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REVIEW

Genome editing (CRISPR/Cas9) in plant disease management: challenges and future prospects

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Abstract

The field of plant pathology has adopted targeted genome editing technology as one of its most crucial and effective genetic tools. Due to its simplicity, effectiveness, versatility, CRISPR together with CRISPR-associated proteins found in an adaptive immune system of prokaryotes have recently attracted the interest of the scientific world. Plant disease resistance must be genetically improved for sustainable agriculture. Plant biology and biotechnology have been transformed by genome editing, which makes it possible to perform precise and targeted genome modifications. Editing offers a fresh approach by genetically enhancing plant disease resistance and quickening resistance through breeding. It is simpler to plan and implement, has a greater success rate, is more adaptable and less expensive than other genome editing methods. Importantly CRISPR/Cas9 has recently surpassed plant science as well as plant disease. After years of research, scientists are currently modifying and rewriting genomes to create crop plants which are immune to particular pests and diseases. The main topics of this review are current developments in plant protection using CRISPR/Cas9 technology in model plants and commodities in response to viral, fungal, and bacterial infections, as well as potential applications and difficulties of numerous promising CRISPR/Cas9-adapted approaches.

Keywords: CRISPR/Cas9, genome editing, plant disease management, plant-pathogen interaction

Introduction

By 2050, there will be at least 9.8 billion people on the Earth, which means that more and more food will be required to feed the growing populations. A wide range of etiological agents (fungi, bacteria, oomycetes, viruses, etc.) can affect crops and cause significant financial damage. Thus, increasing plant resistance is crucial for altering crop production to meet the needs of a growing population (Nejat *et al.* 2017; Dong and Ronald 2019). Disease-resistant plants are becoming more and more desirable and have been successfully generated through the use of CRISPR/Cas9 in plant breeding and plant pathology. It has already been discussed how CRISPR/Cas9 technology is used in plant pathology, particularly for improvement in agriculture (Langner *et al.* 2018; Das *et al.* 2019). However, genetic engineering alters inherited or noninherited genetic material to modify a cell, tissue, or organism's genotype or phenotype. To produce genetic modifications, a specific gene or DNA

sequence is deleted and inserted (Wolter *et al.* 2019). Clustered interspaced palindromic repeats (CRISPR) and CRISPR-associated proteins (Cas, CRISPR/Cas system) have demonstrated significant advantages due to their simplicity and specificity in the regulation of both genetic and nongenetic plant characteristics (Maikova *et al.* 2019). Additionally, the use of CRISPR/Cas9 in plants to combat plant diseases promises to alter the pace and direction of agricultural research. Future research will focus on developing/identifying smaller Cas9 variants with different specificity that may be easier to distribute in cells in an effort to advance the technique. It will be easier to insert new or corrected sequences into genomes if we have a better understanding of the homology-directed repair mechanisms that follow Cas9-mediated DNA cleavage (Doudna and Charpentier 2014). The invention of CRISPR is considered to be one of the most groundbreaking discoveries of recent years in the history of biology, biotechnology, medicine, as well as the pharmaceutical and agricultural industries. The methods developed using CRISPR create new, previously unattainable possibilities that can significantly improve the comfort of life (Blicharska *et al.* 2022).

For many years, one of the main areas of research was how plants interact with populations of bacteria, fungus, and other microbes. The development of highthroughput molecular technology has allowed for a more thorough inventory of the diseases linked to certain crops and it has given insight into how the genotype of the crop and the environment may alter these communities. A host plant and a pathogen interact in a complex way to cause disease, and the resistance/susceptibility response might have multiple components. Natural and artificial mutations may alter how some components interact and prevent the progression of some phases in the infection mechanism (Dracatos *et al.* 2018).

The purpose of this review is for a deeper understanding of CRISPR/Cas9 and its potential applications in order to better understand and manage plant diseases. Thus, we focus on how native CRISPR/Cas systems function as well as the mechanisms driving CRISPR/Cas9 gene editing for the application of this technology in plant diseases.

Evolution of CRISPR/Cas9 Technology

The development of CRISPR as a method for genome editing in the modern era can be attributed to its discovery in the late 1980s (Ishino *et al.* 1987), with a decade of intensive research starting in 2005 (Richter *et al.* 2012).

Researchers from all over the world have contributed to the development of the CRISPR/Cas9 microbial adaptive immune system. CRISPR systems have received a great deal of attention, and Ishino discovered the key CRISPRs in *Escherichia coli* decades ago (Klompe *et al.* 2019). CRISPRs were discovered in *Haloferax mediterranei* in 1993 and subsequently found in a variety of bacterial and archaeal genomes (Faure *et al.* 2019; Guo *et al.* 2019). The discovery of sequence similarities between the spacer sections of CRISPRs and those of bacteriophages, archaeal viruses, and plasmids in the early 2000s provided evidence that CRISPR functions as an immune system (de Oliveira Luz *et al.* 2019). CRISPR/Cas9 systems are bacterial cell immune response mechanisms against viral invasion, according to proportional genomic analysis (Guo *et al.* 2019). An analysis of the iap gene in *E. coli* led to the discovery of the first CRISPR (Maikova *et al.* 2019). The word "CRISPR" was proposed, and it was adopted as the research community worked on these sequences (Jansen *et al.* 2002).

