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REVIEW ARTICLE

Unveiling the Genetic Revolution: A Review of CRISPR-Cas9 in Plant Diseases Management

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ABSTRACT

The emergence of genome editing tools offers unprecedented opportunities for fortifying crop resilience. From its humble origins in bacterial immune systems to its application in plant pathology, CRISPR/Cas9 has revolutionized our understanding of genetic manipulation and disease control. This review explains the principles of genome editing system and explore its potential applications in augmenting resistance against pathogens in model plants and key crops, with a keen eye on rice, wheat, and other agricultural staples. Among the key roles of the sgRNA/cas9 (single guided RNA) complex in enhancing plant immunity and reducing the burden of disease burden, this review focuses on the role of CRISPR Cas9 in the management of Viral, Bacterial and devastating Fungal plant diseases, bolstering agricultural productivity and ensuring food security. CRISPR/Cas9 is enabling precise genetic edits that enhance plant resilience against specific viruses, fungi, and bacteria, marking a revolutionary shift in sustainable plant pathogen management. The paper provided with some key resistance genes of plant diseases resistance.

Keywords: CRISPR/Cas9, Genome Editing, Crop Resilience.

INTRODUCTION

Through the ages, plant breeding has stood out as the foremost method for crafting fresh crop varieties, a legacy dating back to the dawn of domestication. This method has heralded remarkable strides in nourishing global populations and fostering societal progress (Stephens and Barakate, 2017). Crops remain besieged by an array of adversaries' fungi, bacteria, viruses—sowing economic turmoil in their wake (FAO, 2017). Bolstering plant resilience emerges as a pivotal strategy in adapting crop yields to the burgeoning demands of humanity (Fones *et al.*, 2020; Dong and Ronald 2019). While defenses centered on resistant strains and chemical interventions typically wield potency, the relentless adaptability of

plant foes spawn's novel strains impervious to existing countermeasures (Zaynab *et al.*, 2020). Thus, dependable disease management tactics falter as these resilient strains propagate, asserting dominance over vast swathes of land (Langner *et al.*, 2018). Delving into the intricate tapestry of plant-microbe relationships has captivated scholars for generations. Advances in molecular technologies now afford a comprehensive census of crop-associated pathogens, unveiling the sway of environmental nuances and crop genetic makeup on these alliances (Maikova *et al.*, 2019). Disease dynamics unfold through a labyrinthine dance between host and pathogen, where the fine balance of resistance and

susceptibility hinges on multifarious factors (Doehlemann *et al.*, 2017). In the bygone era of pre-genomics, traditional breeding schemes, though fraught with uncertainty and imprecision, endeavored to incorporate natural and induced mutations conferring resistance into elite crop lineages. Notwithstanding their pitfalls, mutation breeding techniques have yielded commendable successes in fortifying crop defenses and birthing novel cultivars. The advent of genomic and transcriptomic revelations heralds a fresh dawn for plant breeders, ushering in an era of precision agriculture. Association genetics, leveraging single nucleotide polymorphisms (SNPs) and allied molecular markers, now chart the course towards identifying quantitative trait loci (QTLs) pivotal in fortifying crops against pathogens (Jinek *et al.*, 2012). Novel breeding methodologies, brimming with potential, beckon researchers across a spectrum of disciplines, from developmental biology to combatting abiotic stress and pathogenic onslaughts (Nelson *et al.*, 2018). Armed with site-directed nucleases, such as the famed CRISPR/Cas9 system, researchers orchestrate precise genetic tweaks (Jansen *et al.*, 2002). This arsenal of molecular scalpel wielders—Meganucleases, Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), and CRISPR/Cas9—empower breeders to sculpt genomes with unparalleled finesse (Cermak *et al.*, 2011). Among these, CRISPR/Cas9 shines brightest, its simplicity, cost-effectiveness, and surgical precision eclipsing its peers, its advent signals a new epoch in crop improvement, offering tantalizing prospects for fortifying crops against pathogenic incursions (Blicharska *et al.*, 2022, Pennisi, 2013). Advances in genetic tools like CRISPR/Cas9 are crucial for agriculture as they enable precise enhancements in crop resilience, essential for combating evolving diseases and pests. This innovation not only supports food security but also reduces reliance on chemical treatments, promoting sustainable farming practices.

