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Review article

Genome Editing for the Development of Rice Resistance against Stresses: A Review

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ABSTRACT

Food security is the most crucial issue faced by humans considering the rising population. Rice, a staple food consumed by nearly 50% of the world's population, faces challenges to meet the consumers' demand to ensure self-sufficiency amidst various abiotic and biotic stresses. Drought, salinity, heat, and infection by bacteria and viruses are the main challenges in rice cultivation. Genome editing technology provides abundant opportunities to implement selective genome modifications. Moreover, it finds the functional implications of different genome components in rice and provides a new approach for creating rice varieties tolerant of stresses. This review focuses on rice production worldwide and challenges faced in rice cultivation, and current genome editing tools available that can be utilised for crop breeding and improvement. In addition, the application of genome editing to develop biotic and abiotic resistance rice varieties is critically discussed.

Keywords: Abiotic stress, biotic stress, genome editing, rice

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INTRODUCTION

For years, plant breeders have used conventional breeding to produce improved crops with desirable traits such as resistance to pests and diseases, enhanced quality and yield, disease resistance, shortened growing seasons, high nutritional content, extended shelf life, and better adaptation to various topographies. However, the conventional

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breeding method gives unpredictable results. Therefore, a more extended period is required to achieve crops with desirable traits. Nowadays, breeders use the molecular breeding method to assist the conventional approach and increase the likelihood of success in breeding programs. In addition, the molecular breeding method reduces time, cost, and workloads compared to the conventional breeding method. There are various branches of molecular breeding for crop improvement study, such as genome editing.

Genome editing is an effective tool for introducing mutations in plants, and the results showed room for trait improvements. Gene editing is a precise and efficient advanced molecular biology technique with targeted modifications at genomic loci (J. Zhang et al., 2018). Examples of genome editing tools are zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats/ CRISPR-associated system (CRISPR/Cas) (Boettcher & McManus, 2015). Using DNA sequence-specific manipulations, many studies shows success in producing desirable traits crops via genome editing.

Global Rice Cultivation and Production

Out of 300,000 edible plant species, only 0.7% is regularly consumed for their nutritional values (Voss-Fels et al., 2019). However, essential commodities contributed to the average global production, including fruits (10%), vegetables (13%), sugarcane (23%), roots and tubers (11%), and oil-bearing crops (11%). In addition, cereal crops such as rice, wheat, and maize are considered essential crops, providing 60% of energy in the human diet.

Apart from wheat, rice is rendered the most vital edible crop in the world. Rice is consumed by more than 3.5 billion globally and is an important income source for families in developing countries. The average global rice production was recorded at 750 million tonnes (Food and Agriculture Organization [FAO], 2020). Thus, the overall area for rice cultivation continuously increases to fulfil a rising global rice demand following the rapid population growth. From 1994 to 2018, the total rice yields increase from 5.39 million tonnes to 7.82 million tonnes. The Asia regions consume 80% of the rice production. Referring to Figure 1, Asian countries such as China, India, Indonesia, Bangladesh, and Vietnam are the world's largest rice producers and consumers (FAO, 2020). However, rice yield loss due to abiotic, biotic stresses, and other factors cause pressure to improve rice yield and quality to ensure self-sufficiency. Therefore, rice quality improvement must be realised to overcome current agriculture issues and improve rice production and farmers' incomes to achieve the zero hunger goal by 2030.

Challenges Faced in Rice Cultivation

Based on the latest United Nations population prospects, the world population is predicted to increase by 34%, from 7.6 billion today to 10 billion in 2050 (Voss-Fels et al., 2019). The current rice

