

REVIEW

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Applications of CRISPR technology in studying plant-pathogen interactions: overview and perspective

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Abstract

Targeted genome editing technology is becoming one of the most important genetic tools and widely employed in the plant pathology community. In recent years, CRISPR (Clustered regularly interspaced short palindromic repeats) and CRISPR-associated proteins discovered in the adaptive immune system in prokaryotes have been successfully reprogrammed into various genome editing tools and have caught the attention of the scientific community due to its simplicity, high efficiency, versatility. Here, we provide an overview of various CRISPR/Cas systems, the derived tools and their applications in plant pathology. This review highlights the advantages of knocking-out techniques to target major susceptibility genes and negative regulators of host defense pathways for gaining resistance to bacterial, fungal and viral pathogens in model and crop plants through utilizing the CRISPR/Cas-based tools. Besides, we discuss the possible strategies of employing the CRISPR-based tools for both fundamental studies on plant-pathogen interactions and molecular crop breeding towards the improvement of resistance in the future.

Keywords: Genome editing, CRISPR, Plant-pathogen interaction, Crop improving

Background

The world's population will reach at least 9.8 billion by 2050, more and more food is needed to provide sufficient nutrients for the rising populations. Crops are susceptible to a larger set of pathogens (fungi, bacteria, oomycetes, viruses, etc.) causing severe economic loss. Thus, the development of plant resistance plays a key role in adjusting crop production to meet the global population requirements (Nejat et al. 2017; Dong and Ronald 2019). Understanding the molecular mechanisms of host-pathogen interactions has been a significant area of investigation in plant pathology for many years.

It's well known that plant and pathogen are locked in a battle of recognition and evasion, in which a multi-layered defense system including both pathogen-associated molecular patterns (PAMPs) -triggered immunity (PTI) and effector-triggered immunity (ETI) has evolved in plants to fight against invading pathogens for survival. Generally speaking, PTI is rapidly activated through the recognition of PAMPs by pattern recognition receptors (PRRs). This basal resistance response, which includes reactive oxygen species (ROS) production, callose deposition and transcriptional reprogramming, usually prevents the invasion of non-adapted pathogens. To counteract this, pathogens secrete effectors to interrupt PTI and modulate host cell physiology, resulting in effector-triggered susceptibility (ETS). However, in resistant plants effectors or its byproducts can be recognized by intracellular immune receptors and induce ETI (a robust resistance response) which is usually associated with localized plant cell death leading to

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pathogen arrest. Some virulent pathogens can overcome the host's ETI response via loss and/or modification of ETI-eliciting effectors as well as meta-effector interactions (Chisholm et al. 2006; Laflamme et al. 2020). Thus, the pathogen and the host play an endless arms race game between them. Despite decades of research, we still have only a limited understanding of the molecular mechanisms of host-pathogen interactions. Therefore, further extensive investigations of host-pathogen interactions, especially the identification of key targets related to defense responses in plants would provide a great opportunity to engineer broad-spectrum and durable resistance in many crops.

Gene loss-of-function mutants and gain-of-function germplasms are important resources for gene function studies and crop genetic improvements (Borisjuk et al. 2019). In recent years, genome-editing (GE) technologies with homing endonucleases (Meganucleases), zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and newly-emerged CRISPR/Cas systems, which enable precise DNA modification of the genome, have greatly transformed the researches on plants (Gaj et al. 2016). The CRISPR/Cas9 system was initially identified as an RNA-mediated adaptive immune system against viral invasion in bacteria and archaea (Rath et al. 2015). Currently, CRISPR/Cas9 has been adapted for genome engineering in diverse eukaryotes due to the simplicity and high efficiency, becoming an alternative to ZFNs or TALENs. To date, CRISPR-based tools have been employed in a wide range of plant-pathogen interaction studies, including host responses to bacteria, fungi, oomycetes, viruses, etc.

CRISPR technology has provided new opportunities to identify important defense-related genes in plants and improve crop resistance to pathogens to ensure food safety and sustainable agricultural development in the future. In this review, we summarize the main features of various CRISPR-based genome editing techniques and address their recent applications in a large number of plant-pathogen interactions. Also, we discuss potential applications of CRISPR/Cas9 tools in defense-related gene dissection and crop improvement.

