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Enhancing Disease Resistance and Yield in Welsh Onion through Marker-Assisted Breeding

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Abstract The production of Welsh onion (*Allium fistulosum* L.), a vital vegetable crop, is often challenged by various diseases that lead to reduced yield and quality, posing a significant threat to agricultural productivity. Enhancing the yield of Welsh onion to meet market demands is also a key objective in agricultural research. This study comprehensively describes the types of molecular markers, including Simple Sequence Repeats (SSRs), Single Nucleotide Polymorphisms (SNPs), and Amplified Fragment Length Polymorphisms (AFLPs), showcasing their potential in precision breeding. It further analyzes methods for identifying disease resistance genes, emphasizing the importance of hybridization and selection, field trials, and performance evaluation in the breeding process. Additionally, the study discusses the application of marker-assisted selection (MAS) in improving Welsh onion yield and explores the technical challenges faced in MAS. Through MAS technology, it is possible to accurately locate and introduce genes for yield and disease resistance, enabling the cultivation of Welsh onion varieties resistant to multiple diseases. This approach not only effectively reduces pesticide use and lowers production costs but also ensures the quality and safety of Welsh onions, achieving high-yield goals. Practical application and technological innovation in this area contribute to the advancement of agricultural biotechnology and provide insights and references for the genetic improvement of other crops.

Keywords Welsh onion; Marker-assisted selection; Disease resistance; Yield; QTL analysis

1 Introduction

Welsh onion (*Allium fistulosum*) is a perennial plant widely cultivated for its culinary uses and nutritional benefits (Kim et al., 2023). It is a staple in many Asian cuisines and is valued for its mild flavor and versatility. Despite its importance, Welsh onion cultivation faces significant challenges, particularly from diseases that can severely impact yield and quality. Effective breeding strategies are essential to enhance disease resistance and improve overall yield in Welsh onion.

Disease resistance is a critical trait in Welsh onion breeding programs. Diseases such as gray mold, caused by Botrytis species, and Fusarium basal rot, caused by Fusarium oxysporum, are major threats to Welsh onion crops. These diseases not only reduce yield but also affect the storage life and marketability of the produce (Kim et al., 2021; Sharma and Cramer, 2023). Traditional methods of disease control, including chemical treatments, are often insufficient and can lead to environmental concerns. Therefore, breeding for disease-resistant varieties is a sustainable and effective approach to mitigate these issues (Poland and Rutkoski, 2016; Scholten et al., 2016).

Yield improvement is another primary objective in Welsh onion breeding. High-yielding varieties ensure better productivity and profitability for farmers. However, achieving high yield while maintaining disease resistance is a complex task, as these traits are often controlled by multiple genes (Poland and Rutkoski, 2016). Marker-assisted breeding (MAB) has emerged as a powerful tool to address this challenge by enabling the precise selection of desirable traits (Pathania et al, 2017).

This study summarizes the latest advancements in marker-assisted breeding for improving disease resistance and yield in Welsh onions, emphasizing the challenges and limitations associated with these breeding strategies. It provides an in-depth understanding of the future directions and potential applications of genomic tools in Welsh onion breeding programs. By integrating findings from various studies, the research offers a comprehensive overview of the role of marker-assisted selection in enhancing disease resistance and yield, thereby aiding in the development of more resilient and productive varieties.

2 Overview of Welsh Onion Diseases

2.1 Common diseases affecting welsh onion

Welsh onions (*Allium fistulosum* L.) are susceptible to a variety of diseases that can significantly impact their yield and quality. Among the most prevalent diseases is Fusarium basal rot (FBR), caused by the soil-borne fungus *Fusarium oxysporum* f. sp. cepae (FOC). This disease is a major concern for onion production worldwide, leading to severe losses in both yield and bulb quality. The infection typically starts at the basal plate of the onion bulb, progressing upwards and causing rot, which can be particularly devastating during storage (Cramer et al., 2021; Sharma and Cramer, 2023). Another significant disease is gray mold, caused by Botrytis cinerea and Botrytis squamosa. This disease affects the leaves and bulbs, leading to reduced productivity and storage life. Control of gray mold is challenging due to the difficulty in managing the pathogens through physical and chemical methods (Scholten et al., 2016; Kim et al., 2021).

Downy mildew, caused by *Peronospora destructor*, is another critical disease affecting Welsh onions. It is considered one of the most destructive fungal diseases for bulb onions, leading to significant yield losses. The disease manifests as yellowish patches on the leaves, which eventually turn brown and necrotic, severely affecting the photosynthetic capability of the plant (Kim et al., 2016; Khrustaleva et al., 2019). Additionally, Welsh onions are also susceptible to Iris yellow spot virus (IYSV), which is transmitted by onion thrips. This virus causes necrotic lesions on the leaves and scapes, leading to reduced plant vigor and yield (Cramer et al., 2021).

