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Harnessing CRISPR Genome Editing to Decode and Engineer Disease Resistance in the Wheat *Zymoseptoria tritici* Patho-system

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Abstract

Zymoseptoria tritici, the cause of *Septoria tritici* blotch (STB), is a major fungal disease of wheat (*Triticum aestivum* L.) that has long been a problem in wheat production and causes considerable losses in wheat yields globally. The hemibiotrophic lifestyle of *Z. tritici* and rapid evolution, genome plasticity and large population size make it difficult to manage the disease and susceptible to breaking deployed resistance mechanisms. Conventional breeding methods, such as quantitative resistance, QTL mapping, marker assisted selection and introgression of resistance genes have been used to improve resistance to STB, but they offer only limited capacity for long-lasting resistance, due to the host's complex genetics and the evolution of the pathogen. However, relatively little is known about the molecular factors that regulate host-pathogen interactions, resulting in limited predictability and durability in resistance. With the advent of the CRISPR/Cas genome editing system, new tools have been developed to dissect gene function and to directly edit genetic elements involved in the virulence of pathogens and the host immune response. This review highlights the use of these CRISPR technologies to reveal molecular determinants of wheat–*Z. tritici* interactions, the validation of *Z. tritici* virulence factors, and the engineering of wheat with resistance-related traits. Wheat polyploidy, gene redundancy, transformation efficiency, off-target effects, pathogen adaptation, and regulatory acceptance are significant challenges for wheat resistance strategies in the future. Future resistance strategies will combine CRISPR genome editing with multi-omics technologies, such as genomics, transcriptomics and proteomics, and with the use of AI systems for predicting the optimization of guide RNAs, characterization of the effectors and identification of resistance genes. The combination of CRISPR, computational biology and precision breeding provide next generation tools for developing sustainable wheat resistance to *Z. tritici* and new fungal threats.

Keywords:

Wheat; *Zymoseptoria tritici*; Septoria tritici blotch*;* CRISPR/Cas9; genome editing; effector biology; disease resistance; precision breeding.

Introduction

Wheat (*Triticum aestivum* L.) is a cereal crop of global importance contributing to human nutrition and agricultural systems and thus food security. Over the last decades, genetic progress and improved agronomic management practices have increased wheat productivity without expanding cropped areas (Shewry, 2026). However, these productivity gains threat from biotic stresses, especially fungal diseases that affect the stability of production. Septoria tritici blotch (STB), caused by *Zymoseptoria tritici*, is a fungal disease of wheat that can reduce yields by 5–50%, depending on the temperate region (Torriani et al., 2015). The life cycle of *Z. tritici* is complex and there is a wide diversity of populations makes it adaptable, persistent and capable of overcoming host resistance (Meile et al., 2025b). The management of *Z. tritici* is based mainly on fungicide treatment and available host resistance but has been challenged by the fungal strains that are resistant to fungicides. There is a high level of genetic diversity within *Z. tritici* populations that allows adaptation to fungicide pressure and can enable the pathogen to overcome deployed resistance genes (Ababa, 2023; Fantaw et al., 2026). While a number of resistance genes have been found in wheat, it has been difficult to develop a durable resistance due to the complexity of the

wheat genome and the ability of the pathogen to evolve rapidly (Meile et al., 2025b). It is therefore crucial to understand the molecular mechanisms involved in the interaction between wheat and *Z. tritici* to find specific targets for improvement of resistance. Advanced functional genomics approaches are needed to identify and validate pathogen genes that are important for virulence and host resistance, and to do so in a timely manner, to achieve this goal. In the past, the genetic manipulation of fungi, known as traditional fungal genetic manipulation, has increased the efficiency of introducing genes, but it is time-consuming to create and validate mutant strains when performing large-scale functional studies (Meyer et al., 2016). CRISPR/Cas9 genome editing is a powerful platform, which allows for accurate, versatile and high-throughput manipulation of fungal genomes. Multiplex CRISPR can be used to simultaneously disrupt multiple genes by coordinated expression of guide RNAs, enabling functional disruption of virulence factors, host/pathogen interactions and potential antifungal targets (Cairns et al., 2016; Pérez-Pizá et al., 2024). In addition to gene knockout, the powerful capabilities of CRISPR systems allow for targeted mutations, regulatory modification, gene fusion generation, and gene expression control. Thus, CRISPR/Cas9 is an emerging precision tool to study fungal biology and create long-lasting disease management solutions. The review is focused on genome editing techniques based on CRISPR for understanding the interaction of wheat with Z. tritici, identification of virulence factors of pathogens, and engineering disease resistance. We also highlight recent progress, ongoing problems, and prospects for using CRISPR technologies in wheat breeding for sustainability.

