Chapter 3
Genome Editing: A Tool from the Vault of Science for Engineering Climate-Resilient Cereals

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Abbreviations

CRISPR	Clustered regularly interspaced short palindromic repeats
HDR	Homologous DNA directed repair
HR	Homologous recombination
NHEJ	Non-homologous end joining
TALEN	Transcription activator-like effector nucleases
ZFN	Zinc finger nucleases

1 Introduction

From the time of understanding of cropping, humans have continuously been focusing on the yield and quality of plant products via various traditional approaches until the green revolution. On the other hand, the human population is increasing continuously, but arable land is constant in dimensions. So, to feed the world population, it is imperative to make efficient use of non-cultivable lands such as pH imbalanced, salt-stressed, drought areas, and marshy lands (Singh et al. 2018; Mehta et al. 2019a). Gene revolution has been possible due to technological revolutions in the field of biotechnology. However, despite countless benefits and qualities in cereal plants, there are a few problems which need to be addressed such as cultivation issues, selective nutrient enrichment, overall nutritional content, and stress tolerance using genetic engineering of genes in the genome of affected cereal crops. GM crops can fight poverty and malnutrition by increasing yield, bio-fortification, reuse of salt-affected areas, overcome energy crisis, pest resistance, pesticide and herbicide tolerance, drought tolerance, efficient CO_2 use, nitrogen use efficiency, and phytoremediation. ISAAA report March 2020 reported an increase in GM crops planting area. In 1996, 1.7 million hectares of land cover were utilized for GM plants, while in 2018, it was 191.7 million hectares. Genetic engineering techniques for cutting and introducing the desired gene sequence involves the use of DNA-based biomolecules such as transcription activator-like effector nuclease (TALEN), zinc finger domain nucleases (ZFNs), CRISPR/Cas9, CRISPR/Cpf1 most importantly, and non-DNA based biomolecules such as guide RNA-based gene editing (Mehta et al. 2020). These genome-editing tools have advantages as well as

limitations (Mehta et al. 2020). Both TALENs and CRISPR/Cas9 allow precise alterations by target specificity. TALENs provides greater freedom in target site selection compared to CRISPR/Cas9. However, the popularity of CRISPR is due to its capability for modifying chromosomal targets at higher frequencies. Simplicity in design and usage makes CRISPR/Cas9 an attractive tool. However, TALENs designing gets streamlined by modules with repeated combinations, thus reducing the cloning time. TALENs is sensitive to cytosine methylation, expensive and targets one site at a time. ZFNs are another class of nucleases that can edit/modify any targeted genomic sequence. However, they have low efficacy and is time consuming. Here, we highlight various targets in cereals to fight biotic and abiotic stresses using gene-editing techniques to boost the productivity and nutrient content of cereals to fight world hunger in an eco-friendly manner.

2 Genetic Engineering Tools for Crop Resiliency

There are various DNA and non-DNA-based techniques for editing the crop's genome, which includes insertion, deletion, or modification of a gene of interest. Many classical techniques are available for the transformation of the edited genes of interest into crops, such as electroporation, *Agrobacterium*-mediated, and nanoparticle-mediated transformation. *The Agrobacterium*-based technique is the most widely accepted because of its simplicity, and a higher integration probability even for low-copy DNA fragments with minimum problems to the host genome (Sindhu et al. 2019). The genetically modified crops created using gene-editing tools have desired characteristics (Griffiths et al. 2005). The transgene may have its origin from any organism having a beneficial trait. It can be from prokaryotes such as bacteria and viruses, or eukaryotes such as fungi and animals. Apart from this, there are other popular modern breeding techniques such as genome-wide association studies (GWAS), genome selection (GS), and marker-assisted selection (MAS) that are being employed by scientists for incorporating useful traits into the crops.

