PHILOSOPHICAL TRANSACTIONS B

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Review

Cite this article: Yin K, Qiu J-L. 2019 Genome editing for plant disease resistance: applications and perspectives. *Phil. Trans. R. Soc. B* **374**: 20180322.
http://dx.doi.org/10.1098/rstb.2018.0322

Accepted: 16 October 2018

One contribution of 19 to a theme issue 'Biotic signalling sheds light on smart pest management'.

Subject Areas:
biotechnology, plant science

Keywords:
genome editing, crop, disease resistance, susceptibility genes, breeding

Author for correspondence:
Jin-Long Qiu
e-mail: qiujl@im.ac.cn

Genome editing for plant disease resistance: applications and perspectives

Kangquan Yin and Jin-Long Qiu

State Key Laboratory of Plant Genomics, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, People's Republic of China

KY, 0000-0002-4627-6585; J-LQ, 0000-0001-8054-2552

Diseases severely affect crop yield and quality, thereby threatening global food security. Genetic improvement of plant disease resistance is essential for sustainable agriculture. Genome editing has been revolutionizing plant biology and biotechnology by enabling precise, targeted genome modifications. Editing provides new methods for genetic improvement of plant disease resistance and accelerates resistance breeding. Here, we first summarize the challenges for breeding resistant crops. Next, we focus on applications of genome editing technology in generating plants with resistance to bacterial, fungal and viral diseases. Finally, we discuss the potential of genome editing for breeding crops that present novel disease resistance in the future.

This article is part of the theme issue 'Biotic signalling sheds light on smart pest management'.

1. Introduction

Agriculture is the basis for the survival and development of society. Sustainable agriculture that achieves food security while minimizing pressure on the environment is the mission of agriculture in the twenty-first century. Diseases are major and important constraints that threaten agricultural development and global food security. Diseases reduce crop yields by adversely affecting plant growth and development and affect the quality of agricultural products in the field and during storage. According to incomplete statistical data, diseases globally reduce crop yields by as much as 20–40% (FAO, http://www.fao.org/news/story/en/item/280489/icode/). At present, the main strategies for controlling crop diseases remain highly dependent on chemical pesticides. However, these pesticides are usually directly or indirectly harmful to humans and to the natural environment [1]. Since pesticides are generally not highly specific, they can also influence other organisms while killing pathogens, thereby disturbing ecological balances. In addition, pathogenic microorganisms may evolve pesticide resistance, which requires the continuous development of new pesticides and/or applications of more pesticides. Therefore, reducing the dependence of food production on chemical pesticides is a key goal to avoid their negative environmental impact, especially in developing countries [2].

Genetic breeding of disease-resistant crop varieties serves as an effective and eco-friendly strategy for sustainable agriculture. Although conventional resistance breeding has been successful over the last few decades, it has several limitations. First, it can be performed only between plants that can mate with each other. Second, it is highly dependent on plant populations that have sufficient genetic variation. Third, it often introduces many traits along with the resistance trait of interest, including traits with undesired effects on yield. Fourth, genetic crosses and segregant progeny selection are generally labour-intensive and time-consuming. Therefore, it is a challenge for conventional breeding to keep pace with continually changing pathogens and increasing food demand, particularly during an era of global climate change [3]. These challenges to our current agricultural practices suggest the need for new technologies. For example, new technologies such as transgenesis have shown to be important in overcoming such challenges and securing world food security

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[4]. In this review, we discuss newly developed genome editing technology and its applications in breeding crops that have enhanced resistance to diseases.

2. Genome editing technology

Genome editing is a way to make precise changes to the genomic DNA of a cell or organism. The core of genome editing technology is the use of sequence-specific nucleases for recognizing specific DNA sequences and producing double-stranded DNA breaks (DSBs) at targeted sites. DSBs are repaired mainly via two pathways: the nonhomologous ending-joining (NHEJ) pathway and the homologous recombination (HR) pathway [5]. In most cases, cells use the NHEJ pathway to repair DSBs. However, NHEJ is error prone and usually results in insertion or deletion mutations. In the presence of a donor DNA template, DSBs are likely to be repaired by the HR pathway, which results in precise base changes or gene replacement. Currently, there are three major types of sequence-specific nucleases for genome editing: zinc finger nucleases (ZFNs) and transcription activators such as effector nucleases (TALENs) and the clustered regularly interspaced short palindromic repeats/CRISPR-associated protein (CRISPR/Cas) system.