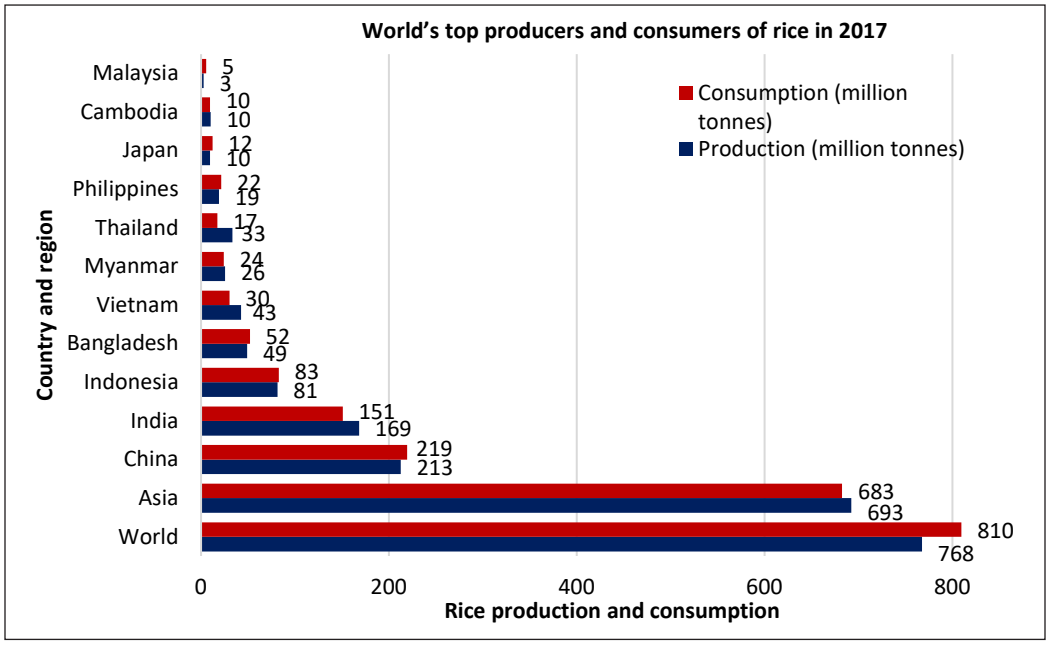

Figure 1. World's top producers and consumers of rice, by region and country (FAO, 2020)

production is low and unlikely to meet the predicted needs and demands in 2050 (Ray et al., 2013). Therefore, the growing global human population may bring challenges in achieving global food security.

Global warming negatively impacted the yield, making it alarming as Earth's atmosphere can influence agricultural activities. Rapid climate changes may initiate food insecurity on a global scale. Recent studies explore the relationship between the ozone decline and other stresses on soybean, maize, wheat, and rice yield globally (Mills et al., 2018). The authors also found that soybean is the most sensitive towards ozone reduction, followed by wheat, maize, and rice. Ozone decline reduces the annual global yield on soybean (12.4%), wheat (7.1%), rice (4.4%), and maize (6.1%) based on stomatal uptake. It was also reported that India, Bangladesh, China, and Indonesia were severely affected due to the ozone decline.

Extreme weathers such as drought can inhibit crop production and yield quality. Over the last three decades, drought effects on rice yield were observed in several countries. Bangladesh losses of 2 million tonnes of rice in 1978-1979 due to the seasonal drought phenomenon. In 2009, Bangladesh was devastated with a 50-60% yield reduction (Climate Change Cell, 2009). About 40% yield losses were reported in several eastern Indian regions and 20% yield losses in Thailand due to extreme drought conditions in 2004 (Pandey & Bhandari, 2009). The direct effect of drought on the rising temperature is concerned as the rice crops are temperature-sensitive. The rising temperature affects the flowering and grain filling stage, inhibiting the rice yield (Alam et al., 2012). With every 2°C temperature

rise, the rice yield decreases from 5 million tonnes per hectare to 4 million tonnes per hectare (Alam et al., 2012). Recent studies in the Mun River Basin, Thailand, rice cultivation area showed that yield decreases between 2% to 10% per 1°C increment. With every 1°C increase in the dry season, the rice yield decreases by 10% (Prabnakorn et al., 2018). Apart from drought and rising temperature, floods can also be equally devastating to rice-producing countries (Alemu & Assaye, 2020; Ettang, 2020).

Salinity stress is another major constraint in rice cultivation since rice is a salt-sensitive crop. Salinity stress in rice had various effects on the growth and development stages of rice. Rice during the flowering stage is most sensitive to salt stress that inhibits the grain yield (Clermont-Dauphin et al., 2010). Salt stress can also reduce the number of tillers, grain weight, and panicle length. Most rice producers in Southeast Asia, such as Vietnam, Thailand, and Bangladesh, face salinity stress problems, especially in areas exposed to seawater (Clermont-Dauphin et al., 2010).