However, the current trend in life sciences is genome engineering using methods such as ZFN, TALEN, and CRISPR with variants such as CRISPR/Cas9, and CRISPR/Cpf1. These are well-known approaches which have been used to develop crops that can tolerate climate variabilities and biological stresses (Liu et al. 2013; Ali et al. 2015; Andolfo et al. 2016; Wang et al. 2016; Li et al. 2017; Kumar et al. 2018). There are other approaches such as RNA silencing using microRNA, trans-acting siRNA, hairpin RNA, and virus-induced gene suppression which reduce the translated product of marked genes (Bernstein et al. 2001). ZFNs, TALENs and CRISPR technologies create dsDNA cuts at particular genomic sequences which are further repaired using either NHEJ (non-homologous end-joining) or HR (homologous recombination). ZFNs and TALENs use engineered proteins along with DNA binding regions, and DNA endonuclease enzymes, whereas CRISPR/Cas utilizes laboratory synthesized sgRNA (synthetic guide RNA) targeted to precise

DNA sequences by base pairing. As far as ZFNs and TALENs are concerned, protein engineering is expensive, laborious, and time consuming, which limit their usage in high-throughput studies. On the other hand, CRISPR/Cas9 technique has circumvented these problems by being cheaper, versatile, less laborious, precise, and most importantly, efficient in genome-editing purposes at multiple levels (Mehta et al. 2020).

2.1 DNA-Based Genetic Engineering Approaches

2.1.1 Transcription Activator-Like Effector Nucleases (TALENs)

These are a variety of DNA-cutting proteinic enzymes especially designed to cleave at specific target DNA sequences. They are formed of the TAL effector DNA-binding domain with DNA cleaving sphere acting as nuclease which is taken from restriction endonuclease FokI. DNA-binding domain has various tandem DNA repeats ranging from 1.5 to 33.5 characteristically. Every repeat has 34 aa stretch with deviation at 12–13th amino acid named repeat variable residues, that is, RVD. These repeats recognize one nucleotide specifically. There are unique RVDs such as IN (Ile Asn), NN (Asn Asn), DH (Asp His) and GN (Gly Asn) which recognize A, G, C, and T, nucleotide bases respectively. Double-strand breaks after nicks are mended by HDR or NHEJ. These repairs result in deletions, insertions, duplications, inversions, transversions, translocations, or point mutations (Wei et al. 2013). The key features are explained in Table 3.1.

2.1.2 Zinc Finger Nucleases (ZFNs)

ZFNs are a type of DNA-cutting enzymes used for genome-editing applications such as duplication, addition, deletion, or substitution of gene sequences, with more precision than conventional plant breeding techniques for improvement of various crop attributes. They also include a DNA-sticking domain (Zinc finger class) and DNA cleavage sphere. DNA binding part of a molecule usually has 3–4 zinc finger proteins conjugated with di-cysteine and di-histidine motifs. These motifs recognize nucleotide triplets based on the residues from the alpha helix. DNA cleaving domain comes from FoKI restriction endonuclease. It dimerizes for DNA cutting in a non-sequence-specific manner and induces dsDNA cuts. The DSBs (double-stranded breaks) are mended by two paths, namely (1) HDR using homologous DNA as a model sequence to reinstate the DSBs for accurate and controllable repairs and (2) NHEJ machinery leads to additions, deletions, or substitutions in the broken dsDNA of host genome (Miller et al. 2007). ZFNs' key attributes are well-documented in Table 3.1.

Table 3.1 Key features of different genetic engineering approaches used nowadays for enhancing crop resiliency

S. No.	Features	ZFNs	TALENs	CRISPR/Cas9
1	Cleavage basis	Protein-based	Protein-based	RNA-based
2	Size	Considerably smaller than Cas9 (+)	Larger than ZFNs (++)	Considerably larger than ZFNs and TALENs (+++)
3	Constituents	Zinc-finger domains, nonspecific FokI nuclease domain	TALE DNA-binding domains, nonspecific FokI nuclease domain	Cas9 protein, crRNAs
4	Catalytic domains	FokI endonuclease domain	FokI endonuclease domain	HNH, RUVC
5	Structural parts	Dimeric	Dimeric	Monomeric
6	Target nucleotide length	18–36	24–59	20–22
7	gRNA requirement	No	No	Yes
8	Forms of action	Induce DSBs in target DNA	Induce DSBs in target DNA	Induce DSBs or ssDNA nicks in target DNA
9	Restriction size	High G	5′T and 3′A	PAM sequence
10	Recognition efficiency of target site	High	High	Very High
11	Mutation rate	High	Low	Very Low
12	Off-target effects	Yes	Yes	Yes, but can be minimized by the selection of unique crRNA sequence
13	Cleavage of methylated DNA	No	No	Yes, but it will be explored more
14	Multiplexing enabled	Highly difficult	Highly difficult	Yes
15	Laboriousness	Yes	Yes	No
16	Technology cost	Very high (£1000–£3000)	High (£40–£350)	Low (£30–£300)
17	First report in plants	Durai et al. (2005)	Christian et al. (2010)	Feng et al. (2013)