2.2 Pathogens and infection mechanisms

The pathogens responsible for these diseases have distinct infection mechanisms. *Fusarium oxysporum* f. sp. cepae (FOC) infects the onion bulbs through the basal plate, where it colonizes the vascular tissues, leading to rot and eventual plant death. The fungus produces conidia that can survive in the soil for extended periods, making it difficult to eradicate once established (Sharma and Cramer, 2023). Botrytis cinerea and Botrytis squamosa, the causative agents of gray mold, infect the plant tissues through wounds or natural openings. These necrotrophic pathogens thrive in humid conditions and produce spores that can spread rapidly, leading to widespread infection (Scholten et al., 2016; Kim et al., 2021).

Peronospora destructor, the pathogen responsible for downy mildew, infects the leaves of Welsh onions by penetrating the stomata. The pathogen produces sporangia that are dispersed by wind and water, facilitating rapid spread under favorable conditions. The infection leads to the formation of characteristic downy growth on the underside of the leaves, which is a key diagnostic feature of the disease (Kim et al., 2016; Khrustaleva et al., 2019). Iris yellow spot virus (IYSV) is transmitted by onion thrips (Thrips tabaci), which acquire the virus while feeding on infected plants and subsequently transmit it to healthy plants. The virus replicates within the thrips and is introduced into the plant tissues during feeding, leading to the development of necrotic lesions (Cramer et al., 2021).

2.3 Economic impact of diseases

The economic impact of these diseases on onion production is significant. Fusarium basal rot (FBR) can cause severe yield losses, with infected bulbs becoming unmarketable due to severe decay. This not only affects the current season’s harvest but also has long-term effects on storage and market sales, as infected bulbs may contaminate healthy ones during storage (Cramer et al., 2021; Sharma and Cramer, 2023). The costs associated with managing FBR, including the use of fungicides and soil treatments, add to the economic burden on growers. The inoculation method shown in Figure 1 provides an effective tool for screening disease-resistant varieties, simplifying the inoculation process and increasing spore inoculation efficiency, which accelerates the development of resistant varieties (Cramer et al., 2021). By utilizing this method, reliance on chemical pesticides can be reduced, enhancing the disease resistance of onions, thereby lowering the economic losses caused by disease and promoting the sustainability of the onion industry.

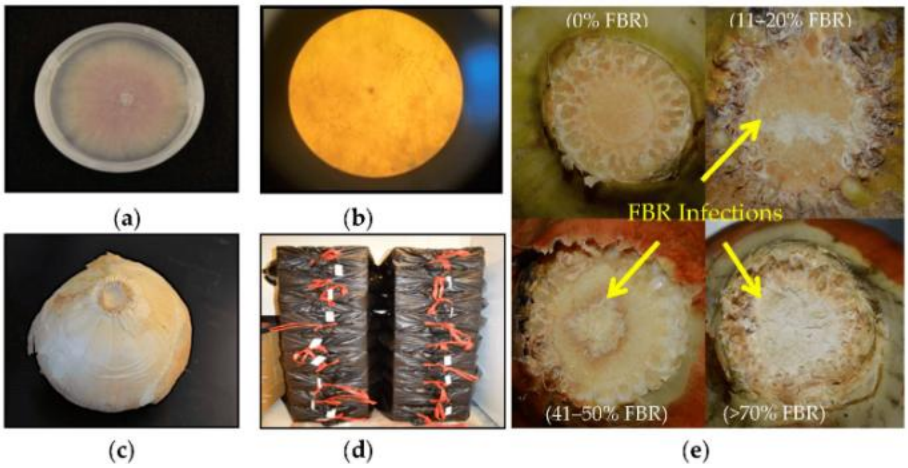

Figure 1 Inoculation method for Fusarium basal rot (FBR) resistant onion bulbs (Adapted from Cramer et al., 2021)

Image caption: (a) FOC (*Fusarium oxysporum* f.sp. cepae) culture inoculated after cutting the basal plate of the onion bulb; (b) Special potato dextrose agar (SPDA) medium containing FOC conidia suspension; (c) Onion basal plate inoculated using an SPDA plug; (d) Onion bulbs initially incubated under high humidity conditions; (e) Visual assessment of FBR infection after 20 days, categorized into four levels based on infection severity: 0%, 11-20%, 41-50%, and over 70% (Adapted from Cramer et al., 2021)

Similarly, gray mold disease caused by Botrytis species results in reduced productivity and storage life of onions, leading to financial losses for farmers. The difficulty in controlling this disease through conventional methods further exacerbates the economic impact (Scholten et al., 2016; Kim et al., 2021).