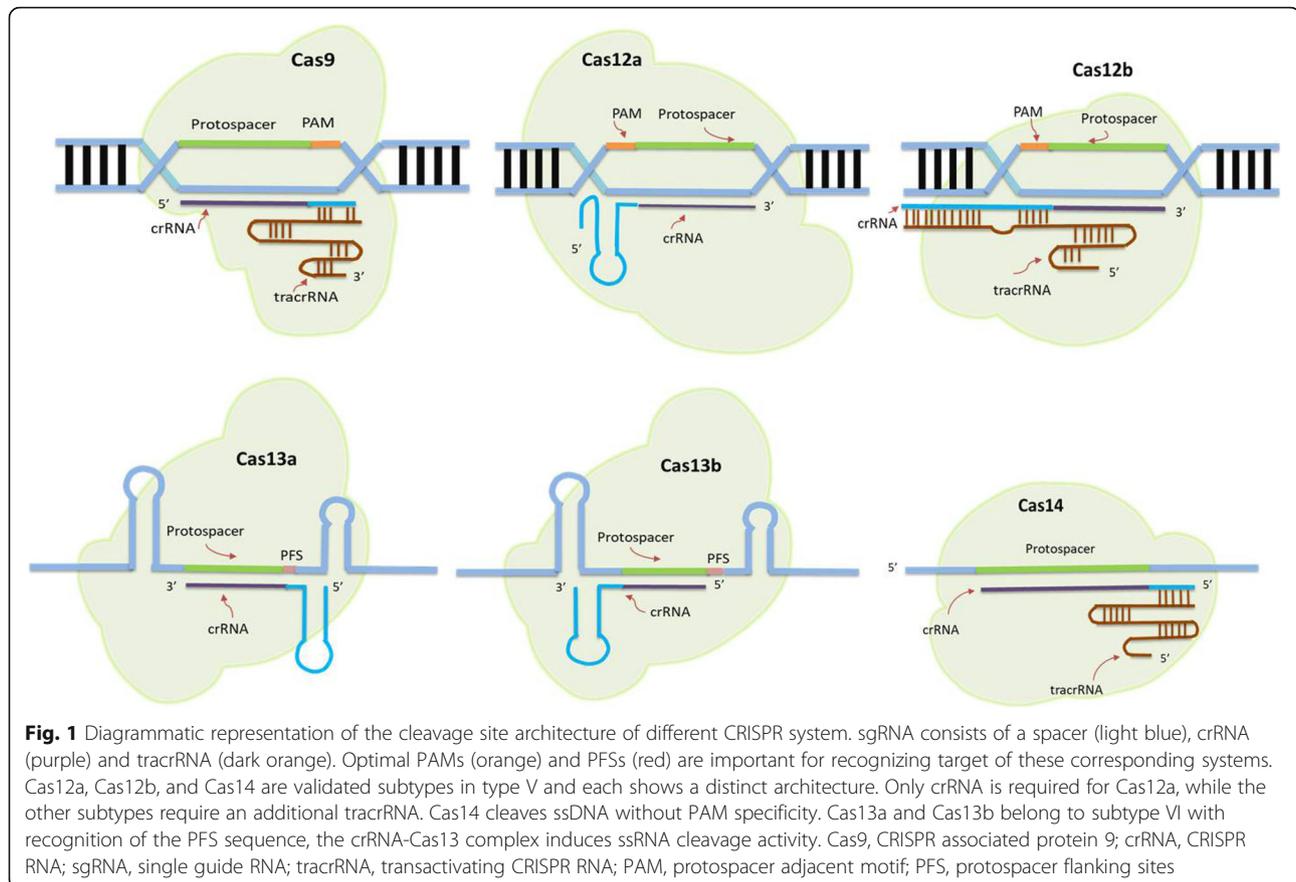
An overview of CRISPR-based tools

Based on phylogenetic, structural, and functional characteristics of Cas proteins, CRISPR/Cas systems are categorized into types I, III and IV (Class 1), and types II, V, and VI (Class 2) with distinct mechanisms of guide RNA biogenesis and target interference (Makarova et al. 2015, 2018; Garcia-Doval and Jinek 2017), in which the Class 2 CRISPR-associated nucleases have been extensively adapted and utilized for nucleic acid manipulation (Garcia-Doval and Jinek 2017; Ji et al. 2019) (Table 1 and Fig. 1).