The CRISPR/Cas system is based on an adaptive immune system that eliminates invasion of foreign plasmids or viral DNA via cleavage within bacteria and archaea [6]. CRISPR/Cas genome editing systems consist of a single guide RNA (sgRNA) and a Cas protein that exhibits nuclease activity. Moreover, gRNA contains a scaffold for Cas protein binding and a user-defined spacer sequence (approx. 20 nt) for genomic sequence targeting. Owing to its simplicity, high efficiency and ease of use, since its first demonstration in mammalian cells [7,8], applications of the CRISPR/Cas9 system have rapidly surpassed those of ZFNs and TALENs in various organisms, including plants [9]. One constraint of the CRISPR/Cas system is the protospacer adjacent motif (PAM)-dependent cleavage of the target sequence. However, many different CRISPR/Cas systems with different PAM specificities (i.e. CRISPR/saCas9, CRISPR/Cpf1 (Cas12a), xCas9) have been identified and engineered beyond CRISPR/Cas9 from *Streptococcus pyogenes* [10]. In addition, RNA targeting CRISPR/Cas systems (i.e. CRISPR/Cas13a (C2c2), CRISPR/Cas13b, CRISPR/Cas13d) also have greatly expanded the CRISPR toolbox [11–13].

3. Editing plants for disease resistance

Several traditional methods have been successfully developed for breeding disease resistance. The pure line method, specifically developed for self-pollinated crops, was used to breed the Kanred variety of wheat, which is resistant to rust [14]. The pedigree method has been widely used for developing disease-resistant varieties controlled by major genes [15]. The backcross method and recurrent selection have been developed for improving elite varieties' resistance to rice blast [16,17]. Interspecific hybrids have been used to generate resistance to cotton rust by transferring resistance genes from *Gossypium anomalum* and *Gossypium arboreum* into *Gossypium hirsutum* [18]. The mutation breeding method has been successfully used to produce many lines resistant to rice blast [15]. However, traditional methods for breeding resistance are time-consuming, and the resistance alleles are sometimes linked to genes that influence plant development [15].

Prerequisites for successful genome editing are known host genome sequences and molecular information about a target gene. Nevertheless, an increasing number of plant species have been fully sequenced. Additionally, intensive genetic and molecular studies have revealed molecular details of plant innate immunity, providing increasing numbers of targets for the prevention and control of pests and diseases. In particular, negative regulators of plant disease resistance, which may be considered host susceptibility (S) genes, represent good targets for genome editing. Therefore, disease resistance is an ideal trait for editing.

4. Genome editing for resistance against bacterial pathogens

Bacterial diseases are very difficult to control, especially after the establishment of epidemics, in part because bacterial pathogens are highly diverse, multiply rapidly, and can be spread in many ways. However, due to the marked effects on elucidating the molecular mechanisms of host–bacterial pathogen interactions, many host plant genes, including some S genes, that participate in these complex processes, have been identified. As S genes may be more durable in the field, they are becoming popular targets for breeding crops that are resistant to bacterial diseases via genome editing.

Rice bacterial blight, a rice vascular bundle disease caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), has high epidemic potential and is one of the major diseases of rice. It causes 10–20% losses of yield [19], but this loss can surpass 50% under conditions favourable to the pathogen (i.e. high humidity) and can sometimes even result in complete loss of yield [20]. *Xoo* secretes TALE (transcription activator-like effector) proteins into host cells through the type III secretion system [21]. Many TALE proteins target S genes and activate their expression to promote successful infection [22,23]. For example, the TALE protein AvrXa7 from the Philippine strain PXO86 binds to the effector-binding element (EBE) in the promoter of *OsSWEET14* (also called *Os11N3*) and activates its expression. *OsSWEET14* encodes a sucrose-efflux transporter and is thus hijacked by PXO86 for transferring sugars from rice cells to sustain pathogen growth and virulence [24]. As OsSWEET14 also plays an important role in plant development, it is not feasible to knock out *OsSWEET14* to provide resistance against *Xoo* without adverse effects. Therefore, a pair of TALENs targeting EBEs in the *OsSWEET14* promoter were designed to disrupt the binding of AvrXa7 but not affecting the developmental function of *OsSWEET14* [25]. PthXo2 is another TALE protein involved in the virulence of some *Xoo* strains [26,27], but its host target gene has not been experimentally determined. However, expression of *OsSWEET13* is promoted by PthXo2, which binds to the *OsSWEET13* promoter in rice. Knockout mutants of *OsSWEET13* have been generated by targeting the coding region via the CRISPR/Cas9 system. Unlike *OsSWEET14*, *OsSWEET13* loss of function did not affect plant development but still provided enhanced resistance to *Xoo* in a PthXo2-dependent manner [28].