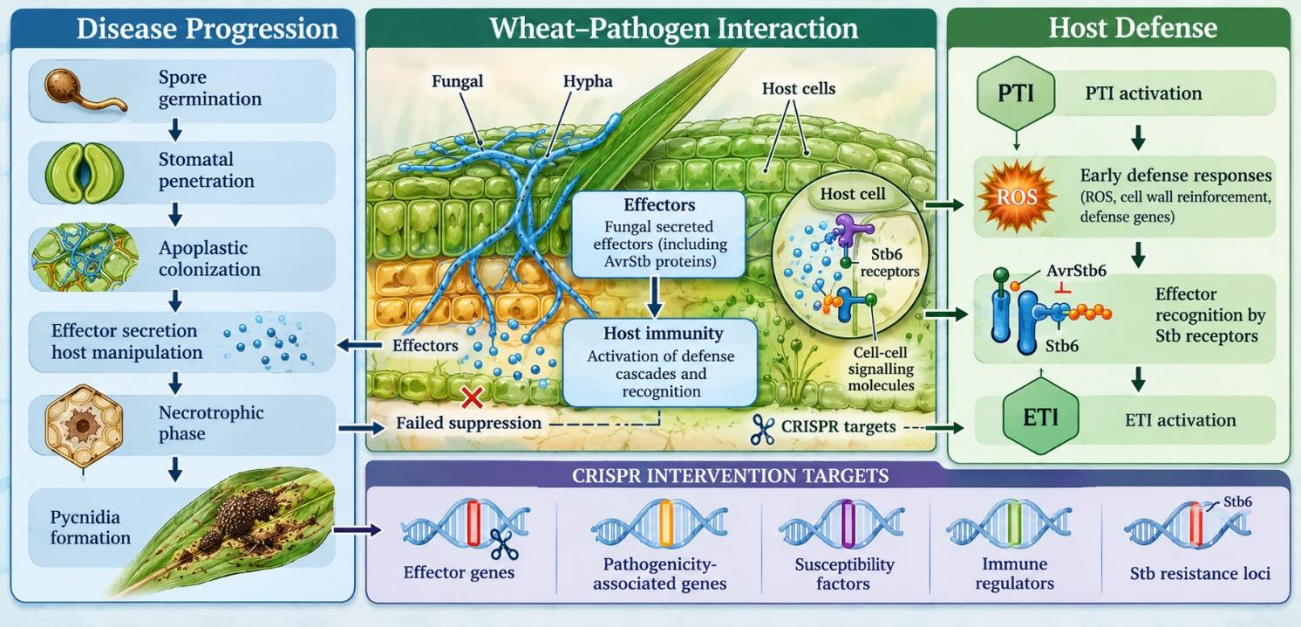
Biology and Molecular Complexity of the Wheat–*Zymoseptoria tritici* Interaction

Zymoseptoria tritici, the cause of Septoria tritici blotch (STB), is a specialized hemibiotrophic fungus that passes through an initial asymptomatic colonization phase followed by a necrotrophic stage leading to disease development. When fungal spores germinate on the wheat leaf surface and invade, they mainly enter through the stomatal openings. *Z. tritici* does not form appressoria or haustoria as most fungal pathogens do, but infects the leaf by entering through the stomata and remains in the apoplastic space between mesophyll cells during initial colonization (Battache et al., 2024; Kettles & Kanyuka, 2016). *Z. tritici* infects cereals and enters a prolonged period of asymptomatic growth with apoplastic, slow-growing and immune-response dampening activities. Successful colonization involves effector proteins which are also responsible for lowering the ability of wheat defense systems to recognize the fungus, such as LysM-containing proteins like Mg3LysM. Once colonized the pathogen switches into a necrotrophic phase, leading to host cell death, release of nutrients and its rapid proliferation, leading to the formation of lesions that are seen as chlorotic and necrotic. In the later stages of infection, fungal hyphae coalesce within the sub-stomatal cavity forming pycnidia, from which pycnidiospores are released through the stomata and can infect again with the help of rain splashes (Duba et al., 2018; Kettles & Kanyuka, 2016). This biphasic infection strategy makes resistance difficult as both resistance to disease and pathogenicity processes have to be understood. The genome of *Z. tritici* is extremely plastic and has a large degree of genetic variation, enabling an extreme degree of evolutionary adaptability. Variation in the pathogen is achieved by sequence polymorphisms, genetic recombination and activity of transposable elements, which leads to an intricate genetic structure of pathogenicity and host adaptation (Amezrou et al., 2024). Recent genome-wide association studies have revealed that quantitative pathogenicity is controlled by multiple genetic factors enabling *Z. tritici* populations to adapt to diverse wheat genotypes and environmental conditions. Dynamic regions of the genome and accessory chromosomes also play a role in the evolution of pathogens, by enabling genetic variation and adaptation. Population studies have shown that there is high genetic differentiation and local adaptation, such as to climatic factors. This evolutionary flexibility enables *Z. tritici* populations to quickly acquire virulence traits and overcome host resistance genes and chemicals. However, the genetic basis of many quantitative pathogenicity traits and adaptation mechanisms remains unresolved, creating a major barrier for targeted resistance improvement. Understanding these evolutionary mechanisms is essential for identifying stable resistance targets and applying CRISPR-based approaches for functional characterization and resistance improvement (Miñana-Posada et al., 2025).