The type II CRISPR/SpCas9 system is the best characterized and widely used for plant targeted genome targeting. It is derived from *S. pyogenes* and originally consists of three components: the SpCas9 nuclease, a trans-activating small RNA (tracrRNA), and a small mature CRISPR RNA (crRNA) (Bhaya et al. 2011). To date, the optimized system only contains two components, a SpCas9 protein and an easily-engineered guide RNA (gRNA), an artificial fusion RNA with both the custom-designed crRNA and the scaffold tracrRNA (Jinek et al. 2012; Cong et al. 2013). SpCas9 is guided by the gRNA to the specific DNA site which contains the sequence complementary to the first 17–20 nucleotides of the gRNA and is followed by an NGG PAM (protospacer adjacent motif). Upon binding, the HNH and RuvC-like nuclease domains of SpCas9 cleaves both strands of the DNA (complementary and non-complementary to the guide RNA) at exactly -3 position upstream of PAM, resulting in a blunt-ended DNA double-strand break (DSB) (Jinek et al. 2012; Hsu et al. 2014). One of the most significant limitations of the CRISPR/SpCas9 application is the requirement of the PAM sequence for DNA target cleavage. Therefore, many Cas9 variants and orthologues with different PAM specificities, such as ScCas9, SaCas9, SpCas9-NG, xCas9, SpCas9(VQR), SpCas9-NRRH, SpCas9-NRCH, etc., have been isolated, greatly expanding the targeting scope of genome-editing tools in plants (Hu et al. 2016, 2018; Ren et al. 2017, 2019; Chatterjee et al. 2018; Hua et al. 2018, 2019; Wang et al. 2020). Other than that, FnCas9 from *F. novicida*, which is unlike ScCas9 and recognizes a YG PAM, was implicated in RNA targeting (Price et al. 2015).

Table 1 Characteristics of different types of CRISPR/Cas systems involved in plant-pathogen interactions

CRISPR system	Cas9	Cas12a	Cas12b	Cas13	Cas14
Type and Class	Type II Class 2	Type V Class 2	Type V Class 2	Type VI Class 2	Type V Class 2
Pre-crRNA processing	No	Yes	No	Yes	Yes
tracrRNA	Yes	No	Yes	No	Yes
PAM/PFS	3', G-rich	5', T-rich	5', T-rich	3', non-G PFS	Independent-PAM
Nuclease domain	RuvC, HNH	RuvC	RuvC	HPEN	RuvC
Substrate	dsDNA	dsDNA	dsDNA	RNA	ssDNA



CRISPR/Cas12 is the second most well-documented CRISPR system. It is classified as type V and has distinct evolutionary origins and structural architecture from Cas9. To date, some RuvC-like domain-containing Cas12 (i.e. Cas12a, b, c, d, e, g, h, i, k, etc.) have been defined with diverse functions, and both CRISPR/Cas12a (Cpf1) and CRISPR/Cas12b (C2c1) have been successfully applied for genome editing in plants (Zetsche et al. 2015; Zaidi et al. 2017; Ming et al. 2020). Cas12a is guided by a single mature crRNA in DNA targeting (Zetsche et al. 2015), whereas Cas12b requires the presence of both tracrRNA and crRNA to function (Teng et al. 2018; Strecker et al. 2019). Both Cas12a and Cas12b recognize a T-rich PAM and generate 4–5 nt long staggered ends distal to the PAM (Yang et al. 2016; Garcia-Doval and Jinek 2017; Teng et al. 2018; Strecker et al. 2019). Cas14, another type V protein isolated from non-culturable archaea, has been identified and reported to cleave single-stranded DNA (ssDNA) without restrictive sequence requirements. Thus, Cas14 is an ideal tool for engineering resistance against ssDNA plant viruses and high-fidelity SNP genotyping due to its sequence-independent and unrestricted cleavage (Harrington et al. 2018; Khan et al. 2019).

