

**A Systematic Review of CRISPR Gene Editing in Enhancing Fusarium Head Blight Resistance in Wheat and Barley**

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**Abstract**

Fusarium Head Blight (FHB) continues to be a critical threat to global agriculture, particularly impacting wheat and barley by drastically reducing yields and contaminating grains with mycotoxins, a significant food safety concern. As the effectiveness of traditional methods dwindles, the imperative to explore advanced genetic interventions becomes ever more pressing. In this systematic review, I explore how CRISPR gene editing technology is being harnessed to fortify wheat against FHB, emphasising the its precise, inheritable genomic modifications. By conducting thorough searches of academic databases, this review aggregates and synthesises empirical evidence that attests to CRISPR’s targeted effectiveness in modifying genes essential for combating FHB. The results collected reveal CRISPR’s variability in efficacy and aims to ascertain the most promising CRISPR technique. The findings indicate that sophisticated CRISPR techniques, such as BSMV-mediated CRISPR and SpCas9 CRISPR delivery, yield more successful mutation outcomes. Additionally, targeting a diverse array of genes reveals numerous phenotypic advantages. Importantly, some of these phenotypic changes show a more direct and reliable correlation to FHB resistance, offering more advantageous outcomes compared to other observed phenotypic changes where they indirectly benefit FHB resistance.

1. **Introduction** 
   1. Background

Wheat and barley, a bedrock of global agriculture for millennia, has been central to human sustenance and cultural traditions across numerous societies (Igrejas, 2020). Esteemed as a dietary staple, this crop feeds billions, providing a food source in 89 nations, from North Africa to Eastern Asia even exceeding grains as important as rice; wheat provides 18% of the total dietary calories and 19% of the proteins globally (Erenstein, 2022; CIMMY, 2022). The demand for both crops is also on an incline, as by 2050 it is predicted that consumers will require a 60% overall production increase for wheat alone. Not only is it vital as a source of food for billions of people it is also a major commodity in international trade and plays a significant role in the global economy, making it a critical crop for the economical agriculture sector (CIMMY, 2020). It is cultivated over a broader expanse of land than any other commercial crop (Enghiad, 2017), showcasing remarkable adaptability to diverse climatic and geographical conditions. This adaptability shows these cereal crops status as one of the most vital crops in the world. Wheats extensive cultivation reflects not only its agronomic flexibility but also its critical role in supporting the economies and food security of a vast array of nations (Enghiad, 2017).

* 1. Fusarium Head Blight

This essential grain faces a relentless challenge against Fusarium Head blight (FHB), a devastating disease, primarily caused by the fungus *Fusarium graminearum*, although other species can produce similar symptoms (Kayim, 2021). This disease is most prevalent in warm, wet weather with high relative humidity during the flowering and early grain formation stages of wheat as shown in Fig.1 and Fig.2. Fusarium head blight affects wheat and barley in several different ways, leading to substantial yield losses and reduced grain quality. In wheat the spikelets are infected and exhibit symptoms of premature bleaching, subsequently spreading up and down the wheat infecting more spikelets (CPN, 2019). The disease also leads to overall weight loss, changes in carbohydrate and protein composition, and the presence of harmful mycotoxins such as trichothecenes, zearelenone (ZEN) and moniliformin (MON), drastically reducing the quality of the crop and posing a serious threat to human and livestock health (Kayim, 2021; Spanic, 2017; El Chami, 2022). The economic implications of FHB can be immense. In the United States alone, FHB resulted in an estimated USD 2 billion loss between 1993 and 2001, and 520 million Canadian dollars (CAD) in Canada in the 1990s (Mcmullen, 2012). More recently between 2015 and 2016, losses have been estimated to be a total of $1.176 billion in the United States, these losses are expected to increase due to the amplification of the frequency and intensity of FHB outbreaks (Fabre, 2020). The dynamic nature of FHB is displayed in Fig. 3 where the process of the disease's life cycle begins with the initial infection of spikelets, kernels, and stems, and ends with the release of airborne spores and conidia dissemination. The process to kernel maturity and harvest, which results in mycotoxin contamination and the pathogen's post-harvest survival in crop leftovers, is then depicted. This emphasises the significance of management techniques used during the wheat production and post-harvest phases to lessen the effects of this widespread illness.

* 1. Disease Management

Managing this disease is far from an easy task, and there are a varied number of traditional approaches including crop rotation, biological control, chemical fungicides, and selective breeding. These however face limitations for various reasons. Selective breeding is hindered by many different factors that include the lack of genetic diversity in the existing wheat gene pool, the transfer of undesirable traits, pathogens evolving to compete with the resistance being bred, and the time taken for this selective breeding is undesirably slow which does not help when competing against the fast evolutionary nature of the fungal pathogens (Megapoku, 2021). The use of chemical fungicides is widely less used due to their being costly, weather dependent and the concern with health and detriment to the environment (Figeuroa, 2017), there is also a concern about overuse, as applying them to a healthy crop field could unnecessarily produce fungicidal-resistant diseases (Mueller, 2021). Additionally, crop rotation is a strategic agronomic practice of farming wherein different crop species are cultivated in succession on the same plot of land, which aims to disrupt the lifecycle of pathogens in the soil by depriving them of their host crops (Mohler, 2009) may be used. However, this has its limitations. It's heavily dependent on time for effectiveness, for example, the control of Septoria leaf spot requires a fallow period of at least two years to significantly reduce fungal presence in the soil. Additionally, there is a risk of disease reintroduction and the potential for pathogens to adapt to alternative host plants, which can compromise the productivity of subsequent crops. The introduction of diverse organic matter into the soil may also inadvertently promote the growth of saprophytic fungi, which can detrimentally impact the crops before the planting of the next sequence (Mohler, 2009). With many more challenges than the ones mentioned, there is a pressing need for advanced solutions in wheat disease management.

* 1. CRISPR Gene Editing

Gene editing technologies, such as CRISPR, TALENs, and ZFNs, have revolutionised the field of crop protection research, holding significant promise for the enhancement of crop traits and protection against diseases like FHB. These tools offer several advantages over traditional breeding and management practices, including greater specificity and efficiency (Gao, 2022; Kelleher, 2020; Savadi, 2017). Among them, CRISPR is lauded for its user-friendly operation and cost-effectiveness, outperforming ZFNs and TALENs, particularly in tasks that require targeting multiple genomic sites simultaneously. This attribute is exceptionally useful for complex genomes such as that of wheat, which is hexaploid and thus presents considerable challenges for genetic manipulation (Howells, 2018; Gupta, 2014). The streamlined nature of CRISPR facilitates the creation of a diverse array of vectors to interact with the wheat genome, offering a robust platform for advancing wheat resilience against FHB. CRISPR's precision in gene editing has been leveraged to target and modify genes that are particularly susceptible to FHB, thereby enhancing resistance. Notably, the utilisation of the Barley stripe mosaic virus to mediate CRISPR-based gene editing has shown potential in conferring FHB resistance, marking a significant advancement in disease management (Chen, 2022; Hicks, 2023). The multiplexing capability of CRISPR is particularly advantageous for complex traits like FHB resistance, which may involve several genes. This allows for the concurrent targeting of multiple genetic loci within a single cell, a strategy that could streamline the development of resistant wheat varieties (Taj, 2022; Gupta, 2014). Additionally, the minimal 20-base pair recognition sequence required of the guide RNA facilitates simpler cloning processes compared to traditional gene targeting methods, enhancing the efficiency of generating specific genetic modifications for FHB resistance (Szczesna, 2010). CRISPR also is a potent tool in modifying genes associated with biotic stress responses, including those implicated in FHB resistance. By knocking down these genes, the tolerance of wheat to this disease can be significantly improved, offering a promising direction for future crop protection strategies (Taj, 2022).