Wheat resistance against *Zymoseptoria tritici* is governed by complex interactions between host immune mechanisms and pathogen-derived virulence factors. Plant defense begins with pattern-triggered immunity (PTI) where conserved fungal molecules such as chitin are recognized by plant immune receptors triggering activation of defense responses, including reactive oxygen species (ROS) production, cell wall reinforcement, and defense signaling pathways (Amezrou et al., 2023). To overcome these responses, *Z. tritici* secretes effector proteins that manipulate host immune responses and promote successful colonization. This gene-for-gene interaction is mediated by Stb (Septoria tritici blotch resistance) genes that activate immune responses and prevent successful pathogen colonization. Several Stb resistance loci have been identified in wheat including Stb6, Stb16q and Stb18, which contribute to isolate-specific resistance against diverse Z. tritici populations. The molecular interaction between Stb genes and fungal avirulence factors is a key determinant of disease outcome. For example, the fungal effector AvrStb9 was shown to interact with the wheat Stb9

resistance gene triggering host immune activation and limiting disease development (Amezrou et al., 2023). However, the durability of these resistance mechanisms is challenged by rapid pathogen evolution including diversification of avirulence genes that enable escape from host recognition. CRISPR-based validation of Stb-associated pathways and effector recognition mechanisms can accelerate identification of resistance determinants that are difficult to resolve through conventional genetics. (Kokhmetova et al., 2024).

Figure 1. Molecular interaction between wheat and *Zymoseptoria tritici* using infection and potential CRISPR intervention points for resistance engineering**.**



The figure illustrates disease progression from spore germination, stomatal penetration, and apoplastic colonization to effector-mediated host manipulation, necrotrophic development, and pycnidia formation. During infection, fungal effectors interact with wheat immune pathways, triggering pattern-triggered immunity (PTI) and effector-triggered immunity (ETI) through AvrStb–Stb recognition mechanisms. These molecular interactions provide potential CRISPR targets, including effector genes, pathogenicity-associated genes, susceptibility factors, immune regulators, and resistance loci for engineering durable STB resistance. Given the complexity of wheat–*Z. tritici* interactions and the constraints of traditional genetics, it is clear that there is a need to have precise functional genomics tools. Breeding and molecular techniques have identified sources of valuable resistance and an understanding of how pathogens adapt, but they are not yet able to explain gene function and create durable resistance. CRISPR genome editing will thus offer a complementary approach to dissect pathogen virulence mechanisms and develop resistance traits.

Conventional Approaches for Understanding and Managing *Z. tritici* Resistance

Conventional breeding has been the foundation of STB-resistance improvement, allowing discovery of STB-resistant germplasm, mapping of resistance associated loci, and introduction of favorable traits into elite wheat germplasm. Multigene resistance (quantitative resistance) has been extensively used as it offers greater stability of protection than single resistance genes. Polygenic resistance, however, often makes it difficult to pinpoint causal genes and to directly validate them. Resistance-associated regions have been identified and deployed via QTL mapping and marker-assisted selection (MAS), with introgression of resistance from a wide variety of germplasm having increased the genetic resources available for wheat improvement (Li et al., 2021). These successes have not transformed the conventional breeding approach, however, because it is limited by its lengthy selection cycles, complex inheritance of resistance traits, environmental interactions, and limited resolution in determining the genes involved in the resistance trait. Z. tritici populations are genetically diverse, have a high level of recombination ability and high evolutionary adaptability, which means that resistance genes can be overcome quite often (Ghaffary et al., 2018; Li et al., 2021; McDonald & Mundt, 2016). Conventional breeding, therefore, is not enough to rapidly discover and to precisely manipulate resistance-associated genes. Prior to the advent of the CRISPR-based methodologies, functional genomics methods such as random mutagenesis, homologous recombination, RNA interference (RNAi), transcriptomics and genome-wide association studies (GWAS) were very helpful in identifying regions of the genome associated with resistance, expression of genes and pathogen virulence mechanisms (Prasad, 2025). However, many of the above methods did not provide a precise targeting capability, had to be tested in many experiments and were hard to scale for systematic gene-function analysis. The wheat hexaploid genome and high degree of gene

