Available online at www.sciencedirect.com

ScienceDirect

Rice Science, 2021, 28(3): −

Review

Genome Editing Strategies Towards Enhancement of Rice Disease Resistance

Rukmini MISHRA[1], WEI Zheng[2], Raj Kumar JOSHI[3], ZHAO Kaijun[2]

([1]*School of Applied Sciences, Centurion University of Technology and Management, Odisha, India;* [2]*National Key Facility for Crop Gene Resources and Genetic Improvement (NFCRI), Institute of Crop Science, Chinese Academy of Agriculture Sciences, Beijing 100081, China;* [3]*Department of Biotechnology, Rama Devi Women's University, Bhubaneswar-751022, Odisha, India*)

Abstract: Rice (*Oryza sativa* L.) serves as the major source of staple food for two-third of the world population. In the past few decades, agricultural productivity has been hugely affected by several biotic stresses. The emerging pests and phytopathogens have reduced the crop yield and quality that has threatened the global food security. Traditional breeding methods, molecular marker-based breeding approaches and use of genetically modified (GM) crops have played a crucial role in strengthening the food security worldwide. However, their usages in crop improvement have been highly limited due to multiple caveats. The advent of genome editing tools like transcriptional activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPR)-associated endonuclease Cas9 (CRISPR/Cas9) have effectively overcome limitations of the conventional breeding methods and are being widely accepted for improvement of crops. Among the genome editing tools, the CRISPR/Cas9 system has emerged as the most powerful tool of genome editing because of its efficiency, amicability, flexibility, low cost and adaptability. Accumulated evidences indicate that genome editing has great potential in improving the disease resistance in model as well as crop plants. In present review, we offer a brief introduction to the mechanisms of different genome editing systems and then discuss recent developments in CRISPR/Cas9 system-based genome editing towards enhancement of rice disease resistance by different strategies. This review also discusses the possible applications of recently developed genome editing approaches like CRISPR/Cas12 (formerly known as Cpf1) and base editors for enhancement of rice disease resistance.

Key words: Genome editing, Rice improvement, CRISPR/Cas9, CRISPR/Cas12a, Base editors, disease resistance

Rice (*Oryza sativa* L.) serves as the significant source of carbohydrate for more than two-third of the world's population. The burgeoning population coupled with climatic change and emergence of new phytopathogens have a drastic effect on global rice productivity. During the last few decades, the traditional breeding approaches including mutation breeding and molecular breeding have significantly contributed in the development of effective disease resistance strategies in rice as required for strengthening global food security. However, these methods are time consuming and labor-intensive. Gene transfer techniques by way of *Agrobacterium*-based transformation and other approaches have significantly improved the yield, quality and disease resistance in plants. However, the biosafety regulations, social and ethical issues related to transgenic crops have always been a major hindrance to public acceptance of these genetically modified (GM) crops (Lusser et al, 2012). In other words, better strategies for higher yielding and stress-resistant rice

Received: 10 April 2020; **Accepted:** 4 August 2020

Corresponding author: ZHAO Kaijun (zhaokaijun@caas.cn)

Peer review under responsibility of China National Rice Research Institute

http://dx.doi.org/

varieties are the need of the hour for increasing rice productivity and ensuring global food security.

Plant diseases are the major constraints that have threaten the agricultural development and global food security. Crops are susceptible to a wide range of pathogens including bacteria, viruses and fungi, which cause significant economic losses (FAO, 2017). The pathogens affect the growth and development of crops which result in huge yield loss thereby creating hurdles for sustainable agriculture. Multiple disease management strategies and risk assessment tools including the usage of disease forecasting protocols, combinatorial usage of chemicals, fungicides and biological control agents, crop rotations and host resistance breeding have been under practice for a very long time (Haq and Ijaz, 2020). However, most of these strategies are inadequate to achieve successes in many pathosystem due to diversity in host range of the phytopathogens, environmental toxicity, discrepancy in the assessment of disease resistance reaction and inefficient disease forecasting systems. Therefore, understanding the molecular mechanism governing the interaction between host and the pathogen is central to the development of an effective disease resistance strategy.

Plants utilize multiple resistance responses towards preventing the colonization by pathogenic microorganisms. On one hand, plant resistance to phytopathogens is typically regulated by the resistance (*R*) genes encoding nucleotide-binding leucine-rich repeat (NB-LRRs) proteins which neutralizes the molecular activity of pathogen effector proteins in the plant cell (Cui et al, 2015). On the other hand, targeted mutation or knockout of susceptibility (*S*) gene(s) that acts as host-pathogen compatibility factor also induces recessive immunity against the adapted phytopathogens (van Schie and Takken, 2014). As majority of plant diseases arise due to compatible interaction between host and the pathogen, altering the *S*-gene(s) that promotes compatibility could be highly significant in the development of broad spectrum and durable molecular breeding strategies for disease resistance (van Schie and Takken, 2014).

In the recent times, the emergence of multiple new breeding techniques including speed breeding platforms, high throughput genotyping and precise genome editing coupled with genetic engineering has successfully generated multiple disease-free high yielding crop varieties (Li et al, 2020). Among them, the genome editing approaches have emerged as the revolutionary tools for crop improvement (Voytas and Gao, 2014). The genome editing tools are represented by sequence specific nucleases (SSNs) that introduce a double stranded DNA break at a specific genomic region thereby inducing the host DNA repair pathways either by homologous recombination (HR) or through non-homologous end joining (NHEJ) (Sander and Joung 2014). While, NHEJ is an error prone method that creates random mutations leading to frame shift and target gene knockout, HR pathway is much more precise resulting in gene replacement or gene knock-in when donor DNA templates are available (Baltes et al, 2015). Nevertheless, these natural processes of DNA repair result in mutation leading to alteration of specific trait. Multiple genome editing tools including Zinc finger nucleases (ZFNs), transcriptional activator-like effector nucleases (TALENs) and more recently developed clustered regularly interspaced short palindromic repeats (CRISPR)-associated endonuclease Cas9 (CRISPR/Cas9) have facilitated the introduction of agronomically important traits in many plant species (Zaidi et al, 2018). Among these platforms, CRISPR/Cas9 system has greater acceptance by the scientific community for its simplicity, high specificity for target cleavage, involvement of no complex protein chemistry and universal applicability (Zaidi et al, 2018). What more, CRISPR/Cas mediated disease resistance has already been reported against multiple phytopathogens including bacteria (Peng et al, 2017), fungi (Wang et al, 2016) and viruses (Zaidi et al, 2016; Chandrasekaran et al, 2016) in different crops including in rice (Sun et al, 2017; Li et al, 2018; Tomlinson et al, 2019) either by target specific modification of the host genes or by precise alteration of the pathogen genome. In the present review, we summarize the different strategies associated with genome editing and their subsequent applications in the improvement of crops with focus on highlighting the advancements of the CRISPR/Cas9 system and its role in conferring disease resistance in rice.

Overview of the genome editing technologies

Genome editing is a novel approach wherein SSNs are used to make precise modifications in the genomic DNA. Creation of a double stranded break (DSB) activates the cell's DNA repair mechanism, either through NHEJ or HDR method. NHEJ incorporates

insertions and deletions (InDel) mutations whereas HR method results in gene insertion or replacement which is much more precise. Mega nucleases such as the I-*Sce*I endonuclease enzyme from yeast constituted the earliest known genome editing system (Paques and Duchateau 2007). However, these are the least efficient among the editing toolbox due to unclear communication between mega nuclease protein residues and corresponding specific target DNA sequence (Hsu et al, 2014). ZFNs are the SSNs that bind DNA through an engineered array of zinc finger motifs (Carroll 2011). A specific zinc-finger entails about 30 amino acids in a conserved ββα configuration. The non-specific cleavage domain FokI is dimeric in nature and as such a pair of ZFNs are designed to bring the FokI monomers close to the specific DNA target for creation of a DSB (Bogdanove and Voytas, 2011) (Fig. 1-A). ZFNs have been successfully used as a genome editing tool in a wide range of crops and model plants including Arabidopsis, tobacco and maize (Cai et al, 2009; Shukla et al, 2009; Osakabe et al, 2010). Yet, there usage as an editing tool is highly limited due to several constraints including off target binding of the zinc-finger motifs and multifaceted interactions between amino-acid residues and the target sequence (Carrol 2011; Voytas 2013).

