Enhancing Crop Resilience: Advances and Challenges in Marker-Assisted Selection for Disease Resistance

Aniket Ramdas Mapari [a*] and Suhel Mehandi [a++]

[a] *Department of Genetics and Plant Breeding, School of Agriculture, Lovely Professional University, Phagwara, Punjab-144002, India.*

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Review Article

ABSTRACT

Marker-assisted selection (MAS) has emerged as a pivotal technique in crop improvement programs, enabling the identification and incorporation of disease-resistant traits in crops. This review highlights the significance of MAS in enhancing disease resistance, the current state of research, and the challenges faced in its implementation. Various case studies, including successful applications in crops such as rice, wheat, and maize, demonstrate the potential of MAS in sustainable agriculture. The review also discusses the integration of MAS with other modern breeding techniques and emphasizes the need for advanced genomic tools for future advancements in this field. The development of advanced molecular markers, high-throughput screening techniques, and the incorporation of genomic selection approaches have all contributed to recent developments in MAS. Even in intricate polygenic situations, these developments have significantly improved t+he precision and speed of discovering disease-resistant characteristics.

[++] *Assistant Professor;*
Corresponding author: E-mail: aniketmapari03@gmail.com;

Case studies involving important crops such as maize, wheat, and rice show how MAS can be successfully applied to create varieties with improved resistance to certain diseases, significantly lowering crop losses and reduced use of pesticides.

These developments have consequences that go beyond the purely technical; they could have a big influence on environmentally friendly agriculture methods, cost effectiveness, and sustainable development. This study addresses the possible future path of MAS in crop breeding and summarizes the major discoveries from current research, highlighting both the successes and difficulties in the field. The body of data highlights MAS as a critical instrument in the toolbox of the contemporary breeder, essential for satisfying the ever-increasing demands of a world that is changing quickly.

1. INTRODUCTION

Plant diseases provide a persistent threat to food security for the world's expanding population, as they can severely damage crop production and quality. Thus, cultivating crops with disease resistance becomes essential to achieving food security and agricultural sustainability [1]. Using conventional breeding techniques, plant breeders have historically concentrated on creating crop types with innate resistance to diseases [2-4]. These techniques usually entail choosing parent plants with desired characteristics, crossing them, and then choosing the best progeny over a number of generations [5]. Even if it works, traditional breeding is frequently a labour- and resource-intensive procedure with limited accuracy and efficiency.

Different molecular markers introduced in breeding programs since 1980 are AFLP, RFLP, and SSR markers [6]. The field of crop breeding has entered a new age with the development of molecular biology and biotechnology. The production of disease-resistant crop varieties has been greatly hastened by the innovative method known as marker-Assisted Selection (MAS) (Collard & Mackill, 2008). Molecular markers, or DNA sequences with a known position on a chromosome, are used in MAS and are linked to desirable qualities like disease resistance. Breeders can use this technique to significantly shorten the breeding cycle and improve selection precision by identifying and choosing plants that exhibit the required features as early as the seedling or even the seed stage [7].

However, it soon became apparent that applying knowledge gained through molecular mapping in real world breeding might not be entirely straightforward. The traits whose underlying genes could easily be tagged with reliable DNA markers – simple, qualitative phenotypes with high heritability – were often the traits that benefited least from MAS technology [8]. more stable and broad-spectrum resistance can be
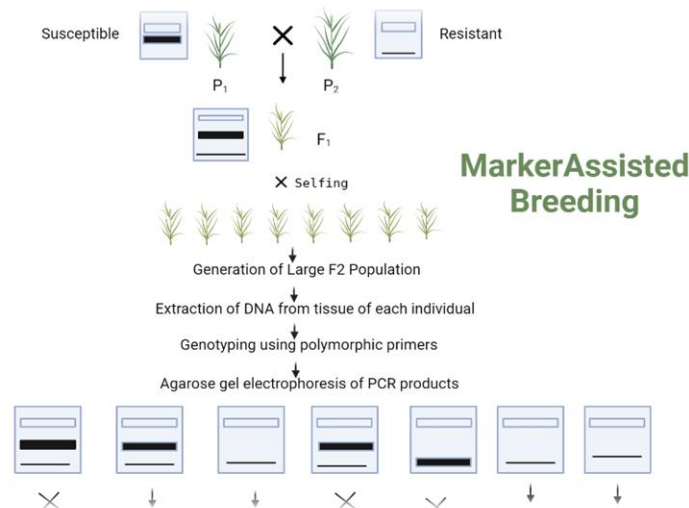

Fig. 1. The figure explains the basic procedure of marker-assisted selection

achieved by breeding varieties resistance for minor quantitative genes. Genomic selection is most convenient approach in molecular plant breeding for developing plant varieties resistant to quantitative disease resistance. Kumari et al.,[9].