Citrus canker is a devastating disease caused by the bacterium *Xanthomonas citri* ssp. *citri* (*Xcc*). *CsLOB1*, a member of the lateral organ boundaries domain (LBD) family of transcription factors, was previously identified as an S gene for

Xcc [29]. The *CsLOB1* promoter contains an EBE that is recognized by the *Xcc* effector PthA4, which activates *CsLOB1* expression to facilitate canker development. In one study, the *CsLOB1* promoter EBE was targeted, whereas another study targeted the coding region of *CsLOB1* via CRISPR/Cas9. Both studies showed that editing *CsLOB1* provided resistance to *Xcc* [30,31]. Surprisingly, although the potential negative effect of mutating *CsLOB1* on plant growth has yet to be determined, the growth status of the *CsLOB1* null mutant was similar to that of wild-type plants [30], suggesting that *CsLOB1* is an ideal candidate for engineering canker resistance in elite citrus varieties.

Despite being one of the most economically important crops worldwide, the production and quality of tomato are still limited by several major pathogens, including *Pseudomonas syringae*, *Phytophthora* spp. and *Xanthomonas* spp. [32]. A recent study revealed that mutation of a single gene in *Arabidopsis*, *DMR6* (*downy mildew resistance 6*), led to increased salicylic acid levels and resistance to several plant pathogens, including bacteria and oomycetes [33]. Interestingly, the tomato ortholog *SlDMR6-1* is also upregulated in response to infection by *P. syringae* pv. tomato and *Phytophthora capsici*. Null mutants of *SlDMR6-1* generated via the CRISPR/Cas9 system showed resistance to *P. syringae*, *P. capsici* and *Xanthomonas* spp. without detrimental effects on tomato growth and development [34]. Together, these results suggest that knocking out *DMR6* may be a promising strategy to confer broad-spectrum disease resistance to plants.

5. Genome editing for resistance against fungal pathogens

Fungal pathogens represent the dominant causal agents of plant diseases and have an enormous impact on agriculture. With diverse lifestyles and high genetic flexibility, they can quickly invade new hosts, break R gene-mediated resistance and generate resistance to fungicides, thus constituting a major challenge in disease control [35]. Recently, genome editing has begun to address this challenge by modifying host S genes.

Powdery mildew is a global fungal disease that infects a wide range of plants. Breeding resistant cultivars is the most effective, economical and eco-friendly approach to control powdery mildew. The traditional method for producing resistant cultivars relies on introducing resistance (via R genes) from alien species into elite varieties by hybridization. Since most of these resistance genes are race specific, their resistance gradually diminishes as new races of wheat powdery mildew evolve in the field. Therefore, breeding wheat varieties with broad-spectrum and durable resistance is highly desired.

The discovery of barley *mlo* (*mildew resistance locus o*) mutants is a major success in plant breeding for broad-spectrum and durable resistance to powdery mildew [36]. Mlo resistance has been widely used in spring barley throughout Europe for nearly 40 years [37]. After cloning the barley *Mlo* gene in 1997, *Mlo* orthologues were found to be evolutionarily conserved in dicots and monocots. Since bread wheat is an allohexaploid, it has three orthologues of barley *Mlo* (*TaMlo-A1*, *B1* and *D1*). Wang *et al*. [38] used TALEN and CRISPR to edit these wheat *Mlo* genes and found that the edited plants exhibited resistance towards the powdery mildew fungus *Blumeria graminis* f. sp. *tritici* (*Bgt*) only when all six copies of *TaMlo* were simultaneously mutated. These results show that these copies of *TaMlo* function redundantly and demonstrate that genome editing is a superior tool for modifying targets within polyploidy genomes. In tomato, *Mlo* knockout mutants were generated by targeting *SlMlo1* with two sgRNAs simultaneously. As expected, the resultant tomato mutants are fully resistant to the tomato powdery mildew fungus *Oidium neolycopersici* [39].