Unlike ZFNs, TALENs are designed by combining a FokI nuclease domain with a transcription activator-like effector (TALE) DNA binding domain (Fig. 1-A). TALEs are secretory proteins from *Xanthomonas* bacteria that are characterized by the presence of a C-terminal acidic activation domain (AD) and nuclear localization signal (NLS) sequence, central DNA binding domain (DBD) and N-terminal translocation signal sequence (Bogdanove et al, 2010). TALEs are primarily responsible for the transcriptional activation of the disease susceptibility genes in host plants. TALENs has been well established in many plant species, including rice, wheat, tobacco and barley (Wang et al, 2014; Li et al, 2016; Blanvillain-Baufume et al, 2017). Although TALENs are qualitatively more effective over ZFNs in terms of high target specificity and low off target activity, the TALE DBDs are represented by extensive repeat structure which acts as

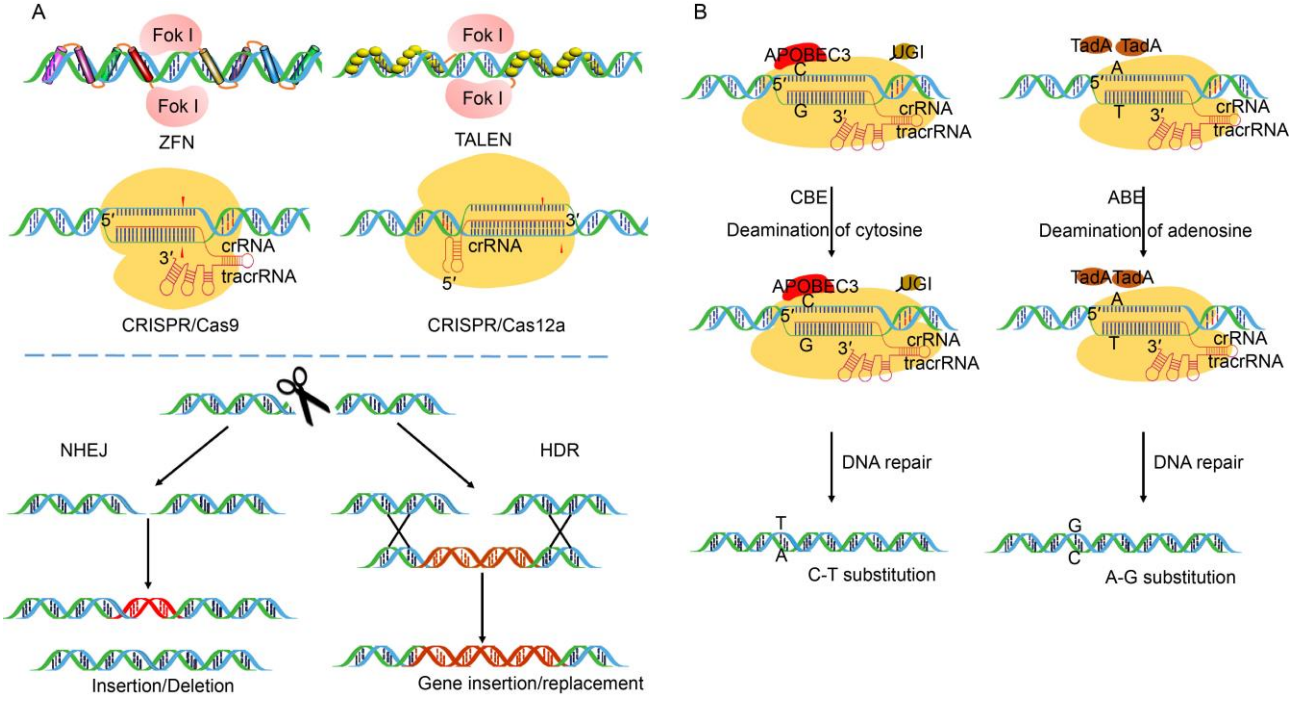

Fig. 1. Sequence specific nucleases used for genome editing.

A, The sequence specific nucleases (ZFNs, TALENs and CRISPR/Cas9, CRISPR/Cas12a) create double-stranded breaks (DSBs) at the target site which is subsequently repaired either by non-homologous end joining (NHEJ) or homologous recombination (HR) by cellular system leading to gene disruption by indels, gene addition or replacement respectively. The cleavage of the targeted DNA is facilitated by FokI endonuclease in ZFNs and TALENs and Cas endonucleases in CRISPR/Cas9 and CRISPR/Cas12a. CRISPR/Cas9 makes use of a 100 nt sgRNA comprising of crisprRNA (crRNA) and trans-acting crispr RNA (tracrRNA) while CRISPR/Cas12a requires only a 40–45 nt long crRNA to facilitate gene editing.

B, Base editing platforms used for target specific single base modification. CBE uses Cas9 nickase (nCas9D10A) together with a cytidine deaminase rAPOBEC1 and an uracil DNA glycosylase inhibitor (UGI) to facilitate cytosine to thymine conversions. ABE consists of Cas9 nickase (nCas9D10A) fused with *E. coli* derived ecTadA (WT)-ecTadA* heterodimer to facilitate adenosine to guanine conversions.

a limitation for their comprehensive use in editing of multiple genomes. In contrast, the type II CRISPR/Cas9 system is the utmost innovative genome editing method that has superseded ZFNs and TALENs due to its efficiency and robustness. CRISPR/Cas9 make use of a DNA endonuclease Cas9 along with a small RNA molecule called single guide RNA (sgRNA) that regulates the Cas9 mediated site specific double stranded break (DSB) at specific targets (Fig. 1-A). The attachment of the Cas9-sgRNA structure to the target DNA and the ensuing cleavage depends on the presence of a protospacer adjacent motif (PAM) sequence (5′-NGG-3′) at the 3′-end of the target site. Hence, the requisite of only different spacer sequences makes CRISPR/Cas9 a very simple and highly effective editing tool that has been greatly exploited in recent years for improvement of model plants and major crops (Ma et al, 2015; Xu et al, 2016; Zhang et al, 2020).

While, trait improvement has been highly fruitful using CRISPR/Cas9, the prerequisite of an NGG PAM sequence has restricted its usage to potential target sites. Lately, several Cas9 variants and homologous proteins, such as Cas9-VRER, Cas9-VQR, Cas9-EQR, Cpf1-RVR, Cpf1-RR and SaCas9, have improved the feasibility of engineering a wide range of Cas9s with improved PAM specificities for genome editing in eukaryotic cells (Klienstiver et al, 2015; Gao et al, 2017). Hu et al (2018) used a phage-assisted continuous evolution process to develop a SpCas9 variant called xCas9 which have broader range of PAM compatibility including NG, GAA and GAT and at the same time greater DNA specificity and lower genome-wide off target activity compared to SpCas9. More recently, novel cytosine and adenine base editors with engineered *Staphylococcus aureus* Cas9 (SaCas9-NG), SpCas9-NG variants have substantially expanded the targetable sites in the rice genome (Hua et al, 2019). Additonally, SpCas9-NG variants have greatly expanded the scope of genome editing in potato and tomato by targeting the non-canonical NGA and NGT PAMs (Veillet et al, 2020). Taken together, an efficient usage of these Cas9 variants would be critical to accelerate rice improvement through enhanced resistance to multiple phytopathogens.

The advent of another class II CRISPR-associated endonuclease, CRISPR/Cas12a or Cpf1 has broadened the horizon for genome editing with greater accuracy and competence (Endo et al, 2016) (Fig. 1-A). Compared to CRISPR/Cas9 system, the CRISPR/Cas12a recognizes T-rich (5′-TTTN-3′ and 5′-TTN-3′) PAMs at the 5′-end of the target site resulting in high cleavage efficiency (Zetsche et al, 2015). Unlike CRISPR/Cas9 which require a 100 nt sgRNA, the CRISPR/Cas12a complex doesn't require a tracrRNA and therefore can facilitate gene editing with only a crRNA of 40–45 nt consisting the repeat and the spacer. Also, the RuvC and nuclease domains of Cas12a cleaves the target and the secondary strand of the DNA at 23 bp and 17 bp downstream of the PAM sequence respectively resulting in staggered ends with 5 bp overhangs (Zetsche et al, 2015). By itself, the development of bigger mutations using CRISPR/Cas12a upsurges the efficiency of HDR mediated donor gene insertion at the specific genomic location. Also, Cas12a simultaneously acts as an RNAse to convert pre-crRNA to crRNA and a nuclease to cleave the dsDNA demonstrating dual enzymatic activity (Zetsche et al, 2015). Therefore, CRISPR/Cas12a has the potential to generate multiple crRNA driven by a single promoter making it simpler than CRISPR/Cas9 system. Moreover, the off-target cleavage by Cas12a is relatively lower than Cas9. These features make CRISPR/Cas12a more advanced over Cas9, making it a potentially important genome editing tool of the future (Zaidi et al, 2017). The Cas12a from *Francisella novicida* (FnCas12a) and its ortholog from a *Lachnospiraceae bacterium* (LbCas12a) and an *Acedomonococcus* sp. BV3L6 (AsCas12a) have been used to introduce targeted mutations in many crops (Endo et al, 2016). Emerging reports have already demonstrated the successful adaptation of CRISPR/Cas12a system in rice improvement (Yin et al, 2017; Li et al, 2018). Yin et al (2017) utilized the CRISPR-LbCpf1 to target the early developmental gene *EPFL9* (*Epidermal Patterning Factor like-9*), a positive regulator of stomatal development in rice. The homozygous mutant plants showed eight-fold reduction in the stomatal density on the abaxial leaf surface. Likewise, Li et al (2018) reported that the donor repair template (DRT) with only the left homologous arm was good enough for precise targeted allelic replacement in the wild type *OsALS* (*Acetolactate synthase*) gene resulting in herbicide resistant rice plants. Most recently, a comparative assessment of three Cas9 (Cas9 D10A nickase, HiFi Cas9 nuclease, WT Cas9 nuclease) and two Cas12a nucleases (LbCas12a and AsCas12a) was carried out to determine their mutation efficiency on a

single target site of the rice *phytoene desaturase (PDS)* gene (Banakar et al, 2020). The study showed that LbCas12a resulted in deletion between 2 bp to 20 bp without the loss of PAM site leading to higher editing efficiency over the Cas9 variants. This suggest the potential of Cas12a to generate specific and heritable targeted mutations in rice and thus can be used as a precise genome editing tool for future rice improvement programmes including in the development of disease resistant rice varieties.