Two distinct research teams proposed the idea that spacer elements serve as remnants of earlier invasions by foreign DNA and protect against phage infection (Bolotin *et al.* 2005; Mojica *et al.* 2005). They pointed out that all spacers have the same end sequence, which is now known as protospacer-adjacent motif (PAM). The transcription of phage spacer sequences into short RNAs (crRNAs), which direct Cas proteins to the target DNA (Brouns *et al.* 2008). Cas9-induced DNA double-strand breaks (DSBs) three nucleotides upstream of PAM were also displayed, as was an interference mechanism based on RNA-mediated DNA targeting (Marraffini and Sontheimer 2008; Garneau *et al.* 2010). The technique was further streamlined by fusing the CRISPR RNA (crRNA) and trans-activating RNA (tracrRNA) to create a single, synthetic guide RNA (Jinek *et al.* 2012). The ability of Cas9 to facilitate homology directed repair with minimal mutagenic activity was reported (Cong *et al.* 2013).

Classification of CRISPR/Cas9 System

The researchers Haft and colleagues made the initial attempt to categorize the CRISPR/Cas9 system (Haft *et al.* 2005). They identified 45 families of CRISPR-related proteins (Cas), which may be broken down into core proteins (Cas1, Cas2, Cas3, Cas4, Cas5, Cas6), eight subtypes of CRISPR/Cas, and the RAMP (repair associated mystery protein) module found in bacterial genomes. According to Makarova *et al.* (2011), CRISPR/Cas9 systems can be broken down into three distinct categories: type I, type II, and type III. These

categories are differentiated by the presence of signature Cas3, Cas9, and Cas10 proteins, in that order (Fig. 1). The existence of additional signature proteins allowed for the categorization of this system into ten distinct subtypes. Depending on the types of signature proteins and CRISPR loci, this three-type classification system is further adjusted into two class-five type classification systems (Makarova *et al.* 2015). The make-up of crRNP complexes is the primary determinant of major distinctions between the various classes of CRISPR. Both the Cas1 and Cas2 genes are present in every kind of CRISPR/Cas9 system (Makarova *et al.* 2011).

A key part of the CRISPR/Cas9 system, which originated from type II CRISPR/Cas9 systems of *Streptococcus pyogenes* (Sampson *et al.* 2013), is a CRISPR-associated endonuclease that is not specific to any particular target (Cas9). Cas9 forms an operational complex by binding with two RNAs – a CRISPR RNA (crRNA) and a trans-activating RNA (tracrRNA) molecule, or a fabricated single-guide RNA (gRNA) – which then acts as an RNA-directed endonuclease and generates a double-strand break (DSB) within the desired DNA sequence. Cas9 also binds with a fabricated single-guide RNA (gRNA (Karvelis *et al.* 2015). The presence of a PAM sequence in the target DNA, which functions as a nucleotide signature and can be recognized by Cas9 for its activity, is the primary characteristic of this RNA-guided DNA cleavage process. This is an essential aspect of the RNA-guided DNA cleavage (Anders *et al.* 2014). The 20-bp DNA sequence that has to be edited needs to be positioned immediately upstream of a PAM sequence that is analogous to the standard form of 5'-NGG in order to make the CRISPR/Cas9 system functional (Shah *et al.* 2013).

There are many different vectors that can be used for genome editing in a variety of organisms. Some examples of these vectors include pRGE31, pRGEB31, lentiCRISPR v2, eSPCas9(1.1), and others. These vectors contain all of the necessary sequences, such as promoters, selection markers, multiple cloning sites, restriction sites, and the coding sequence for the Cas9 protein (Xie and Yang 2013). These vectors have unique restriction sites that allow the guide RNA that has been generated for a particular gene to be cloned into them. This process is then followed by the transformation of plants via Agrobacterium-mediated transformation. Inside plant cells, the guide RNA provides instructions to the CAS9 enzyme, telling it to cleave the target sequence. This, in turn, initiates the DNA mending machinery, such as NHEJ. Due to the fact that these DNA mending systems are prone to errors, they cause frameshift mutations, which in turn lead to site-specific gene editing. These mutations are caused by insertions and deletions (Jiang *et al.* 2013a).