The time constraint issue with conventional breeding was resolved by molecular marker-assisted breeding. The efficient and effective use of molecular markers in crop improvement programs increases the effectiveness of selection accuracy and speeds up the breeding cycle to create new cultivars with desirable traits [9].

With a special emphasis on how marker-assisted selection is being used to help crops become resistant to disease, this review attempts to offer a thorough examination of the most recent developments in this field. It will go through the development of MAS, from its conception to the most recent developments in technology and methodology. Along with a discussion of the difficulties and opportunities facing MAS in crop breeding going forward, a critical analysis of case studies demonstrating the effective application of MAS in important crops should be given. This review aims to provide a thorough overview of the state and promise of MAS in boosting disease resistance, which is a crucial factor in determining the viability of sustainable agriculture and the security of the world's food supply.

2. FUNDAMENTALS OF MARKER-ASSISTED SELECTION

Marker-Assisted Selection (MAS) is a process in plant breeding that uses molecular markers to select for desirable traits in plants, such as disease resistance, without relying solely on visible traits. This technique is rooted in the principles of genetics and molecular biology, integrating traditional breeding approaches with modern biotechnological tools [10]. Markers can be used in targeted introgression of desirable traits from diverse sources, as well as the integration of genotypic and phenotypic data for association mapping, genomic prediction, and selection [11].

2.1 Definition and Principles of MAS

MAS involves the identification and use of DNA sequences, known as markers, which are closely linked to genes of interest. These markers serve as proxies for the traits being selected. The principle behind MAS is that the presence of a marker in a plant's genome can reliably predict the presence of a desired trait, thereby enabling breeders to select for these traits more efficiently and accurately than through phenotypic selection alone [12].

2.2 Types of Markers Used

2.2.1 Simple Sequence Repeats (SSRs)

Also known as microsatellites, SSRs are repeating sequences of 2-6 base pairs of DNA. They are highly polymorphic, making them useful for distinguishing between different genetic variants (Gupta et al., 1996).

2.2.2 Single Nucleotide Polymorphisms (SNPs)

SNPs are the most common type of genetic variation in the genome, involving a change in a single nucleotide. They are abundant and evenly distributed throughout the genome, providing high-resolution genetic information [13].

2.2.3 Others

Besides SSRs and SNPs, other markers like Amplified Fragment Length Polymorphisms (AFLPs) and Sequence Tagged Sites (STSs) have also been used in MAS, though with less frequency due to advancements favouring SSRs and SNPs for their higher efficiency and accuracy.

2.3 Overview of Genetic Mapping

Genetic mapping is a key component in MAS. It involves creating a genome map of a species that locates specific genes and markers. This map is analogous to a roadmap, guiding breeders to the exact location of genes responsible for desired traits [14]. The process begins with the identification of polymorphic markers across the genome. These markers are then associated with phenotypic traits through linkage analysis, typically performed in breeding populations or mapping populations specially designed for this purpose [15].

2.4 Advantages over Conventional Breeding Methods

2.4.1 Enhanced efficiency

By enabling the early selection of desired traits, frequently before the plant reaches maturity, MAS greatly speeds up the breeding process. This speeds up the creation of new kinds and shortens the breeding cycle (Hospital, 2009).

Table 1. Comparison of different DNA markers

Feature and Description	RFLP	RAPD	AFLP	SSR	SNP
Genomic abundance	High	High	High	Moderate to high	Very high
Genomic coverage	Low copy coding region	Whole genome	Whole genome	Whole genome	Whole genome
Expression/inheritance	Co-dominant	Dominant	Dominant/co-dominant	Co-dominant	Co-dominant
Number of loci	Small (< 1000)	Small (< 1000)	Moderate (1000s)	High (1000s–10,000s)	Very high (> 100,000)
Level of polymorphism	Moderate	High	High	High	High
Type of polymorphism	Single base change, indel	Single base change, indel	Single base change, indel	Changes in length repeat	Single base change, indel
Cloning and/or sequencing	Yes	No	No	Yes	Yes
Type of probes/primers	Low-copy DNA or cDNA clones	10 bs random nucleotides	Specific sequence	Specific sequence	Allele-specific PCR primer
PCR-based	Usually no	Yes	Yes	Yes	Yes
Radioactive detection	Usually yes	No	Yes or no	Usually no	No
Reproducibility/reliability	High	Low	High	High	High
Amount of DNA required	Large (5–50 µg)	Small (0.01–0.1 µg)	Moderate (0.5–1.0 µg)	Small (0.05–0.12 µg)	Small (> 0.05 µg)
Genotyping throughput	Low	Low	High	High	High
Cost	Moderate to high	Low	Moderate	Moderate to high	High
Marker index	Low	Moderate	Moderate	Moderate to high	Moderate
Time demanding	High	Low	Moderate	Low	Low
Number of polymorphic per loci	1.0–3.0	1.5–5.0	20.100	1.0–3.0	1.0
Primary application	Genetic	Diversity	Diversity and genetic	All purposes	All purposes