History of CRISPR Cas9 in Phytopathology: The history of CRISPR-Cas9 is a fascinating journey marked by scientific breakthroughs and revolutionary discoveries. The story begins with the recognition of clustered regularly interspaced short palindromic repeats (CRISPR) in the genomes of bacteria and archaea in the late 1980s (Ishino *et al.* 1987; Guo *et al.* 2019). Initially, these repetitive sequences puzzled researchers until their

function as part of a bacterial immune system was elucidated (Dort *et al.*, 2020). In 2012, Jennifer Doudna and Emmanuelle Charpentier pioneered the groundbreaking research that unlocked the potential of CRISPR-Cas9 as a versatile genome editing tool (Doudna and Charpentier 2014). Their work, published in *Science*, demonstrated the ability to program Cas9 to target specific DNA sequences, offering unprecedented precision and efficiency in genetic manipulation (Brouns *et al.*, 2008). This discovery unleashed a wave of excitement across the scientific community, sparking rapid advancements in various fields, including medicine, agriculture, and, notably, plant pathology (Sánchez-Martín and Keller, 2019, Mojica *et al.*, 2009). Plant pathology, the study of plant diseases and their management, has benefited significantly from the application of CRISPR-Cas9 technology (Brouns *et al.*, 2008). Traditionally, controlling plant diseases relied on chemical treatments, breeding resistant varieties, or cultural practices. However, these methods often had limitations in terms of effectiveness, sustainability, or speed of development (Dracatos *et al.*, 2018). CRISPR-Cas9 emerged as a promising alternative, offering precise genetic modifications to enhance plant immunity and resilience against pathogens (Haft *et al.*, 2005). One of the key roles of CRISPR-Cas9 in plant pathology is in elucidating the molecular mechanisms underlying plant-pathogen interactions (Yin and Qiu, 2019). By precisely editing the genomes of both plants and pathogens, researchers can identify genes involved in disease resistance or susceptibility (Fisher *et al.*, 2012). This knowledge provides valuable insights into how pathogens infect plants and how plants defend themselves, paving the way for more targeted approaches to disease management (Borrelli *et al.*, 2018). Furthermore, CRISPR-Cas9 enables the development of genetically engineered crops with enhanced resistance to diseases. By introducing beneficial traits or disrupting pathogen susceptibility genes, researchers can create plants that are less susceptible to infection or better able to withstand disease pressure. This has the potential to reduce the need for chemical pesticides and fungicides, promoting sustainable agriculture practices and minimizing environmental impacts (Doehlemann *et al.*, 2017).

In addition to enhancing disease resistance, CRISPR-Cas9 offers opportunities for improving crop yield and quality.

By precisely editing genes involved in traits such as yield, nutritional content, or stress tolerance, researchers can develop crops that are better adapted to changing environmental conditions or consumer preferences (Nejat *et al.*, 2017). This can contribute to food security and agricultural sustainability, particularly in the face of climate change and growing global population (Das *et al.*, 2019). Despite its immense potential, the use of CRISPR-Cas9 in plant pathology also raises ethical and regulatory considerations. Concerns about unintended off-target effects, potential environmental impacts, and intellectual property rights have prompted discussions among scientists, policymakers, and the public (Miah *et al.*, 2013). Addressing these challenges requires careful risk assessment, transparent communication, and responsible governance to ensure the safe and ethical deployment of CRISPR-Cas9 technology in agriculture (Hsu *et al.*, 2013). In conclusion, the history of CRISPR-Cas9 is a testament to the power of curiosity-driven research and interdisciplinary collaboration (Tyagi *et al.*, 2021). From its discovery in bacterial immune systems to its application in plant pathology, CRISPR-Cas9 has revolutionized our ability to understand and manipulate the genetic code. By harnessing this technology, researchers are poised to address some of the most pressing challenges facing agriculture and global food security in the 21st century. The overview of CRISPR is mentioned in Figure 1. The history of CRISPR-Cas9 is a story of transformative breakthroughs, from the initial discovery of CRISPR sequences in bacteria to its revolutionary application in plant pathology. Identified in the late 1980s, these sequences were found to be part of an adaptive immune system in bacteria, inspiring scientists to explore similar applications for plants. By 2012, Jennifer Doudna and Emmanuelle Charpentier's research on CRISPR-Cas9 established it as a precise genome-editing tool, enabling plant pathologists to target specific genes that influence disease susceptibility. This capability transformed plant disease management by allowing precise modifications to enhance disease resistance, reduce dependency on chemical pesticides, and promote sustainable agriculture. CRISPR has since enabled the development of genetically engineered crops with improved resistance, yield, and quality, while also raising ethical and regulatory questions around its application. Each milestone in CRISPR-Cas9's journey underscores its potential to reshape agriculture, addressing food security

