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Genetic Engineering in Crop Improvement for Diseases on Resistance

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ABSTRACT

This research delves into the progressions made in genetic manipulation, specifically focusing on genome editing techniques like CRISPR-Cas9, to enhance crop resilience against diseases. It underscores the constraints of conventional breeding methods while highlighting the precision and efficacy of contemporary genetic modification tools. The investigation encompasses the identification of resistance genes, gene duplication, vector assembly, plant alteration, and thorough molecular and phenotypic analysis. The discourse also touches upon field experiments and regulatory endorsement procedures to facilitate the advancement and commercialization of robust, disease-resistant crops. By utilizing case studies such as the wheat variety Guinong 29, the study illustrates the potential of genetic engineering in reducing reliance on pesticides, fostering sustainable agriculture, and ensuring global food security. Conventional breeding methods, like backcrossing, are juxtaposed with modern methodologies like CRISPR/Cas9, which allow for accurate genetic modifications. The study sheds light on three categories of site-specific nucleases (SDNs) and their implications on regulatory supervision. Key discoveries consist of successful gene editing in rice and maize, the significance of pattern recognition receptors (PRRs) and nucleotide-binding leucine-rich repeat receptors (NLRs) in pathogen identification, and tactics for modifying susceptibility factors. The research underscores the transformative capacity of genetic engineering in enhancing crop development, accentuating the necessity for regulatory adjustments and societal acceptance to guarantee secure and efficient implementation.

Keywords: Genetic Engineering, Crop Improvement, Disease Resistance, Sustainable Agriculture, Public Perception

1. INTRODUCTION

Genetic modification has become a pivotal instrument in the realm of agriculture, presenting novel resolutions to the inherent obstacles encountered in crop cultivation. A prominent utilization of genetic engineering in agriculture pertains to the enhancement of crops' resistance to diseases (Miedaner, 2016). Traditional methods of breeding have made remarkable progress in developing crop varieties that can withstand diseases. Nonetheless, these techniques typically

involve a lengthy process and are restricted by the presence of resistant genes in the genetic pool of the crop. Genetic modification surpasses these constraints by facilitating the targeted integration of particular genes from various origins, encompassing species that are unrelated, into the genome of the crop (Scott et al., 2016). Numerous pathogens, including bacteria, germs, fungi, and nematode worm, have the ability to cause diseases that result in notable decreases in crop yield and pose a threat to global food safety. The capacity to genetically modify crops to exhibit heightened immunity against such diseases serves not only to protect yields but also to diminish the dependency on chemical pesticides, thereby advocating for more sustainable agricultural approaches. Through the application of methodologies such as genetic manipulation, transgenic on technology, and RNA interference, researchers are able to create crop varieties equipped with strong resistance mechanisms, consequently enhancing both productivity and resilience. This introductory section establishes the fundamental basis for comprehending the pivotal significance of genetic manipulation in enhancing crops for the purpose of combating diseases. It highlights the potential benefits of these advancements, including increased agricultural sustainability, reduced environmental impact, and enhanced food security. As we delve deeper into the specifics of genetic engineering techniques and their applications in disease-resistant crop development, it becomes evident that this field holds the promise of revolutionizing modern agriculture. Genetic engineering has emerged as a pivotal tool in crop improvement, particularly for enhancing resistance to diseases. Traditional breeding methods, while effective, often display a temporal nature and limitations in their ability to finely tune complex gene clusters associated with disease resistance. The emergence of genome editing tools, exemplified by CRISPR-Cas, provides unparalleled accuracy in the manipulation of plant genomes, facilitating specific modifications to bolster disease resistance and other advantageous characteristics (Dhaliwal & Uchimiya, 1999). The application of the CRISPR-Cas9 system demonstrates the advancements in improving crop varieties to resist various pathogens such as bacteria, germs, fungi, and nematode worm. This technology has facilitated the implementation of innovative approaches for imparting confrontation then the creation of mechanisms intended for timely finding of pathogens (Dong & Ronald, 2019). The wheat cultivar Guinong 29 (GN29) represents a successful utilization of genetic modification, showcasing a combination of various resistance genes (such as Pm2, Pm21, Yr26, Lr1, and Lr46) that provide immunity against powdery mold, hoop oxidation, and foliage rust, in addition to genes for stress tolerance and superior baking qualities, illustrating the potential for simultaneous enhancement of multiple characteristics (Rahman et al., 2023). Moreover, transgenic on technologies have played a decisive part in advancing the creation of multiple cultivars that demonstrate resistance towards herbivorous insects, pathogenic viruses, and fungi by incorporating foreign genes and utilizing RNA interference (RNAi). This outcome has led to a significant reduction in the occurrence of yield loss as well as the deterioration of quality (Sun et al., 2019). Despite the achievements in the control of fungal, viral, and insect challenges, the development of resistance to bacterial and nematode diseases is still in the preliminary phase, as recent breakthroughs in RNAi and CRISPR/Cas technologies present encouraging prospects for forthcoming enhancements (Sun et al., 2019). In general, genetic manipulation, especially when utilizing genome editing, offers significant possibilities in the

