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Gene editing for disease resistance in wheat

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Abstract:

Genome editing has emerged as a revolutionary tool for crop improvement, providing precise and efficient modifications to enhance disease resistance. The CRISPR/Cas system, in particular, has gained prominence due to its simplicity, accuracy, and versatility in genome manipulation. This technology enables targeted gene knockout or modification of susceptibility (*S*) genes, conferring enhanced resistance against bacterial and fungal pathogens. Applications of genome editing in wheat and other staple crops have demonstrated its potential to mitigate diseases such as wheat blast and bacterial blight. Moreover, advances in bacterial genome editing hold promise for developing plant-associated microbes with biocontrol capabilities. As genome editing technologies continue to evolve, they offer a sustainable alternative to conventional breeding, ensuring resilient crop varieties and reducing dependence on harmful chemical pesticides. This article highlights the progress and future prospects of genome editing in developing disease-resistant crops and its role in promoting environmentally sustainable agriculture.

Key words: CRISPR/Cas system, Genome editing, Disease-resistant, sustainable agriculture.

Introduction:

Agriculture is the foundation of human civilization, playing a crucial role in sustaining livelihoods and driving economic progress. In the 21st century, the primary goal of agriculture is to ensure food security through sustainable practices that minimize environmental impact. However, one of the major challenges threatening global agricultural productivity and food security is the prevalence of plant diseases. These diseases not only hinder plant growth and development but also significantly reduce crop yields and degrade the quality of agricultural produce, both in the field and during storage. According to the Food and Agriculture Organization (FAO), plant diseases can lead to

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yield losses of up to 20–40% globally, posing a serious risk to food production. Chemical pesticides remain a widely used method for controlling crop diseases. However, their extensive application has raised concerns regarding environmental safety and human health. Many pesticides lack specificity, affecting beneficial organisms and disrupting ecological balance. Additionally, the continuous use of chemical pesticides has contributed to the emergence of resistant pathogen strains, necessitating the development of newer pesticides or increased application rates. This situation underscores the urgent need for alternative, sustainable approaches to disease management in agriculture, particularly in developing nations where environmental and health risks are more pronounced. In recent years, gene editing has emerged as a revolutionary tool in crop improvement, offering precise, efficient, and targeted modifications at the genomic level. This technology has been extensively explored in wheat to enhance resistance against major fungal diseases, such as those caused by *Blumeria graminis* (powdery mildew) and *Fusarium graminearum* (Fusarium head blight). The first application of the CRISPR-Cas system in wheat was demonstrated by Shan et al., marking a significant milestone in plant genome engineering. Gene editing involves the use of advanced molecular tools to modify, insert, or delete specific genetic sequences, thereby enhancing desired traits. Previously, gene-editing techniques such as zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) were widely used. However, the CRISPR-Cas system has gained increasing attention due to its simplicity, efficiency, and precision in genome editing. These methods utilize site-specific nucleases (SSNs) that introduce double-strand breaks at targeted DNA sequences, enabling controlled genetic modifications. Gene editing in plants primarily relies on two strategies: (1) gene knockout, which disrupts the function of specific genes, and (2) gene knock-in, which introduces new or modified genes to confer desired traits. The CRISPR-Cas9 technology, co-developed by Jennifer Doudna and Emmanuelle Charpentier, has revolutionized genetic research, offering new possibilities for disease-resistant crop development. Genetic engineering for disease resistance presents a sustainable and eco-friendly alternative to conventional breeding methods. Traditional resistance breeding, despite its historical success, has several limitations:

1. It is restricted to plants that can naturally interbreed, limiting genetic diversity.
2. It depends heavily on the availability of genetic variations within plant populations.

3. The breeding process often transfers unwanted traits along with the desired resistance genes, potentially affecting yield and other agronomic characteristics.
4. It is a time-consuming and labour-intensive process, requiring multiple generations of selection and backcrossing.

