Plant Gene and Traits 2024, Vol.15, No.4, 174-183
http://genbreedpublisher.com/index.php/pgt

Research Insight Open Access

Cloning, Functional Analysis, and Breeding Application of the Powdery Mildew Resistance Gene *MlWE74* in Wheat

Feng Huang ✉, Xiaoyu Du, Shaokui Zou, Lina Wang, Yulin Han
Zhoukou Academy of Agricultural Sciences, Zhoukou, 466001, Henan, China
✉ Corresponding email: huangfeng0714@163.com

Plant Gene and Trait, 2024, Vol.15, No.4 doi: 10.5376/pgt.2024.15.0018
Received: 22 Jun., 2024
Accepted: 23 Jul., 2024
Published: 31 Jul., 2024

Preferred citation for this article:
Huang F., Du X.Y., Zou S.K., Wang L.N., and Han Y.L., 2024, Cloning, functional analysis, and breeding application of the powdery mildew resistance gene *MlWE74* in wheat, Plant Gene and Trait, 15(4): 174-183 (doi: 10.5376/pgt.2024.15.0018)

Abstract The *MlWE74* gene, a novel powdery mildew resistance gene, has garnered widespread attention for its potential to enhance disease resistance in wheat. This study provides a comprehensive summary of the positional cloning, functional analysis, and breeding application of the wheat powdery mildew resistance gene *MlWE74*. A systematic analysis of the cloning strategy, functional mechanisms, and its application in molecular breeding was conducted, evaluating its contributions to disease resistance improvement and its practical value in modern wheat breeding. The findings reveal that the *MlWE74* gene holds significant potential for use in wheat disease resistance breeding. Its functional analysis and breeding applications will further advance the development of resistant wheat varieties. Future research should focus on in-depth studies of the *MlWE74* gene, combining gene editing and multi-gene resistance strategies to enhance its breeding efficiency. The successful cloning and functional analysis of the *MlWE74* gene offer new perspectives and tools for the development of powdery mildew-resistant wheat varieties. With the advancement of genome selection, marker-assisted breeding, and gene editing technologies, the application of *MlWE74* is expected to greatly improve disease resistance in wheat varieties, contributing to global food security.

Keywords Wheat; Powdery mildew; *MlWE74* gene; Positional cloning; Molecular breeding

1 Introduction

Wheat powdery mildew, caused by the fungal pathogen *Blumeria graminis f.* sp. *tritici* (Bgt), is a significant threat to wheat production worldwide. This disease can lead to substantial yield losses and reduced grain quality, posing a major challenge to global food security (Li et al., 2017; Zhao et al., 2017; Mapuranga et al., 2022). The utilization of genetic resistance is one of the most effective and environmentally friendly strategies to combat this disease. Wild emmer wheat (*Triticum turgidum* var. *dicoccoides*), a progenitor of modern wheat, has been identified as a valuable genetic resource for enhancing disease resistance in common wheat (Huang and Röder, 2004; Zhu et al., 2021).

The powdery mildew resistance gene *MlWE74*, derived from wild emmer wheat accession G-748-M, has shown promise in conferring resistance to this devastating disease. Genetic analysis has revealed that *MlWE74* is a single dominant gene located on the terminal region of chromosome 2BS. The gene is flanked by molecular markers WGGBD412 and WGGBH346 within a 0.25 cM genetic interval, corresponding to a 799.9 kb genomic region in the Zavitan reference sequence (Zhu et al., 2021). The identification and functional analysis of *MlWE74* provide critical insights into the molecular mechanisms underlying powdery mildew resistance and offer potential for its application in wheat breeding programs.

Despite the identification of numerous powdery mildew resistance genes, breeding for durable resistance remains challenging. The rapid evolution of the pathogen and the breakdown of resistance genes necessitate the continuous discovery and deployment of new resistance genes (Li et al., 2020). Traditional breeding methods are often time-consuming and labor-intensive. Therefore, gene-based disease resistance breeding strategies, such as marker-assisted selection (MAS), are essential for the efficient and precise incorporation of resistance genes into elite cultivars (Huang and Röder, 2004; Kang et al., 2020). The development of molecular markers linked to resistance genes, such as the co-segregated marker WGGBD425 for *MlWE74*, facilitates the rapid and accurate transfer of these genes into breeding lines (Zhu et al., 2021).

This study comprehensively analyzes the genetic and molecular basis of the *MlWE74* gene, with a particular focus on its role and signaling pathways in powdery mildew resistance. It discusses relevant research methods and experimental findings, while identifying the main challenges and future directions for the application of *MlWE74* in wheat disease resistance breeding. The study aims to provide a thorough overview of the cloning, functional analysis, and breeding applications of the powdery mildew resistance gene *MlWE74* in wheat.