redundancy further add to the difficulty in precise gene characterization. The constraints posed by these technologies underscored the importance of developing more precise and scalable tools, and accordingly, the development of CRISPR/Cas-based genome engineering, a powerful tool for targeted genome manipulation of virulence factors of pathogens and host immunity factors (Prasad, 2025; Yin et al., 2023)

Table 1. Conventional approaches versus CRISPR-based strategies for STB resistance improvement

Approach	Main contribution	Advantages	Limitations
Conventional breeding	Resistance introgression	Field-proven	Slow, complex genetics
QTL mapping	Resistance locus identification	Genome-level information	Limited causal gene resolution
MAS	Marker-assisted selection	Faster selection	Depends on available markers
GWAS	Candidate gene discovery	Population-level analysis	Functional validation required
CRISPR	Targeted gene manipulation	Precise functional validation	Delivery/regulatory challenges

CRISPR/Cas Genome Editing: Principles and Advances

Functional genomics has been revolutionized by the advent of CRISPR/Cas genome editing, enabling targeted and precise modification of genomic regions through a programmatic approach. CRISPR/Cas systems were evolved from the adaptive immune systems of bacteria and archaea, and have been engineered as effective genome engineering tools for plants for gene knockout, targeted mutation, gene regulation, and improvement of plant traits. CRISPR/Cas systems are also simpler, more flexible and efficient than previous genome editing approaches like the zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), which use conformation-based or sequence-based specificity to direct a protein to a target site (Přibylová & Fischer, 2024). These benefits have driven the functional studies of complex plant genomes which are difficult to approach with standard genetics.

The CRISPR/Cas9 system mainly consists of the Cas9 nuclease and a single-guide RNA (sgRNA), which directs Cas9 to a specific genomic sequence through complementary base pairing. Once bound to the target DNA, Cas9 first identifies a short sequence of DNA called a protospacer adjacent motif (PAM), which varies depending on the type of Cas9 protein that is being used, usually the 5′-NGG-3′ sequence for Streptococcus pyogenes Cas9, which is necessary for target recognition and DNA cleavage (Přibylová & Fischer, 2024). After PAM recognition, the guide RNA anneals with the target DNA sequence to create R-loop structure and activate the Cas9 nuclease activity. The HNH domain and the RuvC nuclease domain of Cas9 make targeted double-strand breaks (DSBs) in opposite strands of the DNA at the target site (Hwarari et al., 2024; Přibylová & Fischer, 2024).

The repair of Cas9-induced DNA breaks occurs mainly through two endogenous repair pathways: non-homologous end joining (NHEJ) and homology-directed repair (HDR). NHEJ is the predominant repair pathway in many plant cells and often introduces small insertions or deletions (indels) resulting in gene disruption or loss-of-function mutations. In crop improvement applications, this property makes NHEJ-mediated knockout particularly valuable for disrupting susceptibility genes and negative regulators of immunity. In contrast, HDR employs a homologous template for precise sequence replacement or targeted gene modification; however, its efficiency is relatively low in plants due to limitations in donor template delivery and repair activity (Hillary & Ceasar, 2024; Vats et al., 2024). Therefore, CRISPR/Cas9 provides a versatile platform for functional characterization of genes involved in plant immunity, pathogen virulence, and resistance improvement.

Conventional CRISPR/Cas9 gene editing is well suited to gene disruption but is hampered by the requirement for DNA double-strand breaks and the low efficiency of HDR. The current limitations of CRISPR/ Cas9 technology, such as dependence on double-strand break and HDR efficiency, have led to the development of next-generation editing technologies. These next-generation systems allow for more accurate genome changes with fewer off-target mutations and can be used to edit genes beyond single knockout (Fantaw et al., 2026).

Base editing is an important breakthrough, allowing for single nucleotide changes without a donor DNA template or the creation of DSBs. Cytosine base editors (CBEs) mediate C→T substitutions through cytidine deamination whereas adenine base editors (ABEs) enable A→G conversions through adenine deamination mechanisms (Hwarari et al., 2024). These methods can be particularly useful for introducing naturally occurring resistance-associated alleles, without creating widespread genomic disruptions. These systems integrate the catalytically modified Cas proteins with nucleotide-modifying enzymes that can be used to precisely edit point mutations and that minimize indel generation from traditional Cas9 editing.