The climates and environmental threats have become substantial, notably during unpredictable extreme weather. Drastic reduction of rice as a staple food will cause mass starvation. In addition, small farmers are at risk of poverty as they lose their source of income or are displaced due to natural disasters like floods (Ettang, 2020). Overcoming or adapting to these challenges will be too overwhelming and challenging for the farmers. Besides, global rice cultivation is at risk of disease from bacteria, fungi, viruses, and pests. Notable diseases that affect global rice production are a blast, sheath blight, and sheath rot caused by fungi infection, bacterial blight disease, and rice tungro disease caused by viral infection (Bunawan et al., 2014; Gnanamanickam, 2009).

Genome Editing Tools

Genome editing technology has been improved rapidly in the last two decades to overcome the shortcomings of conventional breeding methods for future crop improvement. Zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR) are the example of the technology using the engineered site-specific nucleases (SSNs) (Mishra et al., 2018). Owing to the high efficiency and specific mutation at the desired target requiring RNA-guided DNA nucleases, the clustered regularly interspaced short palindromic repeats/CRISPR-associated endonuclease 9 (CRISPR/Cas9) system is the most advanced system using protein-DNA interaction. New advanced and improved genome editing tools were created based on the previous system, clustered regularly interspaced short palindromic repeats/CRISPR-associated endonuclease 12a (CPISPR/Cas 12a), base editing and prime editing in that order (Figure 2).

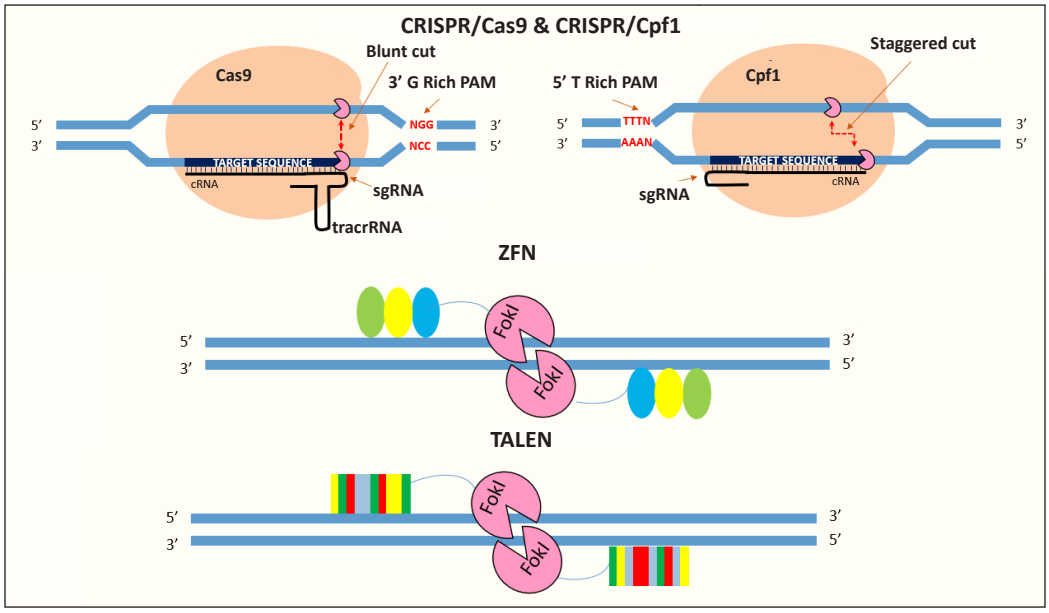

Figure 2. Schematic illustrating the engineered type II CRISPR-Cas9, CRISPR-Cpf1, ZFN, and TALEN system. More recently, the use of clustered regularly interspaced short palindromic repeats (CRISPR) vectors has provided a 'breakthrough' in the technique of specific genome editing to develop mutants
Note. CRISPR/Cas9 = Clustered regularly interspaced short palindromic repeats/CRISPR-associated endonuclease 9; CRISPR/Cpf1 = Clustered regularly interspaced short palindromic repeats/CRISPR-associated endonuclease in *Prevotella* and *Francisella* 1; ZFN = Zinc finger nuclease; TALEN = Transcription activator-like effector nuclease

