB has been used to improve flower size and colour in tulips. Markers associated with these traits help breeders develop tulip varieties with more striking and uniform flower characteristics.

5. CHALLENGES AND LIMITATIONS OF MARKER-ASSISTED BREEDING

Marker-Assisted Breeding, often known as MAB, has brought about a revolution in plant breeding by offering a method that is both more accurate and more effective for the development of new crop types. Nevertheless, despite the fact that it has a great deal of benefits, MAB is not devoid of difficulties and constraints. These difficulties have the potential to have an influence on the efficiency of MAB as well as its broad adoption in a variety of breeding operations. In this article, we will examine in further detail the most significant difficulties and constraints that are linked with marker-assisted breeding

5.1 The Requirements for both Cost and Resources

Developing and confirming genetic markers may be a costly endeavour, especially for crops that have limited genomic resources. This is especially true for crops that are genetically modified. During the procedure, sequencing, the finding of markers, and the construction of accurate genotyping techniques are all taken into consideration.

Initial Investment: The expenses involved with establishing up marker development and genotyping infrastructure might be prohibitive, particularly for breeding operations that are either on a small scale or have limited resources.

The high-throughput genotyping and marker analysis processes need the use of specialized equipment and the application of specialist knowledge, which results in continuing operating expenses.

When it comes to the allocation of resources, MAB necessitates a substantial amount of resources for both the creation of markers and their use in breeding operations.

Labour and Expertise: In order to successfully implement MAB, professional staff and laboratory resources are required. However, it is possible that not all breeding programs have access to these resources (Rafalski 2002).

5.2 The Availability of Markers is Restricted

Availability of valid Genetic Markers for Certain characteristics It is possible that valid genetic markers are not accessible for a number of characteristics, particularly those that are regulated by several genes or those that are not well understood. The use of MAB may be difficult because complex traits, such as yield or multi-gene disease resistance, sometimes lack well-defined markers. This makes it difficult to apply MAB successfully.

Obstacles that are specific to the crop: It may be challenging to locate appropriate markers for some crops, particularly those with genomes that have been poorly sequenced or with minimal levels of genetic information. When it comes to discovering and verifying markers for desirable features, crops that have a smaller amount of genetic resources may have a more difficult time (Turan et al., 2012).

5.3 Marker-Trait Association

Accuracy of Associations between Markers and Traits: Because of the strength and consistency of the relationship between the marker and the trait, the reliability of MAB is dependent on these two factors. In some instances, the relationships between markers and traits may not be very robust or constant across a variety of situations.

Environmental variables: Environmental variables have the potential to impact the expression of characteristics, which in turn may have an effect on the reliability of the markers that are responsible for predicting those traits. In some instances, markers may be related to characteristics via linkage disequilibrium rather than a direct correlation with the trait itself. This is because linkage disequilibrium is different from a direct association. Because of this, it is possible for trait prediction to be inaccurate (Kaushal et al., 2020).

5.4 The Ambiguity of Genetic Characteristics

Numerous key characteristics, such as yield or stress tolerance, are regulated by a number of genes, which are referred to as polygenic features. As a result of the complexity of the underlying genetics, it might be difficult to recognize and make use of markers for polygenic characteristics. Gene Interactions: The application of markers for polygenic characteristics may be made more difficult by the interactions that occur between several genes and environmental variables. Gene Interactions and Epistasis: The interaction between distinct genes, known as epistasis, may have an effect on the expression of traits, which has the potential to make the identification and application of markers more difficult (De Mori and Cipriani 2023).

5.5 Adaptation and the Diversity of Genetic Origins

Reduced Genetic variety an over dependence on marker-based selection might result in a reduction in the genetic variety that is present within breeding operations. It is possible that this will make the individual more susceptible to infections, pests, and shifting environmental circumstances. There is a risk of genetic bottlenecks occurring when there is an excessive concentration on certain markers or characteristics. This may result in a reduction in the total genetic diversity of crop types. Adaptation to Local situations: It is possible that MAB may not always take into consideration local adaptation and environmental uniqueness, both of which can be significant for crop performance in a variety of growing situations (Tyagi et al., 2024).