Downy mildew caused by *Peronospora destructor* is another disease with significant economic implications. The disease can cause severe yield losses, particularly in wet and humid conditions that favor its spread. The cost of managing downy mildew, including the use of resistant cultivars and fungicides, adds to the production costs for growers (Kim et al., 2016; Khrustaleva et al., 2019). Iris yellow spot virus (IYSV) also poses a significant economic threat, as it reduces plant vigor and yield, leading to lower marketable produce. The management of IYSV involves controlling the vector, onion thrips, which can be challenging and costly (Cramer et al., 2021). Overall, the combined impact of these diseases necessitates the development of effective disease management strategies, including the use of resistant cultivars and integrated pest management practices, to mitigate their economic impact on Welsh onion production.

3 Marker-Assisted Breeding Techniques

3.1 Principles of marker-assisted breeding

Marker-assisted breeding (MAB) is a modern plant breeding technique that utilizes molecular markers to select desirable traits in crops. This method significantly accelerates the breeding process by allowing for the early identification of plants that carry beneficial genes, thus reducing the time and resources needed for traditional breeding methods. MAB relies on the identification of genetic markers that are closely linked to the traits of interest, such as disease resistance, yield, and quality. These markers can be used to screen large populations of plants, ensuring that only those with the desired genetic makeup are selected for further breeding.

The principles of MAB involve several key steps: identifying and validating molecular markers associated with the traits of interest, developing high-throughput screening methods, and integrating these markers into breeding programs. For instance, in onion breeding, SNP markers have been identified and validated for their association with disease resistance, which can be used to introgress resistance genes from related species into onion cultivars (Scholten et al., 2016; Kim et al., 2021). This approach not only enhances the efficiency of breeding programs but also enables the development of new cultivars with improved traits in a shorter time frame.

3.2 Types of molecular markers

Simple Sequence Repeats (SSRs), also known as microsatellites, are short, repetitive DNA sequences that are highly polymorphic and distributed throughout the genome. SSR markers are widely used in plant breeding due to their high level of polymorphism, co-dominant inheritance, and ease of detection. In Welsh onion, transcriptome sequencing has identified numerous SSR loci, which can be used to develop polymorphic markers for genetic diversity studies and breeding programs (Yang et al., 2015). These markers are valuable for constructing genetic maps and identifying quantitative trait loci (QTLs) associated with important agronomic traits.

Single Nucleotide Polymorphisms (SNPs) are the most abundant type of genetic variation in genomes and involve a single base pair change. SNP markers are highly valuable in plant breeding due to their abundance, stability, and potential for high-throughput genotyping. In onion breeding, SNP markers have been developed and used to construct molecular linkage maps and identify QTLs for disease resistance (Scholten et al., 2016; Kim et al., 2021). These markers facilitate the precise selection of desirable traits and the efficient introgression of resistance genes from related species into cultivated onions.

Amplified Fragment Length Polymorphism (AFLP) is a DNA fingerprinting technique that detects polymorphisms in DNA sequences by amplifying restriction fragments. AFLP markers are highly reproducible and can generate a large number of markers in a single assay, making them useful for genetic mapping and diversity studies. Although AFLP markers are less commonly used in recent years due to the advent of more advanced technologies like SNPs and SSRs, they have been instrumental in early genetic studies and the development of molecular markers in various crops, including onions (Khosa et al., 2016).

3.3 Application in crop improvement

Marker-assisted breeding has revolutionized crop improvement by enabling the precise selection of plants with desirable traits, thus enhancing the efficiency and effectiveness of breeding programs. In onions, molecular markers such as SSRs and SNPs have been used to identify and introgress disease resistance genes from related species, leading to the development of cultivars with improved resistance to pathogens like Botrytis squamosa and Alternaria porri (Scholten et al., 2016; Chand et al., 2018; Kim et al., 2021). These advancements have not only increased the yield and quality of onion crops but also reduced the reliance on chemical fungicides, promoting sustainable agricultural practices.

The integration of molecular markers into breeding programs has also facilitated the study of genetic diversity and the identification of superior genotypes. For example, the use of SSR markers in Welsh onion has revealed significant genetic diversity among different accessions, which can be exploited to develop new cultivars with enhanced traits (Yang et al., 2015). Similarly, the development of SNP markers has enabled the construction of detailed genetic maps and the identification of QTLs associated with important agronomic traits, providing valuable insights for future breeding efforts (Scholten et al., 2016; Kim et al., 2021). Overall, the application of marker-assisted breeding in crop improvement holds great promise for the development of high-yielding, disease-resistant, and quality-enhanced cultivars.