and environmental sustainability in the face of global challenges. The details of viral resistance via CRISPR/Cas9 has been described in Figure 1.

Viral Resistance via CRISPR/Cas9: Plant viruses pose a significant threat to numerous economically vital staple and specialized crops. Classified into six main groups based on their genome structure, these viruses encompass double-stranded DNA (dsDNA), single-stranded DNA (ssDNA), reverse-transcribing viruses, double-stranded RNA (dsRNA), negative sense single-stranded RNA (ssRNA⁻), and positive sense single-stranded RNA (ssRNA⁺) viruses (Roossinck *et al.*, 2015). Among these, the spotlight often falls on ssDNA geminiviruses in studies involving CRISPR-edited plants for virus resistance (Ji *et al.*, 2015). Geminiviruses, belonging to the expansive Geminiviridae family, inflict significant crop losses globally across various plant families (Zaidi *et al.*, 2017). Their genome replication is facilitated through rolling-circle amplification using a dsDNA replicative form or recombination-mediated replication (Naveed *et al.*, 2023). Among the economically consequential genera is Begomovirus, infecting dicotyledonous plants through vectors like the sweet potato/tobacco/silverleaf whitefly (*Bemisia tabaci*), primarily dwelling within the phloem of infected plants (Abbas *et al.*, 2020). The Begomovirus genome typically comprises one (A, monopartite) or two (A and B, bipartite) components, incorporating a shared region of approximately 220 base pairs (Fondong, 2013). **Tomato Yellow Leaf Curl Virus (TYLCV)** The devastating disease referred to as Tomato Yellow Leaf Curl Virus (TYLCV) induces significant yield losses in tomato crops throughout the world. By altering genes crucial in viral replication and delivery, researchers developed tomato plants with increased resistance to TYLCV through the use of CRISPR-Cas9 (Ali *et al.* 2015; Ali *et al.*, 2018). These genetically altered tomatoes provide tomato growers with a long-term answer because they exhibit less symptoms and better resistance to TYLCV infection (Jiang *et al.*, 2019). **Citrus Tristeza Virus (CTV)** Citrus output is seriously threatened by the *Citrus Tristeza Virus (CTV)*, which results in fruit loss and tree deterioration (Yin *et al.*, 2015). Citrus cultivars resistant to CTV have been created by using CRISPR-Cas9 to target viral genes essential for pathogenicity and replication (Cody *et al.*, 2017). Through the application of precise genetic changes, scientists have generated citrus plants that exhibit enhanced resistance against CTV.