The type VI CRISPR/Cas13 systems function as a ribonuclease and have been divided into four subtypes, including type VI-A (Cas13a), VI-B (Cas13b), VI-C (Cas13c) and VI-D (Cas13d) (Abudayyeh et al. 2016, 2017; Cox et al. 2017; Shmakov et al. 2017; Konermann et al. 2018; Zhang et al. 2018a, 2018b). Cas13 proteins target RNA rather than DNA molecules and are characterized by two distinct HEPN RNase domains and assembled with single crRNA to form a crRNA-guided protein complex for RNA targeting. Similar to PAM for Cas9 and Cas12, 5'- and/or 3'-protospacer-flanking site (PFS) are needed for redirecting the Cas13/crRNA complex to the target site. Cas13 also showed the promiscuous ability of the RNase to cleave collateral RNAs once activated in the presence of template targets. Cas13 proteins are being used to localize, detect, and track RNA molecules of different types.

Single nucleotide polymorphisms (SNPs) are the genetic basis of many important agronomic traits variations (i.e. pathogen resistance, yield) of crop plants. Therefore, a series of base editing tools, including cytidine base editor (CBE) and adenine base editor (ABE), have been developed to achieve specific base substitution in plants (Ren et al. 2017; Yan et al. 2018). It has been engineered

by fusing various cytidine deaminase and/or adenine deaminase to impaired Cas9 (Cas9 nickase or dead Cas9), which induces cytosine-to-thymine (C > T) and adenine-to-guanine (A > G) substitutions in the target region in genome, respectively (Komor et al. 2016; Gaudelli et al. 2017). Besides, prime editors, constructed with Moloney murine leukemia virus reverse transcriptase (M-MLV RT) and Cas9 nickase, have been further developed, enabling all 12 base-to-base conversions as well as indels and insertions (Anzalone et al. 2019; Lin et al. 2020).

Genome editing in plant pathogens

The development of efficient genome-editing techniques, particularly CRISPR/SpCas9, has emerged a broad array of probable uses that could be explored in plant pathogens. The ability to modify plant pathogen genomes offers the possibility to confer desirable phenotypes for numerous purposes (Zhang et al. 2018). Compared to traditional methods for genetic manipulation of the microbial genome which are usually associated with inefficient homologous recombination, the CRISPR/SpCas9 tools are highly efficient and much simpler in some cases. Besides, they provide a high-throughput experimental platform to dissect gene function at the whole-genome level in plant pathogens.

After the first example of genome editing with high efficiency in *Escherichia coli* was reported (Jiang et al. 2013), the application of CRISPR technology has been greatly expanded into other bacterial species such as *Pseudomonas*, *Yersinia*, *Bacillus*, *Streptomyces* and *Corynebacterium* (Liu et al. 2019). For example, CRISPR/SpCas9 and CRISPR/FnCas12a systems have been established in *P. putida* KT2440. Both systems are successful in achieving gene deletions, gene insertions, and gene replacements with efficiency as high as 100% (Aparicio et al. 2018; Sun et al. 2018). Furthermore, a base editing system pnCasPA-BEC was developed by engineering cytidine deaminase APOBEC1 to the Cas9 nickase, which allowed a highly-efficient C > T substitution in *P. aeruginosa*, *P. putida*, *P. fluorescens*, *Staphylococcus aureus* and *P. syringae* (Chen et al. 2018; Gu et al. 2018). Besides, dCas9-based transcription inhibition system (CRISPRi) was recently applied for target gene repression in *Pseudomonas* spp. using a deactivated SpCas9 (Bikard et al. 2013; Tan et al. 2018).

The CRISPR/SpCas9-mediated genome editing technology has been successfully established in a wide range of fungal species, including *Magnaporthe oryzae* (Arazoe et al. 2015; Foster et al. 2018), *Alternaria alternata* (Wenderoth et al. 2017), *Leptosphaeria maculans* (Idnurm et al. 2017), *Fusarium oxysporum* (Wang et al. 2018; Wang and Coleman 2019), *Fusarium graminearum* (Gardiner and Kazan 2018), *Fusarium fujikuroi* (Shi et al. 2019), *Fusarium proliferatum* (Ferrara et al.