Prime editing is a new addition to genome editing, which allows for precise sequence replacement and small insertions and deletions without the need for the generation of double strand breaks. Prime editors have been engineered by fusing a Cas9 nickase with reverse transcriptase and a prime editing guide RNA (pegRNA) that has both the targeting sequence and the synthetic template for the desired genetic changes (Vats et al., 2024). CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) methods rely on catalytically inactive versions of the Cas proteins (dCas proteins) to modulate gene expression without changing the DNA sequence to suppress or activate the targeted gene. These sophisticated CRISPR-based platforms offer important resources for elucidating gene function and for the engineering of complicated traits, such as disease resistance in crop plants (Li et al., 2024). These have been especially useful in regulating quantitative resistance pathways in which total gene disruption may not be desired.

CRISPR-Based Functional Genomics in *Zymoseptoria tritici*

CRISPR/Cas genome editing has provided new capabilities for resolving the molecular mechanisms underlying *Zymoseptoria tritici* pathogenicity. Although genome sequencing and transcriptomic studies have identified hundreds of candidate virulence-associated genes including secreted effectors and pathogenicity-related proteins, functional validation of these candidates has been challenging due to difficulties in targeted gene disruption and extensive gene redundancy. Recent advances in CRISPR-based editing provide a precise approach to connect candidate genes with specific infection-related phenotypes enabling direct investigation of fungal virulence mechanisms (Gomez-Gutierrtz et al., 2026; Meile et al., 2025a). However, transcript abundance and genomic prediction alone cannot establish whether candidate genes directly contribute to infection-related traits.

A major application of CRISPR technology in *Z. tritici* is the functional identification of genes contributing to fungal virulence. The pathogen genome contains a large repertoire of predicted effector genes with hundreds of candidate effectors identified; however, only a limited number have been experimentally confirmed to contribute to disease development (Meile et al., 2025a; Rezvani et al., 2026). CRISPR-mediated gene disruption enables systematic knockout of candidate effector genes allowing researchers to evaluate their contribution to host colonization, immune suppression, and pathogenicity. Potential CRISPR targets include secreted effector proteins, necrosis-inducing proteins, effector clusters, and genes involved in fungal signaling pathways. For example, a CRISPR/Cas9-based transformation system using Cas9–sgRNA ribonucleoprotein complexes successfully generated knockout mutants of the candidate effector gene Mycgr3107904 (Gomez-Gutierrtz et al., 2026). Targeted genome editing was used to directly reveal pathogenicity-associated functions in *Z. tritici*, and this loss resulted in delayed symptom development and decreased virulence.

CRISPR-based knockout enables a direct pathway for determining the gene–phenotype relationships in *Z. tritici*. Mutants can be generated by disrupting individual genes, and by comparing mutant and wild-type pathogens, researchers can identify those that regulate infection processes, such as penetration of the host, apoplastic colonization, sporulation, stress adaptation, and transition to the necrotrophic growth stage. The knockout-to-phenotype approach is one of the biggest strengths of functional genomics using CRISPR, as it directly correlates genetic modifications with infection parameters (Li et al., 2024). Previous functional studies have identified the role of genes in effector secretion, stress responses and infection-associated pathways. The CRISPR disruption of candidate virulence genes is used to scale up the determination of virulence gene essentiality (Hillary & Ceasar, 2024). This strategy is especially useful in *Z. tritici*, which has high effector redundancy and is difficult to analyzed by the single-gene method. This redundancy has made interpretations of single gene studies difficult historically and led to the idea that when one effector is lost, another related effector will provide the same function. Hence, the CRISPR technology offers a strong tool to characterize the genetic determinants of pathogenicity and virulence in fungi and to discover key virulence factors and the molecular mechanisms underlying fungal adaptation to wheat infection.

Effector proteins are important molecular factors involved in the Wheat-*Z. tritici* interaction by controlling host immunity, and promoting fungal colonization. These secreted molecules may modify the signaling of host defence, interfere with immune recognition mechanisms and modify cellular mechanisms necessary for successful colonization. Many effector candidates have been predicted from the genome of *Z. tritici*, but few are studied and characterized for function, underscoring the need for focused gene editing strategies (McDonald & Mundt, 2016). The CRISPR-generated effector deletion mutants

allow a direct approach to study the role of effectors in the disease process and host manipulation. Examples include LysM-containing effectors (e.g. LysM effectors Mg3LysM, Mg1LysM and Mgx1LysM) that enhance virulence through binding to fungal chitin, thus blocking perception by wheat immune receptors (Meile et al., 2025a). Also, avirulence effectors like AvrStb6 show evolution of pathogen effectors and wheat resistance genes. AvrStb6 is recognized by the wheat resistance protein Stb6, which triggers host defence responses, while sequence variation in the effector allows populations of the pathogen to evade recognition by the host (Rezvani et al., 2026). This is one example of the evolutionary arms race between diversification of pathogen effectors and immune recognition by the host.