Zinc Finger Nucleases (ZFNs). ZFNs are generated from mergers of zinc fingers (ZFs) domain linked to *Fok*I endonuclease (Urnov et al., 2010). It is a non-specific restriction enzyme and does not involve sequence recognition (Pernstich & Halford, 2012). Another half of the ZFs fused to the *Fok*I endonuclease is needed for the DNA cleavage domain to be active and cut at a specific DNA sequence. The ZFs recognise and bind to three specific nucleotides. When the ZFN occurs as a dimer, the 3-4 ZFs arrays recognise and bind to the 18-24 bp nucleotides (Petolino, 2015). Nevertheless, some reports that ZFNs exhibited off-target cleavage and improved ZFNs efficiency and precision have been made. Researchers were able to multiply the turnover rate of the ZFN endonuclease by designing variants like sandwiched nucleases attached to one or two artificial zinc-finger proteins (APZs) and adding a single-chain *Fok*I dimer to the complex (Mino et al., 2009).

Transcription Activator-Like Effector Nucleases (TALENs). Like ZFNs, TALENs involve fusing the *Fok*I endonuclease and the 34 amino acids DNA binding repeat domain (M. Zhang et al., 2014). The *Fok*I endonuclease dimerisation with another half of the TALEN will mediate DNA cleaving. The transcriptional activator-like effectors (TALE) are the DNA binding domain for TALENs. TALENs can facilitate one-to-one

pairing of nucleotides, more precise than ZFNs. Moreover, TALENs can achieve sequence specificity in contrast to ZFNs due to their sequence-specific DNA binding domain, derive from proteins excreted by *Xanthomonas* (Joung & Sander, 2013). The DNA specific binding occurs at positions 12 and 13 of each repeat (Mahfouz et al., 2011). Hence, TALENs are more beneficial than ZFNs in terms of protein guided genome editing. TALENs are also less toxic to the cells due to their improved specificity and reduced off-target sites cleavage.

CRISPR/Cas9. CRISPR/Cas9 system involves RNA-DNA interaction to achieve sequence specificity. It is more flexible, reliable, and cost-effective compared to ZFNs and TALENs. The CRISPR system was first discovered in *Escherichia coli* and its role in bacterial resistance to viruses (Doudna & Charpentier, 2014; Jinek et al., 2012). CRISPR/Cas9 can site-specific binding by combining the system with endonuclease Cas9 and utilised in different non-bacterial organisms (Kotwica-Rolinska et al., 2019; Markossian & Flamant, 2016). CRISPR/Cas9 mechanism begins with the site-specific binding of a ~100-nt sequence single guide RNA (sgRNA) to the target sequence of a 5'-NGG-3' protospacer adjacent motif (PAM). The trans-activating CRISPR RNA (tracrRNA) and CRISPR RNA (crRNA) of the sgRNA was designed to assist sequence-specific Cas9 double -stranded DNA (dsDNA) cleavage (Jinek et al., 2012). A CRISPR complex will form and initiate cleaving of the target DNA upon binding, creating a double-strand DNA break (DSBs). CRISPR/Cas9 adopts the homology-directed repair (HDR) while repairing the DSBs. In recent years, CRISPR/Cas9 technology has been applied in rice cultivation for functional analysis and crop improvement studies (Ren et al., 2019; Schachtsiek & Stehle, 2019).

CRISPR/Cas12a. CRISPR/Cas12a is the advanced genome editing tool derived from CRISPR. The CRISPR-associated endonuclease in *Prevotella* and *Francisella* 1 (Cpf1) or Cas12a is an RNA endonuclease, similar to Cas9 nuclease (Zetsche et al., 2017). CRISPR/Cas12a is more beneficial and cheaper than CRISPR/Cas9 system despite the similarity in its mechanism. The synthesised sgRNA attached to the Cas12a nuclease only requires crRNA (~42-nt), enhancing the delivery speed owing to its smaller sgRNA-Cas12a complex (Zetsche et al., 2017). The Cas12a is more versatile than Cas9 as it can prepare the pre-crRNA to crRNA and simultaneously cleaves the DNA double-strand (Nakade et al., 2017). Unlike Cas9, Cas12a recognises T-rich (5'-TTTN-3 or 5'-TTN-3') PAM sites and cleaves DNA at 23-17 bp downstream of the PAM sites generating staggered 5' overhangs. These features prevent the PAM site's disruption, allowing flexibility in choosing the target site, minimising the non-specific mutations at the cut site, and enabling multiplexed genome editing using one vector (S. Li et al., 2019; Zetsche et al., 2017). Within a year of the first report of Cas12a advantages properties, many publications demonstrated its specificity in maize, *Arabidopsis*, rice, and other plants (Malzahn et al., 2019).