In *Arabidopsis*, *EDR1* (*enhanced disease resistance 1*) negatively regulates resistance against the powdery mildew fungus *Erysiphe cichoracearum*, but only slightly affects plant growth [40], suggesting that *EDR1* is an ideal target for improving resistance to powdery mildew. Moreover, similar to *Mlo*, *EDR1* is highly conserved across plant species [40]. The CRISPR/Cas9 system was used to generate *Taedr1* wheat plants by targeting all three homoeologs of wheat *EDR1* simultaneously. The resultant *Taedr1* mutant plants were resistant to *Bgt* but without mildew-induced cell death [41]. As *Arabidopsis edr1* mutant plants are resistant to bacteria and oomycetes, it is reasonable to speculate that *Taedr1* plants might also be resistant to other wheat pathogens.

Rice blast, which is caused by *Magnaporthe oryzae*, is one of the most devastating diseases that affect rice production worldwide [42]. Ethylene responsive factors (ERFs) of the APETELA2/ERF (AP2/ERF) superfamily play pivotal roles in rice adaptation to multiple biotic and abiotic stresses [43]. The expression of *OsERF922* is induced not only by abscisic acid (ABA) and salt but also by *M. oryzae*. Knockdown of *OsERF922* by RNAi (RNA interference) leads to increased resistance to *M. oryzae*, indicating that *OsERF922* is a negative regulator of rice blast resistance [44]. Targeted modification of *OsERF922* using CRISPR/Cas9 generated rice *Oserf922* knockout mutants [45]. These null mutants showed enhanced resistance to rice blast without affecting other major agronomic traits. Therefore, the targeted knockout of negative regulators or/and susceptibility genes via genome editing represents a powerful approach for plant disease resistance breeding.

6. Genome editing for resistance against viruses

Plant viral diseases are difficult to manage because viruses evolve rapidly and because insect vectors are usually involved. During the past three decades, transgenic expression of virus proteins or RNAs has been widely used to improve plant virus resistance, and the resultant resistance is called pathogen-derived resistance. Recently, RNAi induced by double-stranded RNA has also been considered an efficient method to confer resistance against viruses to plants [46]. Genome editing technology provides a new weapon in the arsenal against plant viruses.

Geminiviruses, a group of DNA viruses, comprise more than 360 species [47]. They have a circular, single-stranded DNA genome, which is replicated in the host nucleus via a double-stranded DNA (dsDNA) intermediate [48]. This dsDNA intermediate makes it a target for sequence-specific endonucleases. Previously, an artificial zinc finger protein (AZP) without a nuclease domain was generated to target the replication origin of the Beet severe curly top virus (BSCTV) by blocking the binding of viral replication protein (Rep). Transgenic AZP *Arabidopsis* plants showed increased resistance to BSCTV, and more than 80% of these transgenic

plants exhibited no viral infection symptoms [49]. Similarly, ZFNs have been designed to target a conserved region of the Rep gene of Tomato yellow leaf curl China virus (TYLCCNV) and the Tobacco curly shoot virus (TbCSV). A transient assay in tobacco demonstrated that these ZFNs cleaved the target sequences and inhibited replication of the viruses [50]. TALENs were also used to generate transgenic tobacco with resistance to TbCSV, TYLCCNV and Tomato leaf curl Yunnan virus (TLCYnV) by targeting the Rep genes [51]. More recently, several studies reported the successful use of CRISPR/Cas9 to generate geminivirus resistance in plants. Delivery of gRNAs targeting the intergenic region (IR), coat protein (CP) or Rep in Cas9-expressing tobacco reduced the viral accumulation of several important DNA viruses, including Tomato yellow leaf curl virus (TYLCV) [52,53]. Moreover, these studies also revealed that a gRNA targeting the conserved sequence (TAATATTAC) in the IR region could be used to target multiple geminiviruses simultaneously [52]. Consistently, gRNAs targeting the Rep, CP or IR significantly reduced or abolished disease symptoms against Bean yellow dwarf virus (BeYDV) and BSCTV [54,55].