2.4.2 Precision

By using DNA-based markers, many problems associated with the phenotypic evaluation of traits can be avoided in breeding programmes. DNA markers are especially useful in selecting for polygenic traits that prove difficult to select based on phenotypic assessment alone. S. Rajapakse, Elsevier [16].

2.4.3 Cost-effectiveness

MAS may prove to be more economical in the long term, despite the very high initial cost of molecular markers and equipment. According to Morris et al. [17], it reduces the requirement for extensive field experiments and the expenses related to keeping these trials going across multiple growing seasons.

2.4.4 Encouraging the selection of complex qualities

Multiple inheritance systems (MAS) are especially useful for qualities that are hard to quantify, including late-onset traits, or traits that are influenced by several genes, like disease resistance or drought tolerance (Young, 1999).

2.4.5 Biofortification

MAS is helpful in the development of crop varieties with improved nutritional quality, which is a difficult challenge to accomplish by traditional breeding alone [18].

In conclusion, MAS has developed into a highly valuable instrument in contemporary plant breeding due to its exceptional accuracy, effectiveness, and economy. By combining their capabilities, its integration with conventional breeding techniques constitutes a synergistic strategy that speeds up the production of superior crop types.

3. HISTORICAL PERSPECTIVE

The story of scientific progress in crop breeding is reflected in the slow development of methods and procedures that have defined the history of Marker-Assisted Selection (MAS). From the earliest MAS uses to the present, there have been many important turning points along the way, particularly with regard to improving crop resistance to disease.

3.1 Initial Use of MAS in Crop Breeding

With the introduction of DNA markers and advancements in molecular biology, the idea of MAS started to take shape in the 1980s. The early uses were limited and cantered mostly on important genes that significantly impacted phenotypes. Using markers to select for resistance against diseases like Fusarium and Verticillium wilts, tomato breeding was one of the first effective applications of MAS [14]. This early accomplishment showed how MAS may be used to benefit crops, especially in terms of disease resistance.

3.2 The Development of Methods and Techniques

The variety of markers used for MAS rapidly expanded in the early 1990s. Restriction Fragment Length Polymorphisms (RFLPs) were the most common at first, but more effective markers like SSRs and, subsequently, SNPs gradually displaced them Rafalski, [13]. High-throughput genotyping technology advancements and falling DNA sequencing costs have further helps in the adoption of MAS in breeding operations.

The knowledge of plant genomes increased along with these advancements. An important milestone was reached in 2000 with the completion of the Arabidopsis thaliana genome sequence, which helped in marker identification and served as a reference for understanding plant genetics (The Arabidopsis Genome Initiative, 2000). This information, along with sophisticated computer programs, made linkage mapping and marker identification more efficient.

3.3 Important Markers in MAS for Disease Resistance

A significant advancement in the use of MAS for disease resistance was the creation of rice cultivars resistant to bacterial blight. This demonstrated the method potential in disease fighting, as it was accomplished by finding and introgression the Xa21 gene from wild rice using MAS [19]. After that, MAS was effectively used to create crop variety resistant to several diseases, including late blight resistance in potatoes and rust resistance in wheat. Improvement of most agronomic traits that are of complicated inheritance and economic importance like yield and quality is still a great challenge for MAS including the newly developed GS [20].

The evolution of MAS over time is indicative of an ongoing refining of methods and procedures, propelled by developments in genomics, bioinformatics, and molecular biology. Thanks to these developments, MAS has evolved from a simple trait-tracking tool to a sophisticated method that can handle intricate polygenic disorders. MAS is proof of the effectiveness of fusing science and technology in agriculture as it develops further.

