

# Sustainable Potato Production through MAS and Late Blight Resistance

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**Abstract-** Late blight, caused by *Phytophthora infestans*, continues to pose a significant threat to potato production globally. While traditional breeding methods have been used to create resistant cultivars, these methods can be slow and often face limitations due to the availability of genetic resources. Marker-assisted selection (MAS) provides a more efficient and accurate approach by using molecular markers to identify plants that possess resistance genes. This review offers a thorough overview of MAS for late blight resistance in potatoes, discussing its historical development, genetic foundations, molecular markers, and the steps involved in its application. Key topics include the identification of resistance genes and their corresponding markers, the establishment of PCR conditions for marker amplification, and the combination of MAS with traditional breeding techniques. The review also addresses the challenges and future directions of MAS, emphasizing the importance of ongoing marker development, maintaining genetic diversity, and adapting to changing pathogens. In summary, MAS is a valuable tool for improving late blight resistance in potatoes. By integrating MAS with traditional breeding methods and tackling its challenges, breeders can create cultivars that are more resilient to this destructive disease, thereby supporting sustainable potato production.

**Index Terms-** Late blight, Potato, Marker-assisted selection, Resistance genes, Molecular markers, Breeding, Genetic diversity, Pathogen evolution.

## I. INTRODUCTION

Late blight caused Irish Potato Famine of the 1840s. It is caused by water mold *Phytophthora infestans* which is the member of oomycetes. This disease is most destructive potato disease in world, as it causes complete crop failure. Losses due to late blight have been estimated about Euro 10 billion per year. Highest loss due to late blight have been reported in Sub-Saharan regions (Majeed et al., 2017). Late blight affects in India in most of potato regions causing losses upto 90%. Excessive usage of fungicides has led to resistance in late blight of potato, *P.infestans* is highly diverse pathogen and it is prone to rapid mutation. Most favourable condition for it are warm and humid climate (Arora et al., 2014). The best and most eco-friendly approach to avoid significant damage from late blight is to integrate natural resistance into potato varieties. Since the mid-19th century, there has been considerable selection and breeding aimed at enhancing late blight resistance. Late blight resistance genes have been introduced from wild species such as *S. demissum*, *S. stoloniferum*, and the cultivated varieties *S. tuberosum* subsp. *andigena* and *S. phureja* into common potato varieties across various regions of the world. This highlights the need for potato breeders to explore new sources of resistance found in wild gene pools and to expedite their incorporation into

cultivars using marker-assisted selection. In Marker Assisted Selection breeders identify resistant genes in wild species of potato. Molecular markers such as *Sha*, *R3a2*, *R8b* or *R3a1* are developed that are linked to resistant genes. Monomorphic markers are eliminated considering them to carry improper information. Polymorphic markers in marker-assisted selection (MAS) for late blight in potatoes are DNA sequences that differ among individuals (Kaushik et al., 2013). These differences, known as polymorphisms, help distinguish between plants that have various alleles of genes linked to late blight resistance. By pinpointing plants with certain polymorphic markers, breeders can choose individuals that are more likely to exhibit the desired resistance traits. This approach enables a more efficient and precise selection of resistant plants, speeding up the breeding process and minimizing the time and resources needed to create new potato cultivars that are resistant to late blight.

## II. HISTORIC OVERVIEW OF MAS IN LATE BLIGHT OF POTATO

Marker-assisted selection (MAS) has undergone significant evolution since it first emerged in the late 20th century, driven by technological advancements and a deeper understanding of the genetic foundations of various traits (Śliwka et al., 2013).

The early use of MAS in plant breeding primarily targeted simple traits governed by single genes. There were promising results in employing MAS for traits such as disease resistance and marker-assisted backcrossing. Challenges with Complex Traits: However, the application of MAS to more complex traits, like yield and quality, presented challenges due to their polygenic nature. The initial genetic maps often had low marker density, complicating the identification of markers that were closely linked to the genes of interest. The advent of high-throughput genotyping technologies has led to the creation of dense genetic maps, allowing for more accurate mapping of traits and the identification of markers closely associated with specific genes. Advancements in Marker Technology: The shift from RFLP and AFLP markers to SSR and SNP markers has enhanced the efficiency, accuracy, and cost-effectiveness of MAS (Poonam et al.,2023). MAS has been combined with other breeding methods, such as genomic selection and genome editing, to boost its effectiveness. Application to Complex Traits: Improvements in statistical modelling and computational techniques have made it possible to apply MAS to complex traits, including those affected by multiple genes and environmental influences. MAS has greatly sped up the breeding process by facilitating more efficient selection of individuals with the desired traits. Increased Accuracy: The accuracy of selection has improved thanks to MAS, which offers a more precise evaluation of genetic variation. Development of New Cultivars: MAS has played a key role in the creation of new cultivars.