However incorporating CRISPR/Cas9 technology into wheat genome editing poses distinct challenges that need to be carefully considered for its effective application. One primary concern is the design of CRISPR systems themselves, where the precision in selecting and designing guide RNAs (gRNAs) is crucial. The specificity of these gRNAs must be impeccable to minimise off-target effects, which can disrupt unintended parts of the genome and lead to deleterious traits (Zhang, 2019). Additionally, the polyploid nature of the wheat genome, with multiple copies of each gene, complicates the task of achieving simultaneous edits across all alleles, a necessity for manifesting desired phenotypic changes (Rossato, 2023). Moreover, the efficiency of gene editing is heavily dependent on the method of CRISPR component delivery into plant cells. Traditional methods like Agrobacterium-mediated transformation and biolistic particle delivery face challenges in wheat due to its relatively low rates of callus induction and regeneration. To enhance the applicability of CRISPR in wheat, advancements in these areas are required—improving the accuracy of CRISPR designs to ensure target specificity, optimising callus induction and regeneration techniques for better transformation efficiencies, and deepening our understanding of wheat's genomic architecture (Chen, 2022; Rahim, 2024; ). Such improvements will pave the way for more robust and reliable crop enhancement strategies, turning the theoretical benefits of CRISPR into practical gains in agriculture.

* 1. The Rationale

The rationale behind this review is rooted in the global need for food security, with wheat and barley being an extremely important global crops. With Fusarium Head Blight posing a significant threat to wheat security worldwide, traditional methods have proven to be time-consuming and inefficient (Shude, 2022). CRISPR gene editing offers a promising alternative, with the potential to enhance disease resistance precisely and efficiently with very few downsides (Ma, 2022). However, there are still gaps that persist such as the efficiency variance among CRISPR techniques and the diverse spectrum of proteins and genes that can be targeted. Firstly, research has demonstrated that a range of genes, including *TaRPK1*, *TaPDS*, *TaNFXL1*, and various defence response genes, have shown enhanced resistance to FHB (Rahim 2024; Brauer, 2020). Addressing the efficacy of each gene for enhanced resistance is vital for the advancement of agricultural biotechnology and enhancing FHB resistance in wheat and through gene editing can lead to increased crop yields, reduced use of fungicides, and improved food security (Li, 2021). Furthermore, the knowledge gained can hopefully be applied to other crops and diseases, revolutionising the agriculture sector.

* 1. Aims and SCOPE

This review will systematically evaluate the efficacy of various gene editing techniques in providing resistance to FHB in wheat and barley. It will examine the specific genes that have been targeted by these editing approaches, analysing their roles in promoting FHB resistance. Additionally, it will consider the potential risks and benefits associated with each gene editing method and the individual genes modified. The overall aim is to offer a thorough review of gene editing applications aimed at bolstering FHB resistance in wheat and barley, contributing valuable insights to both the scientific sector and agricultural methodologies. This systematic review will focus on the application of gene editing techniques in the context of FHB resistance in wheat and barley crops. The effectiveness of different gene editing techniques and specific genes that have been targeted for editing will be evaluated and assessed in their role in FHB resistance.

1. **Methods**
   1. Data Synthesis and Analysis

In this systematic review, I adopt a qualitative approach to synthesise the findings from studies investigating the application of CRISPR/Cas9 gene editing for enhancing Fusarium head blight resistance in wheat and barley. Given the diverse nature of the interventions and outcomes across the studies, a narrative synthesis provides the most suitable framework for capturing the complexity and breadth of the research landscape. Each study included in the review was thoroughly examined to identify key themes, methodologies, CRISPR/Cas9 interventions, and their outcomes in terms of resistance to Fusarium head blight. The synthesis process commenced with the coding of qualitative data extracted from the studies, focusing on intervention types, genetic targets, efficacy outcomes, and contextual factors influencing the application and results of CRISPR/Cas9 editing. These coded data segments were then grouped into coherent categories to construct a narrative that reflects the current state of research, identifies commonalities and divergences among the findings, and highlights innovative approaches and significant breakthroughs in the field. Special attention was paid to the methodological rigour of the studies, the specificity and efficiency of the CRISPR/Cas9 edits, and the practical implications of the research for agricultural practices. This qualitative narrative synthesis not only elucidates the diverse strategies employed in CRISPR/Cas9 gene editing for Fusarium head blight resistance but also provides insights into the challenges and opportunities within this rapidly evolving field. By integrating the qualitative data in this manner, the review aims to offer a comprehensive overview of the advances in genetic editing techniques, contributing to the development of more resilient wheat and barley varieties.

* 1. Prisma Review

Our comprehensive search for peer-reviewed literature on CRISPR/Cas9 gene editing in wheat and barley for Fusarium Head Blight resistance concluded with a significant collection of studies. Initially, 892 records were identified through database searches, of which 877 were identified through Mendeley (Mendeley, 2024), supplemented by 15 records from other sources, leading to a robust dataset of studies up until November 11, 2013. The meticulous screening process, adhering to PRISMA 2020 guidelines, refined this to 35 pertinent peer-reviewed studies for inclusion in the eligibility process, excluding reports where the studies were outside the scope of Barley and Wheat. Among the selected studies, two notable instances (Low, 2020; Low, 2022) involved Arabidopsis thaliana rather than barley or wheat. These studies were included due to Arabidopsis's role as a reference organism in plant biology, attributed to its well-characterised genome and the plethora of available genetic tools (Rotaspereti, 2020). However, it's crucial to acknowledge the potential limitations of extrapolating findings from Arabidopsis to cereal crops.

Comparative analyses between Arabidopsis and barley, for example, have uncovered significant disparities in the temporal kinetics and intensity of stress-induced calcium signals, underscoring the species-specific nature of calcium-dependent stress response mechanisms (Giridhar, 2022). The Arabidopsis gene *AtEIN2*, crucial for ethylene signalling and plant defence, has a wheat homolog, Ta*EIN2*, whose suppression via RNAi enhances resistance to Fusarium head blight (FHB), indicating similar defence roles. Similarly, the *At2OGO* gene in Arabidopsis has related homeologs in certain wheat genomes, suggesting related functions despite the absence of a direct ortholog in a certain genome (Low, 2022; Low, 2020; Arif, 2022). This suggests that while Arabidopsis can provide valuable genetic insights, the direct applicability of these findings to wheat and barley, particularly in the context of the *At2OGO* gene, may be constrained by inherent biological differences (Rotasperti, 2020; Calixto, 2015).