2.1.3 CRISPR/Cas9 Approach

CRISPR usage in plants has promised precise and accurate gene editing for targeted crop trait improvements (Arora and Narula 2017). They have been used versatility in almost all model organisms of different origins including plants (Sander and Joung 2014). It has been showcased and used for various attributes in rice, tomato, maize, wheat, woody plants such as apple, poplar, etc., to extended level for

alleviating biotic, abiotic, and other climatic stress issues (Osakabe and Osakabe 2017; Mehta et al. 2020). It involves induction of dsDNA breaks at selective sites in genomic sequences with the help of guided RNA complementary sequences that bind with DNA and Cas endonucleases of CRISPR/Cas system targets and cuts genomic loci of DNA and pair using Watson-Crick base pairing. The breaks are mended by the cell's inner restoration mechanisms involving NHEJ and HR. Cas9 or Cpf1 plays molecular scissor role and RNA marks the address at genomic sequence level, thus guaranteeing precise and accurate cutting for further action. The entire process can be conceptualized as effective designing of Cas nucleases, assemblage of gRNA cassettes, Cas and RNA vector's delivery, screening, selection, efficient gene-editing detection, and plant regeneration with selective traits. Thus, a characteristic trait is extensively utilized in a variety of model cells and organisms for targeted mutagenesis. Thus, it has been widely accessed for achieving functional annotation of various biotic and abiotic stress resilience in genetic elements. However, the efficient delivery of gene-editing tools and components via transformation is a key bottleneck in gene-editing techniques. There is low transformation efficiency in *Agrobacterium*-mediated and particle bombardment-mediated transformation. Alternative strategies such as ex vitro plant composite development can help in efficient functioning of CRISPR/Cas9 and thus help in the elucidation of gene function, generation of valuable traits for yield, and quality improvement which usually gets affected with biotic stresses such as pests and pathogens (bacterial, viral, and fungal) and abiotic stresses. Biological stresses are usually coded by solo gene and gene erasure using CRISPR/Cas9-targeted modification or inactivation of susceptible genes gives protection against pathogens and has proven to produce resistant plants against bacterial, fungal, and viral pathogens (Borrelli et al. 2018). Further, CRISPR/Cas has the ability to edit multiple genes simultaneously (Sasano et al. 2016). Abiotic stress responses are usually controlled by multiple genes because of their complex nature. CRISPR/Cas-based unique allele variants for a non-biological stress-related gene (s) (Shi et al. 2017; Osakabe and Osakabe 2017) or clubbing multiples genes via HDR-induced gene aiming (Devkota 2018) has been used to develop resistance against abiotic stresses. The mutants failed to develop nodules when inoculated with *Sinorhizobium sp.* strain NGR234. Moreover, clubbing CRISPR/Cas with the expression of morphogenic regulators such as BBM/WUS may improve gene-edited plant's regeneration capacity with reduced tissue and genotype dependency.

Programming of CRISPR/Cas9 can be changed using sgRNA sequences and more than one sgRNA can work concurrently using similar Cas9 protein for diverse targets (Wu et al. 2014). However, there is a problem of off-target effects of CRISPR/Cas9, which must be minimized so that new mutants for abiotic and biotic stress response would be generated. One approach is to use optimized sgRNA designing (Montagne et al. 2014). CRISPR-P web software has been exploited for optimized designing of sgRNA as used for more than 20 plant species including cereals (Jain 2015). Numerous vectors and toolkits were also developed to facilitate plant genome editing using CRISPR/Cas9 (Xing et al. 2014). Thus, CRISPR/Cas9 usage in genetic engineering, genomic screening, and transcriptional intonation for

dichotomizing molecular origin behind biotic and abiotic strain response and generating stress-tolerant cereal crop can be used by accessibility of the aforementioned attributes and knowledge. Key features of CRISPR/Cas9 are explained in Table 3.2.