4. RECENT ADVANCEMENTS IN MAS FOR DISEASE RESISTANCE

Significant progress has been made in Marker-Assisted Selection (MAS) for agricultural disease resistance within the last ten years. These advancements, which include high-throughput screening methods, integration with genomic selection, sophisticated molecular markers, and bioinformatics, have significantly improved the effectiveness and reach of MAS.

4.1 High-throughput Screening Methods and Molecular Markers of the Future

Increasingly advanced molecular markers, such Single Nucleotide Polymorphisms (SNPs), which provide higher precision and information density, have become increasingly popular in recent years [21]. The development of next-generation sequencing (NGS) technologies and SNP arrays has made it possible to genotype a large number of SNPs across the genome quickly and affordably, allowing for a more thorough analysis of genetic diversity.

Together with these developments, high-throughput screening methods have been developed. Marker analysis has become much more efficient and less expensive because to technologies like automated DNA extraction and liquid handling systems combined with sophisticated genotyping platforms [22].

4.2 The Integration of Genomic Selection with MAS

A paradigm shift in MAS is represented by genomic selection (GS), which focuses on individual selection according to genomic estimated breeding values (GEBVs) determined from genome-wide marker data. This method works especially well for complicated variables like yield and drought tolerance that are regulated by several genes [23]. Breeders can now select for both major gene-mediated resistance and quantitative trait loci (QTL) that contribute to polygenic resistance by combining the advantages of both methods thanks to the integration of GS and MAS.

4.3 Case Studies Illustrating Effective Application of Marker assisted selection in Diverse Crops

4.3.1 Rice

MAS has been successfully applied to rice to create cultivars resistant to bacterial blight and blast. One significant achievement of MAS is the introduction of the Pi9 gene, which confers resistance to several strains of blast, into well-known rice varieties [24]. The integration of CRISPR/Cas9 technology with traditional breeding methods and other advanced biotechnological tools may further enhance the development of stress-resilient rice varieties with improved productivity and sustainability [25].

4.3.2 Wheat

MAS has been effectively employed by wheat breeding efforts to create wheat types resistant to rust infections. MAS has been successfully used to introgress the Yr15 gene, which provides resistance to stripe rust, into wheat, demonstrating the technique's potential in fighting this important disease [26]. Different abiotic stresses like heat stress tolerance in wheat breeding programs and embracing advancements in molecular genetics and phenotyping technologies for secure and sustainable future of wheat production worldwide [27].

4.3.3 Maize

The presence of MAS in maize has improved the crop resistance to disease like Northern Corn Leaf Blight. The production of maize hybrids with improved disease resistance has been made possible using markers connected to resistance genes, which has improved yield and stability [28].

4.4 Advancements in Data Analysis Tools and Bioinformatics

Advanced MAS techniques have resulted in an explosion of genetic data, which requires complex bioinformatics tools for data

management and analysis. The identification of markers linked to disease resistance, the analysis of massive genomic data, and the prediction of breeding values have all benefited greatly from the development of specialized software and algorithms. In the analysis of genetic association studies and the management of SNP data, programs like TASSEL and PLINK have become industry standards [29].

Finally, the capacities and efficacy of crop breeding programs have been significantly increased by the recent developments in MAS for disease resistance, which have been increased by technological breakthroughs in molecular markers, high-throughput screening, genomic selection, and bioinformatics. These advancements offer new ways to guarantee food security in the face of escalating environmental difficulties by accelerating the breeding process and improving the precision with which disease-resistant cultivars are generated.

5. CHALLENGES AND LIMITATIONS

Marker-Assisted Selection (MAS) has revolutionized crop breeding, but it is having certain challenges and limitations. These include logistical and financial obstacles, particularly in developing nations, as well as limitations on genetic diversity and marker availability in addition to technological and practical concerns.

5.1 Practical and Technical Difficulties in MAS

5.1.1 Complexity of polygenic variables

Although MAS is very successful in controlling variables governed by single genes, it is more difficult to apply to complex, polygenic traits. More complex methods, such as genomic selection, which can be labour-and resource-intensive, are needed for traits regulated by several genes and those impacted by environmental influences [30].

5.1.2 Linkage drag

In multiple inheritance systems, favourable genes are frequently associated with unfavourable genes (linkage drag), which may unintentionally be selected during mating. This is especially important when transferring genes from wild relatives because the target gene may also carry multiple undesired features [12].

5.1.3 Precise marker-trait correlations

The foundation of Marker-Assisted Selection (MAS) is the establishment of trustworthy marker-trait correlations. However, errors may occur as a result of many genes influence or recombination between the trait locus and the marker, which would result in less efficient selection [7].