Genome Editing Technology

Genome editing is a precise method used to modify the genomic DNA of a cell or organism. This technique relies on sequence-specific nucleases to recognize specific DNA sequences and create double-stranded DNA breaks (DSBs) at targeted sites. The repair of these DSBs occurs primarily through two mechanisms: non-homologous end joining (NHEJ) and homologous recombination (HR). NHEJ is the more commonly utilized pathway for DSB repair, though it is error-prone and often results in insertions or deletions. In contrast, the HR pathway enables precise base modifications or gene replacements when a donor DNA template is available. Genome editing employs three major types of nucleases: Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), and the CRISPR/Cas system. The CRISPR/Cas system, which originates from the adaptive immune defense of bacteria and archaea, protects against invading plasmids or viral DNA by cleaving foreign genetic material. CRISPR/Cas genome editing consists of a single-guide RNA (sgRNA) and an active Cas nuclease. The guide RNA (gRNA) contains a customizable spacer sequence (~20 nucleotides) that directs targeting and a scaffold region for Cas protein binding. Since its initial use in mammalian cells, the CRISPR/Cas9 system has rapidly become the preferred genome editing tool across various organisms, including plants, due to its simplicity, high efficiency, and ease of application. However, its dependency on the protospacer adjacent motif (PAM) limits target sequence selection. To overcome this, researchers have developed alternative CRISPR/Cas systems with varying PAM requirements, such as CRISPR/saCas9, CRISPR/Cpf1 (Cas12a), and xCas9. Additionally, RNA-targeting CRISPR systems, including CRISPR/Cas13a (C2c2), CRISPR/Cas13b, and CRISPR/Cas13d, have expanded the CRISPR toolkit, further enhancing genome and transcriptome editing capabilities.

Conventional and Genome Editing Approaches for Disease Resistance in Crops

Traditional breeding methods have been successfully employed to enhance disease resistance in various crops. The pure line selection method, particularly effective for self-pollinated crops, was used to develop the

rust-resistant Kanred wheat variety. The pedigree breeding approach has played a crucial role in developing disease-resistant cultivars controlled by major genes. Additionally, the recurrent selection and backcross methods have been instrumental in strengthening elite rice cultivars against rice blast. Interspecific hybridization has facilitated the transfer of resistance genes from *Gossypium anomalum* and *Gossypium arboreum* into *Gossypium hirsutum*, enhancing resistance to cotton rust. Moreover, mutation breeding has led to the successful development of multiple rice lines resistant to rice blast. However, conventional breeding methods are often time-consuming and can inadvertently introduce genes that influence plant growth and development. The success of genome editing depends on a thorough molecular understanding of target genes and the host genome. With the increasing availability of complete genome sequences for various plant species, along with extensive genetic and molecular studies, the intricate mechanisms of plant immunity have been elucidated. This has opened up numerous possibilities for disease and pest resistance enhancement. In particular, susceptibility (S) genes, which act as negative regulators of plant defense, have emerged as prime candidates for genome editing to improve disease resistance.

Genome Editing for Resistance Against Pathogenic Microorganisms

Bacterial pathogens are highly diverse, rapidly proliferating, and capable of spreading through multiple means, making bacterial disease management particularly challenging, especially once outbreaks occur. However, advancements in understanding plant-pathogen interactions have identified several host genes, including S genes, that play a role in these complex interactions. Genome editing techniques have increasingly focused on modifying S genes to enhance crop resistance to bacterial infections, as they can provide more durable field resistance. Rice bacterial blight, a vascular bundle disease caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo), is a major threat to rice production, with potential yield losses ranging from 10–20%, increasing up to 50% under highly humid conditions. Xoo employs a type III secretion system to introduce transcription activator-like effector (TALE) proteins into host cells, which in turn activate S genes to promote infection. For example, in the Philippine strain PXO86, the AvrXa7 TALE protein binds to the effector-binding element (EBE) in the promoter of *OsSWEET14* (*Os11N3*), inducing its expression. *OsSWEET14* encodes a sucrose-efflux transporter that facilitates sugar movement from rice cells, supporting pathogen growth and virulence. However,

since *OsSWEET14* also plays a critical role in plant growth, completely knocking out this gene to confer resistance could have detrimental effects. Instead, genome editing has been used to modify the EBE within the *OsSWEET14* promoter using TALENs, preventing AvrXa7 binding while preserving its function in plant development. Tomato production, despite its global commercial significance, is severely impacted by major pathogens such as *Pseudomonas syringae*, *Phytophthora* spp., and *Xanthomonas* spp. Recent studies indicate that a mutation in *DMR6* (*Downy Mildew Resistance 6*) in *Arabidopsis* enhances salicylic acid accumulation, leading to broad-spectrum resistance against bacterial and oomycete pathogens. Notably, infection by *P. syringae* pv. *tomato* and *Phytophthora capsici* triggers upregulation of the tomato homolog *SlDMR6-1*. Using the CRISPR/Cas9 genome editing system, researchers developed *SlDMR6-1* knockout mutants in tomato that exhibited resistance to *P. syringae*, *P. capsici*, and *Xanthomonas* spp. without negatively affecting plant growth or development. These findings suggest that *DMR6* knockout could serve as a valuable strategy for conferring broad-spectrum disease resistance in plants.