In combination with the ease of effector sequence variation, a direct link between sequence and biological function can be made with CRISPR-based effector editing, providing insight into immune suppression, host recognition and pathogen adaptation.

Engineering Wheat Resistance via CRISPR

CRISPR-based genome editing has expanded the potential of wheat improvement by enabling targeted modification of genes involved in pathogen recognition, susceptibility, and immune regulation. CRISPR enables targeted modification of specific genetic components underlying resistance-associated traits. Susceptibility (S) gene editing is a promising approach that exploits host factors required for pathogen infection as targets for modification, rather than adding new resistance genes. Given pathogens routinely exploit host proteins and pathways for successful colonization, disrupting these susceptibility factors can provide broader resistance effects and may improve resistance durability depending on pathogen adaptation and host fitness consequences (Samoo, 2026). The most well-known example is TaMLO editing, where simultaneous modification of three wheat MLO homeologs successfully generated resistance and demonstrated the value of multiplex editing in hexaploid wheat (Miñana-Posada et al., 2025). Although demonstrated primarily in other fungal disease contexts, TaMLO editing provides proof-of-concept for susceptibility gene engineering in hexaploid wheat. Similar approaches targeting immune-associated regulators, including TaEDR1 and TaMPK1, have demonstrated potential for enhancing fungal resistance by modifying host defense pathways (Li et al., 2021). Although direct *Z. tritici*-specific susceptibility targets remain to be fully characterized, identifying and editing host factors exploited during infection represents a promising strategy for future STB resistance improvement. CRISPR technology also enables targeted modification of plant immune regulatory networks involved in pathogen recognition and defense activation. The negative regulators are key targets. Immunity, transcription factors, and signaling components like WRKY transcription factors, NAC regulators and MAPK pathway elements that regulate defense gene expression, ROS production, immune responses (Zafar et al., 2026). These regulators are good targets of interest. In wheat, immune regulator modifications have been demonstrated to increase resistance against fungal diseases. The modification of TaEDR1 has been shown to enhance resistance to powdery mildew, for example. MAPK-related regulators in resistance, manipulation of which showed the importance of Fungal resistance signalling systems are connected (Li et al., 2021). Similar approaches could be used to inhibit wheat–Z. tritici interactions by targeting regulators of PTI, ETI and effector-triggered immune responses.

One of the biggest benefits of CRISPR technology is its ability to do multiplex genome editing, which enables multiple genes or pathway to be modified simultaneously. The development of multiplex CRISPR systems has shown that it is possible to direct the activity of Cas9 using multiple guide RNAs. To different genomic regions, thus allowing for complex trait engineering (Cong et al., 2013). This approach is especially crucial in wheat where several homeologous gene copies frequently have to be simultaneously edited to create stable resistance phenotypes. For the wheat–*Z. tritici* pathosystem, durable resistance could be achieved more easily by the association of Stb resistance loci with edited optimized immune regulators and susceptibility factors. In principle, such gene-stacking combinations could be used to achieve quantitative resistance against the pathogen, potentially reducing pathogen levels and providing more adaptive and durable resistance than single resistance strategies (Samoo, 2026). However, ensuring effectiveness of editing and immune pathways should be carefully assessed since excessive alteration of the plant defence network can have a negative impact on plant growth, pathogen adaptation dynamics, or development.

Recent Experimental Advances and Translational Applications

Recent developments in CRISPR gene technology suggest a paradigm shift from gene discovery toward predictive and precision breeding for disease resistance. In *Zymoseptoria tritici*, direct assessment of causative role of genes in disease has been achieved through CRISPR-mediated functional validation of genes related to virulence and effector proteins, providing insights into the mechanisms involved in pathogen adaptation and host colonization. Genome editing Studies have also been carried out in wheat to investigate the potential of modifying susceptibility genes, immune regulators and redundant homeologous genes to enhance fungal resistance. However, these application are still at the concept stage and are undergoing further development. Most of the existing applications, however, remain in the proof-of-concept phase and are still under development for plant pests and diseases. The direct translation of CRISPR-derived resistance strategies