### III. GENETIC BASIS OF LATE BLIGHT RESISTANCE IN POTATO

To hasten the installation of charging stations for electric Several important resistance genes have been identified and mapped to specific chromosomes in potatoes. These genes produce proteins that interact with the late blight pathogen, *Phytophthora infestans*, initiating a defense response (Paluchowska et al.,2022).

Some of the most extensively studied resistance genes include:

- R1: Chromosome 1
- R2: Chromosome 5
- R3: Chromosome 12
- R6: Chromosome 11

These genes offer resistance to various races of *P. infestans*, but their effectiveness can differ based on the specific strain of the pathogen and the environmental conditions. In addition to the key resistance genes, researchers have also pinpointed quantitative trait loci (QTLs) that play a role in late blight resistance. QTLs are specific regions in the genome linked to a quantitative trait, like resistance. They are typically influenced by multiple genes and may have a less pronounced

impact on the trait compared to major genes. Studies focused on QTL mapping have revealed the presence of QTLs for late blight resistance across different chromosomes in potatoes. These QTLs can interact with major resistance genes or other QTLs, affecting the overall resistance level (Collins et al., 1999).

### IV. RESISTANT PHYTOPHTHORA INFESTANS GENES

Hawkes initially identified 232 distinct wild potato species. However, recent research has lowered this figure to 107. These wild potatoes are found across the Americas, stretching from the United States to Chile. Peru boasts the highest diversity of wild potato species, with 93, many of which are considered rare. Mexico also has a significant number of wild potatoes, featuring 36 different species (Paluchowska et al.,2022).

Molecular markers linked to resistance genes can be readily utilized in multiple potato genotypes to accelerate breeding processes. Regardless of the complexity of the genes, potato studies have effectively pinpointed genetic components associated with the desired characteristic. Utilizing molecular markers to identify resistance loci enables early selection, decreasing reliance on lengthy and eco-unfriendly trials. In the past, the majority of molecular markers utilized for mapping were RFLP or AFLP, commonly present exclusively in diploid populations. AFLP is dependable yet costly and time-intensive. Both AFLP and SSR necessitate using polyacrylamide gel electrophoresis, a procedure that is time-consuming and requires hard work (Meksem et al.,1995). These markers are inappropriate for MAS. Nevertheless, SCAR and CAPS are easier to utilize. SCAR markers identify variations in primer locations, resulting in the presence or absence of a band after amplification. CAPS markers utilize restriction enzyme sites for detecting polymorphisms post PCR amplification.

Linkage mapping plays a crucial role in identifying resistance genes and molecular markers. Although the tetraploid nature of potatoes initially hindered mapping efforts, significant progress has been made in creating extensive maps. These maps have pinpointed markers associated with resistance loci, although their application in marker-assisted selection is still evolving. The early maps utilized RFLP markers and revealed similarities between the chromosomes of potatoes and tomatoes (Tiwari et al.,2013). A more recent, high-density map employed AFLP markers. Additionally, another map was developed using diversity array technology, concentrating on the resistant wild species *S. bulbocastanum*. Sequencing and computational analysis have established a framework for anchoring late blight resistance genes. Nowadays, single

nucleotide polymorphisms are the preferred type of molecular marker for monitoring the evolution of *P. infestans*.

### V. MOLECULAR MARKERS LINKED TO LATE BLIGHT RESISTANCE

Molecular markers are unique DNA sequences that differ among individuals and can help identify plants with particular genetic traits, like resistance to late blight in potatoes. They serve as genetic fingerprints, enabling breeders to choose plants that are more likely to carry the desired resistance genes. Molecular markers used for late blight resistance in potatoes should be physically linked to the resistance gene or QTL, polymorphic, highly informative, easy to use, cost-effective, suitable for high-throughput analysis, robust, and specific to the target resistance gene. These characteristics ensure that the markers can accurately identify resistant and susceptible genotypes and provide reliable results for breeding programs. Molecular markers linked to late blight resistance are R3b, R3b2, 45/XI, R3a1, Sha, 148-81R, R3a1, R3a2, R3b1, R8a, R8b and more (RESISTANT GENES AND THEIR ASSOCIATED MOLECULAR MARKER) (Yadav et al.,2013).

Table 1: Late Blight Resistant Genes and Their Associated Molecular Marker Type

Resistance Genes	Molecular Marker Type	Comments
R1	RFLP, SSR, SNP	One of the earliest identified resistance genes.
R2	RFLP, SSR, SNP	Provides resistance to certain races of <i>P. infestans</i> .
R3	RFLP, SSR, SNP	Another important resistance gene, particularly effective in certain potato cultivars.
R6	RFLP, SSR, SNP	Confers resistance to a wide range of <i>P. infestans</i> races
QTLs	RFLP, SSR, SNP	Quantitative trait loci associated with late blight resistance have been identified in different potato populations.