1. **Results**
   1. Genes involved in disease resistance

A total of ten significant genes were revealed of interest, segmented across four key species: four genes were identified within wheat, two genes were associated with Fusarium spp, two were linked to Arabidopsis thaliana, and two genes were discovered in barley. Four significant genes were found in wheat implicated in disease resistance and plant development: *TaNFXL1*, TaPDS, *TaHRC*, and *TaRPK1*. The *TaNFXL1* gene is associated with susceptibility to FHB, as the expression of this gene is induced by deoxynivalenol (DON), a mycotoxin produced by *F. graminearum* (Brauer, 2020; Fabre, 2022). TaPDS and *TaHRC* were both identified by Chen et al. (2022). TaPDS is crucial in wheat for carotenoid biosynthesis, and disruptions can lead to an albino phenotype, so this gene, while not directly linked to FHB resistance, can be extremely beneficial in CRISPR editing studies as a validating marker (Chen, 2022; Howells, 2018). Chen et al. (2022) also investigated *TaHRC*, which suppresses the calcium-mediated immune response to *F. graminearum* infection and facilitates the spread of the disease, thereby contributing to the plant's susceptibility to FHB. The deletion in the *TaHRC* gene has been shown to remove this suppression and thereby enhance resistance (Chen, 2022; Su, 2019). Last, of the Triticum aestivum genes included (Boden, 2023), is *TaRPK1* while it doesn’t directly confer with FHB resistance, does regulate root architecture and abiotic stress responses, thereby potentially modifying root traits and enhance the plant's overall growth and yield under stress conditions, decreasing FHB severity in crop yields. Two different constructs were analysed, both LR-1 and LR-2, which consisted of using different guide RNAs targeting different sites within the gene, during the induced mutagenesis​ (Rahim, 2022, 2024). Only two genes in Barley were found to be significant enough to include. These are MORC genes, *HvMORC1* and *HvMORC6a* and they encode a family of proteins that are important nuclear regulators with critical roles in epigenetic gene silencing and genome stabilisation (Kumar, 2018; Galli, 2022). They also controlled the expression of Pathogenesis-Related (PR) genes, which are integral to the plant's immune response and include a wide array of genes such as those encoding for antimicrobial peptides, enzymes involved in cell wall fortification, and signalling molecules that mediate defence responses. Additionally, these MORC genes regulated the activity of Transposable Elements (TEs), sequences within the genome capable of changing positions and influencing gene expression, maintaining genome integrity by keeping these elements silenced through epigenetic mechanisms (Galli, 2021, 2022; Kumar, 2018). The two Arabidopsis genes were *At2OGO* and *AtEIN2* (Low, 2020, 2022). *AtEIN2* pivotal in the ethylene signalling pathway, influences plant development and defence by regulating leaf senescence. Its barley equivalent, Hv*EIN2*, mirrors *AtEIN2*'s functionality, as evidenced by Hv*EIN2*'s ability to complement *AtEIN2* KO in Arabidopsis, reinstating vulnerability to *F. graminearum* (Low, 2022; Ueda, 2020). *At2OGO* in Arabidopsis, coding for a 2-oxoglutarate Fe(II)-dependent oxygenase, acts as a susceptibility factor for Fusarium head blight (FHB), with knockout mutants showing increased FHB resistance. Its barley equivalent, Hv2OGO, mirrors this function; complementation in Arabidopsis *At2OGO* knockouts with Hv2OGO reinstated FHB susceptibility (Low, 2020; Needham, 2009).

* 1. Observed Improvements

All of the genes in Table.1 were successfully mutated using CRISPR and there were a variety of improvements were observed in each of the above studies, ranging from reduced infected spikelets, enhanced phenotypes, reduced spore production and delays in symptoms. For instance, the editing of the targeted gene *TaRPK1* induced a significant improvement in the modified wheat root architecture, in both constructs LR-1 and LR-2 which was determined using a student t-test, with significance levels set at \*P < 0.05 and \*\*P < 0.01. Both constructs harboured similar results, and both showed statistical significance in overall wheat architecture (Rahim, 2024). In terms of a reduction in infected spikelets the gene *TaNFXL1* was identified as a significant factor in the wheat’s response to *F. graminearum*, the number of symptomatic spikelets per spike was used as the key metric, with a significance level set at \*P < 0.05. The statistical analysis demonstrated that the modifications led to a significant reduction in the number of symptomatic spikelets (Brauer, 2020). Another study led by Chen et al. 2022 showed similar results targeting the gene *TaHRC*, where the number of infected spikelets was also significantly decreased, with significance results set at \*P < 0.001. The last of the Triticum aestivum genes which observed some change was *TaABCC*, where a delay in symptoms was observed, however, no statistical test was administered (Cui, 2017), and *TansLTP9* which displayed a reduction in FHB symptoms by 20-30%, but again there were no statistical tests utilised so we cannot fully determine if its fully significant or not (Cui, 2017). For the barley genes *HvMORC1* and *HvMORC6a*, they exhibited increased resistance to *F. graminearum*, which was evidenced by a significant reduction in the number of necrotic lesions and the amount of fungal DNA in the infected barley leaves and roots, with p-values indicating strong statistical support for the observed differences (p < 0.05) (Galli, 2022). Further, the empirical data showed a decrease in necrotic lesions, with a reduction spanning 1.5- to 2.7-fold across various mutant constructs, compared with the wild-type. This phenotypic resilience was further corroborated by a substantial diminish in fungal DNA loads within infected tissues, as evidenced by quantitative PCR analyses, manifesting statistically significant reductions from 1.4- to 2.1-fold for *F. graminearum*. This resulted in significant phenotypic shifts in root architecture—augmenting root length, depth, volume, and surface area while diminishing root diameter and angle (Galli, 2021, 2022; Kumar, 2018). Finally, for the two Arabidopsis genes, *AtEIN2* knock-out mutants in Arabidopsis demonstrated enhanced *F. graminearum* resistance, with a significant reduction in spore production and fungal growth rate, verified by qPCR analysis showing substantially lower fungal DNA levels compared to wild-type. Statistical validation using t-tests confirmed the improvements as significant (p < 0.05), underscoring the role of *AtEIN2* in pathogen susceptibility. CRISPR/Cas9-induced mutations in the *At2OGO* gene, encoding a 2-oxoglutarate Fe(II)-dependent oxygenase, conferred increased resistance to *F. graminearum* in Arabidopsis thaliana, as evidenced by limited fungal growth and spore production relative to wild-type controls. These enhancements in pathogen resistance were statistically significant, substantiated by t-test analyses (p < 0.05).

* 1. Mutation Success

For the mutation analysis, the mutation success rates were analysed for CRISPR/Cas9 gene editing across various genes targeted for enhancing Fusarium Head Blight resistance in mainly wheat but also Arabidopsis and barley (Fig. 5). Mutation success rates varied from as high as 89.0% in the *HvMORC1* gene to a lower threshold of 26.7% for the *AtEIN2* gene (Low, 2022; Galli, 2022). Notably, genes like TaPDS and *TaHRC* (Chen, 2022) exhibited intermediate success rates of 58.0% and 49.0%, respectively, reflecting a moderate level of editing precision. This study also explored multiplex editing by targeting both TaPDS and *TaHRC* simultaneously, achieving slightly lower but still substantial mutation efficiencies using the BMV-mediated system. The study by Low et al. (2020) on the *AT2OGO* gene in wheat reported an initial mutation success of 57.0%, which intriguingly decreased to 31.0% over the subsequent generation of breeding, which could show the intricate nature of genetic stability over generations of breeding, influenced factors such as off-target mutations, epigenetic factors, and chromatin structure (Louie, 2023; Zhang, 2019). In the study conducted by Rahim et al. (2022) two distinct constructs, LR-1 and LR-2, each harbouring unique guide RNAs tailored for specific genomic targets, targeted the *TaRPK1* gene. The LR-1 construct, predominating with a 44.0% monoallelic mutation rate in one genome, and a 25.0% biallelic mutation rate was contrasted by the LR-2 construct, which facilitated a 40% monoallelic mutation frequency across three different genomes, and a 20.0% biallelic mutation rate. In the research conducted by Galli et al. (2021), significant emphasis was placed on two MORC proteins, *HvMORC1* and *HvMORC6a*, due to their pivotal roles in genome stabilisation, chromatin remodelling, and regulation of gene expression in barley. The CRISPR/SpCas9 system was employed to induce both mono-allelic and bi-allelic homozygous mutations with notable efficiency. Specifically, the transformation of homozygous single mutants achieved mutation efficiencies of 89.0% for *HvMORC1* and 81.0% for *HvMORC6a* when guided by their respective RNAs for mono-allelic mutations. For bi-allelic mutations, the success rates were 23.0% (13 out of 55 plants) for *HvMORC1* and 27.0% (17 out of 64 plants) for *HvMORC6a*. In the study Cui et al. (2017) which is not in figure. 4, a spectrum of mutation efficiencies was observed for two targeted genes, showcasing the variability inherent in CRISPR/Cas9 applications. For TansLTP9, mutation efficiencies ranged from 0 to 11.9%, while TaABCC exhibited a slightly narrower range of 9 to 13%.