Base Editing (BE). BE technology was developed to edit individual base pairs in DNA using base editors. BE can correct single base pairs instead of cutting and replacing the entire DNA sequence, such as CRISPR (Komor et al., 2016). It is commonly used to fix point mutations in the DNA. In addition, it is often applied to correct disease-related mutations in the human genomes (Liang et al., 2017; Zhao et al., 2020). The base editors execute BE consisting of three proteins that can inspect and bind to the target DNA sequences, convert C base to T base via chemical reaction, and prevent the edited T base from being removed. The permanent transition of C-G-to-T-A and A-T-to-G-C can be performed by the cytosine base editors (CBEs) and adenine base editors (ABEs), respectively. The two base editors were engineered together to fuse with a Cas9 nickase (nCas9) and successfully applied in plants to correct single-base changes responsible for phenotypic variations (Zong et al., 2018). As multiple cytosines and adenine replacement are present, unwanted bystander edits may occur, making BE less precise.

Prime Editing. Prime editing is the latest genome editing technology derived from yeast and mammalian cells (Anzalone et al., 2019). Prime editing can edit the genome precisely without using DSBs and a donor DNA template. Unlike BE, prime editing can conduct any base-to-base conversion where modification is limited to C-G-to-T-A and A-T-to-G-C. The technology required an engineered prime editing guide RNA (pegRNA) with a spacer sequence and a prime editor (PE). The spacer sequence is complementary to a primer binding site (PBS) sequence; a reverse transcriptase (RT) template consist of desired edit sequence and one strand of the DNA. In contrast, the PE consists of RT enzyme and Cas9 nickase (Cas9n). Genome editing via prime editing involves reverse transcription. The pegRNA and PE will form a complex and bind to the complementary DNA strand and RT template guided by the pegRNA. Afterwards, Cas9n cuts the PAM- containing DNA strand, generating a flap, and the PBS binds to the 3' cut strand. The RT triggers integrating the desired edit sequence from the RT template region to the PAM-containing DNA strand via reverse transcription. The process produced two redundant single-stranded DNA flaps (the unedited 5' and edited 3' DNA flaps). The edited DNA strand will be integrated into the genome at the cut DNA strand, and the Cas9n will remove the unedited DNA strand. Stable incorporation of the edited sequences into the genome will occur following the repair of the heteroduplex DNA via a cellular DNA repair mechanism. Prime editing can produce multiple and precise nucleotides replacement, unlike base editing, which is incompetent when multiple adenine and cytosine replacement is present (Abdullah et al., 2020). Prime editing technology has a much lower target editing than Cas9 due to the additional two hybridisation steps between DNA and pegRNA and DNA-reverse transcript

templates. Unlike PAM-sequence, prime editing does not require a suitable distance to initiate, making the target scope more flexible. The application of prime editing in plants is limited due to its new technology. However, numerous studies have been conducted to optimise the prime editing tools used for crop improvement. Nevertheless, the recent application of prime editing in plants reported low editing efficiency. C. Lin et al. (2020) reported that prime editing in wheat and rice could generate transversion, substitutions, insertions, and deletions despite lower editing efficiency than base editing. Butt et al. (2020) and R. Xu et al. (2020) also reported low editing efficiency despite being capable of editing at different genome sites and nucleotide substitutions in plants. Thus, plant prime editing is much more flexible and versatile though further optimisation is needed.

Rice Improvement via Genome Editing

The availability of target site-specific mutations or base editing allows researchers to utilise genome editing tools to improve rice cultivation. Extensive studies have been conducted on genome editing in rice and its effect on crop productivity and development, such as metabolisms and stress responses.