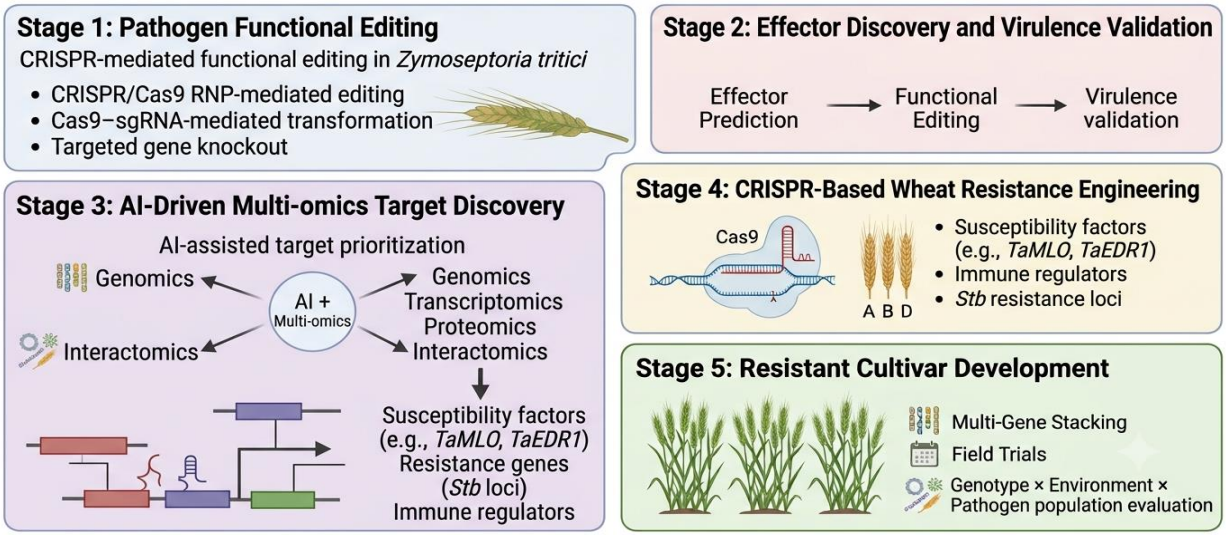
specifically against plant pests and diseases require further investigation. Further testing involving *Z. tritici* needs to be conducted with a wide range of pathogen population and under field conditions.

Table 2. Recent experimental advances and translational applications of CRISPR-based approaches for understanding and engineering fungal resistance in wheat**.**

System	CRISPR approach	Target	Major outcome
Zymoseptoria tritici	CRISPR/Cas9 knockout	Effector and pathogenicity genes	Functional validation of virulence determinants and reduced pathogenicity
Zymoseptoria tritici	Effector gene editing	Secreted effectors (LysM effectors, Avr genes)	Understanding immune suppression and host adaptation
Wheat	Susceptibility gene editing	TaMLO, TaEDR1, TaMPK1	Proof-of-concept for engineering fungal resistance
Wheat	Multiplex CRISPR editing	Multiple homeologous gene copies	Overcoming polyploid genome complexity
Crop–fungal pathogen systems	CRISPR functional screening	Resistance genes and immune regulators	Discovery of genes controlling plant immunity

Effector-guided target discovery, multiplex genome editing, and in situ genome editing into a single strategy. More future approaches will combine effector-guided target discovery, multiplex genome editing and in situ genome editing in a single strategy. Crop varieties that resist diseases, as well as resistance gene stacking and multi-omics-assisted prediction in order to speed up the development of STB Durable and Broad Spectrum resistance.

Figure 2. CRISPR-enabled pipeline for discovery and engineering of durable resistance against *Zymoseptoria tritici*-induced Septoria tritici blotch in wheat



The framework combines pathogen functional editing, effector identification and testing, AI helped with multi-omics target prioritization, and wheat genome engineering using the CRISPR system. Functional characterization of pathogen virulence determinants allows identification of host susceptibility factors, resistance loci and immune regulators that can be targeted for modification by specific manipulation and stacking of genes. Resistance gene will be required for successful deployment field validation and evaluation under various pathogen populations and environmental conditions.

8. Challenges and Limitations of CRISPR Application in Wheat *Z. tritici* System

One of the remaining challenges for wheat resistance engineering using CRISPR is the complexity of the genome. Many genes are hexaploid, meaning that they have three homeologous copies on the A, B and D subgenomes, and functional redundancy often makes multiplex editing necessary to obtain stable phenotypes. Furthermore, *Z. tritici* resistance is complex, involving multiple interactions between Stb resistance genes, susceptibility factors, immune pathways and pathogen effectors, which makes the engineering of durable resistance difficult to achieve (Samoo, 2026). Concurrently introducing several homeologs, however, must be optimized because complete inactivation of gene functions may affect plant growth and development, yield traits, or stress response.