Although the HDR based gene replacement is a feasible approach with CRISPR/Cas9 and CRISPR/Cas12a, the efficiency of template DNA delivery and target insertion is significantly low. To overcome this problem, the base editor technology has emerged as a new and advanced approach for precise nucleotide substitutions in a programmable manner without the requirement of a DSB or donor template (Komor et al, 2016) (Fig. 1-B). The base editors comprise of a catalytically inactive CRISPR-Cas9 domain (dCas9 or Cas9 nickase) and a cytosine or adenosine deaminase domain which converts one base to another. These are capable of making single-base variations or substitutions without creating a double-stranded break in the target DNA, thereby limiting the frequency of InDels. Recently, base editing system has been efficiently used in creating targeted point mutations at multiple endogenous loci in rice and wheat (Li et al, 2018). Most recently, rBE5 (*hAID*Δ-XTEN-Cas9n-UGI-NLS)* base editor was used to target *Pi-d2*, an agriculturally important rice gene that harbour a point mutation modulating the defense response to rice blast fungus (Ren et al, 2018). In the last three years, the cytosine and adenine base editors have been efficiently used for precise base modification (C to T or A to G) in eukaryotic genomes (Zong et al, 2017; Hua et al, 2018; Qin et al, 2019). Base editing tool boxes has been efficiently optimized and demonstrated in several crops including rice, wheat, maize and tomato (Zong et al, 2017; Lu and Zhu 2017; Li et al, 2018; Tang et al, 2019). In course of time, a wide range of ABE and CBE variants have been developed for efficient target specific base modification (reviewed in Mishra et al, 2020). Furthermore, precise base editing in RNA has been realized using a catalytically-inactive Cas13 (dCas13) in association with adenosine deaminase acting on RNA (ADAR) to direct adenosine to inosine conversion (Cox et al, 2017). Cas13 is a type IV CRISPR-linked RNA guided RNAse while ADARs mediates precise editing of the transcripts (Nishikura, 2010). Together, they have been used to develop an RNA editing system named as RNA Editing for Programmable A to I Replacement (REPAIR) that have significant applicability for research in therapeutics and biotechnology (Stafforst and Schneider, 2012). However, it is yet to be utilized in plant system.

Traditional genome editing including CRISPR/Cas9 system involves the delivery of DNA cassettes encoding editing machineries into the host genome. Often, the random integration of the editing cassette results in undesirable genetic changes and off-target effects (Zhang et al, 2018). Further, the introduction of editing cassette into the host genome invokes ethical and regulatory concerns (Jones, 2015). Therefore, scientists are increasing getting aligned to the usage of DNA-free genome editing technologies to minimize the probability of off-target mutations. DNA-free genome editing makes use of the CRISPR/Cas ribonucleoprotein (RNPs) complexes that is delivered into the cell by protoplast transformation or particle bombardment leading to target DNA modification. CRISPR-RNP is an assembly of CRISPR specific guide RNA and Cas9 protein together forming an active enzyme complex (Zhang et al, 2018). The first DNA-free genome edited plants were obtained by transfecting the CRISPR/Cas RNPs into the protoplast of Arabidopsis, tobacco, lettuce and rice (Woo et al, 2015). Likewise, particle bombardment mediated DNA free editing using the CRISPR-Cas9 RNPs have been successfully demonstrated in maize (Svitashev et al, 2016), wheat (Liang et al, 2017) and rice (Toda et al, 2019). In Rice, Cas9-gRNA RNPs has been directly delivered into the zygotic tissues with a mutation efficiency of 14% to 64% (Toda et al, 2019). In maize, CRISPR/Cas9 RNPs have been used to generate both knockout as well as knockin mutants (Svitashev et al, 2016).

Moreover, the CRISPR/Cas-RNPs have relatively high editing efficiency and low off-target mutations compared to CRISPR/Cas system (Liang et al, 2017; Toda et al, 2019). In another study, the base editing has been combined with the DNA-free editing system to facilitate higher frequency of C to T conversion (1.8%) in wheat (Zong et al, 2018). Overall, this transgene free precise editing system has enormous potential for the improvement of rice as well as other important crop species. Although the usage of CRISPR/Cas RNPs is still at its infancy, it can be

effectively explored to accelerate rice crop breeding and the edited products can get wider acceptance in public overcoming the biosafety regulatory hurdles. The readers may refer to several recent reviews for a detail account of the different genome editing platforms and their mechanism of actions towards crop improvement (Mishra et al, 2018; Zhang et al, 2018; Yin and Qiu, 2019; Mishra et al, 2020; Zhang et al, 2020).

Genome editing strategies for rice disease resistance

Various diseases caused by phytopathogenic microorganisms including bacteria, viruses and fungi are the biggest challenge to sustainable rice production. During the latter half of the nineteenth and early twentieth century, host resistance breeding has played a pivotal role in the improvement of yield and other agronomic traits of rice thereby addressing the challenges of feeding the world's growing population. However, traditional methods of resistance breeding are expensive, time consuming and sometimes the resistance allele influences plant growth and development (Miah et al, 2013). Extensive genetic and genomic studies have revealed significant molecular details about rice innate immunity, including a large number of targets for control and inhibition of pathogenic infection. While plants have evolved resistance (*R*) genes that can neutralize pathogen derived virulence proteins or effectors and activate effector triggered immunity (ETI), they also have susceptibility (*S*) genes that are essentially involved in successful pathogenic infection. In context of genome editing, the primary strategy for disease resistance involves knocking out of these *S* genes through the error prone NHEJ pathway-based repair of target DNA (Fig. 2-A). Alternatively, knocking in *R* gene allele to the target site of interest via the HDR mechanism has the potential for resistance development in widely accepted susceptible genotypes (Fig. 2-B and -C). Besides, alleles of certain *R* and *S* genes vary at the single nucleotide level. Thus, precise editing by way of targeted replacement or base editing could generate

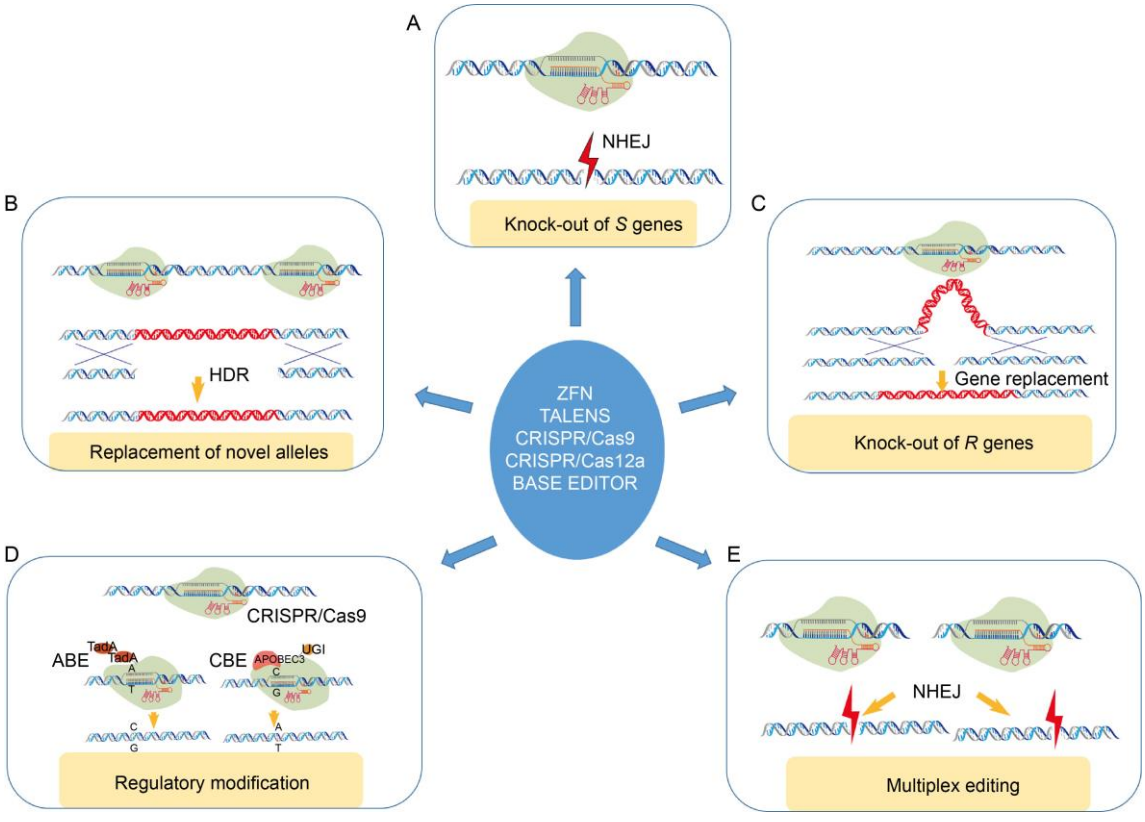

Fig. 2. Genome editing strategies towards disease resistance in plants.
Multiple genome editing platforms could facilitate disease resistance through knockout of susceptibility (*S*) genes (**A**), homology directed replacement of novel alleles **(B)**, knock in of resistance (*R*) genes (**C**), regulatory modification of *R/S* gene expression (**D**) and multiplex editing of resistance and susceptibility factors (**E**).