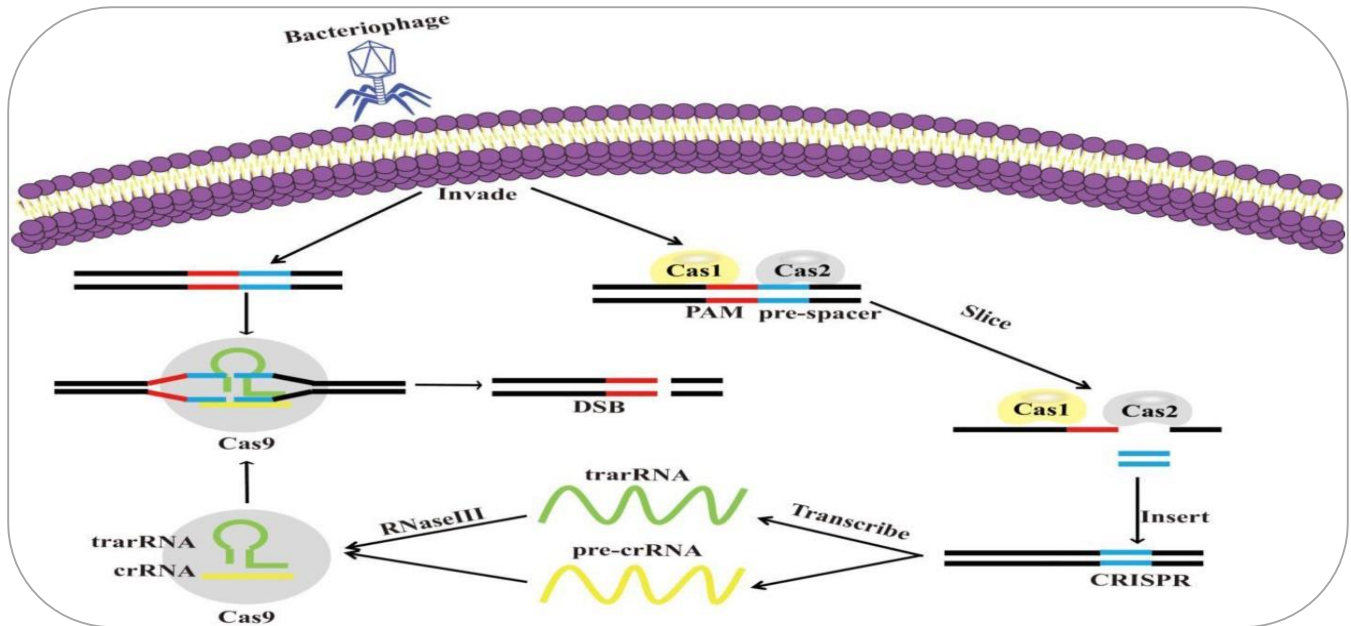


Figure 1. Use of CRISPR Cas9 in management of various Plant Pathogens

Focused on *cotton leaf curl Kokhran virus (CLCuKoV)*, *MeMV*, and various severe and mild strains of TYLCV, the study revealed the generation of virus variants capable of replicating and disseminating upon editing sites within the coding regions by the sgRNA/Cas9 complex (Ellison *et al.*, 2020). Conversely, no novel variants emerged in *N. benthamiana* plants harboring sgRNAs addressing IR sequences. Despite the activation of the non-homologous end joining (NHEJ) machinery, which tends to induce mutations, the targeted IR sequences remained resilient to variant generation. In the realm of viral defense, RNA viruses present a formidable challenge, as the traditional SpCas9 from *Streptococcus pyogenes* has long remained blind to their elusive RNA nature. However, the pursuit of novel nucleases has unearthed a trove of enzymes capable of infiltrating the RNA realm. A groundbreaking report by (Zhang *et al.*, 2018) showcased the first strides in resisting RNA viruses. Employing FnCas9 and RNA-targeting sgRNAs, researchers orchestrated a symphony of defense against *cucumber mosaic virus (CMV)* and *tobacco mosaic virus (TMV)* in *N. benthamiana* and *Arabidopsis* plants. The transgenic guardians exhibited a remarkable reduction of 40–80% in *CMV* and *TMV* accumulation compared to their unaltered counterparts.