2 Positional Cloning of the *MlWE74* Gene

2.1 Positional cloning strategy for the *MlWE74* gene

The positional cloning of the *MlWE74* gene, which confers resistance to powdery mildew in wheat, involves several strategic steps. Initially, the gene was identified in the hexaploid wheat line WE74, derived from wild emmer wheat (*Triticum turgidum* var. *dicoccoides*). Genetic analysis revealed that the resistance is controlled by a single dominant gene, temporarily designated *MlWE74*. The strategy began with bulked segregant analysis (BSA) and molecular mapping, which localized *MlWE74* to the terminal region of chromosome 2BS. This region was flanked by markers WGGBD412 and WGGBH346 within a genetic interval of 0.25 cM, corresponding to a 799.9 kb genomic region in the Zavitan reference sequence (Zhu et al., 2021). Further refinement of the positional cloning strategy involved the use of high-density molecular markers and comparative genomics. This approach has been successfully applied in other studies, such as the mapping of the Pm3b gene in hexaploid wheat, where the combined analysis of genomes from wheat species with different ploidy levels facilitated the establishment of a physical contig spanning the Pm3 locus (Yahiaoui et al., 2004). Similarly, the identification of closely linked markers and the construction of a genetic linkage map were crucial steps in the positional cloning of *MlWE74*.

2.2 Fine mapping based on high-density molecular markers

Fine mapping of the *MlWE74* gene was achieved through the use of high-density molecular markers. This process involved the development and utilization of markers that are closely linked to the resistance gene. In the case of *MlWE74*, the gene was delimited to a 799.9 kb genomic region on chromosome 2BS, flanked by markers WGGBD412 and WGGBH346 (Zhu et al., 2021). The identification of these markers was facilitated by bulked segregant analysis (BSA) and molecular mapping techniques. The importance of high-density molecular markers in fine mapping is underscored by other studies, such as the mapping of the MlIW39 gene, which was localized to a 460.3 kb genomic interval on wheat chromosome arm 2BS using molecular markers (Qiu et al., 2021). Similarly, the fine mapping of the Pm4b gene involved the development of SNP markers from transcriptome sequencing data, which were then used to construct a genetic linkage map (Wu et al., 2018). These examples highlight the critical role of high-density molecular markers in narrowing down the candidate region for the resistance gene and facilitating its eventual cloning.

2.3 Genome sequencing and genome-wide association study (GWAS)

Genome sequencing and genome-wide association studies (GWAS) are powerful tools that complement the positional cloning and fine mapping efforts for the *MlWE74* gene. Genome sequencing provides a comprehensive view of the genetic landscape, allowing for the identification of candidate genes within the mapped region. In the case of *MlWE74*, sequence annotation revealed several candidate genes, including two phosphoglycerate mutase-like genes, an alpha/beta-hydrolases gene, and five NBS-LRR disease resistance genes (Zhu et al., 2021).

GWAS further enhances the identification of resistance genes by associating genetic variants with phenotypic traits across a diverse population. This approach has been successfully applied in the identification of other powdery mildew resistance genes, such as Pm5e, where a rare single nucleotide variant (SNV) within the C-terminal leucine-rich repeat (LRR) domain was found to confer resistance (Xie et al., 2020). Similarly, the use of bulked segregant RNA sequencing (BSR-Seq) in the mapping of the PmPBDH gene demonstrated the utility of combining genome sequencing with association studies to pinpoint resistance genes (Liang et al., 2022).

3 Functional Analysis of the *MlWE74* Gene

3.1 Predicted function and encoded product of the *MlWE74* gene

The *MlWE74* gene, derived from wild emmer wheat (*Triticum turgidum* var. *dicoccoides*), has been identified as a significant contributor to powdery mildew resistance in wheat. Genetic analysis has shown that *MlWE74* is a

single dominant gene located on the terminal region of chromosome 2BS. Sequence annotation within this region has revealed the presence of several candidate genes, including two phosphoglycerate mutase-like genes, an alpha/beta-hydrolases gene, and five NBS-LRR (nucleotide-binding site leucine-rich repeat) disease resistance genes (Zhu et al., 2021). The NBS-LRR genes are particularly noteworthy as they are commonly associated with plant immune responses, suggesting that *MlWE74* likely encodes a protein involved in pathogen recognition and subsequent activation of defense mechanisms.

3.2 Expression patterns and regulatory mechanisms of *MlWE74*

The expression patterns and regulatory mechanisms of *MlWE74* are crucial for understanding its role in conferring resistance to powdery mildew. Studies have shown that the expression of resistance genes can be influenced by various factors, including developmental stage and environmental conditions. For instance, the resistance gene *PmPBDH*, which also confers resistance to powdery mildew, exhibits differential expression at seedling and adult-plant stages, indicating that temporal regulation is a key aspect of its function (Liang et al., 2022). Similarly, the *MlWE74* gene may exhibit stage-specific expression patterns that enhance its effectiveness against *Blumeria graminis f.* sp. *tritici* (Bgt).

Moreover, the regulatory mechanisms of *MlWE74* may involve alternative splicing, a process that generates multiple protein isoforms from a single gene. This has been observed in other resistance genes, such as Pm4, which produces two isoforms with different domain topologies essential for its resistance function (Sánchez-Martín et al., 2021). The alternative splicing of *MlWE74* could potentially generate diverse protein products that enhance its ability to recognize and respond to various pathogen strains.

In addition to alternative splicing, the localization of the encoded protein plays a significant role in its function. For example, the Pm4 protein localizes to the endoplasmic reticulum (ER), where it likely interacts with other components of the immune signaling pathway (Sánchez-Martín et al., 2021). The *MlWE74* protein may similarly localize to specific cellular compartments, facilitating its role in pathogen detection and signal transduction.