In the study conducted by Brauer et al. 2020 there wasn’t an explicit mention of the mutation success however there was data recorded on the expression of *TaNFXL1* in analysing the gene expression data, the relative expression levels offer indirect evidence of CRISPR/Cas9 activity. Figure 6A validates the silencing of the *TaNFXL1* gene in wheat spikelets treated with BSMV-NFXL1, showcasing a notable downregulation of gene expression when compared to the control BSMV0, as confirmed by reverse transcription quantitative PCR (\*P < 0.1, one-tailed t-test). This reduced expression is significantly correlated with a decrease in *F. graminearum* infection rates, indicative of successful VIGS application for potential disease resistance enhancement in wheat. In Figure 6B, *TaNFXL1*-edited plants exhibit a dramatic 60 to 99% reduction in *TaNFXL1* expression relative to their respective azygous controls in the T2 generation.

* 1. CRISPR Methods, Outcomes and Designs

In analysing the efficacy of targeted genes through CRISPR/Cas9 mediated modifications, it is imperative to delve into the nuanced molecular and phenotypic outcomes that underscore the strategic advantage of this gene-editing tool in crop improvement. In the study, Brauer et al. (2022) CRISPR/Cas9 was employed to induce targeted mutations in the *TaNFXL1* gene of wheat cultivar Fielder to enhance resistance against Fusarium head blight. Using specifically designed sgRNAs for Cas9-mediated cleavage, deletions ranging from four to 97 bases were achieved in the gene homologs, leading to a disruption of the *TaNFXL1* gene. For TaPDS and *TaHRC* in Chen et al. (2022) a novel Barley stripe mosaic virus (BSMV)-mediated CRISPR/Cas9 system was developed to facilitate gene editing in wheat, targeting the *TaHRC* and TaPDS genes for Fusarium head blight resistance and albinism, respectively. By bypassing tissue culture, this method enhances the efficiency of gene editing in wheat genotypes with low regeneration capabilities. Utilising Cas9-overexpressing wheat lines and sgRNAs integrated into BSMV vectors, the system achieved inheritable and functional gene edits, evidenced by nucleotide insertions at target sites and confirmed through mutation detection assays and Sanger sequencing. This approach not only demonstrated efficient single and multiplex gene editing but also confirmed the heritability of induced mutations, significantly enhancing FHB resistance in wheat (Zhang, 2022; Chen, 2022; Chen, 2019). When it came to the gene *TaRPK1* in Rahim et al. (2024) CRISPR/Cas9-mediated mutagenesis of the wheat gene was achieved using two constructs, LR-1 and LR-2, each harbouring two different combinations of guide RNAs for targeted gene editing. LR-1 was tailored with gRNAs for specific sites within the D genome of *TaRPK1*, while LR-2 targeted different sites within the A and B genomes. The Agrobacterium-mediated transformation of these constructs into wheat resulted in a notable mutation efficiency of deletions over insertions at the targeted sites, including significant deletions up to 20 base pairs with the LR-1 construct. For the genes *TaABCC* and *TansLTP9* CRISPR/Cas9 was used to introduce targeted mutations in both genes, employing two specifically designed sgRNAs per gene to guide efficient, sequence-specific double-stranded breaks. High-throughput sequencing of PCR-amplified protoplast DNA confirmed successful gene editing, revealing deletions in the target regions of both genes. In the barley studies, CRISPR/Cas9 was employed to target *HvMORC1* and *HvMORC6a*, employing sgRNAs meticulously designed to ensure specificity and minimise off-target effects. Mutants were generated via either successive or simultaneous Agrobacterium-mediated transformations, introducing STOP codons early in the gene sequences to effectively knock out gene function. This approach yielded double-knockout mutants exhibiting significantly enhanced basal expression of PR genes and depression of TEs, correlating with an increased resistance phenotype (Galli, 2021, 2022; Kumar, 2018). Firstly, for the Arabidopsis genes, the *AtEIN2* gene in Arabidopsis was targeted by a sgRNA cloned into the psgR-Cas9-At vector for Agrobacterium-mediated transformation. Resulting mutations, including frameshifts and a 20bp insertion, disrupted the *EIN2* protein function, leading to altered disease resistance, thereby highlighting the crucial role of the gene in ethylene signalling and plant defence (Low, 2022). Then the *At2OGO* gene used a guide RNA designed to exon 3, which resulted in a range of mutations, notably including an 8bp deletion leading to a frameshift mutation and premature termination of the gene product. These targeted mutations enhanced resistance to Fusarium head blight, illustrating the role of this gene in plant susceptibility to pathogens. This CRISPR approach, combining precise gRNA design, and agrobacterium-mediated transformation showed a significant improvement in plant defence (Low, 2020).

* 1. Challenges Presented

Callus induction is pivotal to the transformation of many plant species, whereby the transformed cells can be regenerated into whole plants which can then be used to study gene function or enhance crop traits (Britannica, 2018; Chen, 2022). However, the efficiency of callus induction and regeneration exhibits considerable variability across different wheat genotypes, influenced by intrinsic genetic factors, the specific composition of the growth medium, and environmental conditions in the lab. Such variability poses a substantial hurdle in the application of genome editing for wheat improvement (Chen, 2022; Han, 2011). This was mentioned in Chen et al. (2022) where the variability in callus induction efficiency in the Bobwhite wheat variety limits the application of CRISPR gene editing and successful transformations. In the study by Brauer et al. (2020) the precision of CRISPR/Cas9 gene editing in targeting the *TaNFXL1* gene raises concerns about potential off-target effects, with only two such instances identified, highlighting the need for comprehensive genomic screening to ensure fidelity. Furthermore, the transformation process itself may introduce background genetic changes, complicating the attribution of phenotypic outcomes solely to CRISPR modifications. The experimental design, characterised by a single repetition of key assays, alongside the application of one-tailed and two-tailed t-tests, necessitates a more robust statistical and methodological framework to validate the observed resistance enhancements. Additionally, while *TaNFXL1*'s contribution to Fusarium head blight susceptibility is noted, the underlying exploration of biological pathways associated with FHB resistance, including interconnected signalling cascades and metabolic processes, remain insufficiently explored, warranting further investigation into the gene's role within the plant's broader defence network (Ma, 2022; Ding, 2022). In the study by Cui et al. (2017), they used a protoplast system when testing the CRISPR efficiency which comes with its constraints. As wheat, has a complex genome, the in vitro regeneration of protoplasts into whole plants poses a significant challenge, impeding the achievement of stable, heritable genetic modifications due to limited T-DNA integration and plant development efficiency (Gupta, 2018; Michalski, 2023). Furthermore, the protoplast isolation process may induce genome-wide chromatin relaxation, as observed in Arabidopsis thaliana, potentially affecting the CRISPR/Cas9 editing efficiency and specificity in protoplasts differently than in intact plant cells (Michalski, 2023). A few limitations can be spotted for CRISPR editing in Barley in the study conducted by Galli et al. (2022). Firstly, the use of qPCR for quantifying infection levels provides a measure of fungal biomass but may not fully capture the complexity of plant-pathogen interactions and so is a slightly broader measure of FHB resistance. Also due to Barley’s complexity and the presence of multiple MORC proteins, the possibility of functional redundancy could theoretically mitigate the effects of *HvMORC1* and *HvMORC6a* knockouts (Kumar, 2018). Finally, the role of MORC proteins in RNA-directed DNA methylation (RdDM) and chromatin remodelling, among other processes, suggests that the expression of MORC genes could be influenced by a variety of factors, including developmental stages, environmental conditions, and pathogen virulence factors (Koch, 2017; Xue, 2021). Then finally the studies covering both Arabidopsis genes *At2OGO* and *AtEIN2*, have its drawbacks as, while orthologous genes may exist, their roles in signalling pathways and disease resistance pathways could differ due to species-specific evolutionary adaptations (Low, 2020, 2022), due to wheat and barley having far more complex genomes, primarily due to their larger genome sizes, higher gene counts, and greater proportions of repetitive sequences (Zhang, 2021; Wicker, 2017).