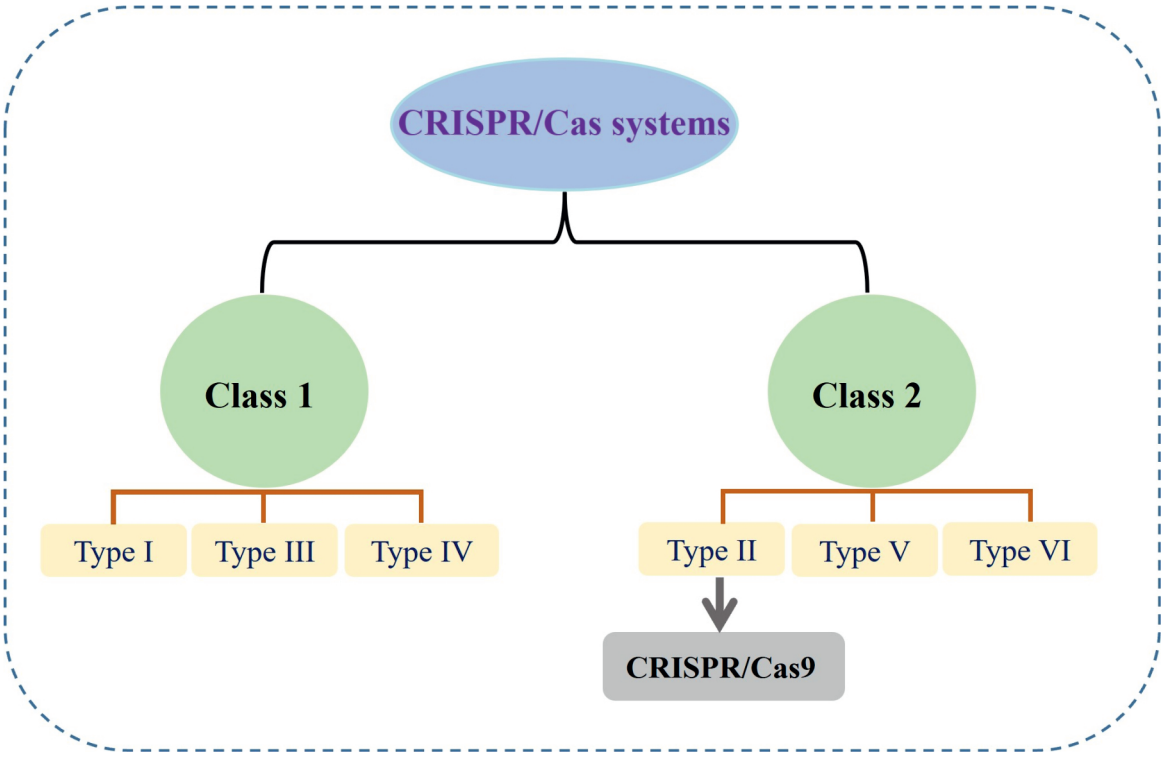

Fig. 1. Classification of CRISPR/Cas9 System

Mechanism of genome editing (CRISPR/Cas9)

Based on an adaptive immune system, the CRISPR/Cas9 system prevents the invasion of foreign plasmids or viral DNA by cleaving it within bacteria and archaea (Marraffini and Sontheimer 2010). sgRNA, a single guide RNA (sgRNA), and the nuclease-active Cas protein make up CRISPR/Cas9 genome editing systems. Additionally, gRNA includes a user-defined spacer sequence (about 20 nt) for targeting genomic sequences as well as a scaffold for Cas9 protein binding. Since its initial demonstration in mammalian cells (Cong *et al.* 2013; Mali *et al.* 2013), applications of the CRISPR/Cas9 system have quickly eclipsed those of zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) in a variety of organisms, including plants. This is due to its simplicity, high efficiency, and ease of use (Pennisi 2013).

Three stages make up the CRISPR/Cas9 system's adaptive immunity: adaptation, expression, and interference. Invading DNA from viruses or plasmids is cut into tiny pieces and inserted into the CRISPR locus as part of the adaptation process. Small RNA (crRNA), which is produced from the transcription and processing of CRISPR loci, directs effector endonucleases to target the viral material by base complementarity (Yosef *et al.* 2012). One Cas9 protein is necessary for Type II CRISPR/Cas9 system DNA interference (Zetsche *et al.* 2015). Cas9 contributes to pre-crRNA processing to crRNA, aids in adaptation, and introduces targeted DSBs under the direction of tracrRNA and double stranded RNA-specific RNase III (Jackson *et al.* 2014; Mulepati *et al.* 2014).

Also exhibited was multiplex genome engineering employing multiple guide RNAs to simultaneously target different genomic regions. By using agroinfiltration and protoplast transfection to target different endogenous genes and transgenes, stable transgenic plants via both nonhomologous end joining (NHEJ) and homologous recombination (HR) processes were produced (Feng *et al.* 2013). Similar to this, three guide RNAs were introduced at different rice genomic loci (Xie and Yang 2013), who also examined the 3–8% mutation efficiency. Off target mutations were also found, however they had less effective genome editing than the matched spot. The use of CRISPR for gene editing is well supported by studies on sorghum (Jiang *et al.* 2013b), wheat (Wang *et al.* 2014), and maize (Liang *et al.* 2014). Several promoters can influence the expression of gRNAs. The CRISPR/Cas9 system is continually being improved for better efficiency and gene targeting precision. The requirement to modify the eukaryotic genome using the CRISPR/Cas9 system has forced the inclusion of nuclear localization signals to one or both ends of the protein. The application of this technique has greatly expanded with the development of orthogonal CRISPR/Cas9 systems (Jiang *et al.* 2013a).

However, the CRISPR/Cas9 system, which uses single-guide RNAs for genome editing, is a straightforward, reliable, and effective method for targeted gene mutagenesis, knockout and knock-in/replacement, as well as transcriptional regulation (Fig. 2). Although it may appear that scientists are just randomly working with plant genomes due to the apparent simplicity of CRISPR/Cas9-mediated editing, the combined power of CRISPR/Cas9 has made it possible to carry out crucial research in an attempt to optimize and adapt crop species, enabling significant advancements in crop improvement.

Using genome editing for plant disease management

There are many potential uses for effective genome editing methods that might be investigated in plant diseases, including CRISPR/Cas9 (Fig. 3). The possibility of conferring desirable phenotypes for a variety of applications exists thanks to the ability to change plant pathogen genomes (Zhang *et al.* 2018c). The CRISPR/SpCas9 tools are far more effective and sometimes even easier than conventional approaches for genetic modification of the microbial genome, which are typically linked with ineffective homologous recombination. Additionally, they offer a high-throughput experimental framework for analyzing gene activity throughout the entire genome of plant diseases.

The application of CRISPR technology has considerably spread into additional bacterial species such as: *Pseudomonas*, *Yersina*, *Bacillus*, *Streptomyces*, and *Corynebacterium* since the first example of genome editing with high efficiency in *E. coli* was described (Jiang *et al.* 2013b; Liu *et al.* 2019).