Table 1. List of genes targeted by genome editing tools for rice disease resistance.

Pathogenic perspective	Target gene	Genome editing tool	Molecular function	References
Resistance to bacterial infection	*OsSWEET13*	TALENs	Enhance resistance to BLB	Li et al, 2012
	OsSWEET13	TALENs	Enhance resistance to BLB	Zhou et al, 2015
	OsSWEET13	TALENs	Enhance resistance to BLB	Blanvillain-Baufume et al, 2017
	Os09g29100	TALENs	Enhance resistance to BLB	Cai et al, 2017
	Os8N3 (*OsSWEET11*)	CRISPR/Cas9	Enhance resistance to BLB	Kim et al, 2019
	OsSWEET11 and *OsSWEET14*	CRISPR/Cas9	Broad spectrum resistance to BLB	Xu et al, 2019
	OsSWEET11, *OsSWEET13* and *OsSWEET14*	CRISPR/Cas9	Broad spectrum resistance to BLB	Olivia et al, 2019
Resistance to fungal infection	*OsERF922*	CRISPR/Cas9	Enhance resistance to blast disease	Wang et al, 2016
	OsSEC3A	CRISPR/Cas9	Enhance resistance to blast disease	Ma et al, 2018
	OsPFT1	CRISPR/Cas9	Resistance to rice sheath blight	Shah et al, 2019
Resistance to viral infection	*eIF4G*	CRISPR/Cas9	Enhanced resistance to tungro disease	Macovei et al, 2018

allelic variants for disease resistance (Fig. 2-B and -D). Then again, editing platforms could be used to induce disease resistance by altering the *cis*-regulatory regions of target genes and quantitative trait loci (QTLs). More than hundred regulatory mutations have been realized in the tomato SlCLV3 promoter using a CRISPR/Cas9 system (Rodriguez-Leal et al, 2017). Such modifications could result in systematic assessment of *cis*-regulatory regions with resistant traits which could enhance rice breeding (Fig. 2-D). Simultaneous editing of several *S* genes or regulatory elements via a multiplex editing platform (Fig. 2-E) could essentially result in broad spectrum disease resistance. Multiple sgRNAs driven by independent promoters could be multiplexed into single Cas9 or Cpf1/sgRNA expression vector using Golden Gate cloning or the Gibson assembly method (Silva and Patron, 2017). A proof of concept study has already demonstrated the feasibility of multiplex gene editing using CRISPR/Cpf1 system (Wang et al, 2017). The advent of multiple editing platforms, particularly the CRISPR/Cas9 technology have facilitated one or more of these strategies towards resistance development against biotic stresses in crops and particularly in rice (Mishra et al, 2018; Yin and Qiu, 2019). The application of CRISPR/Cas tools has mainly been explored in rice against viral infection, followed by efforts to improve fungal and bacterial disease resistance (Table 1). Recent studies demonstrating the power of the genome editing technology in establishing resistance to these pathogen categories are discussed below.

Resistance to bacterial pathogen

Bacterial leaf blight (BLB), caused by γ-proteobacterium *Xanthomonas oryzae* pv. *oryzae* (*Xoo)* is one of the most destructive vascular disease of rice especially in the major rice growing regions of Southeast Asia and sub-Saharan Africa. It is singly responsible for more than 75% of yield loss with millions of hectares of rice affected annually (Varshney et al, 2019). Identification of genetically resistant rice plants and their usage in genomics assisted breeding has been the most effective method for developing *Xoo* resistant varieties. However, the emergence of new and novel *Xoo* types has been the greatest challenge in containing the disease. The *Xoo* pathogenicity in rice is primarily established through the injection of DNA-binding proteins called transcription activator-like effectors (TALEs) that bind to the effector-binding elements (EBEs) in the promoters of the *S* genes in rice (Cohn et al, 2014). TALEs usually target the sugar transporting *SWEET* gene family in rice which are primarily responsible for releasing the sugar into the apoplast as nutrition of the *Xoo* pathogens (Cohn et al, 2014). Multiple TALEs including AvrXa7, TalC, Tal5 and PthXo3 found in different *Xoo* strains all target the *Os11N3* (also known as *OsSWEET14*) gene in rice and therefore considered as the major susceptibility factor associated with bacterial blight infection (Antony et al, 2010; Hutin et al, 2015). Bing Yang and his colleagues from Iowa State University were the first to use the TALEN technology to edit the *OsSWEET14* gene to confer resistance against bacterial blight (Li et al, 2012). TALENs-mediated editing resulted in the development of desired mutations within the *Os11N3* promoter region containing an EBE for AvrXa7, overlapping with another EBE for PthXo3. The edited rice plants with desired homozygous mutation for the 4 bp or 9 bp deletion at the target site were found to be highly

resistant to bacterial blight (Li et al, 2012). The same group also reported the identification of another sucrose transporter gene *OsSWEET13* as the disease susceptibility factor for PthXo2 (TALE) dependent *Xoo* strain (Zhou et al, 2015). The study also identified that the PthXo2 dependent strain induce *OsSWEET13* expression specifically in Indica rice IR24 due to the presence of a mysterious effector binding site not present in the alleles in japonica rice Nipponbare and Kitaake. In another study, a naturally occurring 18 bp deletion in *Oryza barthii* and *O. glaberrima* wild rice species was predicted to prevent the binding of TALEs known to target *OsSWEET14* and confer resistance to BLB (Hutin et al, 2015). The allele named as *xa41(t)*, was found to be resistant against half of the tested *Xoo* strains. More recently, Blanvillain-Baufume et al (2017) used the TALEN constructs to develop an allele library of the *OsSWEET14* promoter region in rice to assess the susceptibility level of edited rice lines carrying mutations in the EBEs of AvrXa7, Tal5 and TalC. While, the rice lines with disruption of the AvrXa7 and Tal5 EBEs resulted in resistance to *Xoo* strains that are dependent on the corresponding TALEs.

CRISPR/Cas9 induced disruption of the TALE EBEs of two susceptibility genes, *OsSWEET11* and *OsSWEET14*, in rice cv. Kitaake was attempted to facilitate broad spectrum BLB resistance in rice (Xu et al, 2019). One of the rice mutant MS14K demonstrated broad spectrum resistance to majority of the *Xoo* strains. Further, two PthXo2-like TALEs were identified as major virulence factors in the compatible *Xoo* strains. As the PthXo2 TALEs primarily target the susceptibility gene *OsSWEET13/Xa25*, an analysis of EBE variants in the *OsSWEET13* promoter across 3000 rice varieties revealed the presence of at least 10 *Xa25*-like haplotypes. CRISPR/Cas9 strategy was further used to introduce InDels in the EBE of the *OsSWEET13* promoter of the MS14K line and a novel rice line with three edited *OsSWEET* EBEs resulted in broad spectrum resistance against all the tested *Xoo* strains (Xu et al, 2019). This suggest that genome engineering of TALEs-susceptibility factor co-evolved loci is crucial for preventing effector triggered susceptibility thereby realizing broad spectrum BLB resistance.

Recently, Kim et al (2019) employed the CRISPR/Cas9 system to knockout rice *Os8N3* (also known as *OsSWEET11*) that acts a susceptibility factor for the *Xoo* strains carrying the TAL effector PthXo1. Desired modifications in *Os8N3* were stably transferred into T_0, T_1, T_2 and T_3 generations without the transferred DNA (T-DNA) through genetic segregation. Not only had the homozygous mutants displayed significantly enhanced resistance to *Xoo* strain, the editing of *Os8N3* also did not affect the agronomic traits and pollen viability in the mutant lines. In another study, Olivia et al (2019) sequenced 856 TALEs across multiple *Xoo* isolates from Asia and Africa distinctively targeting promoters of multiple *OsSWEET* genes (*SWEET11*, *SWEET13* and *SWEET14*) and found the complexity involved in the development of TALE insensitive rice lines. To overcome this difficulty, a CRISPR/Cas9-mediated genome editing system was used to introduce mutations at the promoters of all the three *SWEET* genes at EBEs recognized by various *Xoo* TALEs. Sequence analyses revealed multiple TALE variants for *SWEET13* and *SWEET14* alleles. Introduction of as many as five SWEET promoter mutations into the rice lines Kitaake, IR64 and Ciherang-Sub1 resulted in vigorous and broad spectrum BLB resistance. Most recently, Zhang et al () facilitated significant improvement in rice response to BLB pathogen through usage of a T5 exonuclease (T5exo)-Cas fusion tool box. Compared with CRISPR/Cas9, T5exo-Cas9 or -Cas12a resulted in larger deletion mutations at the targeted site of UPT_{PthXo1} (up-regulated by transcription activator-like effector PthXo1) box of the *OsXa13* gene promoter in edited rice plants. Phenotypic analysis revealed that the T5exo-Cas9 or -Cas12a generated edited rice plants had significant reduction in lesion length and enhanced resistance to BLB as compared to those generated by CRISPR/Cas9 or Cas12a, which might be associated with the factor that T5exo-Cas9 induced larger deletions at the cis-regulatory element (Zhang et al, 2020). All these studies clearly indicate that the genome editing of rice especially with the CRISPR/Cas system has significantly contributed in conferring broad spectrum BLB resistance and these strategies could be effectively used in non-transgenic genomic assisted crop improvement programmes.