4 Identifying Disease Resistance Genes

4.1 Gene mapping and QTL analysis

Gene mapping and quantitative trait loci (QTL) analysis are fundamental techniques in identifying disease resistance genes in crops, including Welsh onion. These methods involve the use of genetic markers to locate regions of the genome associated with resistance traits. For instance, in small-grain cereals and maize, QTL mapping has been instrumental in identifying regions associated with resistance to various diseases such as Fusarium head blight and Northern corn leaf blight (Miedaner et al., 2020). Similarly, in wheat and barley, numerous QTLs have been identified for resistance to diseases like rust and Fusarium head blight, demonstrating the utility of QTL mapping in breeding programs (Miedaner and Korzun, 2012). In Welsh onion, the application of these techniques can help pinpoint specific genomic regions that confer resistance to prevalent diseases, thereby facilitating the development of resistant cultivars through marker-assisted selection (MAS).

The process of QTL mapping involves creating a genetic linkage map using molecular markers such as single nucleotide polymorphisms (SNPs) and simple sequence repeats (SSRs). For example, in oat, six QTLs for adult plant resistance to crown rust were identified using genotyping-by-sequencing, which provided markers closely linked to resistance traits (Nazareno et al., 2023). In maize, a meta-analysis of QTLs for resistance to Fusarium and Gibberella ear rots identified stable QTLs that can be used in breeding programs to enhance disease resistance (Akohoue and Miedaner, 2022). These examples highlight the effectiveness of QTL mapping in identifying resistance genes, which can be applied to Welsh onion to improve its disease resistance profile.

4.2 Functional genomics approaches

Functional genomics approaches, such as transcriptome sequencing and gene expression analysis, provide insights into the molecular mechanisms underlying disease resistance. These techniques help identify candidate genes that are differentially expressed in response to pathogen infection. For instance, transcriptome sequencing in Allium species has led to the identification of SNP markers linked to disease resistance, which are valuable for breeding programs aimed at improving onion cultivars (Scholten et al., 2016). In maize, co-expression analysis has revealed candidate genes associated with resistance to Fusarium and Gibberella ear rots, which can be targeted for functional validation (Akohoue and Miedaner, 2022). Applying similar approaches in Welsh onion can uncover key resistance genes and pathways, facilitating the development of resistant varieties.

Functional genomics also involves the use of advanced techniques such as RNA interference (RNAi) and CRISPR/Cas9 to validate the function of candidate genes. For example, in rice, marker-assisted gene stacking has been used to combine multiple resistance genes into a single cultivar, enhancing its resistance to various biotic and abiotic stresses (Ludwików et al., 2015). This approach can be adapted for Welsh onion to pyramid multiple resistance genes, thereby providing broad-spectrum resistance to multiple diseases. By integrating functional genomics with traditional breeding methods, it is possible to accelerate the development of disease-resistant Welsh onion cultivars.

4.3 Validation of candidate genes

The validation of candidate genes identified through gene mapping and functional genomics is a critical step in confirming their role in disease resistance. This involves the use of techniques such as quantitative PCR, gene knockout, and overexpression studies to assess the impact of these genes on disease resistance. For instance, in oat, candidate genes identified within QTL regions for crown rust resistance were validated using Polymerase Chain Reaction Allelic Competitive Extension (PACE) markers, confirming their association with resistance traits (Nazareno et al., 2023). Similarly, in maize, candidate genes within meta-QTL regions for Fusarium and Gibberella ear rots were validated through transcriptomic data, highlighting their potential for improving disease resistance (Akohoue and Miedaner, 2022).

In Welsh onion, the validation of candidate genes can be achieved by developing specific markers for these genes and assessing their presence in resistant and susceptible lines. This can be complemented by functional studies to determine the role of these genes in the resistance mechanism. For example, SNP markers developed for Allium species have been used to map QTLs for resistance to Botrytis squamosa, providing a basis for marker-assisted breeding (Scholten et al., 2016). By validating candidate genes and incorporating them into breeding programs, it is possible to enhance the disease resistance and yield of Welsh onion cultivars.

5 Enhancing Disease Resistance through Breeding

5.1 Breeding strategies for disease resistance

Conventional breeding techniques have long been employed to enhance disease resistance in crops, including Welsh onion. These methods typically involve selecting and crossbreeding plants that exhibit desirable traits, such as resistance to specific pathogens. However, conventional breeding can be time-consuming and labor-intensive, often requiring multiple generations to achieve significant improvements in disease resistance (Sharma and Cramer, 2023).