* 1. Factors Influencing CRISPR Efficacy

The success of using CRISPR-Cas9 for gene editing in wheat and barley, particularly for developing resistance to Fusarium Head Blight (FHB), relies on a complex array of factors. These include how Cas9 functions, the genomic landscape, the design and precision of the guide RNA (gRNA), the accessibility of the target DNA sequence, the mutation detection methods used, and the inherent characteristics of the targeted gene (Schenke, 2020; Lopos, 2023). The effectiveness of Cas9, which is crucial for making precise cuts in the DNA at specific sites, depends on how well it's expressed and localised within the cell nucleus, which can vary between different types of plant tissues and throughout their growth stages (Zhang, 2019; Schenke, 2023). The genetic environment, especially the state of chromatin and any epigenetic changes, largely determines how accessible the target DNA is to the Cas9-gRNA complex, affecting the outcomes of the editing process. Dense chromatin regions, or heterochromatin, can particularly challenge Cas9's ability to reach and effectively cut the DNA (Lopos, 2023; Schenke, 2023).

The gRNA's efficiency is critical as it guides Cas9 to the exact genetic location that needs editing. Its sequence specificity, including the protospacer adjacent motif (PAM), is essential for ensuring the gRNA targets the correct spot and minimises edits in unintended locations. However, genetic variations like single-nucleotide polymorphisms (SNPs) or small insertions and deletions at the target site can disrupt gRNA binding, leading to changes in the editing results (Howells, 2018; Arndell, 2019). The structure of the gRNA and the DNA sequence it targets may also form complex secondary structures that hinder the Cas9-gRNA complex's formation or interaction with the DNA. Moreover, the physical accessibility of the target DNA, deeply influenced by the chromatin environment around it, including nucleosome placement and DNA methylation, plays a critical role in how effectively the Cas9-gRNA complex can bind and edit the DNA (Lopos, 2023; Schenke, 2023). Techniques for detecting mutations, such as Sanger sequencing, next-generation sequencing (NGS), and PCR-based assays, are vital for both identifying and understanding the mutations caused by CRISPR-Cas9. The choice of detection method affects how well these mutations can be identified and the ability to spot mosaicism or heterozygous mutations in the plants altered by these edits (Arndell, 2019).

The inherent characteristics of the target gene, including gene copy number in polyploid organisms like wheat and barley, and the gene's functional redundancy, also play a role in determining mutation success. Editing a single gene copy might not yield the desired phenotypic trait if other redundant copies can compensate for its function, a scenario that is particularly pertinent in the context of FHB resistance, where multiple genes or gene families may be implicated in the resistance pathway (Matres, 2021; Paudel, 2020). Moreover, the role of the targeted gene in plant growth and development should be carefully considered to prevent unintended phenotypic outcomes (Matres, 2021). In essence, harnessing CRISPR-Cas9 to introduce mutations for FHB resistance in wheat and barley necessitates a comprehensive understanding of these interconnected factors. Fine-tuning each aspect, from the construction and administration of the Cas9-gRNA complex to the screening and evaluation of edited plants, is imperative for attaining high editing precision and efficiency.

1. **Discussion**
   1. Interpretations of Findings and Comparisons

The findings from this literature review underscore the significant advancements in agricultural biotechnology, particularly in the realm of crop disease management through CRISPR/Cas9 gene editing. Among the various CRISPR techniques reviewed, BSMV-mediated CRISPR and SpCas9 CRISPR delivery systems were particularly notable for their high mutation success rates and their ability to enhance Fusarium Head Blight (FHB) resistance in wheat and barley. The genes *TaNFXL1* and *TaHRC* were highlighted as key targets in the studies due to their strong association with disease resistance. *TaNFXL1*, in particular, showed a direct correlation with reduced infection rates in wheat, making it a promising candidate for future genetic enhancements. On the other hand, *TaHRC* also demonstrated significant potential by directly reducing infected spikelets, which are crucial for managing FHB.

Notably, the editing efficiencies varied among the targeted genes within wheat and barley, reflecting the intricate nature of genomic responses to CRISPR/Cas9 interventions. For instance, the mutation success rates, as depicted in Table 1 and Table 2, ranged from high to moderately low across the genes *TaNFXL1*, and *TaRPK1* in wheat, indicating a varied relationship between CRISPR/Cas9 system components and plant genomic architectures. This difference could be down to factors such as the gene TaRPK1, associated with root development as per Rahim et al. 2024, contrasts with *TaNFXL1* in Brauer et al. 2020, which is specialised in disease resistance. This difference could make achieving a successful mutation more challenging (Loewe, 2010). *TaNFXL1* shows the most promise out of the genes reviewed (Table 1) when it comes to phenotypic improvement as it showed a statistically significant reduction in infected spikelets out of the biggest sample size (Table 1). When it comes to FHB resistance the reduction in infected spikelets is a more directly beneficial phenotype compared to improving overall agronomic traits in wheat (Rahim, 2024; Brauer, 2020).

When it comes to the study conducted by Chen et al. 2022 the mutation rate for the *TaHRC* gene were significantly high, and it might be due to the BSMV-mediated CRISPR delivery system that was utilised. The system represents a significant advancement in wheat genome editing, effectively bypassing the challenges associated with traditional tissue culture and transformation methods, which are particularly pronounced in wheat due to low callus induction and regeneration rates (Chen, 2022). This approach enables direct gRNA delivery into a broad range of wheat genotypes, streamlining the process of generating targeted gene edits, as exemplified by the efficient modification of Fhb1, a key gene in Fusarium Head Blight (FHB) resistance. Importantly, the system ensures the heritability of edited traits, allowing for the stable transmission of enhanced disease resistance across generations (Amutha, 2023). Further optimisations, including the incorporation of RNA mobility sequences, have significantly improved the efficiency of this delivery system, reinforcing its potential to facilitate robust and inheritable genetic modifications crucial for crop improvement (Chen, 2022). *TaHRC* is the only other gene reviewed (Table 1), when mutated that significantly reduced the infected spikelets on wheat, which is probably the most favourable phenotypic change as it’s a direct correlation to FHB resistance. However, it did have a significantly smaller sample size so is less reliable than *TaNFXL1*. However the significance results were set at \*P < 0.001 compared to \*P < 0.05 for *TaNFXL1* so it might be not as representative to a population the results are definitely more certain.

The genes *TaABCC* and *TansLTP9* in Cui et al. 2017 both harboured relatively poor results which upon review could be attributed to a high or low GC content within their target sequences (Konstantakos, 2022; Cui, 2017). GC content refers to the percentage of guanine (G) and cytosine (C) bases in a DNA sequence, which can influence DNA's structural properties and stability (Bowers, 2022). Although there is no specific literature pinpointing an optimal GC content for *TaABCC* and *TansLTP9*, it's known that extreme GC levels can affect sgRNA binding efficiency and DNA cleavage by Cas9 (Konstantakos, 2022). Another reason could be down to the isolation of protoplasts in the study Cui et al. 2017 in order to obtain the plant cells, which are cells with their cell wall enzymatically removed, is a delicate process that can affect their viability and stability, making it difficult to maintain them alive for gene editing (Brandt, 2020). Additionally, the CRISPR components are often delivered transiently, which may not allow stable or prolonged expression necessary for effective editing, resulting in variable outcomes (Brandt, 2020). The methods used to deliver these components, such as PEG-mediated transformation, can also vary in efficiency, potentially leading to a subset of protoplasts being edited (Poddar, 2023). Furthermore, the regeneration of protoplasts into whole plants, which is essential to realise the gene editing effects, poses a significant challenge due to the technical difficulties and low efficiency of plant regeneration from protoplasts, especially in wheat (Poddar, 2023; Brandt, 2020).