advancement of disease-resistant crops, thereby playing an essential part in improving the resilience and sustainability of farming practices (Bigini et al., 2021). The paper written by Avci & Sipahi, (2024) identifies the discourse pertains to the enduring objective of agricultural research aimed at attractive the nutritive value, fragrance, visual appeal, and efficiency of crops to address the escalating global demand for food. While conventional approaches such as plant breeding have yielded positive results, they are frequently time-consuming and possess inherent limitations. Recent progressions in molecular biology and hereditary manipulation, especially in genome excision, provide exact mechanisms for altering harvest genetic makeup. These methodologies empower researchers to make specific alterations to a herb's DNA, demonstrating greater efficacy compared to conventional techniques like mutagenesis and transgenesis. Xiao et al., (2024) focuses on a special type of wheat called Guinong 29 (GN29), which is known for its strong resistance to several fungal diseases like crumbly mold and strip rust, and also has good farming traits like high yield and quality. GN29 was experienced with 113 molecular indicators to identify 98 genetic factors related to disease confrontation, pressure lenience, value, and adaptableness, which helped in considerate the genomic base of its elite characters. The study found that GN29 has two significant genes, Pm2 and Pm21, which provide strong confrontation to crumbly mildew, a common fungal disease in wheat.GN29 also has genes like Rht-B1b and Rht-D1a that make the plant shorter, and vernalization genetic factor vrn-A1, vrn-B1, vrn-D1, and vrn-B3 that help the plant adapt to different growing seasons. The study also identified stress tolerance genes Dreb1 and Ta-CRT in GN29, which help the plant withstand harsh conditions like drought, salinity, little malaise, and the attendance of abscisic acid (ABA). Greenwood et al., (2023) discusses how precision genome excision is an influential instrument that lets researchers to make exact variations to the DNA of crops, which can help improve their resistance to diseases. This technology is more accurate and efficient compared to outdated breeding approaches, which can yield several years to achieve similar results. Disease resistance is crucial for crops because diseases can significantly reduce crop yields and quality, leading to food shortages and economic losses for farmers. By enhancing disease resistance, crops can grow healthier and produce more food, which is essential for nourishing the growing worldwide people. The paper highlights several techniques used in accuracy on genome editing, such as CRISPR-Cas9, TALENs, and ZFNs. These techniques allow scientists to target exact genes in the crop's DNA and make precise changes, for example adding, removing, or altering genetic material to improve disease resistance. Yu et al., (2022) investigate the influence of various biotic restrictions, for example infective fungi, germs, bacteria, herbivorous pests, and scrounging nematode worm, on crop yield and quality. These biological stressors present significant obstacles for agricultural professionals and may have adverse impacts on food production and crop quality. The efficacy of traditional management strategies in addressing these biotic constraints is limited. Traditional methods often fall short in offering sufficient protection against pests and diseases, emphasizing the necessity for more advanced solutions.

The problems faced in worldwide agricultural manufacture, including limited arable land, water scarcity, the appearance of new pathogens, and the growth of confrontation in present pathogens, are compounded by the rising worldwide people and the anticipated 100% surge in food request by 2050. While traditional breeding methods remain significant, they are time-consuming and

labor-intensive, necessitating several generations of artificial cultivation to attain desired traits in crops. The article underscores the recent advancement in genome editing technologies, specifically the CRISPR-Cas9 system, that have created new prospects for sustainable agriculture through the growth of disease-resistant crops. The advent of CRISPR-Cas9 and other hi-tech genome editing tools has opened the door to the progression of transgene-free, non-genetically adapted plants, contribution a promising avenue for improving desirable traits in various crop species without the controversies linked to genetically modified organisms (GMOs). Avci & Sipahi, (2024) present a novel strategy to protection harvests against diseases by enhancing the inherent immune system of plants using genetic modification, representing a sustainable approach to guarantee food security. The scholars concentrated on enhancing the tomato plants' resistance toward late disease, a disease triggered by the pathogen Phytophthora infectants, through modifying a specific component of the plant's immune system.

Yin & Qiu, (2019) have clarified the process through which pathogens cause diseases in plants through the activation of specific genes within the plant, referred to as susceptibility (S) genes, facilitating pathogen invasion. The main aim of the researchers was to suppress the expression of these genes in order to boost the plants' resistance to diseases. Their investigation primarily delves into DNA methylation, an epigenetic alteration capable of modulating gene expression without modifying the DNA sequence per se. By directing methylation to particular regions of the plant's DNA, the researchers aspired to suppress the activation of the S genes. Barka & Lee, (2022) elaborate on the transformative impact of novel genome-editing tools on molecular upbringing, aiming to cultivate traits that confer resilience to diverse environmental stresses and diseases in plants, emphasizing precision and efficiency. The principal focus of the discussion is to assess the significant advancements in breeding disease-resistant varieties of three major Solanaceae crops: potato (Solanum tuberosum), tomato (Solanum lycopersicum), and pepper (Capsicum annuum), by manipulating the S genes. A key objective of these genome-editing instruments is to develop plants resistant to diseases by disrupting specific genes recognized as susceptibility genes (S genes), which influence the susceptibility of plants to pathogenic infections. The diseases that impact wheat, arising from a range of pathogens, cause considerable decreases in yield on a global level, thereby affecting the security of food supply. The conventional methods of breeding have encountered challenges in enhancing the resistance of wheat against these diseases, underscoring the need for sophisticated molecular techniques to achieve more favorable outcomes. (Mahmood et al., 2017a) elaborate on a range of molecular indicators like SCAR, RAPD, SSR, SSLP, RFLP, SNP, and DArT, which have been recognized for their efficacy in fortifying wheat resistance to pathogens. The utilization of these markers is indispensable in the formation of wheat cultivars that are unaffected to diseases, facilitated by a variety of breeding initiatives.

The objectives of this study are to investigate and highlight the advancements in genetic modification techniques, particularly genome editing tools like CRISPR-Cas9, for enhancing disease resistance in crops, assess the potential of genetically modified crops to reduce dependency on chemical pesticides, promote sustainable agriculture, and ensure global food security and examine specific case studies, such as the wheat cultivar Guinong 29 (GN29), to illustrate the successful integration of multiple resistance genes and the benefits of precise genetic modifications in crop improvement.

2. SIGNIFICANCE OF THE STUDY

The examination of genetic manipulation in crops for disease resistance is crucial in enhancing global food security by alleviating yield losses from plant diseases, thereby promoting sustainable agricultural practices by reducing dependence on chemical insecticides and mitigating adverse ecological impacts.