Resistance to fungal pathogens

More than 30% of the plant diseases are caused by fungal phytopathogens and necessitate advanced strategies for their sustainable management (Giraud et al, 2010; Sharma et al, 2012). Rice blast caused by the hemibiotrophic filamentous fungus *Magnaporthe*

oryzae is the most widespread and devastating disease of rice. It is a recurrent difficulty for both upland and lowland rice which is highly vulnerable to this fungal phytopathogen from seedling to the adult stage. The frequent occurrence of new races of blast pathogen has resulted in more than 30% of losses in global rice production which is enough to feed 60 million people (Nalley et al, 2016). Utilization of blast resistant cultivars has been the most effective measure for the management of blast pathogen. As of now, more than 100 major blast *R* genes have been identified and 30 of them have been molecularly cloned (Xiao et al, 2019). Major blast *R* genes including *Pi-ta*/*Pi-ta2*, *Pi-z*, *Pi-b*, and *Pi-k*/*h*/*m*/*s* have been deployed in combination with genomic assisted breeding and transgenic programmes for the development of resistant genotypes across the rice producing regions (reviewed in Li et al, 2016). For instance, the recently identified rice *Pigm* locus consists of cluster of genes encoding nucleotide binding site leucine rich repeat (NBS-LRR) receptors that confers resistance to blast fungus *M. oryzae* without compromising the yield trait. The locus is characterized by a pair of epigenetically regulated antagonistic pair of NLR receptors-PigmR and PigmS. While PigmR facilitates broad spectrum resistance, PigmS prevent PigmR homodimerization to suppress resistance and in turn increases seed production (Deng et al, 2017). Likewise, a single base modification in the regulatory region of the bsr-d1 induces blast resistance in rice (Li et al, 2017). However, the current challenge lies in the development of a collection of blast resistance genes that could be used against the incessantly evolving and varied strains of *M. oryzae*. Therefore, precise genome editing tools are the need of the hour for implementation of effective plant resistance in rice.

Enhanced resistance to blast disease has been demonstrated in rice by targeting the ethylene response transcription factor (ERF) gene *OsERF922* via CRISPR/Cas9-targeted knockout (Wang et al, 2016). CRISPR/Cas9 induced Indel mutations were reported in the targeted gene with a mutation frequency up to 42% in the T_0 generation. The allelic mutations were stably transmitted to succeeding generations. Blast resistance screening in six homozygous mutated T_2 plant lines revealed significantly lower lesions as compared to wild-type plants at both the seedling and tillering stages. Further, no significant difference was observed in the agronomic performance of the mutants suggesting that precise editing do not alter other important traits of the plants. Furthermore, the utilization of multiple CRISPR/Cas9 constructs (Cas9/Multi-target-sgRNA) for targeting several sites within *OsERF922* locus resulted in higher number of mutants. These suggest that CRISPR/Cas9 mediated targeting of multiple sites has the potential to increase the mutation efficiency thereby enhancing blast resistance in rice. In another recent report, CRISPR/Cas9 SSN was used to disrupt *OsSEC3A*, a gene encoding exocyst subunit protein to explore its functional role in plant immunity (Ma et al, 2018). *OsSEC3A* has been previously implicated in root hair elongation, pollen germination and defense response in other plant species (Bloch et al, 2016). Two sgRNAs were designed to target the third and tenth exons of the *OsSEC3A* gene. The CRISPR/Cas9 induced mutant displayed improved immune responses coupled with up-regulated expression of pathogenesis-related proteins, salicylic acid synthesis genes, increased levels of salicylic acid, and improved resistance to the rice blast pathogen *M. oryzae*. However, the mutant lines also showed altered growth and agronomic traits including dwarf stature, smaller seedlings, shorter main roots and decreased or impaired plant height, panicle length, tiller number, 1000-grain weight and spikelet fertility as compared to the wild type. The results from this study suggest that multiple approaches to blast resistance in rice could be fructified through precise editing of multifunctional gene(s) associated with defense signaling in rice. Recently, CRISPR/Cas9 has also been adopted to induce mutations in the proline-rich motif of *Pi21* for rice resistance against *M. oryzae* (Li et al, 2019). In this study, a CRISPR/Cas9 vector was designed to facilitate simultaneous targeted mutation of the thermosensitive male sterile 5 (*TMS5*), rice blast *S* gene *Pi21* and BLB *S* gene *Xa13* in the rice line Pinzhan. Three of the generated mutants (LA-230, LA-237 and LA-238) with homozygous frameshift mutations at all three genes (*tms5/pi21/xa13*) displayed characteristics of thermosensitive genic male sterility (TGMS) with enhanced resistance to infections of *M. grisea* and *Xoo* strain PXO99 (Li et al, 2019). Thus, a CRISPR/Cas9 based multiplex gene editing system effectively converted a breeding intermediate Pinzhan into a TGMS line PinzhanS coupled with enhanced resistance to rice blast and bacterial blight thereby significantly accelerating the process of hybrid rice breeding.

Rice sheath blight (ShB) caused by *Rhizoctonia*

solani Kuhn, [Teleomorph stage, *Thanatephorus cucumeris* (Frank) Donk] is the second most important fungal disease of rice after blast that amounts of yield losses in the range of 8%–50% across the tropical rice growing countries of the world (Savary et al, 2000). CRISPR/Cas9 editing has been recently attempted to modify *Oryza sativa Phytochrome and Flowering Time 1* (*OsPFT1*) gene to understand its functional role in ShB resistance (Shah et al, 2019). Previous report has shown that Arabidopsis PFT1 is critically implicated in inducing disease susceptibility by acting as a universal adaptor between RNA polymerase II and DNA bound transcription factors (Backstrom et al, 2007; Thatcher et al, 2009). The CRISPR/Cas9 constructs have been mobilized into the *indica* rice variety ASD16 via *Agrobacterium*-mediated transformation that facilitated mutation in the *PFT1* locus. However, the mutation efficiency and disease resistance affinity of the edited rice lines are yet to be ascertained.

Resistance to viral pathogens

Viral diseases also act as major global constraint in the effort to increase rice productivity. Among the different viral infections, the rice tungro disease (RTD) severely affects rice production in about 3.5 million hectares throughout the major rice producing Asian nations (Chancellor et al, 2006). The disease is basically caused by the interaction of two different viruses namely Rice Tungro spherical virus (RTSV) having a single stranded RNA genome, and Rice Tungro bacilliform virus (RTBV), having a double stranded DNA genome (Hull, 1996). While RTBV develop the primary disease symptoms, RTSV spread the disease by assisting the transmission of RTBV through the green leaf hopper species such as *Nephotettix virescens* and *N. nigropictus* (Hibino and Kabauatan, 1987). Host resistance breeding programmes through the screening of huge rice germplasm collections have resulted in the identification of multiple rice cultivars with specific resistance to either or both of the viruses (Khush et al, 2004). Concentrated molecular research in an indica rice cultivar Utri Merah have demonstrated that the RTSV resistance is controlled by the presence of single-nucleotide polymorphism or deletion affecting the YVV amino acid residues in the translation initiation factor four gamma (*eIF4G*) gene (Lee et al, 2010). As such, it is essential to develop RTSV resistant variety to prevent the secondary spread of this disease. Recently, CRISPR/Cas9 genome editing technology has been successfully used to mutate *eIF4G* gene in the RTSV susceptible rice variety, IR64 to develop new sources of resistance to RTSV (Macovei et al, 2018). Mutation frequency ranging from 36% to 86% was realized and induced mutations were transmitted into the subsequent generation with no detectable modifications in the closest off-target sites. Sequence analysis and pathogen infection assay revealed that in-frame mutation in the amino acid residues adjacent to the YVV residues conferred enhanced RTSV resistance together with improved agronomic parameters such as plant height and grain yield. The stable mutants developed through this study could be released in the RTD prone areas as alternative source of RTSV resistance for controlling the infection and improving the rice productivity.