Different molecular markers often need distinct PCR conditions. Factors like annealing temperature, primer concentration, and magnesium chloride concentration can differ based on the specific sequence and characteristics of the marker. For instance, SSR markers, which rely on repetitive DNA sequences, might need different annealing temperatures than SNP markers, which focus on single nucleotide variations. Moreover, certain markers could be more sensitive to variations in magnesium chloride concentration or primer

design. Thus, it's crucial to optimize PCR conditions for each marker type to ensure reliable and efficient amplification and detection.

### VI. STEPS INVOLVED IN MAS

Plants are grown in field conditions and forward breeding is done i.e. phenotype to genotype breeding (Nahiyan et al., 2018). The presence of symptoms of late blight is visually seen in field than in lab conditions. Leaf sample is crushed in liquid nitrogen and DNA is extracted from methods such as CTAB method. The sample is quantified by spectrophotometer (RNA or protein contamination is checked) (Hermansen et al., 2011).

Table 2: PCR Conditions of Some Markers

Markers	Initial Denaturation	Final Denaturation	Annealing	Initial Extension	Final Extension	Cycles
R3b1	94 °C- 2min	94 °C- 30sec	55 °C- 30sec	72 °C- 30sec	72 °C- 2min	35X
R3b2	94 °C- 2min	94 °C- 30sec	55 °C- 30sec	72 °C- 30sec	72 °C- 2min	35X
R3a1	94 °C- 3min	94 °- 30sec	55 °C- 30sec	72 °C- 30sec	72 °C- 3min	35X
R3a2	94 °C- 3min	94 °- 30sec	55 °C- 30sec	72 °C- 30sec	72 °C- 3min	35X
R8a	94 °C- 2min	94 °C- 30sec	55 °C- 30sec	72 °C- 30sec	72 °C- 2min	35X
R3b1	94 °C- 2min	94 °C- 30sec	55 °C- 30sec	72 °C- 30sec	72 °C- 2min	35X
Sha	95 °C- 4min	95 °- 30sec	60 °C- 30sec	72 °C- 1min	72 °- 5min	35X
R8b	94 °C- 2min	94 °C- 30sec	55 °C- 30sec	72 °C- 30sec	72 °C- 2min	35X

Marker-assisted selection (MAS) is an effective method for speeding up the breeding process of potatoes that are resistant to late blight. This technique utilizes molecular markers associated with late blight resistance genes to identify plants with favourable genotypes, even before any visible symptoms manifest. Genotyping and Marker Analysis consist of choosing suitable markers, amplifying the target DNA regions through PCR (Different PCR conditions of some marker are given in the TABLE-2), and examining the resulting DNA fragments using gel electrophoresis or sequencing to ascertain the genotype at each marker locus (Bormann et al., 2004). A ladder in gel documentation is a very important composition acting as a molecular weight marker. It is a mix of DNA fragments of known sizes from 100 to a few thousand base pairs. The ladder is run along with your unknown DNA

sample on the gel, which in turn provides a reference for estimation of size of your fragments. Resistance Marker Analysis involves comparing the genotypes at the resistance markers with the observed phenotypes to pinpoint markers linked to late blight resistance. Plants that possess resistance marker alleles are selected for breeding, regardless of whether they exhibit visible resistance symptoms. Variety Development includes crossing selected plants with other potato cultivars to merge desirable traits, such as high yield, quality, and late blight resistance (Beketova et al., 2021). The offspring are then assessed for their agronomic performance and resistance to late blight. Promising genotypes are moved on to field trials and ultimately released as new potato varieties.

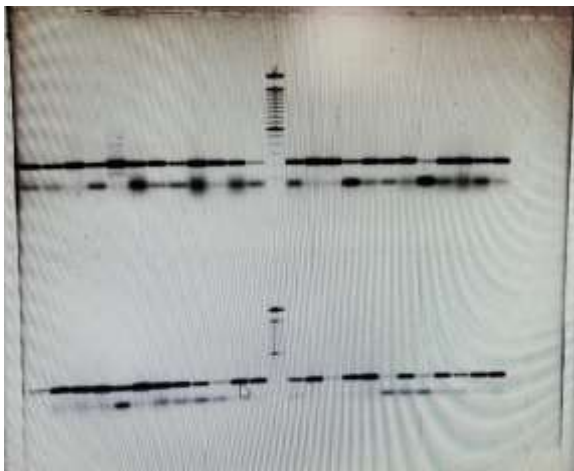


Fig 1: Polymorphic Samples Seen Under Uv Light

## VII. MARKER ASSISTED SELECTION IN NEAR FUTURE

While there are still many questions to address before marker-assisted selection (MAS) becomes a standard practice in potato breeding, we can already make some observations about its initial implementation phase. Specific cross populations, progenitors, and markers may come from studies