3.3 Research on the disease resistance mechanism of *MlWE74*

The *MlWE74* gene, derived from wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*), plays a critical role in providing resistance to powdery mildew caused by *Blumeria graminis* f. sp. *tritici*. The resistance mechanism of *MlWE74* is primarily attributed to its ability to trigger early defense responses in wheat, including the activation of hypersensitive response (HR) and the production of reactive oxygen species (ROS), which are crucial in preventing the spread of the pathogen. Studies have shown that plants carrying *MlWE74* exhibit rapid localized cell death at infection sites, effectively limiting fungal growth (Zhu et al., 2021). Furthermore, *MlWE74* belongs to the NBS-LRR class of disease resistance genes, which are known for recognizing specific pathogen effectors and initiating a cascade of defense signals. Genetic mapping has identified five NBS-LRR genes within the *MlWE74* region, and these genes are likely responsible for the recognition of powdery mildew effectors, triggering a resistance response (Zhang et al., 2015). These findings suggest that *MlWE74* confers broad-spectrum resistance, making it a valuable gene for breeding programs aimed at developing durable resistance to powdery mildew.

3.4 Gene knockout and overexpression experiments

Gene knockout and overexpression experiments have been instrumental in understanding the functionality of *MlWE74*. In gene knockout studies, wheat plants with the *MlWE74* gene knocked out were significantly more susceptible to powdery mildew, indicating that *MlWE74* is essential for the plant's defense against this pathogen. The loss of resistance in these plants was accompanied by reduced ROS production and a weakened hypersensitive response, further confirming *MlWE74*'s role in activating defense mechanisms (Zhang et al., 2009). Conversely, overexpression of *MlWE74* in transgenic wheat lines led to enhanced resistance, with these plants showing stronger and faster defense responses upon infection. Overexpression also resulted in increased accumulation of resistance proteins and defense-related enzymes, indicating that *MlWE74* acts as a key regulator in the plant's immune system. These findings highlight the potential of using *MlWE74* in breeding programs to

develop wheat varieties with improved powdery mildew resistance through marker-assisted selection (MAS) and genetic engineering approaches (Zhang et al., 2015).

4 Application of the *MlWE74* Gene in Wheat Breeding

4.1 Marker-assisted selection breeding of the *MlWE74* resistance gene

Marker-assisted selection (MAS) is a powerful tool in modern plant breeding, allowing for the precise incorporation of desirable traits such as disease resistance (Yang et al., 2008; Miedaner and Korzun, 2012; Fang, 2024). The *MlWE74* gene, derived from wild emmer wheat, has been identified as a key resistance gene against powdery mildew in wheat. Genetic analysis has shown that *MlWE74* is controlled by a single dominant gene, making it an ideal candidate for MAS. The gene has been mapped to the terminal region of chromosome 2BS, flanked by markers WGGBD412 and WGGBH346 within a 0.25 cM genetic interval. The co-segregated marker WGGBD425 is particularly useful for the marker-assisted transfer of *MlWE74* into elite wheat cultivars, facilitating the development of resistant lines with desirable agronomic traits (Zhu et al., 2021).

4.2 Application of transgenic and gene editing technologies in breeding

Transgenic and gene editing technologies offer innovative approaches to enhance disease resistance in wheat. The functional validation of resistance genes like *MlWE74* can be achieved through transgenic assays and gene editing techniques such as CRISPR/Cas9 (Mushtaq et al., 2019). For instance, the Pm5e gene, another powdery mildew resistance gene, was validated using transgenic assays and loss-of-function mutants, demonstrating the potential of these technologies in confirming gene function and facilitating their use in breeding programs (Xie et al., 2020). By applying similar methodologies, the *MlWE74* gene can be precisely edited or introduced into susceptible wheat varieties, thereby conferring resistance to powdery mildew and improving overall crop resilience.

4.3 Innovative utilization of disease-resistant germplasm

The utilization of disease-resistant germplasm is crucial for sustainable wheat production (Kumar et al., 2022). Wild emmer wheat, the source of the *MlWE74* gene, represents a valuable genetic resource for breeding programs aimed at enhancing disease resistance (Zhu et al., 2021). The identification and characterization of resistance genes from diverse germplasm, such as the Pm10V-2 gene from a wheat breeding line, highlight the importance of exploring and utilizing genetic diversity (Ma et al., 2017). By incorporating genes like *MlWE74* into breeding programs, it is possible to develop new wheat varieties with enhanced resistance to powdery mildew, thereby reducing the reliance on chemical fungicides and promoting sustainable agricultural practices.

5 Phenotypic Evaluation and Breeding Performance of the *MlWE74* Gene

5.1 Field performance of wheat varieties resistant to powdery mildew

The field performance of wheat varieties carrying the *MlWE74* gene has shown promising results in terms of resistance to powdery mildew. The gene, derived from wild emmer wheat (*Triticum turgidum* var. *dicoccoides*), has been successfully transferred to hexaploid wheat line WE74, which exhibits strong resistance to the disease. This resistance is controlled by a single dominant gene, *MlWE74*, which has been fine-mapped to a specific region on chromosome 2BS. The geographical distribution of *MlWE74* indicates its prevalence in regions with favorable conditions for powdery mildew, such as Rosh Pinna and Amirim in northern Israel, suggesting its potential effectiveness in similar environments globally (Zhu et al., 2021). Field trials have demonstrated that wheat varieties with the *MlWE74* gene maintain high levels of resistance throughout various growth stages. This is consistent with findings from other studies where resistance genes derived from wild emmer wheat, such as MlIW39, have shown robust performance against multiple isolates of *Blumeria graminis f.* sp. *tritici* (Bgt) (Qiu et al., 2021). The use of marker-assisted selection (MAS) has facilitated the efficient transfer of *MlWE74* into elite cultivars, enhancing their resistance profiles without compromising agronomic performance (Qiu et al., 2021; Zhu et al., 2021).