3. METHODS AND MATERIALS

The methodologies and substances utilized in the application of genetic engineering for enhancing crop resistance to diseases encompass a sequence of methodical and interconnected procedures, starting from the identification of specific genes to the confirmation of transgenic on plants through field experiments. The following outlines the key stages and techniques employed in this process:

3.1. Identification of specific genes that are the focus of study

The first step in genetic engineering for disease resistance is identifying the genes that confer resistance or susceptibility to specific pathogens. This can be accomplished through:

Genomic and Transcriptomic Analysis: High-throughput sequencing methodologies such as RNA-Seq are employed in genomic and transcriptomic investigations to juxtapose the hereditary content and gene expression patterns of plant varieties exhibiting confrontation and susceptibility. By means of bioinformatics examination, genes showing differential expression linked to resistance can be pinpointed.

QTL Mapping and GWAS: The unraveling of genetic sites linked to traits of battling diseases predominantly hinges on tracing Quantitative Trait Loci (QTL) and embarking on Genome-Wide Association Studies (GWAS). These methodologies entail establishing associations between genetic markers and phenotypic information derived from a wide range of plant populations.

Studying Pathogen Effectors: Delving into the importance of pathogen effectors in suppressing plant immunity aids in discovering the specific plant genes that these effectors target. Isolation of plant R genes, which detect particular effectors, can be achieved through functional assays and comparative genomics.

3.2. Cloning genes and crafting vectors

Once the desired genetic elements have been pinpointed, it becomes imperative to replicate and integrate them into fitting carriers to facilitate the metamorphosis of plants:

Gene Cloning: In the realm of Gene Cloning, the targeted gene experiences a surge in numbers through the enchanting process of polymerase chain reaction (PCR) before finding its new home within a cloning vector. Techniques like Gibson assembly, Gateway cloning, and the mystical art of restriction enzyme-mediated cloning are often summoned for this noble quest.

Vector Construction: The cloned gene is then inserted into a binary vector suitable for plant transformation. This vector typically contains a selectable marker gene (e.g., antibiotic resistance) and regulatory elements (e.g., promoters, terminators) to ensure suitable gene expression in the plant.

3.3. Herbal Transformation

The fabricated vector is incorporated into plant cells through a variety of innovative transformation techniques:

Agrobacterium-Mediated Transformation: The integration of the desired gene into the genetic architecture of the plant has been achieved through the use of Agrobacterium tumefaciens, a bacterium that resides in the soil.

Particle Bombardment (Biolistics): This approach comprises the application of a coating on minuscule particles (typically gold

or tungsten) with DNA, followed by their physical delivery into plant tissues. Later, the DNA becomes integrated into the plant genome upon successful penetration.

CRISPR/Cas9 and Other Gene Editing Technologies: To achieve exact genome editing, genetic constructs containing CRISPR/Cas9 elements are integrated into plant cells using techniques like Agrobacterium-mediated delivery or direct transformation methods such as particle bombardment and PEG-mediated protoplast transformation.

3.4. Selection and Regeneration

Transformed cells are selected and regenerated into whole plants:

Selection: Transformed cells are cultured on selective media containing antibiotics or herbicides. Only some cells that have integrated the selectable marker gene (and thus the transgene) will survive.

Rejuvenation: Selected cells are delicately motivated to embark on a journey of renewal, transforming into whole plants through the art of tissue culture techniques. The method involves the application of specific combinations of plant growing regulators to enable the growth of shoots and roots.

3.5. Molecular and Phenotypic Characterization

Regenerated plants are screened to confirm the presence and expression of the transgene:

Molecular Characterization: Techniques such as PCR, Southern blotting, and quantitative real-time PCR (qPCR) are used to confirm transgene integration and copy number. RNA analysis (e.g., RT-qPCR, RNA-Seq) can verify transgene expression levels.

Phenotypic Characterization: Transgenic plants are evaluated for their resistance to diseases using controlled experiments involving the inoculation of pathogens. The assessment includes the observation of disease symptoms, measurement of pathogen levels, and examination of various resistance markers such as hypersensitive response and the expression of defense-related genes.

3.6. Field Trials and Evaluation

Advanced transgenic lines are selected for field trials to undergo real-world evaluation:

Field Trials: Transgenic plants are cultivated within controlled field trials under ambient conditions for the purpose of assessing their agronomic efficiency and resistance to diseases. Adherence to regulatory protocols is imperative to guarantee the environmental integrity.

Data Collection and Analysis: Data on disease incidence, severity, yield, and other agronomic traits are collected and statistically analyzed to determine the effectiveness and stability of the transgene.

3.7. Regulatory Approval and Commercialization

The final step involves obtaining regulatory approval and preparing for commercialization:

Regulatory Approval: Comprehensive safety assessments, including environmental and food safety evaluations, are conducted according to the guidelines of relevant regulatory bodies (e.g., USDA, the FDA, EFSA). Dossiers containing scientific data are submitted for review.

Commercialization: Once sanctioned, genetically modified seeds are manufactured and disseminated to agriculturalists. Ongoing surveillance and supervision initiatives guarantee the sustainable utilization and administration of genetically modified crops.

The procedures and substances utilized for genetic modification in enhancing crops' resistance to diseases entail an interdisciplinary strategy, amalgamating sophisticated molecular methodologies, thorough examination, and adherence to regulations. These measures collectively

ensure the creation of sturdy, secure, and efficient disease-resistant crops that can contribute to sustainable farming practices and food security.

4. RESULTS AND DISCUSSION

The realm of botanical cultivation in the realm of agriculture is primarily characterized by traditional methods that revolve around the union of meticulously selected parent organisms, aiming to generate advantageous novel genetic traits in their offspring. The incorporation of particular characteristics associated with a single gene or a few genes involves a targeted strategy, involving breeding an individual possessing the desired trait with a superior strain. Following this, repeated breeding of the offspring with the superior parent is conducted to remove unwanted genetic components from the trait donor using a method called backcrossing. The realm of genome editing presents a significant opportunity in this context by enabling the direct alteration of the target gene within the elite parental organism (Figure 1). It is worth noting that the current available technology in this sphere surpasses our understanding of the genes that can be effectively modified for beneficial outcomes.