2019), *Sclerotinia sclerotiorum* (Li et al. 2018), *Colletotrichum sansevieriae* (Nakamura et al. 2019), *B. cinerea* (Leisen et al. 2020), *Sporisorium scitamineum* (Lu et al. 2017), *Ustilago virens* (Liang et al. 2018), *Ustilago maydis* (Schuster et al. 2016) and *Ustilago tricophora* (Huck et al. 2019). In fungi, both SpCas9 and the sgRNA can be stably or transiently expressed by polyethylene glycol (PEG)-mediated transformation, *Agrobacterium*-mediated transformation, electroporation, and biolistic transformation (Schuster and Kahmann 2019). Alternatively, the SpCas9/sgRNA ribonucleoprotein (RNP) complex can be assembled in vitro and directly applied in *M. oryzae* and *F. oxysporum* (Foster et al. 2018; Wang et al. 2018).

CRISPR/SpCas9 tools have also been successfully established and applied for genome editing in oomycetes, such as *P. sojae*, *P. capsici*, *P. palmivora* and *P. litchii*. For example, a CRISPR/SpCas9-mediated genome editing method with or without donor DNA has been established for genetic manipulation of *P. sojae* through PEG-mediated protoplast transformation. In these studies, disruption of the RXLR effector Avr4/6 proteins prevented its recognition by the corresponding soybean R protein Rps4 and Rps6 (Dou et al. 2010; Barakate and Stephens 2016; Fang and Tyler 2016). Furthermore, CRISPR/SpCas9 was also used to knockout another RXLR effector gene *PcAvh1* in *P. capsici*. In this study, inoculation of *PcAvh1* mutants on *N. benthamiana* showed that *PcAvh1* was required for the full virulence of *P. capsici* (Chen et al. 2019). CRISPR/SpCas9 system has also been used in *P. palmivora* to generate homozygous PpalEPIC8 mutants via *Agrobacterium*-mediated transformation. In their experiment, mutations in PpalEPIC8 decreased the pathogenicity of *P. palmivora* in papaya fruits, probably by inhibiting the action of papain (Gumtow et al. 2018). In oomycetes (i.e. *P. sojae*, *P. capsici* and *P. litchii*), the CRISPR/SpCas9-mediated genome editing system has been optimized by using the oxathiapiprolin-resistance gene *PcMuORP1* as the selection marker. This has enabled a much higher efficiency for screening of transformants as compared to the conventional selection marker *NptIII* (Kong et al. 2019; Schuster and Kahmann 2019; Wang et al. 2019).

Genome editing for plant disease resistance against bacterial, fungal and oomycete pathogens

During the infection process of bacteria, type III effectors are secreted into the plant cell (Büttner and He 2009). These effectors primarily interrupt the host's defense pathways and/or activate the *S* genes for disease development (Yang et al. 2006; Zaidi et al. 2018). Therefore, both *S* genes and negative regulators of plant innate immune response are good target sites for CRISPR/Cas9-mediated gene editing to improve plant resistance.

For example, knockout of the endogenous *OsSWEET11* gene which is the target of TALEs in rice resulted in significantly enhanced resistance to *X. oryzae* pv. *oryzae* (*Xoo*) without altering pollen development (Kim et al. 2019). TALE-binding elements (EBEs) in the promoter region of both *OsSWEET11* and *OsSWEET14* were also targeted, the resulting rice lines carrying indels conferred robust resistance to most *Xoo* strains (Xu et al. 2019). Moreover, recent studies showed that multiplex genome editing of the promoter regions of *OsSWEET11*, *OsSWEET13*, and *OsSWEET14* in three rice varieties conferred broad-spectrum resistance to *Xoo* (Oliva et al. 2019). CRISPR/SpCas9 and CRISPR/Cas12 have also been used to generate plant resistance to other pathogens. For example, genome editing of the promoter region of the *S* gene *CsLOB1* in citrus, which is the target of TALE from *X. citri* pv. *citri* (*Xcc*), conferred a high degree of resistance to the citrus canker disease (Jia et al. 2016, 2017; Peng et al. 2017). *DMR6* is essential for resistance to downy mildew in *A. thaliana*, knockout of its homolog *SIDMR6-1* in tomato by CRISPR/SpCas9 rendered plant resistant to different bacterial pathogens, including *P. syringae*, *P. capsici* and *Xanthomonas* spp. (Thomazella et al. 2016). Besides, the *SIJAZ2Δjas* tomato germplasm generated by CRISPR/SpCas9 provided resistance to *P. syringae* pv. *tomato* DC3000 without altering its defense response to the necrotrophic fungal pathogen *Botrytis cinerea* (Ortigosa et al. 2019).