Future prospects for enhancing rice disease resistance

Although genome editing approaches depends upon the targeted mutation of *S* genes towards introduction of disease resistance in plants, it might come with a fitness cost. Knocking out *S* genes that encode proteins responsible for pre-penetration structures, defense suppression and replication machinery may lead to phenotypic abnormalities and nutritional deficiency that can affect plant growth and development (van Schie and Takken, 2014). Whereas, *OsSWEET* rice mutants induce resistance through restricted sugar availability for BLB pathogen, it might also lead to reduced plant stature and pollen abortion (Chu et al, 2006). This could be possibly mitigated through targeted editing of different promoter regions of the *S* genes as has been demonstrated for *OsSWEET* gene in rice (Blanvillain-Baufume et al, 2017). Alternatively, instead of fully knocking out an *S* gene, disease resistance can also be developed by introducing synthetic variants of *S* gene identical to the allele that naturally occurs in resistant genotypes (Bastet et al, 2017). Such new allele can induce plant resistance and at the same time demonstrate normal protein functions with no developmental cost. Besides, the recently developed base editing tools can also be used for precise introduction of single base transitions in some *S* genes which vary only at the single nucleotide polymorphism (SNP) level. Moreover, the availability of multiple pathogen inducible promoters and

regulatory elements in plants can be targeted using a pathogen induced CRISPR system for transient switch off of the *S* gene with no compromise in their fitness roles. Such a CRISPR vector system that can exploit the pathogen inducible promoter could be conceptualized to demonstrate their effectiveness towards disease resistance in field crops. On the contrary, the introduction of a custom design sequence into the genome will be more appropriate when specific allelic variants are involved in resistance response. A CRISPR system coupled with the HDR mechanism can indefinitely expand the possibility of knocking in *R* gene allele into the target site of choice for developing disease resistance. Although, HDR is still technically challenging in plants due to low efficiency and lack of multiplexing protocols, CRISPR toolboxes need to be designed and analyzed to expand their applications in resistance breeding for rice improvement.

CONCLUSION

Targeted genome editing technologies have revolutionized the field of agriculture with its wide applications in crop improvement as well as in gene functional studies. The various available editing platforms have the potential to improve rice disease resistance by different strategies. CRISPR/Cas9 system has been demonstrated as one of the most powerful genome editing tools in development of rice varieties with enhanced resistance against bacterial, fungal and viral diseases. The recent development of new Cas9 variants such as Cas9-VQR, Cas9-VRER, Cpf1-RR, Cpf1-RVR and SaCas9 coupled with the advent of novel base editing tools that enables precise genome modifications and the DNA-free genome editing via ribonucleoproteins demonstrate significant promise in the development of future strategies that could greatly help in enhancing the pathogen specific immunity of rice.

ACKNOWLEDGEMENTS

This research was supported by grants from the China Priority Program-Breeding of Seven Major Crops (Grant No. 2017YFD01100100), the Innovation Program of Chinese Academy of Agricultural Sciences (Grant No. 01-ICS) and the Talented Young Scientist Program of China to RM (Grant No. India-17-01). RM is also thankful to the President, Centurion University of Technology and Management for his encouragement and support.

REFERENCES

Antony G, Zhou J H, Huang S, Li T, Liu B, White F, Yang B. 2010. Rice *xa13* recessive resistance to bacterial blight is defeated by induction of the disease susceptibility gene *Os-11N3*. *Plant Cell*, **22**(11): 3864–3876.

Baltes N J, Hummel A W, Konecna E, Cegan R, Bruns A N, Bisaro D M, Voytas D F. 2015. Conferring resistance to Gemini viruses with the CRISPR-Cas prokaryotic immune system. *Nature Plants*, **1**: 15145.

Bastet A, Robaglia C, Gallois J L. 2017. eIF4E resistance: Natural variation should guide gene editing. *Trends Plant Sci*, **22**(5): 411–419.

Banakar R, Schubert M, Collingwood M, Vakulskas C, Eggenberger A L, Wang K. 2020. Comparison of CRISPR-Cas9/Cas12a ribonucleoprotein complexes for genome editing efficiency in the rice Phytoene Desaturase (OsPDS) gene. *Rice*, **13**(1): 4.

Blanvillain-Baufume S, Reschke M, Sole M, Auguy F, Doucoure H, Szurek B, Meynard D, Portefaix M, Cunnac S, Guiderdoni E, Boch J, Koebnik R. 2017. Targeted promoter editing for rice resistance to *Xanthomonas oryzae* pv. *oryzae* reveals differential activities for SWEET14-inducing TAL effectors. *Plant Biotechnol J*, **15**(3): 306–317.

Bloch D, Pleskot R, Pejchar P, Potocký M, Trpkošová P, Cwiklik L, Vukašinović N, Sternberg H, Yalovsky S, Žárský V. 2016. Exocyst SEC3 and phosphoinositides define sites of exocytosis in pollen tube initiation and growth. *Plant Physiol*, **172**(2): 980–1002.

Bogdanove A J, Schornack S, Lahaye T. 2010. TAL effectors: finding plant genes for disease and defense. *Curr Opinion Plant Biol*, **13**(4): 394–401.

Bogdanove A J, Voytas D F. 2011. TAL Effectors: Customizable proteins for DNA targeting. *Science*, **333**:1843–1846.

Cai C Q, Doyon Y, Ainley W M, Miller J C, Dekelver R C, Moehle E A, Rock J M, Lee Y L, Garrison R, Schulenberg L, Blue R, Worden A, Baker L, Faraji F, Zhang L, Holmes M C, Rebar E J, Collingwood T N, Rubin-Wilson B, Gregory P D, Urnov F D, Petolino J F. 2009. Targeted transgene integration in plant cells using designed zinc finger nucleases. *Plant Mol Biol*, **69**: 699–709.

Carroll D. 2011. Genome engineering with zinc finger nucleases. *Genetics*, **188**(4): 773–782.

Chancellor T C B, Holt J, Villareal S, Tiongco E R, Venn J. 2006. Spread of plant virus disease to new plantings: A case study of rice tungro disease. *Adv Virus Res*, **66**: 1–29.

Chandrasekaran J, Brumin M, Wolf D, Leibman D, Klap C, Pearlsman M, Sherman A, Arazi T, Gal-On A. 2016. Development of broad virus resistance in non-transgenic cucumber using CRISPR/Cas9 technology. *Mol Plant Pathol*, **17**(7): 1140–1153.

Chu Z H, Yuan M, Yao L L, Ge X J, Yuan B, Xu C G, Li X H, Fu

B Y, Li Z K, Bennetzen J L, Zhang Q F, Wang S P. 2006. Promoter mutations of an essential gene for pollen development result in disease resistance in rice. *Genes Dev*, **20**(10): 1250–1255.

Cohn M, Bart R S, Shybut M, Dahlbeck D, Gomez M, Morbitzer R, Hou B H, Frommer W B, Lahaye T, Staskawicz B J. 2014. *Xanthomonas axonopodis* virulence is promoted by a transcription activator-like effector-mediated induction of a SWEET sugar transporter in cassava. *Mol Plant Microbe Int*, **27**(11): 1186–1198.

Cox D B T, Gootenberg J S, Abudayyeh O O, Franklin B, Kellner M J, Joung J, Zhang F. 2017. RNA editing with CRISPR-Cas13. *Science*, **358**: 1019–1027.

Cui H T, Tsuda K, Parker J E. 2015. Effector-triggered immunity: from pathogen perception to robust defense. *Annu Rev Plant Biol*, **66**: 487–511.

Deng Y W, Zhai K R, Xie Z, Yang D Y, Zhu X D, Liu J Z, Wang X, Qin P, Yang Y Z, Zhang G M, Li Q, Zhang J F, Wu S Q, Milazzo J, Mao B Z, Wang E, Xie H, Tharreau D, He Z H. 2017. Epigenetic regulation of antagonistic receptors confers rice blast resistance with yield balance. *Science*, **355**: 962–965.

Endo A, Masafumi M, Kaya H, Toki S. 2016. Efficient targeted mutagenesis of rice and tobacco genomes using Cpf1 from *Francisella novicida*. *Sci Rep*, **6**: 38169.

Gao L Y, Cox D BT, Yan W X, Manteiga J C, Schneider M W, Yamano T, Nishimasu H, Nureki O, Crosetto N, Zhang F. 2017. Engineered Cpf1 variants with altered PAM specificities. *Nat Biotechnol*, **35**: 789–792.

Giraud T, Gladieux P, Gavrilets S. 2010. Linking the emergence of fungal plant diseases with ecological speciation. *Trends Ecol Evol*, **25**(7): 387–395.

Hibino H, Cabauatan P Q. 1987. Infectivity neutralization of rice tungro associated viruses acquired by vector leafhoppers. *Phytopathology*, **77**: 473–476.

Hsu P D, Lander E S, Zhang F. 2014. Development and applications of CRISPR-Cas9 for genome engineering. *Cell*, **157**(6): 1262–1278.

Hu J H, Miller S M, Geurts M H, Tang W X, Chen L W, Sun N, Zeina C M, Gao X, Rees H A, Liu D R. 2018. Evolved Cas9 variants with broad PAM compatibility and high DNA specificity. *Nature*, **556**: 57–63.

Hua K, Tao X P, Zhu J K. 2019. Expanding the base editing scope in rice by using Cas9 variants. *Plant Biotechnol J*, **17**(2): 499–504.

Hua K, Tao X P, Yuan F T, Wang D, Zhu J K. 2018. Precise AT to GC base editing in the rice genome. *Mol Plant*, **11**(4): 627–630.

Hull R. 1996. Molecular biology of rice tungro viruses. *Annu Rev Phytopathol*, **34**: 275–297.

Hutin M, Sabot F, Ghesquière A, Koebnik R, Szurek B. 2015. A knowledge-based molecular screen uncovers a broad-spectrum *OsSWEET14* resistance allele to bacterial blight from wild rice. *Plant J*, **84**(4): 694–703.