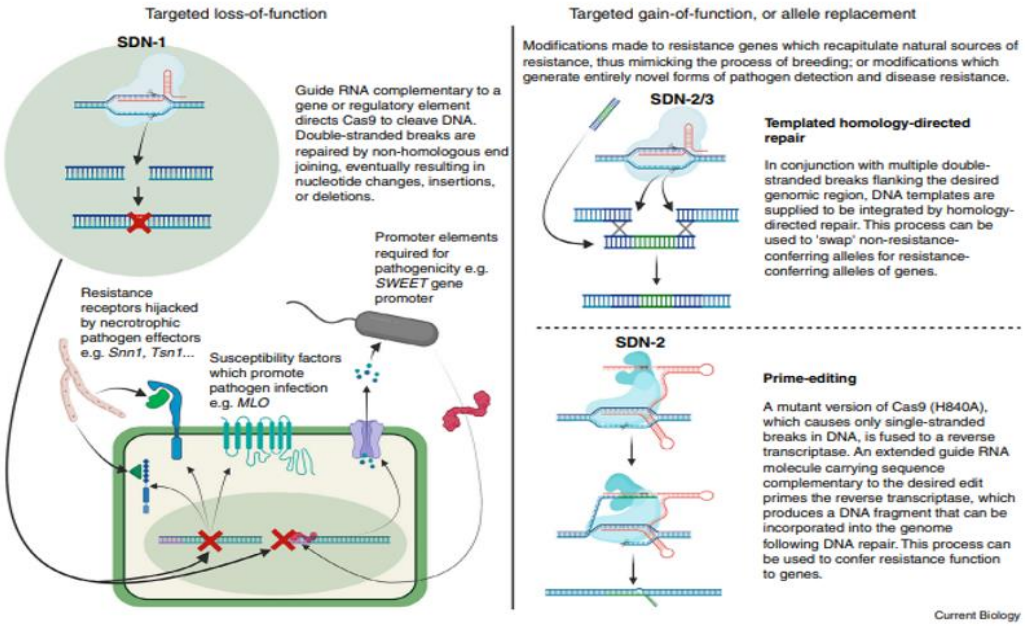

Figure 1: Gene editing for enhanced disease resistance

Genome editing techniques are dependent on the utilization of site-specific nucleases (SDNs) that utilize accurate sequence recognition for delivering a DNA cleaving enzyme to the specified DNA sequence. The predominant choice of technologies in this field is the CRISPR/Cas9 systems, albeit not the sole methods employed. Classically, three categories of genome editing are acknowledged; SDN-1 operates without a DNA template, causing a haphazard, minor genetic alteration at the DNA cleavage location, commonly leading to gene disruption and a null mutation(Parmar et al., 2017). SDN-2 utilizes a brief DNA template to craft accurate sequence modifications in close proximity to the cleavage site (illustrated as prime-editing in Figure 1), facilitated by homology-directed repair (HDR). On the other hand, SDN-3 entails integrating a substantial segment of pattern DNA through HDR at the cleavage site, such

as a extra allele. The distinctions among these classifications carry significant inferences for the rule of the last products, as deliberated below:

Precision and uniform alterations to genetic alleles: Utilizing templates to facilitate SDN-2/3 allele swaps offers a remarkably innovative approach to swiftly integrate disease resistance traits into top-tier crop cultivars. The manipulation of complete genes or gene fragments within their original context allows for the generation of an allele with new functions that are identical to the unchanged wild-type allele. This presents profound consequences for the oversight by regulations or the possible exclusion from regulatory measures of the end product. An important advantage compared to traditional breeding techniques is the avoidance of unintentional genetic material along with the new R allele. Effective allele swaps have been conducted in rice by employing a small gene fragment to introduce the esteemed indica allele of a nitrate transporter (NRT1.1B) into japonica rice within a single breeding cycle (Rashid et al., 2017). While this knowledge is well-suited for incorporating normal alleles similar to traditional breeding methods, it also enables the targeted integration of new sequences at specific genomic loci. For example, in maize, the addition of a new promoter element has significantly boosted ARGOS8 expression, enhancing drought resistance. Similarly, in rice, gene clusters have been accurately placed in the genome using Cas9-mediated homology-directed repair. These specific genomic regions, known as "safe harbors," offer advantages over random gene insertions through genetic engineering as they support genomic integrity and facilitate the design of breeding strategies focused on predetermined integration sites (Bhattacharjee et al., 2024).

Expeditiously implementing genetically engineered resistance genes: Notable advancements in the realm of molecular plant pathology have yielded a comprehensive understanding of the structural biology associated with receptor-ligand interactions. Furthermore, a considerable amount of knowledge exists regarding the diversity of resistance genes across various class at both the genomic and phenotypical levels, which can be a valuable resource for further exploration (Andolfo et al., 2016). The existence of various pools of R genes in natural plant populations is pivotal in conferring effective resistance against diverse pathogens. However, it is important to highlight that this diversity is markedly reduced in high-yielding crop varieties, which come across ongoing challenges from evolving pathogen populations. Genetic engineering is tasked with leveraging the structural insights and inherent diversity of R genes to promptly enhance crop quality.