Host colonization by fungal pathogens is a complex process. To date, much attention has been paid to *S* genes and negative regulators involved in the defense pathway. For example, the mildew resistance locus *O* (*MLO*) is a well-known *S* gene loci originally identified in barley (Jørgensen 1992). CRISPR/SpCas9-mediated knockout of its homologs in wheat (*TaMLOs*) resulted in resistance against the powdery mildew fungal pathogen *Blumeria graminis* f.sp. *tritici* (Wang et al. 2014). Furthermore, knockout of its homologs in tomato (*SLMLO1*) conferred resistance to the powdery mildew fungus *Oidium neolycopersici* (Nekrasov et al. 2017). Likewise, given that *EDR1* plays a negative role in the defense response against powdery mildew in *A. thaliana*, CRISPR/SpCas9 was used to simultaneously knock out three *TaEDR* copies, developing powdery mildew resistance in wheat (Frye et al. 2001; Zhang et al. 2017). Another example is *OsERF922*, which encodes a transcription factor belonging to the APETALA2/ethylene response factor (AP2/ERF) superfamily in rice, and has been reported to act as both positive and negative regulators in plant defense against different pathogens (Langner et al. 2018). Wang and colleagues employed CRISPR/SpCas9 to edit *OsERF922*, and the resulting rice lines carrying different frameshift indels showed enhanced resistance to the rice blast fungus *M. oryzae*

without affecting plant development and other agronomic traits tested (Wang et al. 2016). Recently, CRISPR/SpCas9 has also been used to knock out the *BSR-K1* gene in rice, resulting in resistance to both *M. oryzae* and *Xoo* (Zhou et al. 2018).

Besides the knockout strategy mentioned above, defective *R* gene correction through CRISPR/Cas9-mediated precise base editing is another efficient and time-saving way to improve crop disease resistance. For example, an SNP at position 441 in the recessive allele of *Pi-d2* has been reported to be associated with the resistance to *M. oryzae*. The recessive allele *pi-d2(M441)* in rice results in loss of resistance to the Chinese blast isolate ZB15. On the other hand, incorporation of the dominant *R* gene *Pi-d2(I441)* into the rice variety Digu conferred gene-for-gene resistance to ZB15 (Chen et al. 2006). Based on these findings, Ren and colleagues employed a cytidine base editor rBE5 to rapidly correct the recessive *pi-d2* gene in rice variety Kitaake by introducing a G > A substitution (M4111) into the genome (Ren et al. 2018). This fast and precise genome engineering method can greatly speed up the resistance breeding program as compared to traditional hybrid breeding and marker-assisted selection methods.

As fungal-like eukaryotes, Oomycetes belong to the kingdom Chromista. The most representative pathogenic oomycetes are *Phytophthora* spp., *Pythium* spp., and *Peronospora* spp. CRISPR/Cas tools have been successfully established and applied for controlling plant oomycete disease, which mainly focuses on editing the effectors of Oomycetes, and editing the plants to improve the resistance to oomycetes is limited. The only example came from Fister and coworker who developed a CRISPR/Cas system to target *TcNPR3* by transient expression in *Theobroma cacao* leaves, gene editing in 27% of *TcNPR3* copies was achieved and the gene-edited leaves showed enhanced resistance to the pathogen *P. tropicalis*, and stably genome-edited somatic embryos were obtained via *Agrobacterium*-mediated transformation (Fister et al. 2018). Given that NPR3 and NPR4 serve as redundant transcriptional co-repressors for salicylic acid-responsive defense genes in *A. thaliana*, it's worth editing their homologs in other crops to develop novel resistant germplasm.

Genome editing in the study of plant-virus interactions

The CRISPR/Cas-mediated gene-editing technology has been developed rapidly and has been used to explore effective ways for engineering virus resistance. Virus resistance can be accomplished by (1) targeting host factors that are involved in the replications of the virus or (2) targeting and destroying the viral genome itself and thus preventing the replication of the virus.