5.2 Correlation analysis between disease resistance and agronomic traits

Correlation analysis between disease resistance conferred by the *MlWE74* gene and various agronomic traits is crucial for understanding its overall impact on wheat cultivation. Studies have shown that the introduction of resistance genes like *MlWE74* does not adversely affect key agronomic traits such as yield, plant height, and grain

quality. For instance, the wheat breeding line 10V-2, which carries the *Pm10V-2* resistance gene, has demonstrated superior agronomic performance alongside its resistance to powdery mildew (Ma et al., 2017). This suggests that resistance genes can be integrated into breeding programs without detrimental effects on other important traits. Further analysis has revealed that the presence of resistance genes can sometimes be associated with improved agronomic performance. For example, the Pm5e gene, identified in a Chinese wheat landrace, not only provides resistance to powdery mildew but also contributes to the overall robustness of the plant (Figure 1) (Xie et al., 2020). This indicates that the *MlWE74* gene, similar to other resistance genes, may have a positive correlation with certain agronomic traits, making it a valuable addition to wheat breeding programs (Xie et al., 2020; Zhu et al., 2021).

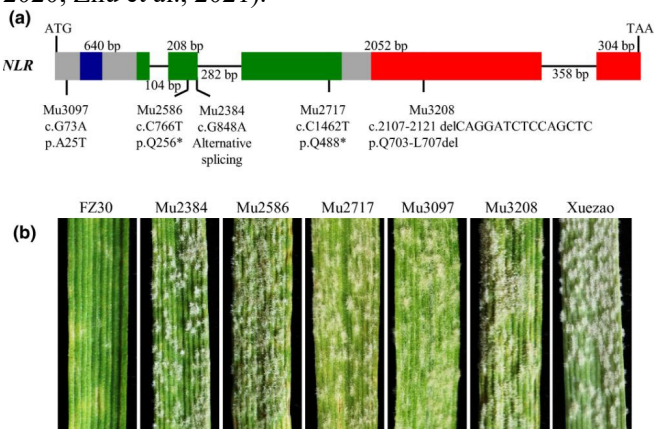

Figure 1 Validation of the candidate *NLR* gene for the powdery mildew resistance gene Pm5e and phenotypes of EMS mutants (Adapted from Xie et al., 2020)

Image caption: (a): Structure of the *NLR* gene and its mutants, showing the gene structure from the start to stop codon. Blue, green, and red represent the coiled-coil (CC-like) domain, nucleotide-binding site (NBS), and leucine-rich repeat (LRR) domain, respectively. Vertical short lines indicate the mutation sites, with the changes in the coding sequence (c.) and the predicted effects on the translated protein (p.) annotated below; (b): Infection phenotypes of resistant variety FZ30, susceptible control Xuezao, and five susceptible mutants 14 days after inoculation with powdery mildew fungus (*Blumeria graminis* f. sp. *tritici*) (Adapted from Xie et al., 2020)

Xie et al. (2020) validated the powdery mildew resistance gene *Pm5e* in wheat through EMS mutants, and their study revealed that multiple mutation sites in *NLR* genes were associated with the loss of disease resistance. In the research, the resistant variety FZ30 showed strong disease resistance, while different mutants, due to mutations at specific gene sites, exhibited increased susceptibility to powdery mildew, indicating that these mutations affected the function of the *Pm5e* gene. This study provides an important molecular foundation for wheat disease resistance breeding. From this, it can be concluded that wheat powdery mildew resistance genes such as *MlWE74* and Pm5e can help in breeding varieties with stable resistance to powdery mildew, reducing the use of pesticides and improving wheat yield and quality.

5.3 Successful cases in breeding programs

Several successful cases in breeding programs highlight the effectiveness of incorporating the *MlWE74* gene into wheat varieties. One notable example is the development of the wheat line WE74, which has been extensively tested and shown to possess strong resistance to powdery mildew due to the *MlWE74* gene (Zhu et al., 2021). The use of molecular markers has been instrumental in tracking the gene during the breeding process, ensuring its successful integration into new cultivars. Another successful case involves the wheat breeding line Yannong 99102-06188, which carries a different resistance gene, *pmYN99102*. This line has shown high resistance to powdery mildew across various growth stages and has been effectively used in breeding programs to develop new resistant varieties (Mu et al., 2022). The success of these programs underscores the potential of resistance genes like *MlWE74* in enhancing the disease resistance of wheat cultivars while maintaining or even improving their agronomic performance (Zhu et al., 2021; Mu et al., 2022).