The *TaABCC* genes however were slightly higher, which could be down to ABC transporters being integral membrane proteins involved in the transport of various molecules across cellular membranes. These proteins typically have critical and well-conserved domains essential for their function. Mutations in these domains are likely to disrupt protein function, which could lead to more detectable phenotypic changes. This increased likelihood of functional disruption makes mutations in ABC transporters easier to detect and quantify compared to mutations in nsLTPs, which may not always result in clear phenotypic changes (Feng, 2022). The CRISPR/Cas9-mediated gene edits in TaABCC and TansLTP9 demonstrated phenotypic changes manifesting as a delay in the onset of symptoms characteristic of Fusarium head blight infection. However, the absence of recorded statistical significance undermines the reliability of these observations. Therefore the linkage between the edited gene and the observable trait change remains speculative. This lack of statistical validation implies that the observed delay in symptoms, while potentially indicative of an underlying genetic modification, cannot be conclusively attributed to the CRISPR/Cas9 edits without further empirical evidence.

For the Arabidopsis genes in studies by Low et al. (2020) and Low et al. (2022) the AtOGO gene had a higher mutation success in the first generation of plants, and the reason could be down to numerous reasons. There was one fewer plant mutated for the AtOGO mutation in the first generation of modified wheat compared to the *AtEIN2* plants. The specifics of the gRNA design weren’t mentioned in Low et al. 2020 which could also be a factor in why it had a higher mutation rate as specificity and efficiency of the guide RNA (gRNA) play a crucial role in determining the success of CRISPR-mediated gene editing. Effective gRNA design, targeting conserved and functional regions of the gene, can lead to higher mutation rates (Cram, 2019). Additionally, the 2OGO gene demonstrated a bolstered immune response in Arabidopsis, which suggests an enhanced ability to resist pathogens. On the other hand, plants modified with the *EIN2* gene exhibited reduced spore production, a phenotype that is probably more attributed to FHB resistance directly. Both mutations were statistically significant and show promise for agricultural applications.

Finally, in Galli et al. 2022 SpCas9-induced bi-allelic and mono-allelic mutations were observed in barley, showing high rates of mono-allelic mutations (81% for *HvMORC6a* and 89% for *HvMORC1*) and relatively lower rates for bi-allelic mutations (27% for *HvMORC6a* and 23% for *HvMORC1*). This could be attributed to the specialised SpCas9 system that was utilised, derived from *Streptococcus* pyrogenes. This system requires only the Cas9 protein and a guide RNA, streamlining the design process (Xu, 2020). Its efficacy is further enhanced by high-fidelity variants which are engineered to minimise off-target effects while maintaining robust on-target activity, ensuring precise genetic modifications (Xu, 2020; Lee, 2018). Moreover, variants like SpCas9 broaden the scope of potential genomic targets, thereby increasing the genes that can be edited for FHB resistance. The system's capacity to multiplex—targeting multiple genes simultaneously—proves particularly advantageous for modifying complex traits such as FHB resistance, which is governed by multiple genes (Guo, 2019; Chen, 2020). This adaptability enables precise customisation of the barley genome, either by knocking out susceptibility genes or enhancing resistance genes (Guo, 2019). Furthermore, despite the absence of detailed sample size data, both mono-allelic and bi-allelic modifications showed statistically significant improvements in reducing fungal growth and the number of necrotic lesions. These results not only demonstrate the potential effectiveness of CRISPR/SpCas9-mediated genetic modifications.

The CRISPR/Cas9 gene-editing technique has marked a significant advance in the field of agricultural biotechnology, with an impact on enhancing disease resistance in crops such as wheat and barley. The review highlights several studies demonstrating variable success rates in gene targeting, reflecting the nuanced interaction between CRISPR components and genomic contexts. Among the successful applications, the BSMV-mediated CRISPR delivery system stands out, especially in studies like Chen et al. (2022), which bypassed the limitations of traditional transformation methods and achieved significant mutation rates in wheat. This system not only improved the efficiency of gene edits but also ensured their heritability, which is crucial for sustainable agricultural practices. Similarly, the SpCas9 system utilised by Galli et al. (2022) for barley showcased high specificity and adaptability, achieving a high rate of mono-allelic mutations which are essential for enhancing Fusarium Head Blight (FHB) resistance. On the other hand, certain CRISPR applications faced challenges, particularly when targeting genes, such as *TaABCC* and *TansLTP9* in Cui et al. (2017) which could be down to the isolation method of plant cells or down to the GC content of the genes.

* 1. Conclusion and Future Directions
     1. Expanding Target Gene Pool

CRISPR/Cas9 technology has ushered in a new era of precision in genome editing. Initial applications have predominantly focused on a relatively narrow set of target genes, primarily those with well-established roles in disease resistance and stress tolerance (Li, 2021). However, there is a burgeoning interest in expanding this target gene pool to address complex traits such as yield, nutrient use efficiency, and quality traits, which are governed by a network of genes and regulatory elements (Lui, 2021). The strategic integration of high-throughput sequencing technologies, genome-wide association studies (GWAS), and advanced CRISPR/Cas9 editing techniques are essential to explore (Naik, 2022; Lui, 2021). High-throughput sequencing technologies have revolutionised our understanding of plant genomes, revealing a wealth of genetic diversity and novel genes potentially associated with agronomically important traits (Yang, 2022). Concurrently, GWAS have emerged as a powerful tool to link genetic variation with phenotypic traits, identifying candidate genes and regulatory elements that could be targeted for crop improvement. These studies have highlighted the complex genetic architecture of traits like yield and nutrient use efficiency, implicating a wide array of genes beyond those traditionally targeted for CRISPR/Cas9 editing (Yang, 2022; Kumar, 2022).

Broadening the spectrum of target genes to include those related to complex traits introduces several challenges. Firstly, the traits are polygenic; multiple genes contribute marginally but cumulatively to the phenotype. This complexity requires a multiplex CRISPR/Cas9 strategy that targets multiple genes at once (Kumar, 2022). Achieving this multiplexing efficiently, especially in polyploid species like wheat, is technically demanding due to the need for accurate sgRNA design and the risk of unintended genetic effects (Rossato, 2023). Additionally, non-coding elements like promoters and enhancers, which are crucial for gene expression, must be accurately targeted by CRISPR/Cas9 to effectively alter trait expression. The roles of these elements in controlling complex traits are still not fully understood (Yang, 2022). To address these challenges and maximise the potential of expanding the target gene pool, integrating CRISPR/Cas9 with omics technologies is indispensable (Yang, 2022). Techniques such as transcriptomics and proteomics shed light on gene functions and the regulatory networks that underpin complex traits, assisting in the selection of target genes for editing. Epigenomics further enhances this approach by mapping the regulatory genome, revealing new targets for epigenetic editing to adjust gene expression (Rossato, 2023).

* + 1. Food Security

This review has highlighted the diverse phenotypic benefits, and the challenges posed by genetic complexity have significant implications for crop science and global food security. The ability of CRISPR to induce precise, inheritable genomic modifications presents a promising avenue for developing crops with enhanced disease resistance, which is crucial for safeguarding yields against pathogenic threats. The review findings highlight sophisticated CRISPR techniques, such as BSMV-mediated CRISPR and SpCas9 CRISPR delivery, which have yielded successful mutation outcomes and phenotypic advantages in combating FHB. These advancements in CRISPR technology could be pivotal in addressing the challenges of food security by ensuring the stability and quality of wheat and barley, which are staple crops for billions of people worldwide (CIMMY, 2020). The targeted modification of genes essential for disease resistance not only improves crop resilience but also reduces the reliance on chemical fungicides, contributing to more sustainable agricultural practices (Figeuroa, 2017). This approach aligns with the broader goals of crop science to develop varieties that can withstand various biotic stresses, thereby enhancing food security in the face of climate change and a growing global population (CPN, 2019).

**Annex A: Search Strategy**

**A.1 Search Strategy**

The search strategy for this systematic review was meticulously designed to identify relevant literature across a variety of databases, including Mendeley, PubMed, Scopus, and Google Scholar. Selected keywords and Boolean operators were used to construct search phrases tailored to our research focus. These phrases included “CRISPR/Cas9”, “gene editing”, “Fusarium Head Blight”, “Barley Resistance”, and “wheat resistance”. To ensure a comprehensive capture of relevant research, parameters such as publication date, language, and article type were applied to the search process. The goal of this strategy was to guarantee an exhaustive and unbiased collection of data pertinent to the study's objectives.