PRRs — external detectors of molecular patterns: The responsibility of pattern recognition receptors (PRRs) lies in the identification of conserved molecular patterns, specifically pathogen-associated molecular patterns (PAMPs), serving as indicators of infection caused by a diverse array of pathogens. The original concept implied the ubiquitous presence of both PAMPs and their corresponding PRRs in all pathogens and hosts, respectively; however, in reality, there exists variability on both ends of this recognition process (Kamthan et al., 2016). This variability presents an opportunity for host manipulation through the introduction of novel capabilities for PRR recognition in plant species. An exemplification of this phenomenon is observed in the transfer of the Brassicaceae Ef-Tu RECEPTOR gene across plant families, enabling Solanaceous plants to detect bacterial elongation factor Tu, thus

bolstering their resistance against various phytopathogenic bacteria. Similarly, the Ralstonia solanacearum bacteria have crafted a unique flagellin protein that evades detection from the PRR FLAGELLIN RECEPTOR 2 (FLS2) in various species, yet it is acknowledged by the FLS2 receptor in soybeans. Through the incorporation of soybean FLS2 and a co-receptor gene into two Solanaceous species, their immunity against Ralstonia solanacearum was notably enhanced. Consequently, the natural variations observed in PRRs provide a novel avenue for enhancing disease resistance by broadening the spectrum of pathogen molecules that can be identified. Instead of completely replacing an entire receptor, it may be practical to modify individual receptors to enhance their recognition capabilities (Lee et al., 2016). The Nicotiana benthamiana receptor RXEG1 exhibits a remarkable ability to discern the xyloglucanase XEG1 produced by the oomycete pathogen Phytophthora sojae. The arrangement of RXEG1 combined with XEG1 uncovers unique protrusions at the beginning and end regions that engage with and hinder the operational trench of XEG1, leading to the emergence of the RXEG1 island domain. Disruption of vital bonding locations within RXEG1 or XEG1 at these junctions eliminates their connection and the effectiveness of RXEG1. Certain bonding locations endure in XEG relatives from Phytophthora sojae and Phytophthora parasitica, enabling RXEG1 to identify these altered proteins. The existence of island domains presents an enticing objective for manipulating receptors and editing genomes, conceivably necessitating only slight adjustments in amino acids to modify ligand specificity (Figure 2).

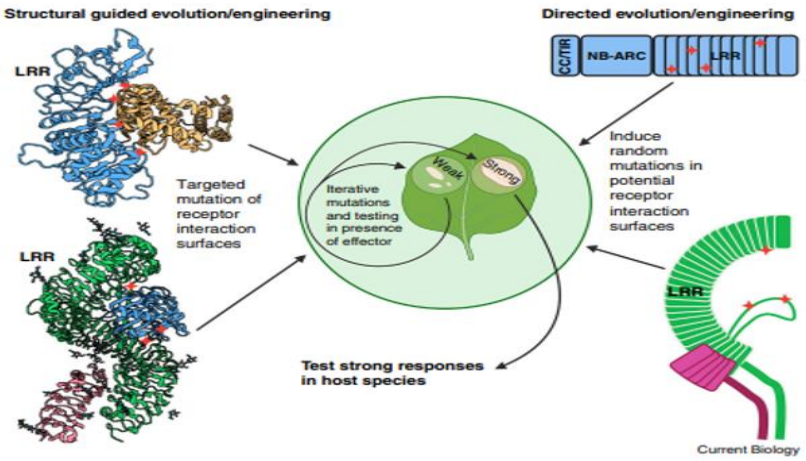

Figure 2: Plant immune receptors structure

Figure 2 illustrates the process of structurally guided and directed evolution to enhance plant immune receptors. Receptor genes are engineered to alter effector binding specificity, improving disease resistance. This involves inducing random mutations and screening for interactions, followed by validation through transient expression in plant tissue. Examples include the intracellular NLR Sr35 with AvrSr35 and the extracellular receptor RXEG1 with XEG1 and BAK1. The illustration highlights these complexes and their interactions, created using BioRender.com.

NLRs — intracellular receptors with built-in flexibility: NLRs represent a class of proteins characterized by a modular structure,

enabling them to recognize their effector ligands intracellularly subsequent to emission by the pathogen. The proteins show a unique composition, with an amino-terminal area, usually a coiled-coil (CC) or Toll/interleukin-1 receptor/resistance (TIR) enzyme domain, a central nucleotide binding (NB) area, and carboxyterminal leucine-rich repeats (LRR) (Shrawat & Armstrong, 2018). These ID areas are supposed to imitator the targets of effectors' virulence, which show noteworthy diversity and polymorphism among diverse pathogens, unlike PAMPs. Examination of effector proteins' configurations has revealed common structural features among sequence-diverse effector proteins, potentially facilitating the fine-tuning of NLR proteins' recognition specificity.

In accordance with their roles as resistant receptors in both plants and animals, NLRs function as multipurpose protein outlines that can be adapted for the detection of various ligands (Figure 2). An illustration of this phenomenon can be observed in the case of wheat Sr35, which confers immunity against fungal rust infection by recognizing the effector AvrSr35. This process of recognition involves a direct interaction that engages the carboxy-terminal segment of the Sr35 LRR domain. Identifying AvrSr35 is hindered due to the replacement of crucial interacting residues with those from an inactive Sr35 variant, TaSh1.Conversely, the creation of hybrid receptors that incorporate the Sr35 LRR domain utilizing TaSh1 or the barley analog HvSh1 as scaffolds facilitates the efficient detection of AvrSr35. Therefore, the alteration of the LRR-encoding regions of R genes emerges as a feasible strategy for boosting resistance (Figure 2). The activation of Sr35 in a pentameric resistance complex is explained by the effector-triggered disruption of the NB domain, resulting in the switch of ADP with ATP, which then stabilizes the active conformation of the protein (Tyagi et al., 2020).