Prospectives

The drawbacks of traditional resistance breeding may be greatly mitigated via genome editing. Firstly, by using genome editing, a target gene in elite varieties may be changed directly, avoiding the mating process. Second, once the target gene is identified, it does not depend on plant populations with high levels of genetic diversity; all that is needed is the target gene's sequence information. Third, linkage drag issues can be avoided because genome editing won't bring about modifications outside of the intended locations. Fourth, genome editing resistance breeding accelerates progress by eliminating the need for genetic crossings and segregant progeny selection. Nowadays, deliberate mutagenesis of genes known as "S genes," which adversely control defence, is used to create the majority of disease-resistant crops against non-viral diseases. Genome editing can produce desirable S gene mutants in most plants of interest for breeding without taking into account species barriers by taking use of the functional conservation of S genes across plant species. It is anticipated that more S genes will be identified, increasing the number of potential targets for genome editing. Null mutations in S genes, however, can occasionally have a negative impact on normal growth since S genes are frequently involved in the growth and development of plants. Their applications may be hampered by this occurrence. Three elements make up the disease triangle, which is the fundamental

paradigm of plant pathology: a virulent pathogen, a vulnerable host, and a favourable environment. Only when all three elements are present together can disease result. Therefore, any of the three components might be restricted by genome editing, breaking the cycle of plant–pathogen interactions and controlling illness. There is a warning about the connection between a rising climate and fungal wheat disease, namely wheat blast, which is caused by the fungus *Magnaporthe oryzae* pathotype *triticum* (MoT). The article "The Production Vulnerability of Wheat towards Wheat Blast Disease Under Climate Change" The potential for a 13% reduction in wheat yield exists with Blast of Wheat. South America and Africa are the most susceptible continents. It's the recently developed illness. By eliminating the gene causing blast sickness, strains resistant to this illness can be created by gene editing. Rapid trait modification in wheat crops will be possible thanks to gene editing technologies. It is among the finest methods since infections are always changing and it is often monogenic. Traditional approaches don't work well for finding novel disease resistance genes and how new cultivars or types incorporate them. In order to prevent Pseudomonas aeruginosa gut infections, a probiotic Escherichia coli strain was recently genetically modified to lyse itself upon sensing the N-acyl homoserine lactone generated by the human pathogen. This process released an antibiofilm enzyme and a toxin. A major chitinase from Serratia marcescens, expressed via the endophytic Pseudomonas fluorescens chi-A gene, efficiently protected bean seedlings against infection with *Rhizoctonia solani*. This can be done by using CRISPR technology to change other plant-associated bacteria's enzyme encoded genes to produce specific activities. Thus, the use of bacterial genome editing to treat plant diseases appears promising. All things considered, genome editing has developed into a potent tool for breeding disease resistance and studying molecular plant–microbe interactions. Genome editing will undoubtedly be used more frequently to create plants resistant to a wider range of diseases and to speed up the breeding process for strong, broad-spectrum resistance. These advancements in genome editing will surely help ecologically friendly agriculture.

Conclusion

Genome editing has transformed the landscape of plant disease resistance breeding by offering precise, efficient, and targeted modifications at the genetic level. Unlike traditional breeding approaches, genome editing allows direct modifications of elite varieties without extensive genetic crosses, reducing breeding timelines and preserving desirable agronomic traits. The targeted alteration of S genes has proven effective in

conferring resistance to multiple pathogens while maintaining plant growth and productivity. Additionally, genome editing technologies provide new avenues for engineering beneficial microbes to combat plant diseases, further broadening their application in sustainable agriculture. As research continues to identify new disease-resistance genes and optimize genome editing techniques, this technology is poised to revolutionize modern crop improvement efforts. By integrating genome editing into breeding programs, agriculture can shift towards more environmentally friendly practices, enhancing global food security while mitigating the impacts of climate change on crop production.

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