However, conventional approaches to breeding resistance take a long time, and the resistance alleles are occasionally connected to genes that affect plant development (Miah *et al.* 2013). The BSR-K1 gene in rice has recently been deleted using CRISPR/SpCas9, conferring resistance to both *Magnaporthe oryzae* and *Xoo*. A second effective and speedy method to increase crop disease resistance, in addition to the knockout technique previously discussed, is faulty R gene rectification by CRISPR/Cas9-mediated precise base editing (Zhou *et al.* 2018).

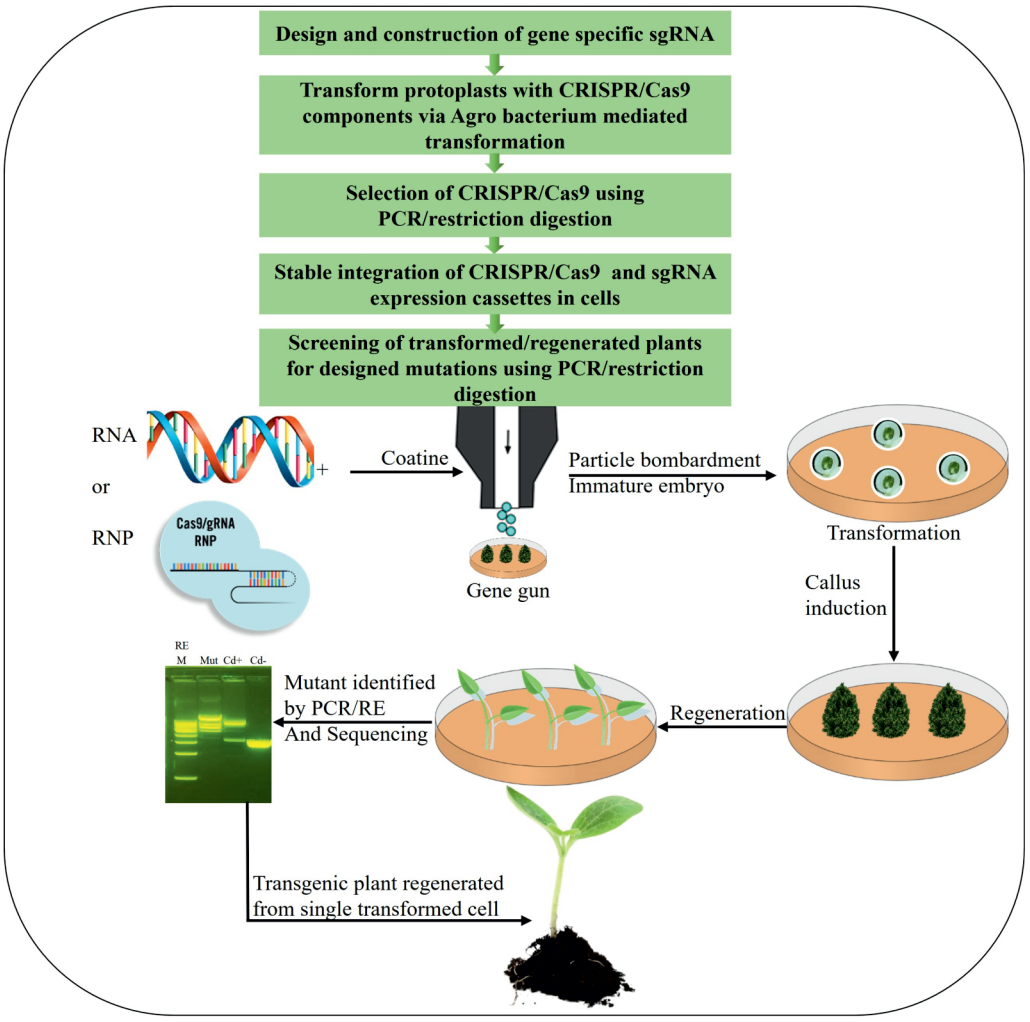

Fig. 2. Mechanism of CRISPR/Cas9 based genome editing in plants (concept adopted from Mushtaq *et al.* 2021)