6. IMPLEMENTATION AND ACCEPTANCE OF THE CHANGE

The implementation of MAB faces a number of technical challenges, including the need for specific equipment and technical skills. There is a possibility that breeding programs who do not have access to these resources may have difficulty adopting MAB.

Training and Infrastructure: In order to successfully execute MAB, it is required to have enough training and infrastructure, both of which may be missing in some locations or institutions. Some breeders and farmers may be hesitant to embracing new technologies, such as MAB, owing to their experience with molecular methods or their adherence to old procedures. Additionally, some breeders and farmers may be resistant to change (Sharma et al., 2021).

7. CONSIDERATIONS REGARDING ETHICAL AND REGULATORY ISSUES

Challenges posed by Regulations the use of molecular markers and genetically chosen plants may be subject to regulatory scrutiny, which may have an effect on the rate at which new kinds are released and the ease with which they are released.

Compliance: The process of navigating the regulatory requirements for marker-assisted varieties may be difficult and time consuming. There may be ethical considerations associated with the alteration of genetic material and the use of molecular markers, both of which have the potential to affect how the general public views and accepts the technology (Mendoza and Loeb 2022).

8. FUTURE PROSPECTS OF MARKER-ASSISTED BREEDING

In spite of the fact that Marker-Assisted Breeding (MAB) has achieved significant progress in the field of plant breeding, its potential is still enormous and constantly expanding. The future of MAB contains tremendous chances that might further alter agriculture. These prospects are only going to increase as technology continues to advance and more information is obtained. A comprehensive analysis of the possibilities for the future of MAB is as follows:

8.1 Developments in technology Related to Genomics

In the field of high-throughput genotyping, it is anticipated that the development of technologies that allow for high-throughput genotyping would considerably improve the effectiveness of MAB. Recent developments in sequencing technology, such as next-generation sequencing (NGS), may ultimately make it possible to conduct genetic analysis that is both more thorough and extensive at a lower cost. Genomic Selection: As a result of the combination of high-throughput genotyping with genomic selection, breeders will be able to make more accurate predictions about the performance of breeding lines, especially for complex characteristics that are regulated by several genes. CRISPR and Gene Editing: The combination of MAB with CRISPR and other gene-editing technologies will make it possible to make precise adjustments to certain genes, which will speed up the process of developing new kinds with specified characteristics.

Precision Breeding: Gene editing will be a useful addition to MAB since it will make it possible to precisely modify genes that are related with desired characteristics. This will result in breeding results that are far more refined and efficient (Oliver and Li 2012).

8.2 Improved Methods for Locating Markers

Improved Marker Technologies: In the future, there will be breakthroughs in marker technologies that will make it easier to identify and apply new markers that are connected with a larger variety of characteristics. The development of molecular markers such as single nucleotide polymorphisms (SNPs), insertion-deletion polymorphisms (INDELs), and simple sequence repeats (SSRs) is included in this category.

Trait Mapping: Developments in marker technologies will make it possible to conduct more extensive trait mapping, which will make it possible to identify markers for qualities that are now difficult to target.

Functional Genomics: The methodologies that are used in functional genomics will be helpful in understanding the functions that certain genes play and the connections that they have with one another, which will ultimately lead to the creation of more accurate markers for complex characteristics (Bidyananda et al., 2024).

8.3 Combining Phenotyping with Integration

Enhanced Capability to Correlate Genetic Markers with Phenotypic features The incorporation of MAB with sophisticated phenotyping technologies, such as remote sensing, drones, and imaging systems, will result in an improvement in the capability to correlate genetic markers with phenotypic features. Phenotyping with Precision: The development of more advanced phenotyping methods will result in the collection of more precise data on the expression of traits, which will enable improved validation and application of genetic markers.