As viruses do not harbour ribosomes in their virions, the synthesis of viral proteins relies on host translation machinery. In plants, eIF4E and its isoforms are translation initiation factors that are involved in the multicomponent translation complex, which recruits ribosomes to the 5′ untranslated regions (UTRs) of mRNAs, and are also required for the translation of viral proteins [56]. Genetic screening for *Arabidopsis* mutants that have enhanced resistance to Turnip mosaic virus (TuMV), an RNA virus, found that a loss-of-function mutation in the *eIF(iso)4E* gene led to TuMV resistance [57]. Moreover, knockout of *Arabidopsis eIF(iso)4E* with CRISPR/Cas9 resulted in resistance to TuMV but did not affect plant vigour [58]. Therefore, plant *eIF4E* genes are probably ideal targets for generating broad-spectrum virus resistance via genome editing. Cucumber *eif4e* mutants harbouring mutations at two sites in the *eIF4E* gene generated by CRISPR/Cas9 were resistant to CVYV (Cucumber vein yellowing virus), ZYMV (Zucchini yellow mosaic virus) and PRSV-W (Papaya ring spot mosaic virus-W) [59]. In cassava, there are five genes encoding eIF4E proteins, of which only nCBP-1 (novel cap-binding protein-1) and nCBP-2 associate with VPgs (viral genome-linked proteins). Consistently, only the *ncbp-1*/*ncbp-2* double mutants generated through CRISPR/Cas9 showed delayed and attenuated symptoms after infection with CBSV (Cassava brown streak virus) [60].

The majority of plant viruses have RNA genomes. Thus, CRISPR/Cas systems that normally cleave double-stranded DNA are unable to directly target viral RNA genomes. However, the advent of new CRISPR/Cas systems, especially those that can target RNA, open up new possibilities for developing RNA virus-resistant plants. FnCas9 was found to target bacterial RNA and was then used to target and inhibit a human ssRNA virus directly with an engineered gRNA [61]. Following this strategy, FnCas9 and sgRNA that target CMV (cucumber mosaic virus) and TMV (tobacco mosaic virus) were expressed in tobacco and *Arabidopsis*, and the transgenic plants showed reduced virus accumulation and attenuated disease symptoms. Interestingly, RNA binding, not cleavage activity of FnCas9, was required for viral inhibition [62]. CRISPR/Cas13a (C2c2) appears to be an even more powerful editing tool that directly targets single-stranded RNA for modifications. CRISPR/Cas13a (C2c2) targeting the viral RNA genome led to resistance against TuMV in tobacco [63]. Although it has been reported that, upon target RNA binding, Cas13a's ribonuclease activity will be activated and will cleave collateral RNAs in a non-specific manner [11], expression of crRNAs targeting viruses did not cause cell death in tested plants [63].

7. Perspectives

Genome editing has great potential to overcome the limitations of conventional resistance breeding. First, a target gene in elite varieties can be directly modified by genome editing, thus bypassing the mating procedure. Second, if the target gene is determined, it is independent of plant populations with sufficient genetic variation; only sequence information of the target gene is required. Third, genome editing will not introduce changes beyond the target sites, thus avoiding the potential problems of linkage drag. Fourth, resistance breeding with genome editing does not require genetic crosses and segregant progeny selection, thus driving rapid advancements.

Currently, most disease-resistant crops against non-viral pathogens are generated via genome editing through targeted mutagenesis of genes that negatively regulate defence, the so-called S genes (table 1). By exploiting the functional conservation of S genes across plant species, genome editing can generate desired S gene mutants in most plants of interest for breeding without considering species barriers. It is expected that additional S genes will be discovered, which will provide more targets for genome editing. However, S genes are often involved in plant growth and development, and null mutations in S genes sometimes cause adverse effects on normal growth. This phenomenon may well hinder their applications. The central paradigm of plant pathology, the disease triangle, includes three factors: a virulent pathogen, a susceptible host and a favourable environment. Disease occurs only when the three factors are present at the same time. Thus, genome editing could be applied to constrain any of the three factors, thereby interrupting plant–pathogen interactions to control disease.