Resistance against Abiotic Stress. Abiotic stresses are the severe development pressures predicted to deteriorate with anticipated climate change (Pereira, 2016). Many studies have focused on understanding the plants' molecular basis reaction with the environmental factors in recent decades. Over a few decades, approximately 100 genes are identified in rice, contributing to abiotic stress response (F. Wang et al., 2016). Various methods, such as identifying multiple genes/pathways and regulatory networks implied in stress responses, have been solved. The growing production of abiotic stress-tolerant rice demonstrates a successful crop yield improvement via genome editing (Table 1).

Drought. Regarding the importance of abiotic stress, it was proven that deactivation and degradation of the mediator of OsbZIP46 deactivation and degradation (MODD) protein mediate (OsbZIP46) inhibit ABA signalling, increasing the rice resistance to drought. This method can enhance drought tolerance in rice plants by producing MODD knock out mutants (Tang et al., 2016). Liao et al. (2019) reported that changing leaf physiology like rolled leaf genotype reduces the water loss rate, enhancing drought tolerance response in rice. Through CRISPR/Cas system, *SEMI-ROLLED LEAF 1* (*SRL1*) and *SEMI-ROLLED LEAF 2* (*SRL2*) rice mutants increase drought tolerance and improved survival rate during the seedling stage. CRISPR/Cas9 mediated knockout of *OsmiR535* study showed that *Osmir535* mutant enhanced rice seedlings survival rate after dehydration stress, suggesting its potential as genetic editing target for drought tolerance (Yue et al., 2020).

Table 1
The improved trait of genome editing technology for the development of stress-tolerant rice varieties

Application	Genome editing tool	Targeted gene	Reference
Drought tolerance	CRISPR/Cas9	*OsMODD*	Tang et al. (2016)
	CRISPRi	*OsNAC14*	Shim et al. (2018)
	CRISPR/Cas9	*SRL1*, *SRL2*	Liao et al. (2019)
	CRISPR/Cas9	*OsAAA-1*, *OsAAA-2*	Lu et al. (2020)
	CRISPRa dCas9	*AREB1*	Paixão et al. (2019)
	CRISPR/Cas9	*OsmiR535*	Yue et al. (2020)
Salinity tolerance	CRISPRi	*OsSIT1*	C.-H. Li et al. (2014)
	CRISPR/Cas9	*OsmiR535*	Yue et al. (2020)
	CRISPR/Cas9	*OsRR9*, *OsRR10*	W.-C. Wang et al. (2019)
	CRISPR/Cas9	*OsRR22*	A. Zhang et al. (2019)
Cold tolerance	CRISPR/Cas9-HDR	*OsCTB4α*	Z. Zhang et al. (2017)
	CRISPR/Cas9-HDR	*OsCOLD1*	Y. Ma et al. (2015)
	CRISPR/Cas9	*OsPIN5b*, *GS3*, and *OsMYB30*	Y. Zeng et al. (2020b)
Bacterial blight resistance	TALEN	*OsSWEET14*	Blanvillain-Baufumé et al. (2017)
	TALEN	*OsSWEET11*, *OsSWEET14*	Z. Xu et al. (2019)
	CRISPR/Cas9	*OsSWEET14*	Zafar et al. (2020)
	TALEN	*Os11N3* (*OsSWEET14*)	T. Li et al. (2012)
	CRISPR/Cas9	*OsSWEET14*	X. Zeng et al. (2020a)
	CRISPR/Cas9	*SWEET11*, *SWEET13*, *SWEET14*	Oliva et al. (2019)
	CRISPR/Cas9	*Os8N3* (*OsSWEET11*)	Kim et al. (2019)
	CRISPR/Cas9	*Xa13*	C. Li et al. (2020)
	CRISPR/Cas9	*TMS5*, *Pi21*, and *Xa13*	S. Li et al. (2019)
Bacterial leaf streak resistance	TALEN	*Os09g29100*	Cai et al. (2017)
Rice blast resistance	CRISPR/Cas9	*OsERF22*	F. Wang et al. (2016)
	CRISPR/Cas9	*OsSEC3A*	J. Ma et al. (2018)
Rice tungro resistance	CRISPR/Cas9	*eIF4G*	Macovei et al. (2018)