**A.2 Inclusion and Exclusion Criteria**

In the process of literature curation for this systematic review, I initially screened titles and abstracts, rigorously excluding any records not directly pertinent to the genetic enhancement of Fusarium Head Blight (FHB) resistance in wheat and barley through CRISPR/Cas9 gene editing. The exclusion criteria were designed to sift out studies unrelated to CRISPR/Cas9 technology, those not involving wheat or barley, and research not focused on FHB or its resistance mechanisms. Subsequently, the remaining records underwent a comprehensive review, and inclusion was contingent upon satisfying any of the following criteria: (1) empirical studies that demonstrated the application of CRISPR/Cas9 gene editing specifically for enhancing FHB resistance in wheat or barley; (2) investigations that elucidated the molecular mechanisms by which CRISPR/Cas9 edits conferred enhanced resistance to FHB; (3) studies that provided predictive models or equations assessing the potential impact of CRISPR/Cas9-mediated genetic modifications on FHB resistance; or (4) studies that convey insight on the virulence factors and the pathogenicity of Fusarium Head blight on Wheat. Each study meeting these inclusion criteria was meticulously catalogued and analysed, contributing valuable insights towards addressing the core objectives of this review.

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**Figure 1**

Fig. 1. illustrates the progression of Fusarium Head Blight (FHB) infection across three wheat cultivars with varying resistance levels, as documented by Chen et al. (2023). Panels G and I depict the resistant cultivar Sumai3 (R) and the susceptible cultivar Annong8455 at 15 days post-anthesis (DPA), respectively. The resistant strand exhibited a golden colour which associates with healthy maturity. In comparison the vulnerable strand was observed to have a chalky pink colour suggestive of Fusarium symptoms.



**Figure 2**



Fig.2 from Hagerty et. al (2023) shows two kernels in their “soft dough stage” which is the beginning stages of maturity before harvest (Zhang, 2021). The kernel infected with fusarium head blight (top left) is “ghosted” and withered compared to a healthy uninfected kernel (bottom right).

**Figure 3**

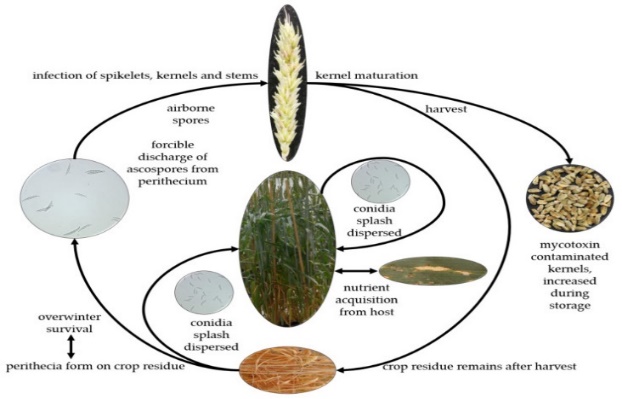


Fig.3 illustrates the life cycle of the Fusarium spp. responsible for Fusarium Head Blight (FHB) in wheat, as reported in the study by Alisaac et al. (2023).

**Figure 4**

Fig.4 Shows a PRISMA flow diagram conforming to the PRISMA 2020 checklist (Page, 2021) with eleven pivotal observations taken from seven studies, offering a comprehensive summary of CRISPR/Cas9 gene editing interventions applied to wheat and barley, using PRISMA This table catalogues the specific genes targeted, the modifications enacted, the success rate of the modifications, and the observable improvements in Fusarium Head blight Resistance. The resultant improvements vary from reduced infected spikelets to improved phenotypic traits that are conducive to resistance.

Reports sought for retrieval.

(n = 71)

Records identified through Mendeley (n= 877)

Other sources (n=15)

Records removed *before screening*:

Records removed because it’s not in English (n=821)

Full text articles screened for eligibility.

(n = 35)

Reason reports not included in final analysis (n=27):

Not enough significant outcomes

**Identification of studies via databases and registers**

**Identification**

**Screening**

Reason reports excluded before for before eligibility check (n=36):

Studies outside the scope of Barley and Wheat

CRISPR/Cas9 not used.

No FHB mentioned.

Studies included in review:

Mendeley studies (n = 4)

SCOPUS and Google scholar (n= 3)

**Included**

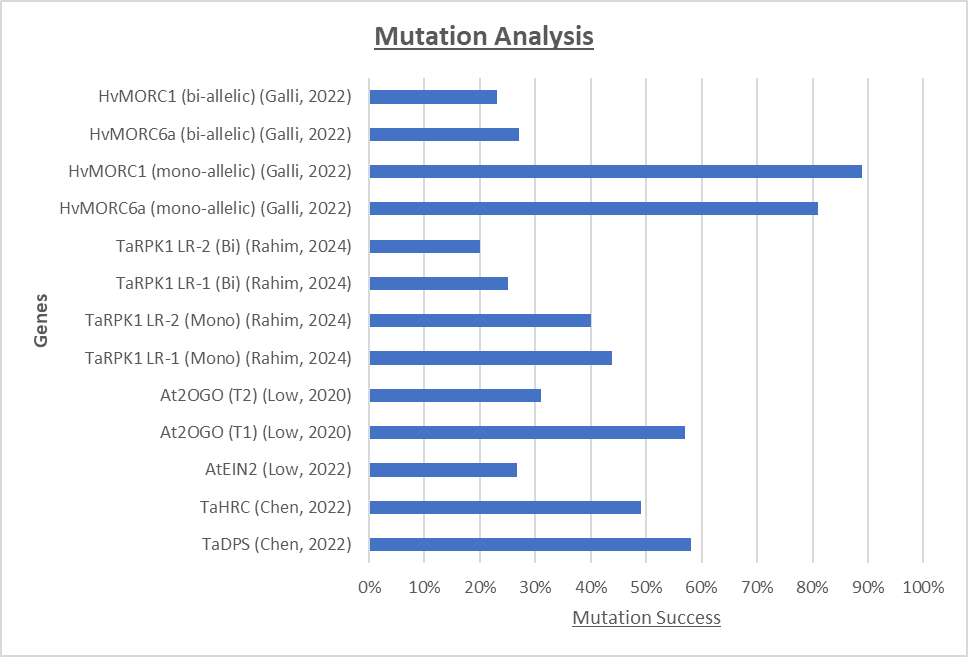
**Eligibility**

**Table 1**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Table 1.**  **Gene Editing Outcomes and Efficiencies in Wheat and Barley for Fusarium Head Blight Resistance** | | | | | | | |
|  | | | | | | | |
| **Observation** | **Study** | **Gene** | **What was modified?** | **Mutation success** | **sample size** | **What was improved** | **Statistical Significance** |
| 1 | (Brauer, 2020) | *TaNFXL1* | Wheat | N/A | 15-22 | After 8 days there was a reduction in the infected spikelets | Yes |
| 2 | (Chen, 2022) | *TaDPS* | Wheat | 58% | 187 | Albino phenotype produced allowing for a visible marker for successful mutation | No |
| 3 | (Chen, 2022) | *TaHRC* | Wheat | 49% | 5 | Reduction in the infected spikelets | Yes |
| 4 | (Low, 2022) | *AtEIN2* | Arabidopsis | 27.00% | 15 | Reduced spore production | Yes |
| 5 | (Low, 2020) | *At2OGO* | Arabidopsis thaliana | T1 = 57% & T2= 31% | T1=14 & T2=13 | Improved defence response | Yes |
| 6 | (Rahim, 2024) | *TaRPK1* (LR-1) | Wheat | 44% | 16 | Plant height, Number of effective Tillers, Spike length, Grain weight, Root Traits | Yes |
| 7 | (Rahim, 2024) | *TaRPK1* (LR-2) | Wheat | 40% | 15 | Plant height, Number of effective Tillers, Spike length, Grain weight, Root Traits | Yes |
| 8 | (Galli, 2022) | *HvMORC1* | Barley | 89% | N/A | Reduction in fungal growth and the number of necrotic lesions | Yes |
| 9 | (Galli, 2022) | *HvMORC6a* | Barley | 81% | N/A | Reduction in fungal growth and the number of necrotic lesions | Yes |
| 10 | (Cui, 2017) | *TaABCC* | Wheat | 9-13% | 17 | Delay in onset of symptoms | No |
| 11 | (Cui, 2017) | *TansLTP9* | Wheat | 0-11.9% | 5 | Delay in onset of symptoms | No |
| \**Mutation Success*: Represents the percentage of targeted mutations achieved through CRISPR/Cas9 gene editing across various genes, indicating the efficiency of the editing process.  \**Sample Size*: Refers to the number of samples or plants analysed for each gene to assess the mutation success and the observed improvements.  \**What Was Improved*: Highlights the key phenotypic or resistance traits enhanced in wheat and barley because of the CRISPR/Cas9-mediated gene editing, including but not limited to reduced infected spikelets, enhanced plant architecture, delayed disease symptoms, and increased resistance to Fusarium Head Blight (FHB).  \**Statistical Significance*: Denotes the results of statistical tests performed to validate the observed improvements, thereby affirming the efficacy of gene editing in enhancing FHB resistance.  \**T1 and T2:* First generation and second generation of modified plants respectively. | | | | | | | |