5.2 Genetic Diversity and Marker Availability Restrictions

5.2.1 Decreased genetic diversity

In MAS, the use of select breeding lines and a small number of markers may result in a decrease in genetic diversity. The long-term viability and adaptation of crop types may be hampered by this decline in variety, particularly in light of shifting environmental conditions [31].

5.2.2 Marker availability

There are sometimes insufficient markers associated with significant features, particularly for less researched crops. Research and genetic mapping expenditures necessary for the development of these markers can be prohibitive, especially for orphan crops [32].

5.3 Barriers to Trade and Logistics in Developing Nations

5.3.1 Expensive costs

The initial MAS setup might be unaffordable, especially when it comes to the equipment and technology needed for genotyping. For underdeveloped nations, where funding for these kinds of initiatives may be scarce, this is especially problematic [33].

5.3.2 Technical proficiency

Molecular genetics, genomics, and bioinformatics training and experience are prerequisites for MAS. The execution of MAS projects may be hampered in developing nations by a lack of experts workers in these fields [34].

5.3.3 Infrastructure and accessibility

In addition to being expensive, underdeveloped nations frequently lack the laboratory and computer resources necessary for performing MAS. The unavailability of the recent

technologies and knowledge might also be a major obstacle [35].

In conclusion, even though MAS has a lot of potential for crop breeding in the future, its greater acceptance and efficacy depend on resolving these issues and constraints. To fully realize the potential of MAS, efforts must be made to create high-throughput genotyping tools that are affordable, to increase the genetic base utilized in breeding programs, and to strengthen infrastructure and capability, particularly in developing nations.

6. IMPACT AND IMPLICATIONS

Crop breeding that incorporates Marker-Assisted Selection (MAS) has significant effects on global agricultural practices and policies, food security, production, quality, and sustainable agriculture.

6.1 Contribution to Food Security and Sustainable Agriculture

By facilitating the creation of crop varieties that are more resistant to pests and diseases and, consequently, less dependent on chemical pesticides, MAS makes a substantial contribution to sustainable agriculture [36]. This is consistent with the ideas of sustainable agriculture, which place a strong emphasis on preserving productivity while reducing negative effects on the environment. Additionally, by accelerating the growth of crops that are resilient to a range of biotic and abiotic stresses, MAS improves food security by guaranteeing steady and higher productivity in a variety of dynamic environmental settings [37].

6.2 Effects on Crop Quality and Yield

6.2.1 Enhanced yield

Increasing crop yield is one of Marker-Assisted Selection main objectives. MAS aids in the development of cultivars that can sustain high levels of yield even in challenging circumstances by selecting for characteristics like stress tolerance and disease resistance (Liu et al., 2013). For instance, the production of rice varieties with noticeably greater yields than traditional varieties has resulted from the application of MAS in disease resistance breeding [38].

6.2.2 Better quality

MAS is essential in raising the standard of agricultural products. This entails boosting physical characteristics like grain size and appearance, which are crucial for market acceptability, as well as nutritional properties like biofortified crops with more vitamins and minerals [39].

6.3 Consequences for International Agriculture Policies and Practices

6.3.1 Adoption of precision agriculture

Using cutting-edge technology to maximize field-level management with regard to crop farming is the foundation of precision agriculture, of which MAS is a crucial component. MAS promotes a more focused approach to crop development and management by offering more precise and effective breeding techniques (Fischer et al., 2009).

6.3.2 Policy development

To be successful, MAS needs policies that are in Favor of it, especially those that deal with funding for research, intellectual property rights, and, where relevant, genetically modified organism (GMO) regulations. To promote the benefits of MAS while resolving ethical and safety concerns, governments and international entities must take into account the consequences of MAS in their agricultural policy [40].

6.3.3 Capacity building and technology transfer

Capacity building and technology transfer are crucial for expanding essential for MAS to have a worldwide influence, particularly in developing nations. The technological divide in MAS applications can be closed with the support of policies that encourage technology transfer and cooperation between developed and underdeveloped countries [35].

In conclusion, there are wide-ranging effects and ramifications of MAS in crop breeding. MAS is essential in tackling some of the most important issues facing modern agriculture, including improving crop production and quality, strengthening food security, and supporting sustainable agriculture practices. However, a mix of advancements in science, favourable legislation, and international cooperation are necessary for its successful implementation and widespread adoption.