R genes, such as NB-LRR (nucleotide-binding, leucine-rich repeat) genes, from wild species are useful resources for transferring resistance to related elite varieties. Since the key differences between R genes from wild species and elite varieties are sometimes limited to single nucleotide variations [64], newly developed base editors (BEs) are especially preferable for generating specific base changes in elite varieties [65]. Stacking of multiple R genes is required for broad-spectrum and robust disease resistance. Moreover, recent work on synthetic immune receptors has highlighted a new direction for breeding crops resistant to phylogenetically divergent pathogens [66]. CRISPR/Cas9-mediated knock-in might facilitate this kind of genetic engineering [67]. Kim *et al.* [68] recently reported that modification of the proteolytic cleavage site with the host decoy protein PBS1 provided resistance to new pathogens. As *PBS1* is highly conserved in soybean, barley and other crop species, genome editing can be used to modify endogenous *PBS1* genes precisely to detect multiple pathogen proteases.

Resistance breeding via genome editing is not limited to gene disruption or replacement; it also includes gene regulation. For example, compared with the *indica* rice plants, *japonica* rice plants carrying *Xa3* showed an enlarged resistance

Table 1. Genome editing technologies developed for disease resistance in plants. Artificial zinc finger protein (AZP), in-del (Insertion and deletion), n.d. (not determined), Beet severe curly top virus (BSCTV), Tomato yellow leaf curl China virus (TYLCCNV), Tobacco curly shoot virus (TbCSV), Tomato yellow leaf curl virus (TYLCV), Tomato leaf curl Yunnan virus (TLCYnV), Beet curly top virus (BCTV), Merremia mosaic virus (MeMV), Bean yellow dwarf virus (BeYDV), Cotton leaf curl Kokhran virus (CLCuKoV), Tomato yellow leaf curl Sardinian virus (TYLCSV), Cucumber vein yellowing virus (CVYV), Zucchini yellow mosaic virus (ZYMV), Papaya ring spot mosaic virus W (PRSV-W), Turnip mosaic virus (TuMV), Cassava brown streak virus (CBSV), Cucumber mosaic virus (CMV), replication associated protein (Rep), intergenic region (IR), coat protein (CP), long intergenic region (LIR).

disease	target gene	nuclease	mutation	pathogen	host plant	ref.
				bacteria		
rice bacterial blight	*OsSWEET14*/promoter	TALEN	In-dels	*Xanthomonas oryzae* pv. *oryzae*	rice	[25]
rice bacterial blight	*OsSWEET13*/exon	CRISPR/Cas9	deletions	*Xanthomonas oryzae* pv. *oryzae*	rice	[28]
bacterial speck, phytophthora blight, bacterial spot	*SlDMR6-1*/exon	CRISPR/Cas9	deletions	*Pseudomonas syringae* pv. *tomato*, *Phytophthora capsici*, *Xanthomonas* spp.	tomato	[34]
citrus canker	*CsLOB1*/exon	CRISPR/Cas9	In-dels	*Xanthomonas citri* subsp. *citri*	citrus	[30]
citrus canker	*CsLOB1*/promoter	CRISPR/Cas9	deletions	*Xanthomonas citri* subsp. *citri*	citrus	[31]
				funguses		
powdery mildew	*TaMLO*/exon	TALEN	In-dels	*Blumeria graminis* f. sp. *tritici*	wheat	[38]
powdery mildew	*TaEDR1*/exon	CRISPR/Cas9	In-dels	*Blumeria graminis* f. sp. *tritici*	wheat	[41]
powdery mildew	*SlMlo1*/exon	CRISPR/Cas9	deletions	*Oidium neolycopersici*	tomato	[39]
rice blast	*OsERF922*/exon	CRISPR/Cas9	In-dels	*Magnaporthe oryzae*	rice	[45]
				viruses		
DNA viral disease	replication origin	AZP	n.d.	BSCTV	*Arabidopsis*	[49]
DNA viral disease	Rep	ZFN	n.d.	TYLCCNV and TbCSV	tobacco	[50]
DNA viral disease	Rep	TALE	n.d.	TbCSV, TYCCNV and TLCYnV	tobacco	[51]
DNA viral disease	IR, CP, *RCRII*	CRISPR/Cas9	In-dels	TYLCV, BCTV, MeMV	tobacco	[52]
DNA viral disease	LIR, Rep	CRISPR/Cas9	In-dels	BeYDV	tobacco	[54]
DNA viral disease	IR, CP, Rep	CRISPR/Cas9	In-dels	BSCTV	tobacco and *Arabidopsis*	[55]
DNA viral disease	IR, CP, Rep	CRISPR/Cas9	In-dels	CLCuKoV, TYLCV, TYLCSV, MeMV, BCTV-Logan, BCTV-Worland	tobacco	[53]
RNA viral disease	*eIF4E*/exon	CRISPR/Cas9	deletions	CVYV, ZYMV, PRSV-W	cucumber	[59]
RNA viral disease	*eIF(iso)4E*/exon	CRISPR/Cas9	In-dels	TuMV	*Arabidopsis*	[58]
RNA viral disease	*nCBP-1&nCBP-2/exon*	CRISPR/Cas9	In-dels	CBSV	cassava	[60]
RNA viral disease	ORF1a, ORFCP and 3′-UTR	CRISPR/Cas9	no cleavage	CMV	tobacco and *Arabidopsis*	[62]
RNA viral disease	GFP, Hc-Pro and CP	CRISPR/Cas13a	n.d.	TuMV	tobacco	[63]