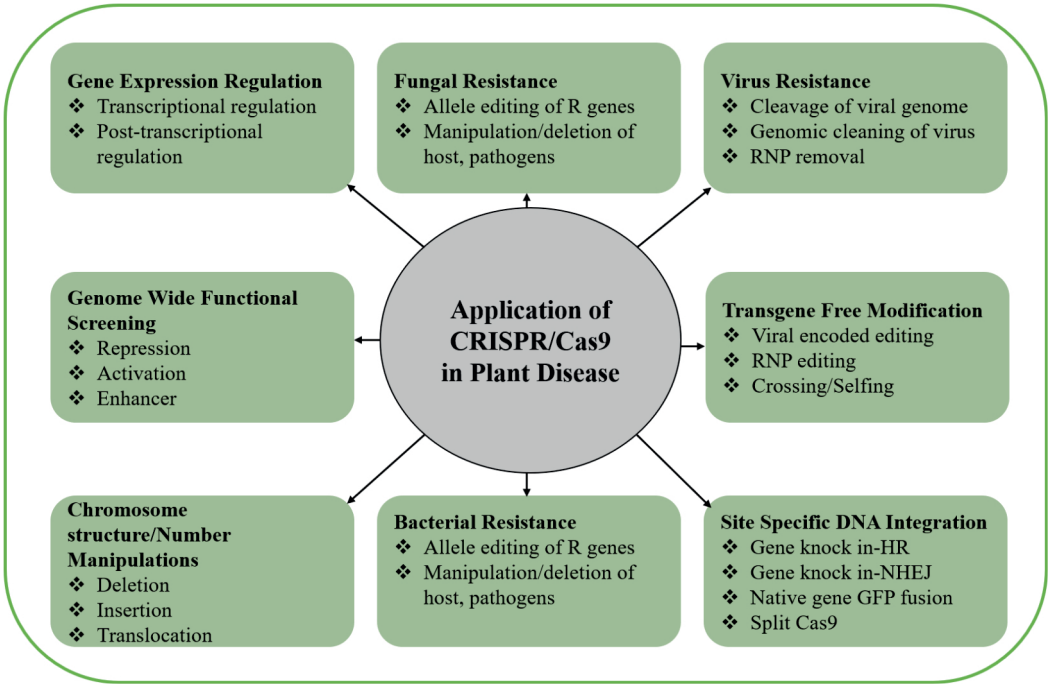

Fig. 3. Applications of CRISPR/Cas9 system in plant disease management

Genome editing for resistance against fungal pathogens

Based on the present understanding of the molecular pathways implicated in plant-pathogen interaction, several techniques have been developed to improve fungal resistance in plant species. These have been identified as potential candidate genes and gene products involved in plant resistance to fungus, and they are now the top targets for CRISPR/Cas9 genome editing (Borrelli *et al.* 2018). Mycotoxins, which are secondary metabolites produced by mycotoxigenic fungi and harmful to both humans and animals when they are consumed in tainted food and feed, are another major concern. The most common root causes of plant diseases, fungi, have a significant negative impact on agriculture. They pose a significant problem in disease control due to their varied lives and high genetic flexibility, which enable them to swiftly invade new hosts, break R gene-mediated resistance, and develop fungicide resistance (Doehlemann *et al.* 2017). By altering host S genes, genome editing has recently started to overcome this problem. A variety of plants are affected by the widespread fungal disease known as powdery mildew. Therefore, it is particularly desirable to create wheat cultivars with lasting and broad-spectrum resistance. A significant breakthrough in the breeding of plants for broad-spectrum and long-lasting resistance to powdery mildew was the discovery of barley mlo (mildew resistance locus o) mutants (Lyngkjær *et al.* 2000). They discovered that only when all six copies of TaMlo were simultaneously mutated the edited plants exhibit resistance to the powdery mildew fungus *Blumeria graminis* f. sp. *tritici* (Bgt) using wheat Mlo genes through TALEN and CRISPR (Wang *et al.* 2014). Negative regulators and S genes that are engaged in defense pathway have received a lot of research. *B. graminis* f. sp. *tritici*, a fungus that causes powdery mildew, was resistant to its homologs in wheat (TaMLOs) after being knocked out using the CRISPR/SpCas9 system (Wang *et al.* 2014). *M. oryzae*, which causes rice blast, is one of the most damaging diseases to harm rice production globally (Dean *et al.* 2012). The APETELA2/ERF (AP2/ERF) superfamily's ethylene responsive factors (ERFs) are essential for the ability of rice to react to a variety of biotic and abiotic stressors (Mizoi *et al.* 2012). Not only is *M. oryzae* able to promote the expression of OsERF922, but also ABA, salt, and salt-free conditions. OsERF922 is a negative regulator of rice blast resistance because it increases resistance to *M. oryzae* when it is knocked down by RNAi (RNA interference) (Liu *et al.* 2012).

Additionally, the CRISPR/SpCas9-mediated genome editing technology has been successfully established in a wide range of fungal species, including *Alternaria alternate* (Wenderoth *et al.* 2017), *Leptosphaeria maculans* (Idnurm *et al.* 2017), *Fusarium oxysporum* (Wang *et al.* 2018), *F. graminearum* (Gardiner and Kazan 2018), *F. fujikuroi* (Huck *et al.* 2019). Both SpCas9 and the sgRNA can be expressed in fungi in a stable or transitory manner by the use of polyethylene glycol (PEG), Agrobacterium, electroporation, and biolistic transformation (Schuster and Kahmann 2019). As an alternative, *M. oryzae* and *F. oxysporum* can be treated with the SpCas9/sgRNA ribonucleoprotein (RNP) complex once it has been synthesized in vitro (Foster *et al.* 2018; Wang *et al.* 2018).

Genome editing for resistance against bacterial pathogens

Bacterial pathogens are extremely varied, multiply quickly, and can spread in a variety of ways, which makes it challenging to control bacterial infections, especially when epidemics have been established. Just a few hundred of the bacterial species that exist on Earth are responsible for agricultural harm, which frequently manifests itself as various diseases (Schloss and Handelsman 2004). In general, bacteriological plant management relies on genetic resistance, agronomic methods, and biocontrol chemicals to prevent and exclude the pathogen from the plant (Kerr 2016). One of the most common diseases of rice is rice bacterial blight, a vascular bundle disease caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo). It causes yield losses of 10–20% (Ou 1985), but under conditions that are favorable to the pathogen (i.e., high humidity), this loss can exceed 50% and occasionally even result in a complete loss of yield (Mew *et al.* 1993). Through the type III secretion system, Xoo secretes TALE (transcription activator-like effector) proteins into host cells (Makino *et al.* 2006). Numerous TALE proteins target S genes and increase their expression to support infection success (Doyle *et al.* 2013). For instance, the effector-binding element (EBE) in the promoter of OsSWEET14 (also known as Os11N3) binds to the TALE protein AvrXa7 from the Philippine strain PXO86 and triggers its expression. PXO86 uses OsSWEET14, which encodes a sucrose-efflux transporter, to steal sugars from rice cells in order to support pathogen development and virulence (Chen *et al.* 2012). It is not possible to knock out OsSWEET14 to provide resistance against Xoo without having negative effects because OsSWEET14 also plays a significant role in plant development. In order to prevent AvrXa7 from attaching to the OsSWEET14 promoter while maintaining OsSWEET14's ability to function normally during development, a pair of TALENs that target EBEs were created (Li *et al.* 2012).