Big data and artificial intelligence: The use of big data and artificial intelligence (AI) in plant breeding will make it possible to conduct an analysis of enormous datasets in order to determine connections between genetic markers and phenotypes. This will make it possible to

conduct MAB that is both more accurate and more efficient.

Artificial intelligence and machine learning algorithms will improve the analysis of genetic and phenotypic data, which will optimize marker selection and breeding choices. This will be referred to as data-driven breeding (Murray and Thompson 1980).

8.4 Increased Number of Trait Targets

Complex Trait Improvement: In the future, improvements in MAB will make it possible to target more complex characteristics, such as yield stability, adaptability to several environments, and nutritional quality.

Multi-Trait Breeding: Recent developments in marker technology will make it possible to select numerous traits simultaneously, which will result in the creation of crop varieties that have enhanced overall performance.

Abiotic Stress Tolerance: Through the use of MAB, there will be a greater emphasis placed on the enhancement of crop tolerance to a variety of abiotic stressors, including salt, drought, and temperature extremes.

When it comes to climate resilience, MAB will play a significant part in the development of crops that are able to endure the difficulties that are presented by climate change. This will ensure both food security and agricultural sustainability (Rafalski 2002).

8.5 Access to and Collaboration on a Global Scale

International Cooperation: The exchange of genetic resources, markers, and information will be made easier by increased international cooperation between breeding programs and research institutes from different countries.

In order to improve the accessibility and implementation of MAB across a variety of areas and crops, worldwide databases will be established. These databases will include information on genetic markers and traits.

Construction of Capacity: Efforts to construct capacity and infrastructure in developing nations will make it possible for MAB to be adopted by a wider audience and will help to the growth of agriculture on a global scale.

Transfer of Knowledge: The application of MAB in locations that have limited access to sophisticated breeding technology will be supported by training programs and the exchange of resources (De Mori and Cipriani 2023).

8.6 Sustainability and Ethical Considerations are the Sixth Point

Sustainable Breeding Practices: In the future, MAB techniques will have an emphasis on sustainability. This will include the creation of varieties that use less inputs, such as water and fertilizers, and have a smaller effect on the environment. MAB will contribute to the creation of crops that are more resource-efficient and ecologically friendly. This will be accomplished via the use of natural resources.

Ethical and Regulatory Frameworks: The construction of ethical and regulatory frameworks will guarantee the appropriate use of MAB technologies and address problems connected to genetic modification and biodiversity. These frameworks will also promote the maintenance of biodiversity. Acceptance by the Public Being able to engage with stakeholders and the general public in order to resolve ethical issues and improve acceptance of MAB will be essential for the organization's continuing success (Zhang 2024).

8.7 Breeding that is Both Individualized and Regionalized

Customized Breeding Solutions: Developments in MAB will make it possible to come up with more individualized and regionally specialized breeding solutions, which will cater to the particular requirements and preferences of various communities and different places. In order to assist the creation of crop varieties that are better matched to the environmental circumstances and agricultural methods of the local area, MAB will be implemented. In order to satisfy the requirements of the market, breeding programs will devote a growing amount of attention to the preferences of consumers. These preferences will include characteristics like as flavour, appearance, and nutritional content (Lander and Botstein 1989).

9. CONCLUSION

Marker-assisted breeding has revolutionized horticultural crop development by enabling the

precise and efficient selection of traits at the genetic level. Its application in disease resistance, stress tolerance, yield improvement, and aesthetic quality has already made a significant impact on the horticulture industry. While challenges remain, advancements in genomics, gene editing, and breeding technologies promise to further enhance the effectiveness of MAB in the future. As the demand for sustainable, high-quality crops continues to grow, MAB will play an increasingly vital role in meeting these needs and addressing the challenges of global agriculture.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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