Marker-assisted selection (MAS) has revolutionized the breeding process by enabling the identification and selection of disease-resistant traits at the molecular level. MAS utilizes molecular markers linked to resistance genes, allowing breeders to screen for these markers in early plant development stages. This accelerates the breeding process and increases the precision of selecting resistant varieties. For instance, the development of SNP markers associated with resistance to gray mold disease in onions has facilitated the selection of resistant lines, significantly reducing the breeding period (Scholten et al., 2016; Kim et al., 2021). Additionally, MAS has been successfully applied in breeding programs to develop varieties resistant to various diseases, such as Fusarium basal rot (Sharma and Cramer, 2023) and Botrytis squamosa (Scholten et al., 2016).

Backcross breeding is another effective strategy for enhancing disease resistance. This method involves crossing a resistant donor parent with a recurrent parent, followed by repeated backcrossing with the recurrent parent while selecting for the resistance trait. Marker-assisted backcross breeding further refines this process by using molecular markers to track the presence of resistance genes, ensuring the retention of desirable traits from the donor parent while maintaining the genetic background of the recurrent parent (Pathania et al., 2017). This approach has been instrumental in developing disease-resistant varieties with improved agronomic performance.

5.2 Crossbreeding and selection

Crossbreeding and selection involve hybridizing different plant varieties to combine desirable traits, such as disease resistance and high yield. This method has been particularly useful in introducing resistance genes from related species into cultivated varieties. For example, the introgression of resistance genes from Allium roylei and Allium fistulosum into onion cultivars has been facilitated by the identification and validation of SNP markers, enabling efficient selection of resistant hybrids (Scholten et al., 2016). This approach not only enhances disease resistance but also broadens the genetic base of the breeding population, contributing to the development of more resilient varieties.

5.3 Field Trials and performance evaluation

Field trials and performance evaluation are critical components of the breeding process, providing essential data on the effectiveness of selected traits under real-world conditions. These trials involve testing the performance of breeding lines in various environments to assess their resistance to diseases and overall agronomic performance. For instance, artificial inoculation and mature bulb screening have been used to evaluate the resistance of onion cultivars to Fusarium basal rot, leading to the identification of advanced selections with improved resistance (Figure 2) (Sharma and Cramer, 2023). Such evaluations ensure that the developed varieties not only possess disease resistance but also meet the agronomic and quality standards required for commercial production.

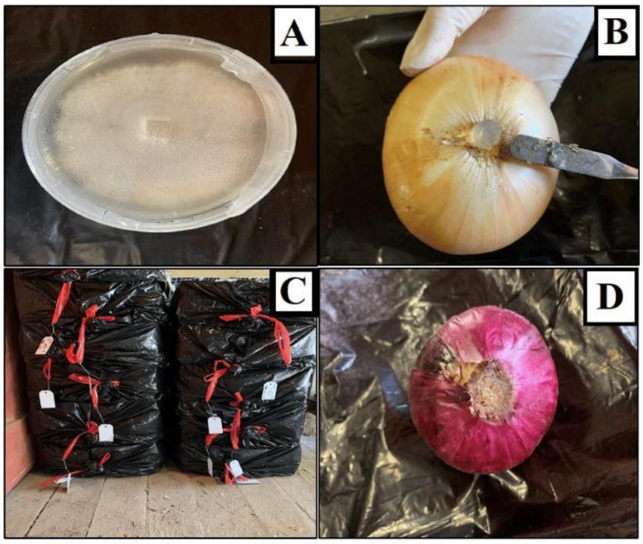

Figure 2 Artificial inoculation method for selecting Fusarium basal rot (FBR) resistant onion bulbs (Adapted from Sharma and Cramer, 2023)

Image caption: (A) Culture of *Fusarium oxysporum* f. sp. cepae (FOC); (B) Artificial inoculation process where PDA (potato dextrose agar) medium containing spores is inserted into the transversely cut basal plate of the onion bulb; (C) Incubation process of the inoculated onion bulb, with high humidity conditions within 24 hours promoting disease development; (D) A susceptible onion bulb scored at 9, showing pronounced symptoms of basal rot (Adapted from Sharma and Cramer, 2023)

The artificial inoculation screening for FBR-resistant onion bulbs is a critical step in developing disease-resistant varieties. The method used by Sharma and Cramer (2023) involves directly inoculating the pathogen onto the onion basal plate and creating a high-humidity environment to promote disease development, thus assessing the disease resistance of onion varieties. This screening method accelerates the breeding of resistant varieties, enhances disease resistance in agricultural production, reduces reliance on chemical control, and lowers production costs. The resistant varieties selected through this method can effectively minimize the economic losses caused by FBR in onion production.