spectrum and increased resistance to *Xoo*. This enhanced resistance results from the increased expression of *Xa3* in *japonica* rice [69]. The maize *Hm2* gene also provides dosage-dependent resistance to leaf spot and ear mould disease, and the nature of this dosage dependence is based on the transcription level of *Hm2* [70]. By contrast, cotton *GhLMMD* gene dosage regulates programmed cell death and immunity, and downregulation of *GhLMMD* resulted in resistance to *Verticillium dahlia* infection [71]. Since the recently developed CRISPR activation (CRISPRa) or CRISPR interference (CRISPRi) approaches can specifically regulate target genes and enable multiplex gene regulation, they can be used to modulate the above and other similar genes for breeding crops that have broad-spectrum resistance in the future. Moreover, if the target genes have upstream open reading frames, their mRNA translation can be fine-tuned by genome editing [72].

Recently, a probiotic *Escherichia coli* was genetically engineered to lyse itself upon perception of *N*-acyl homoserine lactone secreted by the human pathogen *Pseudomonas aeruginosa*, thus releasing a toxin and an antibiofilm enzyme to prevent *P. aeruginosa* gut infection [73]. An endophytic *Pseudomonas fluorescens* expressing the *chiA* gene, a major chitinase from *Serratia marcescens*, effectively protected bean seedlings from infection with *Rhizoctonia solani* [74]. This can be achieved by CRISPR technology to modify other enzyme-encoded genes of plant-associated bacteria for customized activities. Therefore, genome engineering of bacteria shows promise for combating plant pathogens.

Altogether, genome editing has become a powerful tool for both molecular plant–microbe interactions and disease resistance breeding. We will certainly witness more applications of genome editing in generating plants that are resistant to various pathogens and accelerating breeding for robust and broad-spectrum resistance. Undoubtedly, environmentally sustainable agriculture will benefit from these developments in genome editing.

Data accessibility. This article has no additional data.

Authors' contributions. K.Y. and J.-L.Q. conceived and wrote the article. All authors gave final approval for publication.

Competing interests. We declare we have no competing interests.

Funding. This work was supported by grants from the National Key Research and Development Program of China (2016YFD0100602) to J.-L.Q., the National Transgenic Science and Technology Program (2016ZX08010-002) to J.-L.Q., the Chinese Academy of Sciences (XDB11030500), the National Natural Science Foundation of China (31672015) to J.-L.Q. and the National Natural Science Foundation of China (31700314) to K.Y.

Acknowledgements. We thank John Mundy for the critical reading of the manuscript. We apologize to the authors for the omission of their work that could not be included in this paper due to space constraints.

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