Similarly, the saga of protection continued with Cas13a (Aman *et al.*, 2018) harnessed this RNA-guided ribonuclease to besiege the *turnip mosaic virus (TuMV)* RNA genome. By targeting key genomic regions, including

the green fluorescent protein (GFP) and the coat protein (CP), researchers achieved significant repression of TuMV replication and spread in tobacco leaves. Notably, Cas13's innate capacity to process pre-CRISPR RNA paved the way for enhanced multiplex targeting, elevating the potency of the antiviral arsenal (Aman *et al.*, 2018). However, the quest for viral immunity poses regulatory challenges, as the maintenance of transgenes in crop genomes subjects them to the scrutiny of unveiled cucumber plants fortified against *potyviruses* by mutating the eIF4E gene. Through meticulous backcrossing, nontransgenic eIF4E mutants emerged, impervious to viral onslaughts from the Potyviridae family. Similarly, (Pyott *et al.*, 2016) and (Macovei *et al.*, 2018) engineered site-specific mutations in the eIF (iso)4E locus, endowing *Arabidopsis* and rice plants with robust resistance against *TuMV* and *rice tungro spherical virus (RTSV)*, respectively. Remarkably, these edited warriors exhibited no discernible defects in growth or development, signaling a promising path towards sustainable viral resistance (Macovei *et al.*, 2018). In the perpetual struggle against viral adversaries, CRISPR/Cas emerges as a beacon of hope, offering a versatile toolkit for fortifying crops against RNA viral incursions. Whether through molecular interference or genetic fortification, researchers continue to chart new frontiers in the age-old battle between hosts and viruses.

Within the vast tapestry of bacterial species that populate our planet, only a select few hundred wield the potential to

wreak havoc upon crops, often exhibiting a diverse array of taming these phytopathogenic bacteria proves to be a formidable challenge, owing to their knack for stealthy, asymptomatic infections and the limited arsenal of effective agrochemicals at our disposal. Typically, managing bacterial plant diseases entails a multifaceted approach, encompassing preventive measures, genetic fortification, agronomic practices, and the strategic deployment of biocontrol agents (Kerr, 2016).

Bacterial Resistance: With tailored approaches to increase crop resilience and reduce production losses, CRISPR-Cas9 technology has become known as an exciting option in the management of plant maladies brought on by bacterial pathogens (Xu *et al.*, 2019). Potatoes, tomatoes, citrus fruits, and other crops are among the many crops that are seriously impacted by pathogenic bacteria such as *Xanthomonas* and *Pseudomonas* species, which have an effect on the world's food production (Oliva *et al.* 2019).

Transforming the genomes of plants to confer resistance to bacterial pathogens is one way that CRISPR-Cas9 has been utilized to manage plant diseases. Crop plants' inherent defenses against bacterial infections have been strengthened by scientists. Plants can acquire enhanced resistance to bacterial infections, enhancing crop yields and lessening the severity of disease, by carefully altering particular genomic sequences linked to pathogen recognition or host vulnerability. Furthermore, CRISPR-based diagnostics offer rapid and precise detection of bacterial pathogens in plants, enabling early disease identification and targeted intervention. Portable CRISPR-based platforms can detect unique DNA signatures of bacterial pathogens directly from plant samples, facilitating timely disease management and preventing further spread (Zeng *et al.*, 2020). The concept of CRISPR Cas9 editing methods and its tools have been mentioned in Figure 2 (Mushtaq *et al.*, 2021).