6 Improving Yield through Marker-Assisted Breeding

6.1 Yield-related traits and QTLs

Yield-related traits in crops are often complex and polygenic, meaning they are controlled by multiple genes and influenced by environmental factors. Quantitative trait loci (QTLs) have been identified as key genetic factors associated with these traits. For instance, wild species of crop plants have been utilized to map QTLs for yield, revealing that these QTLs are distributed across almost all chromosomes, with some regions being more frequently associated with yield traits. This mapping has shown that QTLs for yield and related traits often co-locate, suggesting linkage or pleiotropic effects, and many of these QTLs are stable across different environments and genetic backgrounds (Swamy and Sarla, 2008). In maize, studies have identified and located QTLs associated with grain yield and 24 yield-related traits, demonstrating that different genomic regions contribute to yield through various subsets of these traits. The gene action for these traits varies, with some showing dominant or overdominant effects, while others exhibit mainly additive gene action (Collard et al., 2005).

The identification and mapping of QTLs are crucial for understanding the genetic basis of yield-related traits. Advanced backcross QTL analysis has been particularly useful in identifying favorable QTL alleles from wild species while minimizing the impact of unwanted alleles. This approach has been applied successfully in crops like rice and tomato, where stable and consistent major effect yield-enhancing QTLs have been identified. These QTLs are prime targets for marker-assisted selection (MAS), although their context-dependency remains a challenge (Swamy and Sarla, 2008). In maize, the use of molecular markers such as isozymes and restriction fragment length polymorphisms (RFLPs) has enabled the discrimination of individual gene effects, facilitating the elucidation of the numbers and genomic distribution of QTLs involved in yield expression (Collard et al., 2005).

6.2 Breeding for high yield

Breeding for high yield involves the strategic use of identified QTLs to enhance the genetic potential of crops. Marker-assisted selection (MAS) is a powerful tool in this regard, allowing breeders to select for favorable alleles associated with high yield. The integration of QTLs from wild species into cultivated varieties has shown promise in improving yield. For example, QTLs identified from wild relatives of rice and tomato have been successfully incorporated into breeding programs, resulting in yield improvements (Swamy and Sarla, 2008). In maize, the identification of QTLs associated with grain yield and related traits has provided valuable insights for breeding programs. The significant associations between marker loci and yield traits highlight the potential for MAS to enhance yield through the manipulation of these QTLs (Collard et al., 2005).

The process of breeding for high yield using MAS involves several steps, including the identification of QTLs, validation of their effects in different genetic backgrounds and environments, and the incorporation of these QTLs into breeding lines. The use of advanced backcross QTL analysis has been effective in identifying yield-enhancing QTLs from wild species, which can then be introgressed into elite cultivars. This approach not only improves yield but also helps in maintaining genetic diversity within the breeding pool (Swamy and Sarla, 2008). In maize, the use of molecular markers has facilitated the precise manipulation of QTLs, enabling breeders to enhance yield by selecting for specific genomic regions associated with high yield and its component traits (Collard et al., 2005).

6.3 Evaluation of yield performance

The evaluation of yield performance in breeding programs is essential to ensure that the selected QTLs and breeding strategies result in tangible yield improvements. This involves field trials and phenotypic assessments to measure the actual yield and its stability across different environments. In crops like rice and tomato, QTLs derived from wild species have been evaluated in multiple environments, demonstrating their stable and consistent effects on yield. Such evaluations are crucial for confirming the utility of these QTLs in breeding programs (Swamy and Sarla, 2008). In maize, the evaluation of yield performance has shown that different genomic regions contribute to yield through various traits, and the effects of these regions can vary depending on the genetic context and environmental conditions (Collard et al., 2005).

Field trials and phenotypic evaluations provide the necessary data to validate the effectiveness of MAS in improving yield. These evaluations help in identifying the most promising QTLs and breeding lines, which can then be advanced in the breeding pipeline. The use of molecular markers in these evaluations allows for the precise tracking of QTLs and their effects on yield, facilitating the selection of the best-performing lines. In maize, the evaluation of yield performance has highlighted the importance of considering both the genetic and environmental factors influencing yield, ensuring that the selected QTLs provide consistent yield improvements across different conditions (Collard et al., 2005).

7 Integrating Disease Resistance and Yield Improvement

7.1 Combining traits through breeding programs

Combining disease resistance and yield improvement in Welsh onion (*Allium fistulosum* L.) through breeding programs involves the integration of both classical and modern genetic techniques. Classical breeding methods, such as recurrent backcrossing and multi-stage selection, have been traditionally used to introduce and combine desirable traits, including disease resistance and high yield. Recurrent backcrossing is particularly effective for introducing single major genes for disease resistance, while multi-stage selection allows for the simultaneous improvement of multiple traits, including yield and resistance (Miedaner, 2016). The use of molecular markers has revolutionized these breeding programs by enabling the precise targeting of genes and reducing the time required to recover the genome of the recurrent parent (Miedaner, 2016; Padula et al., 2022).