Jones H D. 2015. Regulatory uncertainty over genome editing. *Nat Plants*, **1**: 14011.

Khush G S, Angeles E, Virk P S, Brar D S. 2004. Breeding rice for resistance to tungro virus at IRRI. *SABRAO J Breed Genet*, **36**(2): 101–106.

Kim Y A, Moon H, Park C J. 2019. CRISPR/Cas9-targeted mutagenesis of Os8N3 in rice to confer resistance to *Xanthomonas oryzae* pv. *oryzae*. *Rice*, **12**: 67.

Kim H, Kim S T, Ryu J, Kang B C, Kim J S, Kim S G. 2017. CRISPR/Cpf1-mediated DNA-free plant genome editing. *Nat Commun*, **8**: 14406.

Kleinstiver B P, Prew M S, Tsai S Q, Topkar V V, Nguyen N T, Zheng Z L, Gonzales A P W, Li Z Y, Peterson R T, Yeh J R J, Aryee M J, Joung J K. 2015. Engineered CRISPR-Cas9 nucleases with altered PAM specificities. *Nature*, **523**: 481–485.

Komor A C, Kim Y B, Packer M S, Zuris J A, Liu D R. 2016. Programmable editing of a target base in genomic DNA without double stranded DNA cleavage. *Nature*, **533**: 420–424.

Lee J H, Muhsin M, Atienza G A, Kwak D Y, Kim S M, De Leon T B, Angeles E R, Coloquio E, Kondoh H, Satoh K, Cabunagan R C, Cabauatan P Q, Kikuchi S, Leung H, Choi I R. 2010. Single nucleotide polymorphisms in a gene for translation initiation factor (eIF4G) of rice (*Oryza sativa*) associated with resistance to rice tungro spherical virus. *Mol Plant-Microbe Interact*, **23**(1): 29–38.

Li W, Deng Y W, Ning Y S, He Z H, Wang G L. 2020. Exploiting broad-spectrum disease resistance in crops: From molecular dissection to breeding. *Annu Rev Plant Biol*, **29**: 575–603.

Li C, Zong Y, Wang Y P, Jin S, Zhang D B, Song Q N, Zhang R, Gao C X. 2018. Expanded base editing in rice and wheat using a Cas9-adenosine deaminase fusion. *Genome Biol*, **19**: 59.

Li S F, Shen L, Hu P, Liu Q, Zhu X D, Qian Q, Wang K J, Wang Y X. 2019. Developing disease-resistant thermosensitive male sterile rice by multiplex gene editing. *J Integr Plant Biol*, **61**(12): 1201–1205.

Li S Y, Zhang X, Wang W S, Guo X P, Wu Z C, Du W M, Zhao Y D, Xia L Q. 2018. Expanding the scope of CRISPR/Cpf1-Mediated genome editing in rice. *Mol Plant*, **11**(7): 995–998.

Li W T, Zhu Z W, Chern M, Yin J J, Yang C, Ran L, Cheng M P, He M, Wang K, Wang J, Zhou X G, Zhu X B, Chen Z X, Wang J C, Zhao W, Ma B T, Qin P, Chen L H, Wang Y P, Liu J L, Wang W M, Wu X J, Li P, Wang J R, Zhu L H, Li S G, Chen X W. 2017. A natural allele of a transcription factor in rice confers broad-spectrum blast resistance. *Cell*, **170**(1): 114–126

Li T, Liu B, Chen C Y, Yang B. 2016. TALEN-mediated homologous recombination produces site-directed DNA base change and herbicide-resistant rice. *J Genet Genom*, **43**(5): 297–305.

Li Y, Xiao J H, Chen L L, Huang X H, Cheng Z K, Han B, Zhang Q F, Wu C Y. 2016. Rice functional genomics research: Past decade and future. *Mol Plant*, **11**(3): 359–380.

Li T, Liu B, Spalding M H, Weeks D P, Yang B. 2012. High-efficiency TALEN-based gene editing produces disease-resistant rice. *Nat Biotechnol*, **30**: 390–392.

Liang Z, Chen K L, Li T D, Zhang Y, Wang Y P, Zhao Q, Liu J X, Zhang H W, Liu C M, Ran Y D, Gao C X. 2017. Efficient DNA-free genome editing of bread wheat using CRISPR/Cas9

ribonucleoprotein complexes. *Nat Commun*, **8**: 14261.

Lusser M, Parisi C, Plan D, Rodriguez-Cerezo E. 2012. Deployment of new biotechnologies in plant breeding. *Nature Biotechnol*, **30**: 231–239.

Lu Y M, Zhu J K. 2017. Precise editing of a target base in the rice genome using a modified CRISPR/Cas9 system. *Mol Plant*, **10**(3): 523–525.

Ma J, Chen J, Wang M, Ren Y L, Wang S, Lei C L, Cheng Z J, Sodmergen. 2018. Disruption of OsSEC3A increases the content of salicylic acid and induces plant defense responses in rice. *J Exp Bot*, **69**(5): 1051–1064.

Ma X L, Zhang Q Y, Zhu Q L, Liu W, Chen Y, Qiu R, Wang B, Yang Z F, Li H Y, Lin Y R, Xie Y Y, Shen R X, Chen S F, Wang Z, Chen Y L, Guo J X, Chen L T, Zhao X C, Dong Z C, Liu Y G. 2015. A robust CRISPR/Cas9 system for convenient, high-efficiency multiplex genome editing in monocot and dicot plants. *Mol Plant*, **8**(8): 1274–1284.

Macovei A, Sevilla N R, Cantos C, Jonson G B, Slamet-Loedin I, Cermak T, Voytas D F, Choi I R, Chadha-Mohanty P. 2018. Novel alleles of rice eIF4G generated by CRISPR/Cas9-targeted mutagenesis confer resistance to Rice tungro spherical virus. *Plant Biotechnol J*, **16**(11): 1918–1927.

Miah G, Rafii M Y, Ismail M R, Puteh A B, Rahim H A, Asfaliza R, Latif M A. 2013. Blast resistance in rice: A review of conventional breeding to molecular approaches. *Mol Biol Rep*, **40**: 2369–2388.

Mishra R, Joshi R K, Zhao K J. 2018. Genome editing in rice: recent advances, challenges, and future implications. *Front Plant Sci*, **9**: 1361.

Mishra R, Joshi R K, Zhao K J. 2020. Base editing in crops: Current advances, limitations and future implications. *Plant Biotechnol J*, **18**(1): 20–31.

Nalley L, Tsiboe F, Durand-Morat A, Shew A, Thoma G. 2016. Economic and environmental impact of rice blast pathogen (*Magnaporthe oryzae*) alleviation in the United States. *PLoS One*, **11**(12): e0167295.

Nishikura K. 2010. Functions and regulation of RNA editing by ADAR deaminases. *Annu Rev Biochem*, **79**: 321–349.

Olivia R, Ji C H, Atienza-Grande G, Huguet-Tapia J C, Perez-Quintero A, Li T, Eom J S, Li C H, Nguyen H, Liu B, Auguy F, Sciallano C, Luu V T, Dossa G S, Cunnac S, Schmidt S M, Samet-Loedin I H, Cruz C V, Szurek B, Frommer W B, White F F, Yang B. 2019. Broad-spectrum resistance to bacterial blight in rice using genome editing. *Nat Biotechnol*, **37**(11): 1344–1350.

Osakabe K, Osakabe Y, Toki S. 2010. Site-directed mutagenesis in Arabidopsis using custom-designed zinc finger nucleases. *Proc Natl Acad Sci U S A*, **107**(26): 12034–1239.

Paques F, Duchateau P. 2007. Meganucleases and DNA double-strand break-induced recombination: Perspectives for gene therapy. *Curr Gene Ther*, **7**(1): 49–66.

Peng A H, Chen S C, Lei T G, Xu L Z, He Y R, Wu L, Yao L X, Zou X P. 2017. Engineering canker-resistant plants through CRISPR/Cas9-targeted editing of the susceptibility gene CsLOB1 promoter in citrus. *Plant Biotechnol J*, **15**(12): 1509–1519.

Qin L, Li J, Wang Q, Xu Z, Sun L, Alariqi M. et al. 2019. High efficient and precise base editing of C•G to T•A in the allotetrapoid cotton (*Gossypium hirsutum*) genome using a modified CRISPR/Cas9 system. *Plant Biotechnol J*, 1: 12.

Ren B, Yan F, Kuang Y J, Li N, Zhang D W. Zhou X P, Lin H H, Zhou H B. 2018. Improved base editor for efficiently inducing genetic variations in rice with CRISPR/Cas9-guided hyperactive hAID mutant. *Mol Plant*, **11**(4): 623–626.

Rodriguez-Leal D, Lemmon Z H, Man J, Bartlett M E, Lippman Z B 2017. Engineering quantitative trait variation for crop improvement by genome editing. *Cell*, **171**(2): 470–480.

Sander J D, Joung J K. 2014. CRISPR-Cas systems for editing, regulating and targeting genomes. *Nat Biotechnol*, **32**(4): 347–355.

Savary S, Willocquet L, Elazegui F A, Castilla N P, Teng P S. 2000. Rice pest constraints in Tropical Asia: Quantification of yield losses due to rice pests in a range of production situations. *Plant Dis*, **84**(3): 357–369.