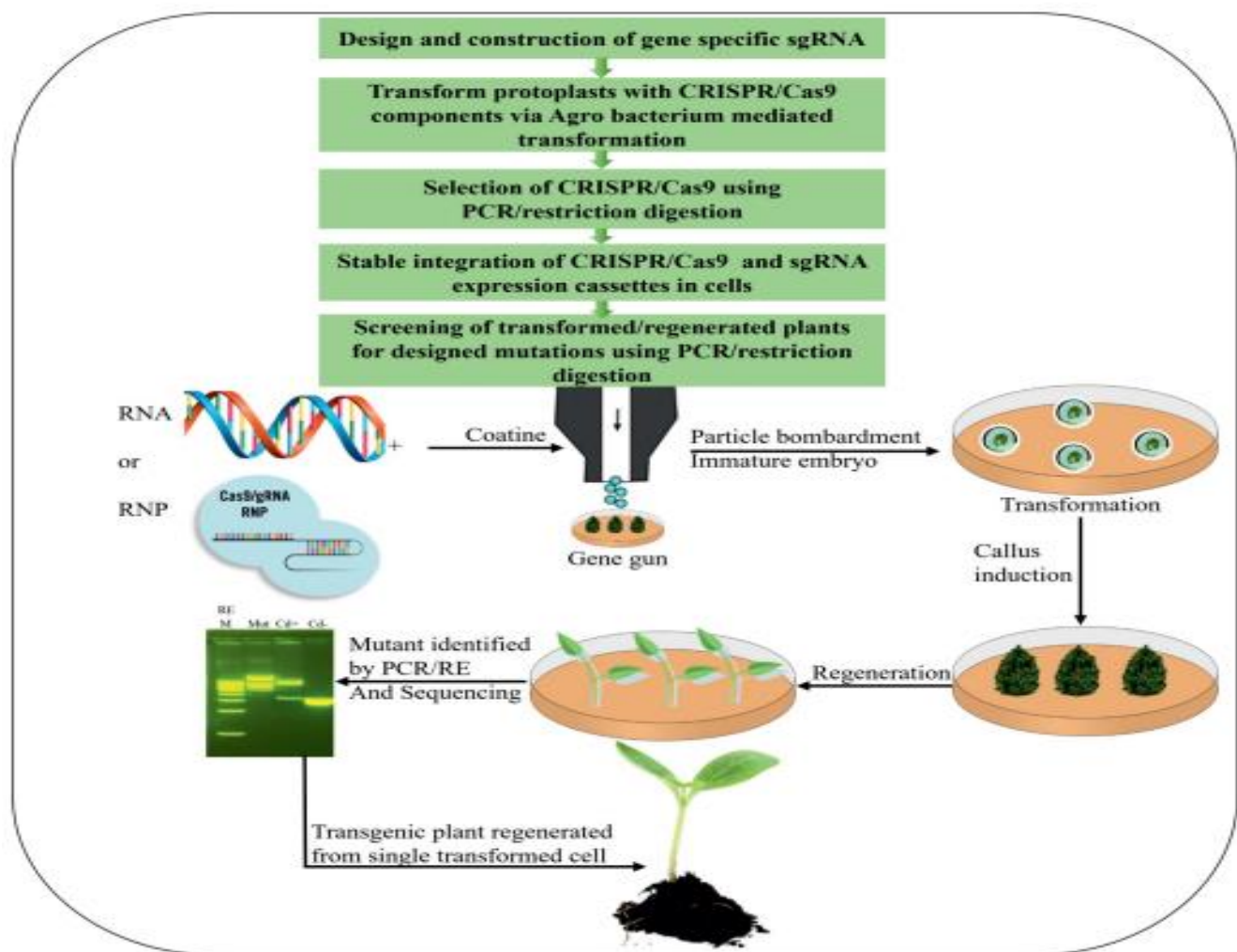


Figure 2. Mechanism of CRISPR/Cas9 based genome editing in plants (concept adopted from Mushtaq *et al.* 2021).