Marker-assisted selection (MAS) and genomic selection (GS) are two modern techniques that have significantly enhanced the efficiency of breeding programs. MAS allows for the identification and selection of specific genes or quantitative trait loci (QTL) associated with disease resistance and yield, thereby facilitating the pyramiding of multiple resistance genes and yield-related traits (Miedaner and Korzun, 2012; Cramer et al., 2021). GS, on the other hand, enables the selection of multiple traits directly from the genome using high-throughput genotyping platforms, thus accelerating the breeding process and improving the accuracy of selection (Miedaner and Korzun, 2012; Miedaner, 2016). These advanced techniques have been successfully applied in various crops, including onions, to develop cultivars with enhanced disease resistance and improved yield (Khosa et al., 2016; Sharma and Cramer, 2023).

7.2 Multi-trait selection

Multi-trait selection is a critical approach in breeding programs aimed at improving both disease resistance and yield in Welsh onion. This approach involves the simultaneous selection of multiple traits, which can be challenging due to the potential for negative correlations between traits. However, the use of molecular markers and genomic tools has made it possible to overcome these challenges by providing detailed genetic information that can guide the selection process (Miedaner and Korzun, 2012; Miedaner, 2016). For instance, the identification of SNP markers linked to disease resistance and yield traits in onion has facilitated the efficient introgression of these traits into breeding lines (Collins et al., 2018).

The integration of multi-trait selection with marker-assisted breeding has shown promising results in various crops. For example, the use of MAS in wheat and barley has enabled the successful selection of multiple disease resistance genes and yield-related traits, leading to the development of high-performing cultivars (Miedaner and Korzun, 2012). Similarly, in onions, the application of MAS and genomic selection has resulted in the identification of germplasm with improved resistance to Fusarium basal rot and enhanced seedling vigor, which are critical traits for yield improvement (Taylor et al., 2019; Sharma and Cramer, 2023). These advancements highlight the potential of multi-trait selection in developing Welsh onion cultivars with superior disease resistance and yield.

7.3 Case studies of successful integration

Several case studies demonstrate the successful integration of disease resistance and yield improvement in onion breeding programs. One notable example is the development of onion cultivars with resistance to Fusarium basal rot (FBR). Through artificial inoculation and selection, researchers have identified and advanced populations with improved FBR resistance, which has been validated over multiple years of testing. The use of conidial inoculation methods has accelerated the breeding process by enabling the precise identification of resistant individuals, which are then used to develop new cultivars with enhanced resistance and yield (Sharma and Cramer, 2023).

Another successful case study involves the use of molecular markers to improve disease resistance and yield in onion. Researchers have identified SNP markers linked to resistance genes in related Allium species, such as Allium roylei and Allium fistulosum, and have successfully introgressed these genes into onion cultivars (Collins et al., 2018). This approach has led to the development of onion lines with improved resistance to diseases like Botrytis squamosa and Downy mildew, as well as enhanced yield traits (Scholten et al., 2016; Collins et al., 2018). These case studies underscore the effectiveness of integrating molecular tools and traditional breeding methods to achieve significant improvements in disease resistance and yield in Welsh onion.

8 Technical Challenges in Marker-Assisted Breeding

8.1 Limitations of molecular markers

One of the primary limitations of molecular markers in marker-assisted breeding (MAB) is their effectiveness in detecting quantitative trait loci (QTL) with small effects. While molecular markers are highly effective for traits controlled by single major genes, their utility diminishes when dealing with complex traits governed by multiple minor genes. This is particularly relevant in the context of disease resistance, where quantitative resistance often involves numerous genes with small individual effects. For instance, in wheat and barley breeding, although many QTLs for disease resistance have been identified, their small individual effects and the prevalence of QTL-background interactions hinder the broad implementation of marker-assisted selection (MAS) (Miedaner and Korzun, 2012). Additionally, the development and validation of markers that are applicable across different breeding populations remain a significant challenge, as demonstrated in the case of lupin breeding for anthracnose resistance (Yang et al., 2008).

Another limitation is the cost and labor associated with developing and validating molecular markers. High-throughput genotyping platforms and next-generation sequencing technologies have reduced these costs to some extent, but they still represent a significant investment, particularly for smaller breeding programs. Moreover, the integration of MAS into practical breeding programs is often constrained by the availability of diagnostic markers and the economic return on investment. For example, in small-grain cereal breeding, the low return on investment and the lack of diagnostic markers have been cited as major constraints (Miedaner and Korzun, 2012). Despite these challenges, advancements in genomic selection (GS) and the development of high-throughput genotyping platforms hold promise for overcoming some of these limitations in the future (Poland and Rutkoski, 2016; Collins et al., 2018).