6 Interaction Between *MlWE74* and Other Disease Resistance Genes and Its Synergistic Breeding Strategy

6.1 Research on the interaction between *MlWE74* and other powdery mildew resistance genes

The *MlWE74* gene is a crucial resistance gene against powdery mildew in wheat, and its interaction with other resistance genes has been the focus of many studies. Research indicates that combining *MlWE74* with other powdery mildew resistance genes such as *Pm21*, *Pm4a*, and *Pm2* can significantly enhance the overall resistance in wheat. These interactions are facilitated by the gene's ability to activate diverse defense pathways, complementing the actions of other resistance genes. For instance, a study on pyramiding different resistance genes found that wheat lines combining *Pm2*, *Pm4a*, and *Pm21* showed higher levels of resistance compared to lines with a single gene (Wang et al., 2001). The synergistic effect of *MlWE74* with other genes stems from its ability to trigger both early and late-stage defense mechanisms. This includes hypersensitive responses at the site of infection, which limits pathogen spread, and systemic acquired resistance, which boosts the plant's overall immunity. Such interactions demonstrate that *MlWE74* plays a central role in enhancing resistance when combined with other genes, making it an excellent candidate for multi-gene resistance strategies in wheat breeding (Liu et al., 2000).

6.2 Multi-gene pyramiding breeding strategy

Pyramiding multiple resistance genes is a powerful breeding strategy aimed at enhancing the durability and breadth of disease resistance in wheat (Wang et al., 2018). Marker-assisted selection (MAS) has enabled the efficient stacking of multiple powdery mildew resistance genes, including *MlWE74*, *Pm21*, *Pm4a*, and *Pm2*. Studies show that wheat varieties carrying pyramided genes exhibit broader spectrum resistance and increased durability compared to those carrying single resistance genes. A successful example of this approach is the pyramiding of *Pm21* and *Pm4a* in elite wheat cultivars, resulting in high resistance levels to powdery mildew under various environmental conditions (Pietrusińska and Czembor, 2017). Incorporating multiple genes like *MlWE74* into breeding programs is particularly valuable because each gene may target different stages of the pathogen's life cycle or activate different defense pathways. This creates a more comprehensive and durable resistance, reducing the likelihood of the pathogen overcoming the resistance. MAS has been instrumental in tracking these genes in breeding populations, ensuring that the pyramided plants possess the desired combinations of resistance traits (Dong et al., 2014).

6.3 Relationship between resistance durability and pathogen evolution

The durability of resistance genes like *MlWE74* is closely linked to the evolutionary dynamics of the pathogen, *Blumeria graminis* f. sp. *tritici*. Resistance genes that are deployed as single factors are often quickly overcome by pathogen evolution, as the pathogen adapts to the specific defense mechanisms activated by the gene. However, multi-gene pyramiding, where multiple resistance genes are combined, has been shown to delay the breakdown of resistance. This is because the pathogen must simultaneously overcome multiple defense strategies, which significantly reduces the likelihood of resistance being eroded over time (Stirnweis et al., 2014). In the context of pathogen evolution, genes like *MlWE74*, when combined with others, create a more resilient system that can adapt to changes in pathogen virulence. Continuous monitoring of pathogen populations and understanding their evolutionary pressures are critical for maintaining resistance durability. By using pyramiding strategies, the arms race between host plants and pathogens can be managed more effectively, ensuring long-term protection against diseases like powdery mildew (Huang and Röder, 2004).

7 Future Research Directions and Challenges

7.1 In-depth functional study of the *MlWE74* gene

The *MlWE74* gene, identified in wild emmer wheat and transferred to hexaploid wheat line WE74, has shown promising resistance to powdery mildew. However, further functional studies are essential to fully understand the mechanisms by which *MlWE74* confers resistance. Fine mapping has identified several candidate genes, including NBS-LRR disease resistance genes, which could be pivotal in the map-based cloning of *MlWE74* (Zhu et al., 2021). Future research should focus on characterizing these candidate genes through gene expression analysis, protein function studies, and gene knockout experiments to elucidate their roles in powdery mildew resistance.

7.2 Addressing genetic complexity in disease resistance breeding

Breeding for disease resistance in wheat is inherently complex due to the polygenic nature of traits like powdery mildew resistance. The resistance conferred by *MlWE74* is controlled by a single dominant gene, but integrating this gene into elite cultivars requires careful consideration of genetic background and environmental interactions (Zhu et al., 2021; Bapela et al., 2023). The use of high-throughput genotyping and marker-assisted selection (MAS) can enhance the efficiency of breeding programs by enabling the precise transfer of resistance genes with minimal linkage drag (Figure 2) (Bapela et al., 2023; Jiang, 2024). Additionally, multi-environment trials are necessary to ensure the stability and effectiveness of resistance across different growing conditions.

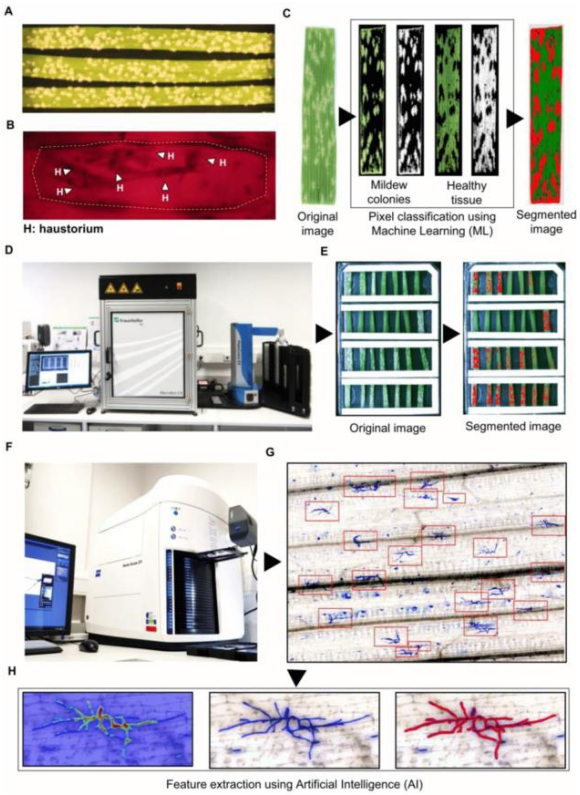

Figure 2 Precision phenotypic analysis of wheat resistance to powdery mildew using Machine Learning (ML) and Artificial Intelligence (AI) techniques (Adapted from Bapela et al., 2023)