The bacteria *Xanthomonas citri* ssp. *citri* is the deadly cause of citrus canker (Xcc). The gene (Xcc S) for lateral organ boundaries domain (LBD) family transcription factor CsLOB1 was previously discovered (Hu *et al.* 2014). The Xcc effector PthA4 recognizes an EBE in the CsLOB1 promoter, activating CsLOB1 expression to promote canker formation. One study used CRISPR/Cas9 to target the CsLOB1 promoter EBE, while another used the technique to target the CsLOB1 coding area. Both studies demonstrated that altering CsLOB1 resulted in Xcc resistance (Jia *et al.* 2017; Peng *et al.* 2017). The growth status of the CsLOB1 null mutant was surprisingly similar to that of wild-type plants (Jia *et al.* 2017), indicating that CsLOB1 is an excellent candidate for engineering canker resistance in premium citrus varieties, even though the potentially adverse effect of mutating CsLOB1 on plant growth has not yet been determined. Even though tomatoes are one of the most economically significant crops in the world, they are nonetheless subject to several serious diseases, including *Pseudomonas syringae* and *Xanthomonas* spp. (Schwartz *et al.* 2015). It is interesting to note that the tomato orthologue SlDMR6-1 similarly experiences an upregulation in response to *P. syringae* pv. *tomato* and *Phytophthora capsici* infection. Through the use of the CRISPR/Cas9 system, SlDMR6-1 null mutants demonstrated resistance to *P. syringae*, *P. capsici*, and *Xanthomonas* spp. without impairing tomato growth and development (de Toledo Thomazella *et al.* 2016). Type III effectors are secreted into the plant cell during the bacterial infection process (Büttner and He 2009). The main functions of these effectors are to disrupt the host's defense mechanisms and/or activate the S genes to cause illness (Zaidi *et al.* 2018). Therefore, CRISPR/Cas9-mediated gene editing is an effective method to target both S genes and the negative regulators of plant innate immune response as well as to increase plant resistance.

Genome editing for resistance against plant viruses

Because viruses change quickly and are typically spread by insects, viral infections in plants are challenging to control. Transgenic production of viral proteins or RNAs has been extensively employed over the past three decades to enhance plant virus resistance; the resulting resistance is known as pathogen-derived resistance. Recently, RNAi generated by double-stranded RNA has also been thought to be an effective way to provide plants with virus resistance (Ding and Voinnet 2007). They are divided into six main groups based on the characteristics of their genomes: 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. The double-stranded DNA (dsDNA) group does not include plant viruses (Roossinck *et al.* 2015). RNA genomes are present in the majority of plant viruses. Therefore, viral RNA genomes cannot be directly targeted by CRISPR/Cas9 systems since they generally cut double-stranded DNA. However, the introduction of fresh CRISPR/Cas9 systems, particularly those that can target RNA, presents fresh opportunities for creating plants immune to RNA viruses. After discovering that FnCas9 targets bacterial RNA, it was used to directly target and suppress a human ssRNA virus using an engineered gRNA (Price *et al.* 2015).

The ssDNA geminivirus genomes have been the focus of the majority of investigations using CRISPR-edited plants for virus resistance (Baltes *et al.* 2015). In terms of economic importance, Begomovirus is the most significant genus of geminiviruses. Begomoviruses are primarily associated with the phloem of infected plants and spread to dicotyledonous plants via the sweet potato, tobacco, and silverleaf whitefly (*Bemisia tabaci*) (Gilbertson *et al.* 2015). Their genome is divided into two halves (A and B, bipartite), or one part (A, monopartite), with a common region of about 220 base pairs (Fondong 2013). Genome editing technology adds a new tool to the plant virus arsenal. In the past, a synthetic zinc finger protein (AZP) without a nuclease domain was created to target the Beet severe curly top virus (BSCTV) replication origin by preventing the binding of viral replication protein (Rep). More than 80% of transgenic AZP Arabidopsis plants had higher resistance to BSCTV and showed no signs of viral infection (Mino *et al.* 2006).

Similar to this, ZFNs have been created to target a conserved region of the Rep gene of the Tobacco curly shoot virus and the Tomato yellow leaf curl China virus (TbCSV). These ZFNs cleaved the target sequences and prevented viral replication, according to a transient experiment on tobacco (Chen *et al.* 2014). Turnip mosaic virus (TuMV), an RNA virus, is resistant to Arabidopsis mutants that have been identified through genetic testing. TuMV resistance was shown to be caused by a loss of function mutation in the eIF(iso)4E gene (Lellis *et al.* 2002). Additionally, CRISPR/Cas9 deletion of Arabidopsis eIF(iso)4E produced TuMV resistance without affecting plant vigor (Pyott *et al.* 2016). Therefore, plant eIF4E genes are probably the best candidates for genome editing to create broad-spectrum viral resistance. Cucumber eif4e mutants with CRISPR/Cas9-induced mutations at two locations in the eIF4E gene were immune to the cucumber vein yellowing