**Figure 5.**

Figure.5 Shows a Bar chart of each mutation success observed using CRISPR mediated gene editing aiming to enhance fusarium head blight resistance. The first generation of plants and the second generation of plants are labelled (T1) and (T2) respectively (Low, 2020). A monoallelic mutation (Mono) affects only one allele of a gene, while a biallelic (Bi) mutation involves changes in two or more alleles (Rahim, 2024; Zhang, 2019)



**Figure 6.**

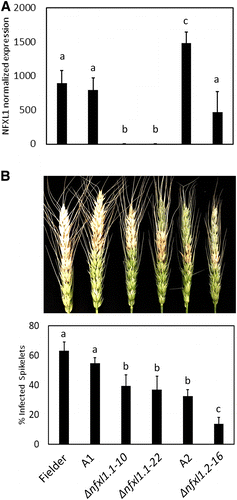
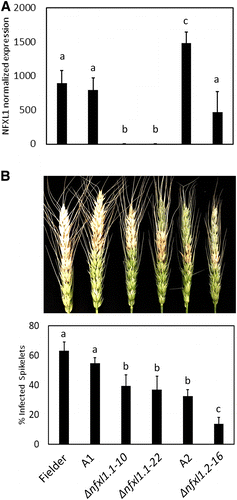
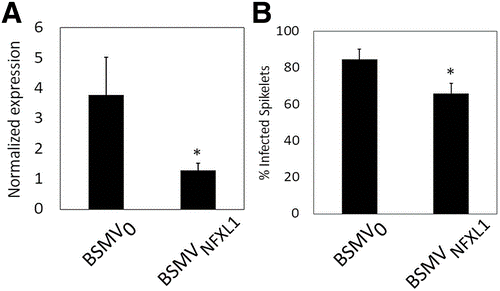
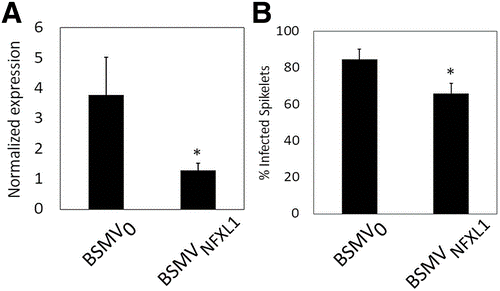


Fig. 6 from Brauer et al. 2020 shows 6A and 6B. In 6A plants were subjected to virus-induced gene silencing against those treated with a control virus (BSMV0) without a wheat-targeting sequence. Gene silencing efficacy is confirmed in the BSMV/*TaNFXL1*-treated plants, where normalised expression levels of the *TaNFXL1* gene, relative to housekeeping genes, indicate successful suppression. The asterisk (\*) denotes statistically significant differences in gene expression compared to the BSMV0 control. 6B illustrates the expression levels of the *TaNFXL1* gene across different wheat lines to assess the impact of CRISPR editing. The x-axis categories include Fielder (Control), which represents baseline expression in unedited Fielder wheat plants; Azygous A1 (A1) and Azygous A2 (A2), sibling plants from two transformation events that did not inherit CRISPR modifications, serving as comparators to isolate the effects of CRISPR edits; and nfxl1.1-10, nfxl1.1-22, nfxl1.2-16, which are CRISPR-edited lines with confirmed deletions in the *TaNFXL1* gene.



|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Table 2**  **Table 2. CRISPR/Cas9 Techniques and Design Specificities for Targeted Gene Mutagenesis in Wheat and Barley** | | | | | |
| Observation | Study | Gene | CRISPR technique | CRISPR outcomes | CRISPR design |
| 1 | (Brauer, 2020) | *TaNFXL1* | Targeted mutagenesis of six homeologs of *TaNXL1* gene | Deletions in the *TaNFXL1* region | Two Single Guide RNAs with CAS9 protein |
| 2 | (Chen, 2022) | *TaPDS* | Targeted mutagenesis of *TaDPS* using BSMV-mediated gRNA delivery system` | N/A | Single guide RNA into BSMV |
| 3 | (Chen, 2022) | *TaHRC* | Targeted mutagenesis of *TaHRC* using BSMV-mediated gRNA delivery system` | Knockout of *TaHRC* | Single guide RNA into BSMV |
| 4 | (Low, 2022) | *AtEIN2* | RNA Guided Mutagenesis | Frameshift mutation of *AtEIN2* | 20-Nucleotide guide RNA sequence |
| 5 | (Low, 2020) | *At2OGO* | RNA Guided Mutagenesis | Frameshift mutation of 2OGO | N/A |
| 6 | (Rahim, 2024) | *TaRPK1*  *(LR-1)* | Two Guide RNA Targeted Mutagenesis at genome D | Deletions of *TaRPK1* regions | Combined sgRNA1 and sgRNA2 |
| 7 | (Rahim, 2024) | *TaRPK1*  *(LR-2)* | Two Guide RNA Targeted Mutagenesis at genomes A, B and D | Deletions of *TaRPK1* regions | Combined sgRNA1 and sgRNA3 |
| 8 | (Galli, 2021, 2022) | *HvMORC1* | CRISPR/SpCas9 system inserts STOP codons into the opening of *HvMORC1* | Double Knockout of MORC genes | Generation of *HvMORC1*-guided RNA with no potential off-target sites |
| 9 | (Galli, 2021, 2022) | *HvMORC6a* | CRISPR/SpCas9 system inserts STOP codons into the opening of *HvMORC6a* | Double Knockout of MORC genes | Generation of *HvMORC6a*-guided RNA with no potential off-target sites |
| 10 | (Cui, 2017) | *TaABCC* | single-guide RNAs (sgRNAs) that introduce double-stranded breaks | Deletions for the *TaABCC* target regions | Two sgRNAs (ABCC-sgRNA-1 and ABCC-sgRNA-2) were designed to target the *TaABCC* gene. |
| 11 | (Cui, 2017) | *TansLTP9* | single-guide RNAs (sgRNAs) introducing double-strand breaks | Deletions for the *TansLTP9* target regions | Two sgRNAs, named nsLTP9.4-sgRNA-1 and nsLTP9.4-sgRNA-2, were designed to target different loci on the *TansLTP9* gene. |
| \*CRISPR Technique: Specifies the gene editing approach utilised, detailing the type of CRISPR system and delivery method.  \*CRISPR Outcomes: Describes the resultant genetic changes from the editing process.  \*CRISPR Design: Indicates the construct or arrangement of CRISPR components and how they were designed to achieve the desired gene edits. | | | | | |