The Mla gene family in cereals, such as barley and wheat, demonstrates a wide range of pathogen recognition abilities through direct interaction with pathogen effectors via the LRR domain. Barley Mla proteins detect powdery mildew effectors without sequence homology, while wheat Mla protein Sr50 recognizes the AvrSr50 effector, providing rust resistance (Liu et al., 2021). Additionally, barley Mla3 and rice Mla-like gene RYMV3 confer resistance to rice blast fungus and rice yellow mottle virus, respectively. Despite high sequence similarity, wheat Mla genes Sr50 and Sr33 recognize different rust strains, revealing diverse recognition mechanisms. Research on these genes shows that altering key variable sites in Sr33 to match Sr50 enables new effector recognition. To ensure durable resistance, employing multiple R genes and expanding NLR gene diversity through artificial evolution are essential, as demonstrated by directed evolution experiments on potato NLR genes Rx and R3a (Bushnell et al., 1998). The resultant evolved forms display novel gratitude capabilities towards effectors from related strains of potato germ X (PVX) and the more indistinctly related poplar medley germ (Thakur et al., 2012).

Transforming identity - altering NLRs by incorporating recognition domains: Some NLRs come equipped with non-canonical integrated domains (NLRIDs) that play a role in aiding the identification of effectors through direct interactions. Most NLR-IDs function as detectors for effectors, working in conjunction with a supporting NLR that handles immune signaling tasks (See Figure 3). By differentiating between the functions of detector and executor, it is feasible to finely tune effector recognition specificity while preserving signaling capabilities (Mahmood et al., 2017b). These studies on plant immune receptors such as Pik1-Pik2 in rice and RRS1-RPS4 in Arabidopsis demonstrate the

adaptability of these proteins through the manipulation of integrated domains (IDs). Pik-1 contains a heavy metal-associated (HMA) domain between its CC and NB domains, which is absent in Pik-2. Different Pik-1 alleles bind to M. oryzae effectors with varying affinities. These affinities can be altered by modifying the HMA domain through structure-guided mutagenesis. RGA5 features an HMA domain at the carboxy-terminal end of its LRR domain. It recognizes two M. oryzae MAX effectors, AvrPia and Avr1-CO39. Mutagenesis can potentially make RGA5 recognize a third effector, AvrPib, but this may compromise recognition of the other two effectors. Incorporating key residues from Pik-1's HMA domain into RGA5 enables it to detect AvrPik-D without losing its ability to recognize AvrPia and Avr1-CO39. Exchange and adjustment of IDs show significant adaptability. RGA5-type rice genes have evolved up to nine distinct ID types naturally. Substituting Pik-1's HMA domain with nanobodies that bind fluorescent proteins (FPs) can trigger resistance against PVX strains engineered to produce FPs. This implies that IDs can be extensively modified or replaced while retaining immune functionality. Synthetic R genes effective in laboratory conditions might not perform well in agricultural crops, indicating a need for careful consideration in real-world applications.

These findings underscore the potential for engineering plant immune receptors for improved disease resistance, although practical agricultural implementation may require further refinement.

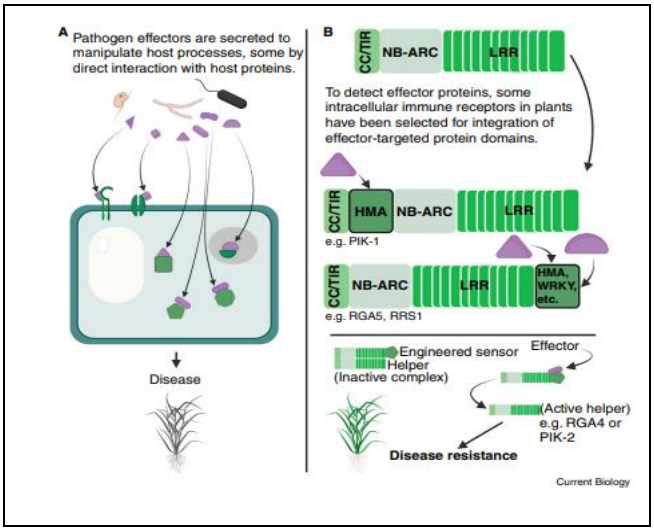

Figure 3: Engineered disease resistance

Figure 3 Engineered disease resistance in plants involves modifying intracellular immune receptors to bind pathogen effectors or using effector-binding nanobodies, triggering an immune response. This method expands plant recognition of pathogen effectors, enhancing immunity. Assessments in planta validate the effectiveness of these engineered sensor proteins in conferring resistance.

Targeted mutation of susceptibility factors: Effector recognition in plants often leads to localized cell death to hinder biotrophic pathogens, which rely on living tissue. However, this defense is exploited by necrotrophic pathogens like Parastagonospora nodorum, which release effectors such as SnTox1 and SnToxA. These effectors, recognized by receptors Snn1 and Tsn1, respectively, trigger cell death that benefits the

necrotroph. Naturally occurring and chemically induced mutations in Tsn1 can prevent the detection of SnToxA, reducing susceptibility. Specific inactivation of these immune receptors through SDN-1 can also eliminate susceptibility to such pathogens (Figure 1). Host genes targeted by effectors can be modified to alter disease susceptibility. Bacterial pathogens like Xanthomonas use transcription-activator-like effectors (TALEs) to enhance host susceptibility genes. In rice, TALEs from Xanthomonas oryzae pv. oryzae activate SWEET sugar transporter genes, promoting bacterial leaf blight. Resistance was increased by editing TALE binding sites in these genes. Similarly, the wheat Lr67 gene, encoding a modified sugar transporter, provides resistance to fungal rusts and powdery mildew. Barley expressing Lr67 also shows resistance to these diseases. Genome editing to replicate such modifications in sugar transporter genes could enhance resistance to biotrophic pathogens. Mutant variants of the barley MLO gene provide broad-spectrum resistance against powdery mildew, demonstrating a loss-of-susceptibility strategy. Despite some yield penalties and leaf cell death, this resistance has lasted over three decades. Traditional breeding identified optimal MLO alleles in elite genetic backgrounds to create resistant barley cultivars. In wheat, gene-editing targeted all three MLO genes, quickly producing resistant plants but with early leaf senescence. This issue was mitigated by overexpressing a sugar transporter gene. Such strategies highlight the potential to correct adverse phenotypes from S gene knockouts through additional genome edits. Other S gene targets for editing include eIF4 for viral replication and DMR6 for biotrophic infections.