and collaborations within breeding programs that are exploring this technology. MAS will focus on selected genes and alleles, allowing breeders to evaluate how much easier it will be to breed for various single traits using this method. As the technology advances and more results and experiences are gathered, it will be possible to lessen the labor demands of traditional breeding in favour of resources dedicated to MAS (Trognitz et al., 2004). Breeding programs might either reduce the size of field trials while maintaining overall output or explore a greater number of cross combinations, tapping into a broader range of genetic resources to enhance the program's output in terms of increased diversity or a higher number of new, high-performing cultivars. The adoption of MAS could also strengthen collaboration between breeders and researchers, especially in identifying genes and alleles that are crucial for developing desired traits, as well as in designing various markers and creating efficient application methods. This collaboration could pave the way for the broader use of MAS across many qualitatively inherited traits and help adapt effective screening methods for quantitative traits. Given the significant advancements in molecular biology and genetics over the past thirty years, it is reasonable to anticipate substantial progress in conventional plant breeding in the coming decades. Late blight of potato is controlled by multiple genes making it difficult to identify and track all relevant markers.

A limited genetic base in potato breeding programs can restrict the variety of resistance genes available for marker-assisted selection (MAS). This limitation can impede the creation of cultivars with lasting resistance, as a single resistance gene might be vulnerable to evolving pathogens. To tackle this challenge, breeders should aim to incorporate genetic diversity from wild potato species or other related sources. Late blight pathogens are in a constant state of evolution, leading to the emergence of new races that can bypass existing resistance genes (Stefan et al., 2024). This reality demands the ongoing development of new markers and resistance sources to ensure the effectiveness of MAS. It also highlights the necessity of integrated disease management strategies that combine MAS with other control methods, such as crop rotation and fungicide applications. To fully harness the advantages of marker-assisted selection (MAS), it is crucial to integrate it with traditional breeding techniques. A balanced approach can help mitigate the limitations of each method while capitalizing on their strengths. For instance, MAS can be employed to identify and select individuals with specific resistance genes, while traditional breeding can be utilized to merge these genes with other desirable traits, such as yield and quality. The application of genetically modified organisms (GMOs) in MAS can provoke ethical dilemmas, especially in areas with stringent regulations. Public attitudes toward GMOs can differ significantly, and negative perceptions can impact the acceptance of these technologies.

Benefits of Marker-Assisted Selection (MAS) for Late Blight Resistance in Potatoes

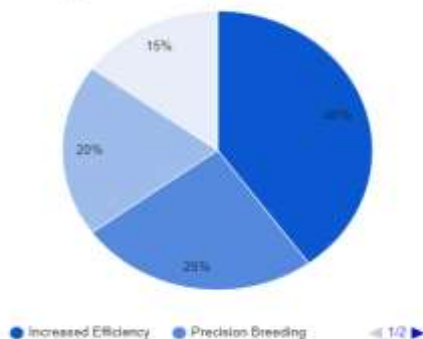


Fig 2: Increased Efficiency- 40 %, Precision Breeding-25 %, Durability-20 %, Reduced Costs-15%

## VIII. CONCLUSION

Marker-assisted selection (MAS) has become an important tool in potato breeding, especially for improving resistance to late blight. By using molecular markers associated with resistance genes, breeders can quickly identify and select plants with the desired traits, speeding up the creation of new cultivars. The presence of a wide range of resistance genes, including those from wild potato species, enhances the effectiveness of MAS in tackling the persistent challenges of late blight. Recent advancements in MAS include the creation of high-density genetic maps, the application of informative molecular markers, and the combination of MAS with other breeding strategies. These developments have made it possible to apply MAS to complex traits like late blight resistance, which is affected by multiple genes and environmental conditions. Although MAS provides considerable advantages, it is crucial to address the challenges that come with this technology, such as the ongoing need for marker development, the risk of limited genetic diversity, and the changing nature of late blight pathogens. By thoughtfully considering these issues and taking a strategic approach, breeders can effectively use MAS to develop potato cultivars that are more resilient to late blight, ensuring sustainable potato production. In summary, MAS offers a promising path for improving late blight resistance in potatoes. By merging the capabilities of molecular genetics with traditional breeding practices, breeders can create cultivars that are better equipped to combat this serious disease, ultimately supporting food security and agricultural sustainability.

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