Image caption: A: Powdery mildew colonies of the reference genome isolate Bgt_96224 on the susceptible wheat variety Chinese Spring; B: Haustoria structures of multiple powdery mildew fungi revealed in wheat epidermal cells via optical microscopy; C: Pixel classification phenotype analysis of powdery mildew leaf coverage using machine learning (ML); D: The fully automated high-throughput image acquisition system Macrobot 2.0 at IPK, Germany; E: Example of ML-assisted feature extraction from images taken by Macrobot 2.0; F: The high-performance Zeiss AxioScan.Z1 microscope used for automated microscopic phenotype acquisition; G, H: Convolutional neural network (CNN)-assisted computational visualization of powdery mildew microcolonies and associated fungal structures (Adapted from Bapela et al., 2023)

Bapela et al. (2023) applied advanced machine learning (ML) and artificial intelligence (AI) technologies in precision breeding for wheat resistance to powdery mildew. With the assistance of ML and convolutional neural networks (CNN), researchers were able to accurately characterize the colony coverage and microcolony structures of powdery mildew fungi, enhancing the efficiency and accuracy of phenotypic analysis. These automated and high-throughput phenotyping tools contribute to accelerating the progress of disease-resistant breeding and provide strong technical support for improving disease resistance in crops such as wheat.

7.3 Global promotion and application of the *MlWE74* gene in wheat breeding

To maximize the impact of the *MlWE74* gene, it is crucial to promote its use in global wheat breeding programs. This involves not only the transfer of *MlWE74* into diverse wheat cultivars but also the dissemination of knowledge and resources related to this gene. The co-segregated marker WGGBD425 identified in the fine

mapping study can facilitate the marker-assisted transfer of *MlWE74* into elite cultivars, thereby accelerating the breeding process (Zhu et al., 2021). Collaborative efforts among international research institutions and breeding programs can help in the widespread adoption of *MlWE74*, ultimately contributing to global food security by enhancing wheat resistance to powdery mildew.

8 Concluding Remarks

The powdery mildew resistance gene *MlWE74*, derived from wild emmer wheat (*Triticum turgidum* var. *dicoccoides*), has been successfully transferred to hexaploid wheat line WE74. Genetic analysis has shown that *MlWE74* is a single dominant gene located on the terminal region of chromosome 2BS. Through bulked segregant analysis (BSA) and molecular mapping, *MlWE74* was delimited to a 0.25 cM genetic interval, corresponding to a 799.9 kb genomic region in the Zavitan reference sequence. This region contains several candidate genes, including two phosphoglycerate mutase-like genes, an alpha/beta-hydrolases gene, and five NBS-LRR disease resistance genes, which are crucial for the map-based cloning of *MlWE74*.

The identification of co-segregated markers, such as WGGBD425, facilitates the marker-assisted transfer of *MlWE74* into elite wheat cultivars, enhancing their resistance to powdery mildew. This gene's geographical distribution, primarily in the Rosh Pinna and Amirim regions of northern Israel, underscores its adaptation to environments conducive to powdery mildew occurrence. The successful cloning and functional analysis of the *MlWE74* gene represent a significant advancement in the fight against powdery mildew in wheat. The integration of *MlWE74* into breeding programs through marker-assisted selection (MAS) can lead to the development of wheat varieties with enhanced resistance to powdery mildew, thereby reducing the reliance on chemical fungicides and promoting sustainable agricultural practices.

Furthermore, the detailed molecular mapping and understanding of the *MlWE74* gene's structure and function provide a foundation for the isolation of additional resistance genes and the development of robust molecular markers. These markers are essential for pyramiding multiple resistance genes, which can offer durable and broad-spectrum resistance to powdery mildew and other diseases. In the long term, the application of *MlWE74* in breeding programs has the potential to significantly boost global wheat production by mitigating yield losses caused by powdery mildew. This will not only enhance food security but also contribute to the economic stability of wheat-producing regions worldwide. The continued exploration and utilization of wild wheat genetic resources, such as those from wild emmer wheat, will be crucial in addressing the challenges posed by evolving pathogen populations and changing environmental conditions.

Acknowledgments

GenBreed Publisher extend the sincere thanks to two anonymous peer reviewers for their feedback on the manuscript of this study.

Funding

This study was funded by the Henan Modern Agricultural Industry Technology System Construction Special Project (HARS-22-1-Z7).

Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Bapela T., Shimelis H., Terefe T., Bourras S., Sánchez-Martín J., Douchkov D., Desiderio F., and Tsilo T., 2023, Breeding wheat for powdery mildew resistance: genetic resources and methodologies-a review, Agronomy, 13(4): 1173. https://doi.org/10.3390/agronomy13041173

Dong N., 2014, Molecular marker assisted pyramid breeding of powdery mildew resistance gene *Pm21* and *Pm13*, Journal of Triticeae Crops, 34(12): 1639-1644.