Fungal Resistance: Plant fungal disease management may benefit from the use of CRISPR-Cas9, a gene editing technique known for its transformative power. Due to their destructive effects on crop losses, fungus pathogens represent serious dangers to the world's food security (Kamoun *et al.*, 2015). The use of fungicides, which are traditional techniques of managing fungal infections, has drawbacks in terms of environmental effect and the emergence of resistant strains. An exact and focused method of fighting these infections is provided by CRISPR-Cas9 (Navet and Tian, 2020). Scientists have the ability to modify plants to have increased resistance to fungus infections through the use of CRISPR-Cas9. In order to provide immunity against fungal diseases, this procedure entails altering particular genes within the plant genome (Li *et al.*, 2020). For example, genes linked to fungal disease susceptibility have been effectively targeted by researchers, strengthening plant defenses. Furthermore, fungal genes necessary for virulence can be modified thanks to CRISPR-Cas9, which prevents the fungi from. Moreover, CRISPR-based approaches offer the potential for developing environmentally sustainable solutions (Kralova *et al.*, 2021). Unlike chemical pesticides, which can harm beneficial organisms and contaminate soil and water, CRISPR-mediated resistance is intrinsic to the plant and poses minimal environmental risks. While issues like off-target effects and regulatory concerns do exist, CRISPR-Cas9 dealing with plant fungal infections has immense potential to improve sustainable agriculture and ensure food security (Lu *et al.*, 2020). To fully utilize CRISPR technology and protect global crop output, more research and development in this domain are essential (Schenke and Cai, 2020).

Plant fungal diseases which represent a risk to global agriculture can be successfully controlled with CRISPR-Cas9. Researchers have altered susceptibility genes in wheat to increase resistance to powdery mildew, disrupted virulence-related genes of *Phytophthora infestans* to address Fusarium wilt in bananas, and reduced late blight in tomatoes (Li *et al.*, 2020). Furthermore, rice's resistance to *Magnaporthe oryzae*'s rice blast has been strengthened using CRISPR-mediated changes (Nekrasov *et al.* 2017). These uses demonstrate CRISPR-Cas9's potential for crop engineering. One notable target for CRISPR/Cas9-mediated editing is the MLO gene, implicated in susceptibility to powdery mildew in several plant species (Zhang *et al.*, 2017)

Studies have demonstrated successful targeting of MLO loci in bread wheat, tomato, and grapevine, resulting in enhanced resistance to fungal infections (Wang *et al.*, 2014).

CONCLUSION

In summary, the investigation of CRISPR/Cas9 technology represents an important turning point in the continuing story of plant breeding and disease control. The advent of precise genome editing tools presents unprecedented prospects to strengthen crop resilience as humankind struggles to feed expanding populations in the face of environmental and disease adversity. Our knowledge of genetic modification and disease management has been completely transformed by CRISPR/Cas9, from its modest beginnings in bacterial immune systems to its use in plant pathology. With the use of this technology, scientists may examine the complex molecular interactions that occur between diseases and plants, determining the genetic foundation of resistance and susceptibility. Within the domain of viral infections, CRISPR/Cas9 offers a formidable defense against both DNA and RNA viruses. By using focused genome editing, scientists can interfere with. With its proactive approach, food safety and environmental stewardship are promoted while crop losses are minimized and the need for chemical treatments is reduced. CRISPR/Cas9 has great promise for creating crops with improved resistance to catastrophic fungal diseases in the face of fungal threats. Researchers can create crops that are resistant to fungal diseases by focusing on susceptibility genes and altering virulence factors, thereby preserving global food security and sustainability. But with CRISPR/Cas9 becoming widely used in agriculture, it is important to carefully explore the ethical, legal, and societal ramifications. To guarantee this technology is deployed safely and ethically, open communication, sound governance, and thorough risk assessment are necessary. CRISPR/Cas9 is essentially the perfect example of how science, technology, and agriculture are coming together to create resilient crops and sustainable food systems. While navigating the plant-microbe complications.

Limitations of CRISPR\ Cas9 and Future perspectives: While CRISPR-Cas9 technology has significantly advanced plant pathology and disease management, several future limitations may affect its broader adoption and effectiveness. One key concern is the potential for unintended off-target effects, where

CRISPR-Cas9 may inadvertently alter non-target genes, potentially leading to unanticipated changes in plant traits. Additionally, there are regulatory and ethical challenges around genetically edited crops. Some regions still classify gene-edited plants under strict GMO regulations, complicating their commercialization and

public acceptance. Another limitation lies in addressing the diversity of pathogen types, as CRISPR-Cas9's current applications are more advanced for DNA-based pathogens, leaving RNA viruses and some fungal pathogens less accessible to effective CRISPR-based interventions.

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