8.2 Genetic diversity and marker availability

Genetic diversity within breeding populations is crucial for the success of marker-assisted breeding programs. However, the availability of molecular markers that are polymorphic and informative across diverse genetic backgrounds is often limited. This is particularly challenging in crops like onion, where the biennial life cycle, cross-pollinated nature, and high inbreeding depression complicate the characterization and breeding of improved traits (Khosa et al., 2016). The identification and validation of single nucleotide polymorphism (SNP) markers in related species, such as Allium roylei and Allium fistulosum, have been instrumental in facilitating introgression breeding in onion, but the process remains complex and resource-intensive (Scholten et al., 2016).

Furthermore, the development of markers that are broadly applicable across different breeding populations requires extensive validation. For example, in the case of lupin breeding for anthracnose resistance, multiple candidate markers were generated and validated across various cultivars to ensure their wide applicability before being converted into a routine implementable form (Yang et al., 2008). This process underscores the importance of having a diverse and well-characterized germplasm collection to identify and validate effective markers. The integration of molecular and conventional breeding approaches, supported by next-generation sequencing technologies, is essential for overcoming these challenges and enhancing the genetic diversity and marker availability in breeding programs (Jiang, 2013; Khosa et al., 2016).

8.3 Managing marker-assisted breeding programs

Managing marker-assisted breeding programs involves several logistical and technical challenges. One of the key challenges is the coordination and integration of molecular and phenotypic data. Effective marker-assisted selection (MAS) requires precise phenotyping to validate the association between markers and target traits. This necessitates robust phenotyping protocols and infrastructure, which can be resource-intensive. For instance, in rice breeding, the successful pyramidization of multiple resistance genes required meticulous phenotyping and molecular analysis at each step to monitor the transfer of target alleles (Ludwików et al., 2015). The integration of phenotypic and genotypic data is crucial for the success of MAS and requires careful planning and execution.

Another challenge is the need for continuous monitoring and updating of breeding strategies to adapt to changing pathogen populations and environmental conditions. The durability of resistance conferred by major resistance genes can be compromised by the rapid evolution of pathogen races. Therefore, breeding programs must incorporate strategies to combine major and minor resistance genes to achieve durable resistance. This involves the use of marker-assisted backcrossing (MABC) and gene pyramiding techniques to stack multiple resistance genes into a single cultivar (Thomson et al., 2009; Pathania et al., 2017). The management of such complex breeding programs requires a multidisciplinary approach, involving molecular biologists, geneticists, and plant breeders, to ensure the successful implementation of MAS and the development of improved crop varieties.

9 Concluding Remarks

The systematic review of the literature on enhancing disease resistance and yield in Welsh onion through marker-assisted breeding has highlighted several key findings. Marker-assisted selection (MAS) and genomic selection (GS) have emerged as powerful tools in plant breeding, offering significant advantages over traditional methods. MAS has been particularly effective in cases where disease resistance is controlled by one or a few major genes, as it allows for precise and efficient selection of desirable traits. However, for traits governed by multiple minor genes, GS has shown greater promise due to its ability to handle complex genetic architectures and provide more durable resistance.

The importance of integrated breeding approaches cannot be overstated. Combining MAS with traditional phenotypic selection and other molecular tools can accelerate the development of disease-resistant varieties while maintaining high yield and quality. For instance, the use of molecular markers linked to cytoplasmic male sterility in Welsh onion has demonstrated the potential to significantly reduce the labor and cost associated with breeding programs. Additionally, the development of markers for specific diseases, such as gray mold in onions, underscores the utility of these tools in addressing specific breeding challenges.

Future breeding programs should focus on the continued integration of advanced genomic technologies with conventional breeding methods. The development of high-throughput genotyping platforms and the application of next-generation sequencing can further enhance the efficiency and precision of breeding efforts. Moreover, the validation and implementation of markers across diverse breeding populations will be crucial for ensuring the broad applicability and success of these approaches. Emphasizing the development of markers for both major and minor resistance genes will provide a more comprehensive strategy for disease resistance breeding.

In conclusion, the integration of MAS and GS into Welsh onion breeding programs holds great potential for enhancing disease resistance and yield. By leveraging the strengths of both traditional and modern breeding techniques, future programs can achieve more robust and sustainable improvements in crop performance. Continued research and innovation in this field will be essential for meeting the growing demands of agriculture and ensuring food security.

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Conflict of Interest Disclosure

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