Table 2. Genome editing technologies developed for disease resistance in plants

Crops	Causal organism	Disease/symptoms	Targeted gene	Reference
Wheat (*Triticum aestivum*)	*Blumeria graminis* f. sp. *tritici*	powdery mildew disease	TaMLO-A1	Wang *et al.* (2014)
Rice (*Oryza sativa*)	*Xanthomonas oryzae*	bacterial blight of rice	OsSWEET11, OsSWEET14	Jiang *et al.* (2013b)
	Magnaporthe oryzae	rice blast disease	OsERF922	Wang *et al.* (2016)
Arabidopsis (*Arabidopsis thaliana*)	Potyvirus (TuMV)	turnip mosaic virus disease	eIF(iso)4E	Pyott *et al.* (2016)
Mexican cotton (*Gossypium hirsutum*)	Begomovirus	cotton leaf curl disease	CLCuD IR and Rep regions	Iqbal *et al.* (2016)
Cucumber (*Cucumis sativus*)	Cucumber vein yellowing virus (Ipomovirus), potyviruses Zucchini yellow mosaic virus	ring spot disease, vein yellowing disease	eIF4E	Chandrasekaran *et al.* (2016)
Tobacco (*Nicotiana benthamiana*)	Bean yellow dwarf virus (BeYDV)	leaf thickening, chlorosis, curling	BeYDV	Baltes *et al.* (2014)
	Tomato yellow leaf curl virus, Beet curly top virus	leaf curl disease	TYLCV-IR, RCA regions	Ali *et al.* (2015)

virus (CVYV), zucchini yellow mosaic virus (ZYMV), and papaya ring spot virus (PRSV-W) diseases (Chandrasekaran *et al.* 2016).

Current applications in plant diseases

The most important issue is facing a rapidly expanding global population. In order to face this issue, CRISPR/Cas9 technology is being developed in order to improve crop quality and, to a certain extent, boost crop productivity as well. Natural elements of healthy ecosystems include plant pathogenic viruses, bacteria, oomycetes, and fungi, but due to mismanagement, globalization, climate change, and other factors, many of these species are found as the potential of emerging infectious diseases (EIDs) which pose a threat to plant ecosystems (Fisher *et al.* 2012).

Plant pathologists are now exploring CRISPR/Cas9 for the mitigation of diseases in both hosts and pathogens (Dort *et al.* 2020). There are many potential uses for effective genome editing methods that might be investigated in plant diseases, including CRISPR/Cas9. Applications of CRISPR/Cas9 against plant diseases can potentially alter the speed and direction of agricultural research. For technology to be used in human gene therapy, special strategies for quick and secure delivery of CRISPR/Cas9 and its guide RNAs to cells and tissues are also essential (Doudna and Charpentier 2014). To date, most of the CRISPR/Cas9 research in plant pathology has concentrated on creating systems in the hosts, namely engineering for disease resistance in plants. Plant virus pathosystems provide the best illustration of the pathogen-gene method. The most popular method for combating CRISPR/Cas9-mediated virus resistance is a transgenic strategy in which a viral DNA sequence is utilized to construct the sgRNA and then inserted into the plant genome using the CRISPR/Cas9 system (Ali *et al.* 2016; Zhang *et al.* 2018a). Targeting plant susceptibility (S) genes, a varied group of genes with various functions that ultimately make plants more vulnerable to invading pathogens, has been a major focus of CRISPR/Cas9--mediated disease resistance utilizing the plant-gene strategy. The proteins that the S genes express can be divided into two categories: those that function as pathogen effector molecule targets and those that act as negative regulators of immunity, reducing the plant immune response in specific situations (Langner *et al.* 2018). The plant-gene approach to CRISPR/Cas9 virus engineering entails creating the sgRNA to target a region of the plant genome utilized by the virus for replication (Makarova *et al.* 2018).

However, the designing of sgRNAs to target S genes in these systems has pimarily focused on producing host knockout mutants that the pathogen effectors find challenging to recognize (Langner *et al.* 2018; Das *et al.* 2019). These plants can be utilized outside of the GMO regulatory framework due to the capacity of CRISPR/Cas9 to produce extremely specific disease-

resistant mutants that do not contain any foreign DNA (Kanchiswamy 2016; Makarova *et al.* 2018). Additionally, it permits targeted genetic alterations to be done within the framework of endogenous genome, preventing haphazard insertion of transgenes from unrelated species, and lowering the possibility of any unwanted downstream consequences brought on by the presence of foreign DNA (Kim *et al.* 2014; Kanchiswamy 2016)

Moreover, the use of CRISPR/Cas9 to engineer pathogen resistance in plants is a promising strategy for reducing disease outbreaks and is also of interest in producing avirulent strains and for understanding how these species interact with their plant hosts to cause disease (Dort *et al.* 2020). Genes that code for the effector proteins released by pathogens during host interactions make up a significant subset gene for pathogenicity. All types of plant infections include effectors, which are a remarkably complex group of molecules. They serve a variety of purposes, such as promoting infection, impairing the plant immune system, and getting nutrients from host tissues (Toruño *et al.* 2016). These are the burning issues for the CRISPR/Cas9 because of their widespread presence and prominent function in plant-pathogen interactions. The complexity of plant-pathogen interactions serves as a reminder for scientists hoping to use genetic engineering techniques to create disease-resistant plants such as targeting an effector with CRISPR/Cas9 which may hinder the pathogen, but depending on the effector's recognition pathway, it may also have unintended consequences for the plant host (Fang and Tyler 2016). Systems that have coevolved over millions of years are difficult to disassemble, thus it is important to take into account their complexity if CRISPR/Cas9 is to be employed as a tool to control plant disease outbreaks (Dort *et al.* 2020).