Note. CRISPR/Cas9 = Clustered regularly interspaced short palindromic repeats/CRISPR-associated endonuclease 9; CRISPRi = Clustered regularly interspaced short palindromic repeats interference; CRISPRa dCas9 = Clustered regularly interspaced short palindromic repeats activation defective CRISPR-associated endonuclease 9; CRISPR/Cas9-HDR = Clustered regularly interspaced short palindromic repeats/CRISPR-associated endonuclease 9-homology directed repair; TALEN = Transcription activator-like effector nuclease; *OsMODD = Oryza sativa mediator of OsbZIP46 deactivation and degradation*; *OsNAC14 = Oryza sativa Nascent polypeptide-Associated Complex (NAC) domain-containing protein 14*; *SRL1 = SEMI-ROLLED LEAF 1*; *SRL2 = SEMI-ROLLED LEAF 2*; *AREB1 = Abscisic acid-responsive element binding protein1*; *OsSIT1 = Oryza sativa salt intolerance 1*; *OsRR9 = Oryza sativa response regulator 9*; *OsRR10 = Oryza sativa response regulator 10*; *OsRR22 = Oryza sativa response regulator 22*; *OsCOLD1 = Oryza sativa chilling tolerance divergence 1*; *OsPIN5b = Oryza sativa PIN protein 5B*; *GS3 = Grain size 3*; *OsMYB30 = Oryza sativa Myb transcription factor 4 paralog*; *Xa13 = Xanthomonas oryzae* pv. *oryzae resistance 13*; *OsERF22 = Oryza sativa ethylene response factor 22*; *OsSEC3A = Oryza sativa subunit of the exocyst complex 3A*; *eIF4G = Eukaryotic translation initiation factor 4 gamma 1*

Salinity. Plant abiotic stress tolerance can be easily improved by generating CRISPR-Cas9-mediated knockout/knockdown of harmful regulatory genes. It is observed via editing of *Oryza sativa SRFP1* and *Oryza sativa response regulator 22* (*OsRR22*). The *SRFP1* is a negative salinity tolerance gene where new mutants produced via CRISPR/Cas9 will increase salinity tolerance (Fang et al., 2015). A. Zhang et al. (2019) reported that *OsRR22*-induced mutations enhanced salinity tolerance compared to wild-type plants during the seedling stage.

Cold. Cold stress can majorly affect the growth and rice yield. A recent study identified *Oryza sativa Myb transcription factor 4 paralog* (*OsMYB30*) as a gene associated with cold tolerance (Y. Zeng et al., 2020b). The *OsMYB30* Nipponbare mutants demonstrated enhanced cold tolerance compared to wild type.

Resistance against Biotic Stresses. Rice is susceptible to various biotic stresses resulted in poor productivity and quality. Biotic stresses such as insect pests, fungi, bacteria, and nematodes (Singh et al., 2020) can cause devastating diseases such as rice blight, rice tungro, rice blast, and rice sheath rot (Gnanamanickam, 2009). Strategies to mitigate rice diseases include chemical control, varietal resistance breeding, biological control, cultural practice, and genetically modified plants with disease resistance traits (Abo & Sy, 1997). Numerous studies have successfully developed rice resistance towards biotic stress diseases by applying and understanding advanced genome editing tools such as CRISPR.

Bacterial Blight. Bacterial blight in rice is caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), a Gram-negative bacterium (Niño-Liu et al., 2006). Attempts on enhancing rice resistance towards blight disease were conducted by editing the promoter of the *SWEET* gene family (Blanvillain-Baufumé et al., 2017; Z. Xu et al., 2019; Zafar et al., 2020). The transcription activator-like effectors (TALEs) target promoter effector-binding elements (EBEs) of *SWEET11*, *SWEET13*, and *SWEET14* belonging to *SWEET* family clade III (Yang et al., 2006) when the plants are infected with *Xoo*. Since *SWEET* genes encode for sugar efflux transporters, induction of these genes hijacks the sugar export into the apoplast, fulfilling the nutritional requirement of the pathogen (Cohn et al., 2014). Besides that, bacterial blight-resistant rice can be achieved by editing the *Xanthomonas oryzae* pv. *oryzae resistance 13* (*Xa13*) promoter via CRISPR/Cas9 (C. Li et al., 2020). The *Xa13* gene in rice is a fully recessive resistance allele of *Os-118N3*, a disease-susceptibility gene against bacterial blight (Antony et al., 2010). Multiplex gene editing of *TMS5*, *Pi21*, and *Xa13* via CRISPR/Cas9 can enhance the rice resistance to blight disease (S. Li et al., 2019).