Regulation and approval of genetically engineered technologies: The development and adoption of new technologies, particularly advanced genome-editing techniques, face prolonged and complex challenges influenced by social, political, and market acceptance. Social acceptance is particularly intricate due to cultural and historical perceptions. Establishing markets and encouraging investment are essential to overcoming these hurdles. The regulatory landscape for genetically modified organisms (GMOs) varies significantly, guided by frameworks like the UN Cartagena Protocol on Biosafety, which aims to ensure the safety of GMOs with a cautious, precautionary approach. Despite initial debates, GM crop cultivation has grown substantially over the past 25 years, with 173 countries endorsing the protocol as of July 2020.

Monsanto (now Bayer) developed genetically modified crops like Round Up Ready and Bt cotton, incorporating transgenes from Agrobacterium tumefaciens and Bacillus thuringiensis. Gene-editing modifications fall into three categories based on repair mechanisms and the use of external templates. The simplest type, SDN-1, creates random mutations indistinguishable from the wild type, leading to regulatory exemptions in many regions. Regulations on genetic engineering (GE) vary globally, with countries like the US, Canada, Australia, and Japan allowing SDN-1 plants to be cultivated without the restrictions applied to GM crops. In the US, the SECURE rule exempts biotechnologically developed products that only involve natural genetic variations. This rule also permits the unregulated growth of cisgenic GM plants. Current GE crops in the US include high oleic soybean oil, herbicide-tolerant canola, waxy corn, and non-browning mushrooms. In contrast, the European Union, Switzerland, and New Zealand enforce strict regulations, while Russia and China are moving towards more lenient policies. Social acceptance is crucial for GE technology approval but is challenging to assess due to its complexity and newness. Consumer surveys, despite difficulties in design and

interpretation, indicate greater openness towards GE than GM, especially when the benefits are clear, such as increased pest resistance. Public education and regulatory adaptations are essential to fostering broad acceptance of GE technology.

5. CONCLUSION

Genetic engineering has revolutionized crop enhancement, especially in developing disease resistance. Advanced molecular biology techniques enable scientists to insert specific genes that confer immunity to various pathogens, including bacteria, viruses, fungi, and nematodes. This approach overcomes the limitations of traditional breeding methods, allowing for the rapid creation of disease-resistant crops, which are vital for global food security. The advantages of genetically modified disease-resistant crops are numerous. They have the possible to suggestively reduce the dependence on chemical pesticides, thereby reducing production expenses, mitigating environmental repercussions, and bolstering agricultural sustainability. Moreover, these crops can contribute to consistent yields even when confronted with evolving pathogen challenges, ensuring a dependable food source amidst a changing climate. Nevertheless, the deployment of genetically engineered crops is not devoid of obstacles. Regulatory barriers, public perceptions, and ethical considerations must be adeptly navigated to garner societal approval and guarantee the safe application of this technology. Furthermore, a comprehensive evaluation of the potential ecological ramifications of genetically modified organisms (GMOs) is imperative to avert unintended outcomes. Despite these obstacles, the continual progress in genetic engineering methodologies, such as CRISPR/Cas9 and other gene editing tools, holds potential for even more precise and effective development of disease-resistant crops. These innovations, in conjunction with traditional breeding techniques and contemporary biotechnological approaches, have the capacity to expedite the creation of robust crop varieties. In conclusion, genetic engineering for disease resistance in crops signifies a critical advancement in agricultural science. It furnishes potent mechanisms to combat the enduring menace of plant diseases, thereby fostering more sustainable agricultural practices and fortifying global food security. Ongoing research, clear regulatory frameworks, and active public engagement will be crucial in fully realizing the benefits of this technology for the advancement of humanity.

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REFERENCES

1. Ali, Q., Yu, C., Hussain, A., Ali, M., Ahmar, S., Sohail, M. A., Riaz, M., Ashraf, M. F., Abdalmegeed, D., Wang, X., Imran, M., Manghwar, H., & Zhou, L. (2022). Genome Engineering Technology for Durable Disease Resistance: Recent Progress and Future Outlooks for Sustainable Agriculture. In *Frontiers in Plant Science* (Vol. 13). Frontiers Media S.A. https://doi.org/10.3389/fpls.2022.860281
2. Andolfo, G., Iovieno, P., Frusciante, L., & Ercolano, M. R. (2016). Genome-editing technologies for enhancing plant disease resistance. *Frontiers in Plant Science*, *7*, 1813.
3. Avci, U., & Sipahi, H. (2024). Genome Editing of Gene Families for Crop Improvement. In *A Roadmap for Plant Genome Editing* (pp. 3–15). Springer Nature Switzerland. https://doi.org/10.1007/978-3-031-46150-7_1
4. Barka, G. D., & Lee, J. (2022). Advances in S gene targeted genome-editing and its applicability to disease resistance breeding in selected Solanaceae crop plants. *Bioengineered*, *13*(6), 14646–14666.