7. FUTURE DIRECTIONS AND POTENTIAL

In crop breeding, Marker-Assisted Selection (MAS) will be entwined with new technologies, extending its reach to include a greater variety of crops and disease, combining with other breeding strategies, and negotiating moral and legal dilemmas.

7.1 New Technologies: Their Prospects

7.1.1 CRISPR-Cas frameworks

Crop breeding now offers more options thanks to the development of CRISPR-Cas genome editing. In contrast to conventional MAS, which depends on genetic diversity that occurs naturally, CRISPR has the ability to deliberately cause desired mutations. With the use of this technique, disease resistance traits that are absent from the gene pool could be produced, providing MAS with a novel supplement (Scheben et al., 2017).

7.1.2 Artificial Intelligence (AI) in MAS

In order to handle the enormous volume of genomic data produced by MAS, AI and machine learning are becoming essential. The effectiveness of MAS systems can be increased by AI algorithms' ability to forecast breeding combination performance and optimize selection procedures [41].

7.2 MAS Extension to a Greater Variety of Crops and Disease

Historically, important crops like rice, maize, and wheat have been the main recipients of MAS applications. Expanding its use to underutilized or "orphan" crops, which are essential for food security in many regions of the world, is a growing trend, nevertheless. Likewise, the range of disease that MAS treats is expanding [42]. Major, well-known diseases are becoming less of a focus due to newly emerging, regionally specialized diseases, which can have disastrous effects on regional agriculture (Varshney et al., 2012).

7.3 Integration with Additional Breeding Techniques and Tools

7.3.1 Combining MAS with conventional breeding

An integrated strategy is being used to take advantage of the benefits of both MAS and traditional breeding. Using conventional breeding to achieve polygenic characteristics, this hybrid approach enables the effective selection of traits controlled by major genes through MAS [17].

7.3.2 Genetic Differencing (GS)

Especially promising is the MAS/GS connection. Although MAS works well for qualities that are regulated by a single gene, GS provides a comprehensive breeding strategy by predicting the performance of complex traits driven by numerous genes [33].

In conclusion, new technological developments and growing applications will characterize the future of MAS in crop breeding. A new era in crop improvement is marked by the application of MAS to a wider range of crops and diseases, its integration with other breeding strategies, and the emergence of technologies such as AI and CRISPR. To fully utilize these developments for global agriculture, it will be necessary to navigate the related ethical and legal environments [43,44].

8. CONCLUSION

The field of crop breeding has seen tremendous change in recent decades due to the invention of Marker-Assisted Selection (MAS), which has ushered in a new era of efficiency and precision in the production of agriculturally significant traits, most notably disease resistance. The breadth and efficacy of MAS in crop breeding have been expanded by the important advancements in molecular markers, high-throughput screening, integrating MAS with genomic selection, and the creation of state-of-the-art bioinformatics tools that have been emphasized in this review.

These developments are significant because they help solve some of the most important issues facing agriculture today, including as increasing crop yields, strengthening disease resistance, and guaranteeing food security in the face of a changing climate and an expanding global population. Breeders can now select for desirable traits more quickly and accurately thanks to the use of high-throughput genotyping technologies and the switch to more complex genetic markers like SNPs. Similar to this, a more thorough approach to breeding—especially for complex, polygenic traits—has been made possible by the combination of MAS with genomic selection and the application of cutting-edge computational methods.

Future prospects for MAS in crop breeding appear promising and full of possibilities. New technologies that promise to further transform MAS include CRISPR gene editing and artificial intelligence (AI) in genomic analysis. These technologies will make MAS more accurate, efficient, and applicable to a wider range of crops and traits. There are intriguing opportunities to increase agricultural resilience and variety through the application of MAS to underutilized crops and its integration with other breeding techniques.

But in order to fully realize the promise of MAS, it will be necessary to overcome obstacles including the need for more technology breakthroughs, deal with ethical and legal issues, and provide fair access to these technologies, especially in developing nations. The importance of MAS for ensuring food security around the world cannot be overstated, and its continuous development and use will be essential to the creation of resilient, productive, and sustainable agricultural systems everywhere.

In summary, MAS is a key component of contemporary crop breeding, and its developments provide hope and answers to some of the most pressing problems facing agriculture around the world. Its future, entwined with appropriate application and technological innovation, is expected to be crucial in creating a world that is more food secure.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc have been used during writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology.

Details of the AI usage are given below:

1. Quilbot: Used for summarization during abstract writing.
2. ChatGPT: Used for changing the structure of sentences.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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