Bacterial Leaf Streak. Bacterial leaf streak (BLS) is a widespread disease in rice-growing regions globally, causing 10% to 20% yield reduction and 40% yield loss

(Niño-Liu et al., 2006). However, BLS can be mitigated by enhancing the resistance to *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*) using TALEN editing of the Tal7 binding site of the *Os09g29100* gene promoter (Cai et al., 2017). Tal7 binds to two EBE sites of *Os09g29100* promoter, which encodes for *cyclin-D4-1* and activation of *cyclin-D4-1* suppressing the *avrXa7-Xa7* mediated rice's defence.

Rice Blast. Rice blast disease is caused by the fungus *Magnaporthe oryzae* (Couch & Kohn, 2002), leading to a 10% to 30% loss in rice production annually (Sakulkoo et al., 2018). Genome editing tools can effectively enhance rice resistance against rice blast disease. Targeted mutation of ERF transcription factor gene, *Oryza sativa ethylene response factor 22* (*OsERF22*) via CRISPR/Cas9, can enhance rice blast resistance in its knockout mutants (F. Wang et al., 2016). The mutant plants contained insertion or deletion mutations at the target site while maintaining the agronomic traits compared to the wild-type plants. Meanwhile, disruption of the *OsCE3A* by CRISPR/Cas9 demonstrates enhanced resistance to *M. oryzae* alongside the increased level of salicylic acid. *Oryza sativa* subunit of the exocyst complex 3A (OsSEC3A) is a subunit of the exocyst protein complex that interacts with OsSNAP32 protein involved in rice blast resistance (J. Ma et al., 2018; Mishra et al., 2021).

Rice Tungro. Rice tungro disease (RTD) is caused by the combination of two distinct viruses, *Rice tungro spherical viruses* (RTSV) and *Rice tungro bacilliform viruses* (RTBV) (Hibino et al., 1979). This disease frequently occurs in South and Southeast Asian countries (Bunawan et al., 2014), causing a $ 1.5 billion loss annually (Dai & Beachy, 2009). IR64 rice variety is known to be susceptible to RTD (Macovei et al., 2018). However, using CRISPR/Cas9, *IR64* mutant of the *eukaryotic translation initiation factor 4 gamma 1* gene (*eIF4G*), demonstrating resistance to RTD.

CONCLUSION

Rice production faces various challenges and threats, mainly climate change, abiotic, and biotic stressors leading to unsustainable development and yield losses. Genome editing has been used to address the limitations of conventional breeding methods in developing high yield and quality rice varieties to meet the growing consumer demands. The genome editing tool is cost-effective, accurate, time-saving, reliable, and robust compared to the conventional methods. However, several aspects need to be reviewed to utilise the tools properly. Low editing efficiency issues such as undesired off-target events/ mutations can be solved by developing high precision guide RNAs and understanding improved Cas9 variants. CRISPR technique requires optimum plant tissue culture and transformation systems. However, both systems depend on the species, genotype, and challenging commercial rice varieties where the systems are not optimised.

Moreover, further data validation is needed on genome-edited rice trait improvement in both controlled and field environments to understand the potential effects on the rice in the natural environment. Field trials and observation is crucial to evaluate the genome-edited plants' performance in the natural environment. Ethical and public acceptance of genome editing over transgenic methods needed to be addressed. Typically, genome-edited crops are associated with genetically modified organisms (GMOs) issues. Debates on the uncertain safety and process of these products cause public unrest and rejection of genome-edited crops. Education among the public of its safety and advantages is needed, especially in Asian countries where rice cultivation is dominant and threatened by abiotic or biotic stresses. Preserving the effective disease resistance plants developed through genome editing over time also poses a tremendous challenge. Future studies on multiplex genome editing strategies to target multiple disease resistance trait genes are desirable. Genome editing tools will likely transform the future of crop improvement and achieving zero hunger goals by securing feed for a growing population.

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