Challenges in plant diseases

Although CRISPR/Cas9 technology is a straightforward and reliable method for changing a plant's genome to increase its immunity, it is nevertheless accompanied with several difficulties. The ability of guide RNA to match sequences with places in genome other than the target site gives CRISPR/Cas9 its intrinsic ability to create remote targets (Hsu *et al.* 2013). To reduce off-site targeting, the precision with which the Cas9 targets a desired sequence must be optimized (Majeed *et al.* 2018). Additionally, modified Cas9 proteins are being created to identify other PAMs (Agudelo *et al.* 2020). There have also been new Cas12a and Cas13a of CRISPR/Cas9 nucleases from different bacterial Type II systems that target DNA and single-stranded RNA, respectively (Koonin *et al.* 2017). Although they share some characteristics with Cas9, these two systems outperform Cas9 in specific situations, especially in plant disease by using different ways to cleave target nucleotide and process pre-crRNA (Langner *et al.* 2018). The occurrence of unintended changes (translocations, inversions, massive deletions, and insertions) as a result of the intricate endogenous pathways that repair the double-stranded DNA by Cas nucleases is another drawback of the CRISPR/Cas9 system (Kosicki *et al.* 2018). Furthermore, Cas9-induced DSBs can be harmful to cells, triggering cell-death pathways and lowering the efficiency of transformation and editing (Roy *et al.* 2018). Nuclease-deficient Cas9 proteins have been created and joined to other proteins, such as deaminases and recombinases, to accomplish base editing and site-specific recombination, overcoming these DSB-related restrictions (Standage-Beier *et al.* 2019). The necessity of the PAM sequence adjacent to the protospacer DNA, which is used by the Cas9 complex in conjunction with the complementary sgRNA region to recognize and cleave the target DNA sequence, is the main drawback of employing the original *Streptococcus pyogenes* CRISPR/Cas9 (SpCas9) (Jinek *et al.* 2012). The PAM sequence 5′-NGG-3′, where N can be any of the four nucleotide bases, is recognized by the SpCas9 complex (Jinek *et al.* 2012). Although most genomes have this three-base-pair region, its necessity restricts the genes that can be targeted, especially when trying to examine genes engaged in extremely specialized pathways of interest (Langner *et al.* 2018). Furthermore, studies have demonstrated that CRISPR/Cas9 can identify different PAM sequences, which raises the possibility of off-target alterations (Zhang *et al.* 2014b).

Future prospects in Plant Disease Management

Plant virologists, geneticists, and molecular biologists have a chance to use CRISPR/Cas9 to create crops with improved yields and disease resistance. Despite the fact that genetic engineering has undergone a revolution, there are still several flaws that must be corrected in order to effectively modify plants for the benefit of humanity. Expression levels of cas9 and gRNA affect how precisely CRISPR/Cas9 editing works in plants. Its effectiveness is also greatly influenced by the target site's sequence composition (such as the amount of GC present), as well as the secondary structure of the target-gRNAs (Majeed *et al.* 2018). Because of CRISPR/Cas9, we can peek into the future of diverse genome editing, which will bring powerful and effective results.

With the development of CRISPR/Cas9 technology, gene editing in plants, particularly crops, has undergone a significant revolution. Designing elite and superior crops will be made easier by investigating the basic biology of plant development and stress response. By removing only the desired gene from a wild type species and specifically inserting the gene at a specific site, CRISPR/Cas9 has great promise for the future of creating designer plants. As a result, this opens up numerous opportunities for plant breeders to create designer plants (Arora and Narula 2017). The newly developed CRISPR/Cas9 RNP system avoided the need to rely on the ability of the target cell to translate Cas9 and its likely encounter with gRNA. To tackle rice blast disease, CRISPR/Cas9 sequence-specific nuclease editing is a successful strategy (Wang *et al.* 2016). Additionally, the cytidine deaminase enzyme and Cas9 can combine to provide high throughput, allowing for high-efficiency emendation of target codons in rice (Li *et al.* 2016). Crop protection through genetic modification offers a promising option with no visible effects on human health or the environment in an era characterized by political and social pressure to limit the use of pesticides.

Conclusions

In the fields of crop improvement and functional genomics, genome editing is quickly becoming the most widely utilized and adaptable technology. As this technology is tailored to function in a wider variety of species, the continuous development of CRISPR/Cas9 technology in plant pathosystems will serve to improve its already impressive level of efficacy. To this day, the majority of CRISPR/Cas9 research in plant pathology has concentrated on agricultural pathosystems. On the other hand, forest pathology has seen very little or no study. CRISPR/Cas9 should be used in plant disease management immediately; at the very least, it should be used to improve our understanding of host--pathogen interactions; however, ideally, it should be used to begin integrating it into crop improvement programs in order to generate more effective disease resistance strategies for long-term sustainability of forests. In order to facilitate the speedy development of this technology and to make these crops acceptable for consumption by the general public, the regulations governing transgenic crops were also significantly streamlined. In addition to these societal and technical obstacles, the CRISPR technique was utilized for the very first time to change the genomes of plant species. Therefore, the application of genome editing on a significant scale for the purpose of improving crop yields is already a reality. The progress being made in genome editing raises a number of ethical questions that need to be addressed on a massive scale by both researchers and society as a whole. In conclusion, the CRISPR/Cas9 system and its derivatives offer a fresh opportunity to investigate the intricate topic of the interactions between plant pathogens and host organisms. We anticipate that the CRISPR/Cas9 technologies will make a significant contribution in the future to the process of deciphering the interaction between plant and pathogen and designing disease-resistant plants that are both long-lasting and resistant to a wide range of diseases. This will occur in tandem with the ongoing changes in agricultural production activities and plant disease systems.

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