Fang J., 2024, Breeding 5.0: AI-driven revolution in designed plant breeding, Molecular Plant Breeding, 15(1): 27-33.
https://doi.org/10.5376/mpb.2024.15.0004

Huang X., and Röder M., 2004, Molecular mapping of powdery mildew resistance genes in wheat: a review, Euphytica, 137: 203-223.
https://doi.org/10.1023/B:EUPH.0000041576.74566.d7

Jiang C., 2024, Genetic mechanisms of crop disease resistance: new advances in GWAS, Plant Gene and Trait, 15(1): 15-22.
https://doi.org/10.5376/pgt.2024.15.0003

Kang Y., Zhou M., Merry A., and Barry K., 2020, Mechanisms of powdery mildew resistance of wheat - a review of molecular breeding, Plant Pathology, 69: 601-617.
https://doi.org/10.1111/ppa.13166

Kumar S., Jacob S., Mir R., Vikas V., Kulwal P., Chandra T., Kaur S., Kumar U., Kumar S., Sharma S., Singh R., Prasad S., Singh A., Singh A., Kumari J., Saharan M., Bhardwaj S., Prasad M., Kalia S., and Singh K., 2022, Indian wheat genomics initiative for harnessing the potential of wheat germplasm resources for breeding disease-resistant, nutrient-dense, and climate-resilient cultivars, Frontiers in Genetics, 13: 834366.
https://doi.org/10.3389/fgene.2022.834366
PMid:35846116 PMCid:PMC9277310

Li J., Yang X., Li Y., Niu J., and He D., 2017, Proteomic analysis of developing wheat grains infected by powdery mildew (*Blumeria graminis* f.sp. *tritici*), Journal of Plant Physiology, 215: 140-153.
https://doi.org/10.1016/j.jplph.2017.06.003
PMid:28646681

Li M., Dong L., Li B., Wang Z., Xie J., Qiu D., Li Y., Shi W., Yang L., Wu Q., Chen Y., Lu P., Guo G., Zhang H., Zhang P., Zhu K., Li Y., Zhang Y., Wang R., Yuan C., Liu W., Yu D., Luo M., Fahima T., Nevo E., Li H., and Liu Z., 2020, A CNL protein in wild emmer wheat confers powdery mildew resistance, The New Phytologist, 228(3): 1027-1037.
https://doi.org/10.1111/nph.16761
PMid:32583535

Liang X., Xu H., Zhu S., Zheng Y., Zhong W., Li H., Niu L., Wu L., Zhang L., Song J., He H., Liu C., and Ma P., 2022, Genetically dissecting the novel powdery mildew resistance gene in the wheat breeding line PBDH1607, Plant Disease, 106(8): 2145-2154.
https://doi.org/10.1094/PDIS-12-21-2771-RE
PMid:35108069

Liu J., Liu D., Tao W., Li W., Wang S., Chen P., Cheng S., and Gao D., 2000, Molecular marker-facilitated pyramiding of different genes for powdery mildew resistance in wheat, Plant Breeding, 119: 21-24.

Ma P., Xu H., Xu Y., Song L., Liang S., Sheng Y., Han G., Zhang X., and An D., 2017, Characterization of a powdery mildew resistance gene in wheat breeding line 10V-2 and its application in marker-assisted selection, Plant Disease, 102(5): 925-931.
https://doi.org/10.1094/PDIS-02-17-0199-RE
PMid:30673391

Mapuranga J., Chang J., and Yang W., 2022, Combating powdery mildew: advances in molecular interactions between *Blumeria graminis* f. sp. *tritici* and wheat, Frontiers in Plant Science, 13: 1102908.
https://doi.org/10.3389/fpls.2022.1102908
PMid:36589137 PMCid:PMC9800938

Miedaner T., and Korzun V., 2012, Marker-assisted selection for disease resistance in wheat and barley breeding, Phytopathology, 102(6): 560-566.

Mu Y., Gong W., Qie Y., Liu X., Li L., Sun N., Liu W., Guo J., Han R., Yu Z., Xiao L., Su F., Zhang W., Wang J., Han G., and Ma P., 2022, Identification of the powdery mildew resistance gene in wheat breeding line Yannong 99102-06188 via bulked segregant exome capture sequencing, Frontiers in Plant Science, 13: 1005627.
https://doi.org/10.3389/fpls.2022.1005627
PMid:36147228 PMCid:PMC9489141

Mushtaq M., Sakina A., Wani S., Shikari A., Tripathi P., Zaid A., Galla A., Abdelrahman M., Sharma M., Singh A., and Salgotra R., 2019, Harnessing genome editing techniques to engineer disease resistance in plants, Frontiers in Plant Science, 10: 550.
https://doi.org/10.3389/fpls.2019.00550
PMid:31134108 PMCid:PMC6514154

Pietrusińska A., and Czembor J., 2017, Pyramiding winter wheat resistance genes (*Pm21* + *Pm34*) of powdery mildew of cereals and grasses (*Blumeria graminis* f. sp. *tritici*) Piramidowanie genów odporności (*Pm21* + *Pm34*) pszenicy ozimej na mączniaka prawdziwego zbóż i traw (*Blumeria graminis* f. sp. *tritici*), Progress in Plant Protection, 57: 41-46.