Shah P R, Varanavasiappan S, Kokiladevi E, Ramanathan A, Kumar K K. 2019. Genome editing of rice *PFT1* gene to study its role in rice sheath blight disease resistance. *Int J Curr Microbiol App Sci*, **8**(6): 2356–2364.

Sharma T R, Rai A K, Gupta S K, Vijayan J, Devanna B N, Ray S. 2012. Rice blast management through host plant resistance: Retrospect and prospects. *Agric Res*, **1**(1): 37–52.

Shukla V K, Doyon Y, Miller J C, Dekelver R C, Moehle E A Worden S E, Mitchell J C, Arnold N L, Gopalan S, Meng X D, Choi V M, Rock J M, Wu Y Y, Katibah G E, Gao Z F, McCaskill D, Simpson M A, Blakeslee B, Greenwalt S A, Butler H J, Hinkley S J, Zhang L, Rebar E J, Gregory P D, Urnov F D. 2009. Precise genome modification in the crop species *Zea Mays* using zinc finger nucleases. *Nature*, **459**: 437–441.

Silva N V, Patron N J. 2017. CRISPR-based tools for plant genome engineering. *Emerg Top Life Sci*, **1**(2): 135–149.

Stafforst T, Schneider M F. 2012. An RNA-deaminase conjugate selectively repairs point mutations. *Angew Chem Int Ed Engl*, **51**: 11166–11169.

Sun Y W, Jiao G A, Liu Z P, Zhang X, Li J Y, Guo X P, Du W M, Du J L, Francis F, Zhao Y D, Xia L Q. 2017. Generation of high-amylose rice through CRISPR/Cas9-mediated targeted mutagenesis of starch branching enzymes. *Front Plant Sci*, **8**: 298.

Svitashev S, Schwartz C, Lenderts B, Young J K, Cigan M A. 2016. Genome editing in maize directed by CRISPR-Cas9 ribonucleoproteins complexes. *Nat Commun*, **7**: 13274.

Tang X, Ren Q R, Yang L J, Bao Y, Zhong Z H, He Y,Liu S S, Qi C Y, Liu B L, Wang Y,Sretenovic S, Zhang Y X, Zheng X L, Zhang T, Qi Y P, Zhang Y. 2019. Single transcript unit CRISPR 2.0 systems for robust Cas9 and Cas12a mediated plant genome editing. *Plant Biotechnol J*, **17**(7):1431–1445.

Thatcher L F, Manners J M, Kazan K. 2009. *Fusarium oxysporum* hijacks COI1-mediated jasmonate signaling to promote disease development in Arabidopsis. *Plant J*, **58**(6): 927–939.

Toda E, Koiso N, Takebayashi A, Ichikawa M, Kiba T, Osakabe K,

Osakabe Y, Sakakibara H, Kato N, Okamoto T. 2019. An efficient DNA- and selectable-marker-free genome-editing system using zygotes in rice. *Nat Plants*, **5**: 363–368.

Tomlinson L, Yang Y, Emenecker R, Smoker M, Taylor J, Perkins S, Smith J, MacLean D, Olszewski N E, Jones J D G. 2019. Using CRISPR/Cas9 genome editing in tomato to create a gibberellin-responsive dominant dwarf DELLA allele. *Plant Biotechnol J*, **17**: 132–140.

Ul Haq I, Ijaz S.. 2020. Plant disease management strategies for sustainable agriculture through traditional and modern approaches. Sustainability in plant and crop protection. doi:10.1007/978-3-030-35955-3.

van Schie C C N, Takken F L W. 2014. Susceptibility genes 101: how to be a good host. *Annu Rev Phytopathol*, **52**: 551–581.

Varshney R K, Godwin I D, Mohapatra T, Jones J D G, McCouch S R. 2019. A SWEET solution to rice blight. *Nat Biotechnol*, **37**: 1280–1282.

Veillet F, Perrot L, Guyon-Debast A, Kermarrec MP, Chauvin L, Chauvin JE, et al. 2020. Expanding the CRISPR toolbox in *P. patens* using SpCAS (-NG variant and application for gene and base editing in Solanaceae crops. *Int J Mol Sci*, 21: 1024.

Voytas D F. 2013. Plant genome engineering with sequence-specific nucleases. *Annu Rev Plant Biol*, **64**: 327–350.

Voytas D F, Gao C X. 2014. Precision genome engineering and agriculture: opportunities and regulatory challenges. *PLoS Biol*, **12**(6): e1001877.

Wang M G, Mao Y F, Lu Y M, Tao X P, Zhu J K. 2017. Multiplex gene editing in rice using the CRISPR-Cpf1 system. *Mol Plant*, **10**(7): 1011–1013.

Wang F J, Wang C L, Liu P Q, Lei P L, Hao W, Gao Y, Liu Y G, Zhao K J. 2016. Enhanced rice blast resistance by CRISPR/Cas9-targeted mutagenesis of the ERF transcription factor gene OsERF922. *PLoS One*, **11**(4): e0154027.

Wang Y P, Cheng X, Shan Q W, Zhang Y, Liu J X, Gao C X, Qiu J L. 2014. Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nat Biotechnol*, **32**: 947–951.

Xiao W M, Yang Q Y, Huang M, Guo T, Liu Y Z, Wang J F, Yang G L, Zhou J Y, Yang J Y, Zhu X Y, Chen Z Q, Wang H. 2019. Improvement of rice blast resistance by developing monogenic lines, two-gene pyramids and three-gene pyramid through MAS. *Rice*, **12**(1): 78.

Xu Z Y, Xu X M, Gong Q, Li Z Y, Li Y, Wang S, Yang Y Y, Ma W X, Liu L Y, Zhu B, Zou L F, Chen G Y. 2019. Engineering broad-spectrum bacterial blight resistance by simultaneously disrupting variable TALE-binding elements of multiple susceptibility genes in rice. *Mol Plant*, **12**(11): 1434–1446.

Xu R F, Yang Y C, Qin R Y, Li H, Qiu C H, Li L, Wei P C. 2016. Rapid improvement of grain weight via highly efficient CRISPR/Cas9-mediated multiplex genome editing in rice. *J Genet Genom*, **43**(8): 529–532.

Yin K Q, Qiu J L. 2019. Genome editing for plant disease resistance: applications and perspectives. *Philos Trans R Soc Lond B Biol Sci*, **374**: 20180322.

Yin X J, Biswal A K, Dionora J, Perdigon K M, Balahadia C P, Mazumdar S, Chater C, Lin H C, Coe R A, Kretzschmar T, Gray J E, Quick P W, Bandyopadhyay A. 2017. CRISPR-Cas9 and CRISPR-Cpf1 mediated targeting of a stomatal developmental gene *EPFL9* in rice. *Plant Cell Rep*, **36**(5):745–757.

Zaidi S S E A, Mahfouz M M, Mansoor S. 2017. CRISPR-Cpf1: A new tool for plant genome editing. *Trends Plant Sci*, **22**(7): 550–553.

Zaidi S S E A, Mukhtar M S, Mansoor S. 2018. Genome editing: targeting susceptibility genes for plant disease resistance. *Trends Biotechnol*, **36**(9): 898–906.

Zaidi S S E A, Tashkandi M, Mansoor S, Mahfouz M M. 2016. Engineering plant immunity: Using CRISPR/Cas9 to generate virus resistance. *Front Plant Sci*, **7**: 1673.

Zetsche B, Gootenberg J S, Abudayyeh O O, Slaymaker I M, Makarova K S, Essletzbichler P, Volz S E, Joung J, van der Oost J, Regev A, Koonin E V, Zhang F. 2015. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. *Cell*, **163**(3):759–771.

Zhang Q W, Yin K Q, Liu G W, Li S N, Li M G, Qiu J L. 2020. Fusing T5 exonuclease with Cas9 and Cas12a increases the frequency and size of deletion at target sites. *Sci Chin Life Sci*, doi: 10.1007/s11427-020-1671-6.

Zhang Y, Pribil M, Palmgren M, Gao C X, 2020. A CRISPR way for accelerating improvement of food crops. *Nat Food*, **1**: 200–205.

Zhang Y, Massel K, Godwin I D, Gao C X, 2018. Applications and potential of genome editing in crop improvement. *Genome Biol*, **19**(1): 210.

Zhou J H, Peng Z, Long J Y, Sosso D, Liu B, Eom J S, Huang S, Liu S Z, Cruz C V, Frommer W B, White F F, Yang B. 2015. Gene targeting by the TAL effector PthXo2 reveals cryptic resistance gene for bacterial blight of rice. *Plant J*, **82**(4): 632–643.

Zong Y, Song Q N, Li C, Jin S, Zhang D B, Wang Y P, Qiu J L, Gao C X. 2018. Efficient C to T base editing in plants using a fusion of nCas9 and human APOBEC3A. *Nat Biotechnol*, **36**: 950–953.

Zong Y, Wang Y P, Li C, Zhang R, Chen K L, Ran Y D, Qiu J L, Wang D W, Gao C X. 2017. Precise base editing in rice, wheat and maize with a Cas9-cytidine deaminase fusion. *Nat Biotechnol*, **35**: 438–440.