The eukaryotic translation initiation factor 4E (eIF4E), also known as a cap-binding protein, has been proven as a susceptibility factor in plant-virus interactions. Disruption of *eIF4E* gene results in innate immunity against many potyviruses (virus belonging to the family of *Potyviridae*) in various plant species. For example, CRISPR/SpCas9-mediated *eIF4E*-edited cucumber plants exhibited resistance to zucchini yellow mosaic virus (ZYMV), papaya ringspot mosaic virus-W (PRSMV-W) and cucumber vein yellowing virus (CVYV) (Chandrasekaran et al. 2016). Moreover, mutagenesis of *eIF4E* alleles in cassava and *A. thaliana* by CRISPR/SpCas9 system reduced cassava brown streak disease symptom and turnip mosaic virus (TuMV) infection, respectively (Pyott et al. 2016; Gomez et al. 2019). Similarly, CRISPR/SpCas9 has also been used to mutate *eIF4G* in rice and generated resistance to rice tungro spherical virus (RTSV) (Macovei et al. 2018). Recently, a C > G conversion (N176K) was introduced into the wild-type *eIF4E1* in *A. thaliana* by cytidine base editor, conferring resistance to clover yellow vein virus (CIYVV) (Bastet et al. 2019).

Geminiviruses, a group of circular single-stranded DNA viruses, cause acute damage to economically important crops, like tomato, sugar beet, and pepper (Langner et al. 2018). Many studies have been carried out to directly target geminiviral genomic DNA with CRISPR/SpCas9 (Kalinina et al. 2020). Constructs containing SpCas9 and sgRNAs which targeted the *Rep* (replication-associated protein) gene and the intergenic region (IR) in viral genomes of beet severe curly top virus (BSCTV) and bean yellow dwarf virus (BeYDV) were transformed into *N. benthamiana* and *A. thaliana*, respectively. The resulting plants exhibited high levels of resistance to the targeted virus (Baltes et al. 2015; Ji et al. 2015). Similarly, *N. benthamiana* and tomato plants expressing SpCas9 and sgRNA specific for CP (coat protein) or *Rep* sequences of tomato yellow leaf curl virus (TYLCV) exhibited significant virus resistance (Tashkandi et al. 2018). More studies reported that the CRISPR/SpCas9 system targeting MP, CP and any other conserved regions of viral genomes established wheat dwarf virus (WDV) resistance in barley and banana streak virus (BSV) resistance in banana, respectively (Kis et al. 2019; Tripathi et al. 2019).

RNA viruses can be also directly targeted by Cas protein (i.e. Cas13a and FnCas9) which target RNA molecules rather than DNA. For example, Aman and colleagues employed CRISPR/Cas13a to target and degrade the viral RNA of TuMV in *N. benthamiana* (Aman et al. 2018). Furthermore, Zhang and colleagues used CRISPR/FnCas9 to degrade the viral genome of cucumber mosaic virus (CMV) and tobacco mosaic virus (TMV) in transgenic *N. benthamiana* and *A. thaliana* plants, respectively (Zhang et al. 2018c). Similar

approaches have been successfully applied to produce resistance to potato virus Y (PVY) in tobacco and resistance to rice stripe mosaic virus (RSMV) and southern rice black-streaked draft virus (SRBSDV) in rice (Zhang et al. 2019). Although CRISPR/Cas has been successfully employed to inhibit the viral growth in transgenic plants, the potential risks of viruses escaping the CRISPR/Cas9 cleavage and leading to loss of resistance caused by the fast-evolving virus are under concern. Mehta and colleagues reported that between 33 and 48% of edited virus genomes evolved a conserved single nucleotide mutation which conferred resistance to CRISPR/Cas9 cleavage, resulting in failed resistance to the geminivirus during glasshouse inoculations (Mehta et al. 2019).

Conclusions and future directions

Today, the CRISPR/Cas system is the most extensively used technology for targeted genomic editing compared to other GE technologies, and has been developed and applied in a large number of host plants and plant pathogens for dissecting the molecular mechanisms underlying the plant-pathogen interactions and for improving host resistance against bacteria, fungi, oomycetes, DNA viruses, and RNA viruses. The CRISPR/Cas system is a valuable tool for creating gene loss-of-function and gain-of-function mutants and for understanding plant-pathogen interactions as well as reducing the damage caused by the destructive pathogens in agricultural application.