Qiu L., Liu N., Wang H., Shi X., Li F., Zhang Q., Wang W., Guo W., Hu Z., Li H., Ma J., Sun Q., and Xie C., 2021, Fine mapping of a powdery mildew resistance gene *MlIW39* derived from wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*), Theoretical and Applied Genetics, 134: 2469-2479.
https://doi.org/10.1007/s00122-021-03836-9
PMid:33987716

Sánchez-Martín J., Widrig V., Herren G., Wicker T., Zbinden H., Gronnier J., Spörri L., Praz C., Heuberger M., Kolodziej M., Isaksson J., Steuernagel B., Karafiátová M., Doležel J., Zipfel C., and Keller B., 2021, Wheat Pm4 resistance to powdery mildew is controlled by alternative splice variants encoding chimeric proteins, Nature plants, 7: 327-341.
https://doi.org/10.1038/s41477-021-00869-2

Stirnweis D., Milani S., Brunner S., Herren G., Buchmann G., Peditto D., Jordan T., and Keller B., 2014, Suppression among alleles encoding nucleotide-binding-leucine-rich repeat resistance proteins interferes with resistance in F_1 hybrid and allele-pyramided wheat plants, The Plant Journal, 79(6): 893-903.
https://doi.org/10.1111/tpj.12592
PMid:24942051

Wang F., Zhang M., Hu Y., Gan M., Jiang B., Hao M., Ning S., Yuan Z., Chen X., Chen X., Zhang L., Wu B., Liu D., and Huang L., 2022, Pyramiding of adult-plant resistance genes enhances all-stage resistance to wheat stripe rust, Plant Disease, 107(3): 879-885.
https://doi.org/10.1094/PDIS-07-22-1716-RE

Wang X., Chen P., and Zhang S., 2001, Pyramiding and marker-assisted selection for powdery mildew resistance genes in common wheat, Acta genetica Sinica, 28(7): 640-646.

Wu P., Xie J., Hu J., Qiu D., Liu Z., Li J., Li M., Zhang H., Yang L., Liu H., Zhou Y., Zhang Z., and Li H., 2018, Development of molecular markers linked to powdery mildew resistance gene *Pm4b* by combining SNP discovery from transcriptome sequencing data with bulked segregant analysis (BSR-Seq) in wheat, Frontiers in Plant Science, 9: 95.
https://doi.org/10.3389/fpls.2018.00095
PMid:29491869 PMCid:PMC5817070

Xie J., Guo G., Wang Y., Hu T., Wang L., Li J., Qiu D., Li Y., Wu Q., Lu P., Chen Y., Dong L., Li M., Zhang H., Zhang P., Zhu K., Li B., Deal K., Huo N., Zhang Y., Luo M., Liu S., Gu Y., Li H., and Liu Z., 2020, A rare single nucleotide variant in Pm5e confers powdery mildew resistance in common wheat, The New Phytologist, 228(3): 1011-1026.
https://doi.org/10.1111/nph.16762
PMid:32569398

Yahiaoui N., Srichumpa P., Dudler R., and Keller B., 2004, Genome analysis at different ploidy levels allows cloning of the powdery mildew resistance gene *Pm3b* from hexaploid wheat, The Plant Journal, 37(4): 528-538.
https://doi.org/10.1046/j.1365-313x.2003.01977.x

Yang H., Renshaw D., Thomas G., Buirchell B., and Sweetingham M., 2008, A strategy to develop molecular markers applicable to a wide range of crosses for marker assisted selection in plant breeding: a case study on anthracnose disease resistance in lupin (*Lupinus angustifolius* L.), Molecular Breeding, 21: 473-483.

Zhang D., Ouyang S., Wang L., Cui Y., Wu Q., Liang Y., Wang Z., Xie J., Zhang D., Wang Y., Chen Y., and Liu Z., 2015, Comparative genetic mapping revealed powdery mildew resistance gene *MlWE4* derived from wild emmer is located in same genomic region of *Pm36* and *Ml3D232* on chromosome 5BL, Journal of Integrative Agriculture, 14: 603-609.
https://doi.org/10.1016/S2095-3119(14)60774-7

Zhang L., Hua W., Guan H., Li G., Zhang H.T., Xie C., Yang Z., Sun Q.X., and Liu Z., 2009, Molecular mapping of powdery mildew resistance gene *MlWE29* in wheat originated from wild emmer (*Triticum turgidum* var. *dicoccoides*), Acta Agronomica Sinica, 35: 1791-1797.

Zhao J., Xu C., Xu J., Huang L., Zhang D., and Liang D., 2017, Forecasting the wheat powdery mildew (*Blumeria graminis* f. Sp. *tritici*) using a remote sensing-based decision-tree classification at a provincial scale, Australasian Plant Pathology, 47: 53-61.
https://doi.org/10.1007/s13313-017-0527-7

Zhu K., Li M., Wu H., Zhang D., Dong L., Wu Q., Chen Y., Xie J., Lu P., Guo G., Zhang H., Zhang P., Li B., Li W., Dong L., Wang Q., Zhu J., Hu W., Guo L., Wang R., Yuan C., Li H., Liu Z., and Hua W., 2021, Fine mapping of powdery mildew resistance gene *MlWE74* derived from wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) in an NBS-LRR gene cluster, Theoretical and Applied Genetics, 135: 1235-1245.
https://doi.org/10.1007/s00122-021-04027-2
PMid:35006335