Although there have been rapid advances in CRISPR technology, its application in wheat is still constrained by several technical challenges such as transformation efficiency, genotype-dependent regeneration, off-target modifications, and variable editing frequencies. New techniques such as base editing and prime editing have improved editing accuracy by minimizing double-strand breaks, but efficiency, accessibility of target DNA, and delivery methods remain significant challenges to widespread adoption (Samoo, 2026). These strategies also

need to be optimized for efficient delivery and access to target site in complex crop genomes.

The high genetic diversity and rapid evolution of *Z. tritici* pose major barrier to the establishment of durable resistance through CRISPR. Furthermore, host resistance to single host factors can create selection pressure, accelerating pathogen adaptation. Attempts to control a single host factor could also create selection pressure and hasten pathogen adaptation. Single-gene strategies are limited by pathogen's ability to overcome host resistance through effector variation, genome plasticity, and population adaptation. Therefore, a combination of approaches, such as susceptibility gene editing, immune pathway modification and stacking of resistance genes will probably be essential for future resistance engineering.

In addition to biological and technical issues, regulatory frameworks and public acceptance will also affect the deployment of CRISPR-edited wheat. Commercialization may be influenced by differences in genome-editing regulations across countries, concerns about genome-edited crops, and consumer perception. For the successful adoption of wheat varieties with CRISPR-based disease resistance, transparency in safety assessment, clarity in regulatory pathways, and effective communication will be critical.

Future Perspectives: Integrating CRISPR with AI and Multi-Omics

The application of multi-omics approaches and prediction systems based on artificial intelligence (AI) in the future development of CRISPR-based resistance strategies against *Zymoseptoria tritici* will likely depend on the integration of genome editing. Genomic method, such as genome wide association studies (GWAS) and population-scale mapping, can help identify resistance-associated loci, candidate susceptibility genes and genetic variants in wheat pathogen interactions. Likewise, transcriptomic techniques such as RNA sequencing (RNA-seq) can reveal genes, immune processes, and infection-induced changes in gene expression in response to a pathogen, enabling the identification of specific genes that control resistance-related pathways and could be targeted for editing. However, the fact that gene expression changes occur does not necessarily indicate gene function, highlighting the need for CRISPR-based validation. Proteomic methods can unveil interactions between fungal effectors and wheat immune proteins, which can prioritize genome- editing targets for experimental testing. The combination of the effector–host interaction studies with validation of these interactions using the CRISPR approach can accelerate the identification of virulence determinants and resistance mechanisms in the wheat–*Z. tritici* pathosystem. Moreover, artificial intelligence and machine learning methods can improve precision breeding using CRISPR by predicting guide RNA efficiency, reducing off-target effects, recognition of functional effectors and prioritizing resistance-associated genes. However, the successful integration of AI and multi-omics approach will require high-quality datasets, accurate biological validation and enhanced computational models to translate predictions into experimentally validated resistance traits. Thus, a novel precision breeding approach that combines AI-aided predictions, multi-omics discoveries and CRISPR genome editing could be developed for the rapid creation of wheat cultivars that are resistant to new and emerging fungal pathogen

Conclusion

The high genetic diversity, rapid evolution, and ability of *Zymoseptoria tritici* to circumvent resistance mechanisms deployed in wheat severely limit sustainable wheat production. While traditional breeding has provided useful sources of resistance and played an important role in the management of STB, these methods are limited by the complexity of wheat genetics, limited knowledge of resistance determinants and the continuous evolution of STB. CRISPR genome editing is a precision tool for the functional analysis of pathogen virulence and targeted wheat resistance engineering. CRISPR-based validation is possible for effector genes, pathogenicity determinants, and infection-associated pathways in *Z. tritici*. In wheat, modifying susceptibility factors, regulators of plant immunity, and resistance-associated genes and pathways provide opportunities for functional validation. Careful attention should be paid to biological, technical and regulatory constraints to achieve durable resistance. The development of future tools will rely on the combination of CRISPR with multi-omics assays and computational tools, such as artificial intelligence, for enhanced target discovery and RNA optimization, and prediction of resistance-associated genes. The combination of CRISPR-based precision engineering, biological discovery through omics technologies and AI-assisted prediction provides a promising platform for engineering wheat cultivars that are resistant to *Z. tritici* and other new and emerging fungal pathogens.

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