CRISPR/Cas-based tools can be used for single and multiple gene knockouts. Moreover, CRISPR/Cas-based tools can also be used to create high-throughput mutant libraries, providing a powerful approach to accelerate gene function studies on plant resistance and pathogen pathogenesis. Therefore, the potential players involved in plant-pathogen interactions, such as resistance gene family, receptor-like kinase gene family, transcriptional factor gene family and differentially expressed transcripts corresponding to pathogen challenge, would be recommended to be targeted on a large scale by the highly-efficient CRISPR/Cas tools, and the crucial defense-related genes can be identified, characterized and further utilized in agricultural application.

SNPs and quantitative trait locus (QTL), abundant forms of genetic variation among individuals in crop species, are responsible for diverse pleiotropic phenotypes, including crop resistance. To date, crucial SNPs and SNP-typed QTLs have been identified in associated with a quite number of resistance and resistance-related genes in many crops, such as *Pi-ta*, *Pi-d2*, *bsr-d1*, *bsr-k1*, *Xa4*, *Xa5*, *CsSGR*, etc. CRISPR/Cas9-mediated base editors and prime editors can be employed to perform precise genome editing of SNPs and SNP-typed QTLs easily in cash crops, endowing multiple resistance to

pathogens. Recently, the base-editing-mediated gene evolution (BEMGE) method was developed (Kuang et al. 2020). This novel crop breeding method can artificially evolve any endogenous gene *in planta* with a tiled sgRNA library corresponding to the target genomic locus. Thus, BEMGE is a promising method for the transformation of any functional gene related to plant defense response.

In conclusion, the CRISPR/Cas system and its derivatives provide a novel opportunity to explore the complex area of plant-pathogen interactions. Along with the continuous changes in agricultural production activities and plant disease systems, we expect that the CRISPR technologies will make a big contribution in deciphering the interaction between plant and pathogen and designing durable and broad-spectrum disease resistance plants in the future.

Abbreviations

ABE: Adenine base editor; BEMGE: Base-editing-mediated gene evolution;; BeYDV: Bean yellow dwarf virus; BSCTV: Beet severe curly top virus; BSV: Banana streak virus; CBE: Cytidine base editor; CMV: Cucumber mosaic virus; CIYWV: Clover yellow vein virus; CVYV: Cucumber vein yellowing virus; CRISPR: Clustered regularly interspaced short palindromic repeats;; CRIS PRi: dCas9-based transcription inhibition; crRNA: CRISPR RNA; CP: Coat protein; DSB: DNA double-strand break; EBES: TALE-binding elements; ETI: Effector-triggered immunity; ETS: Effector-triggered susceptibility; GE: Genome-editing; gRNA: Guide RNA; IR: Intergenic region; *MLO*: Mildew resistance locus O; M-MLV RT: Moloney murine leukemia virus reverse transcriptase; PAM: Protospacer adjacent motif; PAMPs: Pathogen-associated molecular patterns; PEG: Polyethylene glycol; PFS: Protospacer-flanking site; PRRs: Pattern recognition receptors; PRSMV-W: Papaya ring spot mosaic virus-W; PTI: PAMP-triggered immunity; PVY: Potato virus Y; QTL: Quantitative trait locus; Rep: Replication-associated protein; RNP: Ribonucleoprotein; ROS: Reactive oxygen species; RSMV: Rice stripe mosaic virus; RTSV: Rice tungro spherical virus; SNP: Single-nucleotide polymorphism; SRBSDV: Southern rice black-streaked draft virus; ssDNA: Single-stranded DNA; TAL: Transcription activator-like; TALENs: Transcription activator-like effector nucleases; TMV: Tobacco mosaic virus; tracrRNA: Trans-activating small RNA; TYLCV: Tomato yellow leaf curl virus; TuMV: Turnip mosaic virus; WDV: Wheat dwarf virus; *Xcc*: *Xanthomonas citri* subsp. *citri*; ZFNs: Zinc-finger nucleases;; ZYMV: Zucchini yellow mosaic virus

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Authors' contributions

GG, FY, BR, KY, DY, XZ and HZ wrote the manuscript. GG drew the figures. KY finished the Tables. All authors read and approved the final manuscript.

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Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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