https://doi.org/10.1080/21655979.2022.2099599

5. Bhattacharjee, M., Meshram, S., Dayma, J., Pandey, N., Abdallah, N., Hamwieh, A., Fouad, N., & Acharjee, S. (2024). Genetic Engineering: A Powerful Tool for Crop Improvement. In *Frontier Technologies for Crop Improvement* (pp. 223–258). Springer.
6. Bigini, V., Camerlengo, F., Botticella, E., Sestili, F., & Savatin, D. V. (2021). Biotechnological resources to increase disease-resistance by improving plant immunity: A sustainable approach to save cereal crop production. *Plants*, *10*(6), 1146.
7. Bushnell, W. R., Somers, D. A., Giroux, R. W., Szabo, L. J., & Zeyen, R. J. (1998). Genetic engineering of disease resistance in cereals. *Canadian Journal of Plant Pathology*, *20*(2), 137–149. https://doi.org/10.1080/07060669809500419
8. Dhaliwal, H. S., & Uchimiya, H. (1999). Genetic engineering for disease and pest resistance in plants. *Plant Biotechnology*, *16*(4), 255–261.
9. Dong, O. X., & Ronald, P. C. (2019). Genetic engineering for disease resistance in plants: recent progress and future perspectives. *Plant Physiology*, *180*(1), 26–38.
10. Greenwood, J. R., Zhang, X., & Rathjen, J. P. (2023). Precision genome editing of crops for improved disease resistance. In *Current Biology* (Vol. 33, Issue 11, pp. R650–R657). Cell Press. https://doi.org/10.1016/j.cub.2023.04.058
11. Kamthan, A., Chaudhuri, A., Kamthan, M., & Datta, A. (2016). Genetically modified (GM) crops: milestones and new advances in crop improvement. *Theoretical and Applied Genetics*, *129*, 1639–1655.
12. Lee, S., Whitaker, V. M., & Hutton, S. F. (2016). Mini review: potential applications of non-host resistance for crop improvement. *Frontiers in Plant Science*, *7*, 997.
13. Liu, X., Ao, K., Yao, J., Zhang, Y., & Li, X. (2021). Engineering plant disease resistance against biotrophic pathogens. *Current Opinion in Plant Biology*, *60*, 101987.
14. Mahmood, S., Lakra, N., Marwal, A., Sudheep, N. áM, & Anwar, K. (2017a). Crop genetic engineering: An approach to improve fungal resistance in plant system. *Plant-Microbe Interactions in Agro-Ecological Perspectives: Volume 2: Microbial Interactions and Agro-Ecological Impacts*, 581–591.
15. Mahmood, S., Lakra, N., Marwal, A., Sudheep, N. M., & Anwar, K. (2017b). Crop genetic engineering: An approach to improve fungal resistance in plant system. In *Plant-Microbe Interactions in Agro-Ecological Perspectives* (Vol. 2, pp. 581–591). Springer Singapore. https://doi.org/10.1007/978-981-10-6593-4_23
16. Miedaner, T. (2016). Breeding strategies for improving plant resistance to diseases. *Advances in Plant Breeding Strategies: Agronomic, Abiotic and Biotic Stress Traits*, 561–599.
17. Parmar, N., Singh, K. H., Sharma, D., Singh, L., Kumar, P., Nanjundan, J., Khan, Y. J., Chauhan, D. K., & Thakur, A. K. (2017). Genetic engineering strategies for biotic and abiotic stress tolerance and quality enhancement in horticultural crops: a comprehensive review. *3 Biotech*, *7*, 1–35.
18. Rahman, S. U., McCoy, E., Raza, G., Ali, Z., Mansoor, S., & Amin, I. (2023). Improvement of soybean; A way forward transition from genetic engineering to new plant breeding technologies. *Molecular Biotechnology*, *65*(2), 162–180.
19. Rashid, B., Tariq, M., Khalid, A., Shams, F., Ali, Q., Ashraf, F., Ghaffar, I., Khan, M. I., Rehman, R., & Husnain, T. (2017). Crop improvement: new approaches and modern techniques. *Plant Gene and Trait*, *8*.
20. Scott, P., Thomson, J., Grzywacz, D., Savary, S., Strange, R., Ristaino, J. B., & Korsten, L. (2016). Genetic modification for disease resistance: a position paper. *Food Security*, *8*, 865–870.
21. Shrawat, A. K., & Armstrong, C. L. (2018). Development and application of genetic engineering for wheat improvement. *Critical Reviews in Plant Sciences*, *37*(5), 335–421.
22. Sun, L., Nasrullah, Ke, F., Nie, Z., Wang, P., & Xu, J. (2019). Citrus genetic engineering for disease resistance: Past, present and future. *International Journal of Molecular Sciences*, *20*(21), 5256.
23. Thakur, A. K., Chauhan, D. K., Parmar, N., Verma, V., & Parmar, Y. S. (2012). ROLE OF

GENETIC ENGINEERING IN HORTICULTURAL CROP IMPROVEMENT-A REVIEW. In *Agri. Reviews* (Vol. 33, Issue 3). www.arccjournals.com/indianjournals.com

24. Tyagi, S., Kesiraju, K., Saakre, M., Rathinam, M., Raman, V., Pattanayak, D., & Sreevathsa, R. (2020). Genome editing for resistance to insect pests: an emerging tool for crop improvement. *ACS Omega*, *5*(33), 20674–20683.
25. Xiao, B., Qie, Y., Jin, Y., Yu, N., Sun, N., Liu, W., Wang, X., Wang, J., Qian, Z., Zhao, Y., Yuan, T., Li, L., Wang, F., Liu, C., & Ma, P. (2024). Genetic basis of an elite wheat cultivar Guinong 29 with harmonious improvement between multiple diseases resistance and other comprehensive traits. *Scientific Reports*, *14*(1). https://doi.org/10.1038/s41598-024-64998-2
26. Yin, K., & Qiu, J.-L. (2019). Genome editing for plant disease resistance: applications and perspectives. *Philosophical Transactions of the Royal Society B*, *374*(1767), 20180322.
27. Yu, H., Wang, Y., Fu, F., & Li, W. (2022). Transgenic Improvement for Biotic Resistance of Crops. In *International Journal of Molecular Sciences* (Vol. 23, Issue 22). MDPI. https://doi.org/10.3390/ijms232214370

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