2.1.4 CRISPR/Cpf1

One other budding variant of CRISPR is CRISPR/Cpf1 which is CRISPR (Prevotella and Francisella 1) and was recently found to be a unique RNA-mediated site-specific class 11 type V nuclease. It was first used to engineer a mammalian cell (Mahfouz 2017). Cpf1 endonuclease has a RuvC-type nuclease domain but it does not have the HNH domain as in Cas9. Cpf1 produces double-stranded breaks with sticky or staggered ends, not blunt ends as in Cas9. Thus, it enables gene expurgation, inclusions, or substitution via HDR-homology-directed repair (Zetsche et al. 2015; Mehta et al. 2020). Cpf1 provides multiple rounds of cutting as opposite to Cas9 where a single cleavage occurs, and it cuts DNA at a specific distance from protospacer adjacent motif (PAM). Cpf requires shorter CRISPR RNA and utilizes a T-rich PAM instead of G-rich PAM as in the Cas9 system. So, Cpf1 can explore better and increase the number of plant genome editions (Stella et al. 2017). Cpf1 has increased target specificity than Cas9 in plants compared to animals (Tang et al. 2017).

2.1.5 CRISPRi

This variant of CRISPR utilizes catalytically inactive Cas9 (dCas9) which disrupts the gene's function via gene intercession (Qi et al. 2013). CRISPRi technology essays the role of a perturbation tool for sequence-specific suppression of transcription in small prokaryotic organisms as well as complex eukaryotic organisms (Huang et al. 2016). Earlier, it was invented for transcriptional interference to silent gene expression, but nowadays, it is being exploited for transcriptional activation and epigenetic modifications such as exploiting the functionality of gene methylation or chromatin modifications for abiotic stress responses. It is highly specific and precise in nature along with a little off-target effects (Domingue et al. 2016). It was found that when dCas9 gets merged with Kruppel associated box (KRAB), the gene expression level was reduced equal to approximately 99% in human cells (Gilbert et al. 2013). Hence, this technique can be well applied in plants for stress-responsive genes using CRISPR-mediated and synthetically driven transcriptional activator or repressor molecules (Piatek et al. 2015). Transcriptional activation got enhanced if dCas9 was fused with a transcriptional activator (Gilbert et al. 2013). CRISPRi can regulate a particular target gene's efficiency along with enhancing its effect (Lo and Qi 2017).

Table 3.2 Review of literature highlighting the key examples of genome editing in improving resistance to various biotic stresses in cereals

Cereals	Targeted gene	Genome-editing technique	Molecular function related to disease	Promotor used	Efficiency/ mutations	Type of editing	Cultivar	References
Rice	*11N3/SWEET14*	TALENs	Bacterial blight	Ubi1, CaMV35S	13.5%	Disruption in promoter region	Kitake	Li et al. (2012)
	SWEET11 and *SWEET14*	CRISPR/Cas9	Bacterial blight	*Os*U6	–	Promoter disruption	–	Jiang et al. (2013)
	OsMPK5	CRISPR/Cas9	Fungal blast, bacterial blight	U3 or U6	3–8%	Indel	Nipponbare	Xie and Yang (2013)
	SWEET13	TALENs	Bacterial blight	–	–	Knock-out	IR24	Zhou et al. (2015)
	ERF922	CRISPR/ Cas 9	Fungal blast disease	*Os*U6a	42%	Indels in ORF	Kuiku131	Wang et al. (2016)
	SWEET14	TALENs	Bacterial blight	Ubi1	Up to 51%	Gene disruption	Kitaake	Blanvillian-Baufum et al. (2017)
	09g29100	TALENs	Bacterial leaf streak	Ubi and 35S	–	Base editing	–	Cai et al. (2017)
	Xa10-Ni and *Xa23-Ni*	TALENs	Bacterial blight	–	–	Knock-out	Nipponbare	Wang et al. (2017)
	SEC3A	CRISPR/Cas9	Fungal blast disease	*Os*U3/U6	–	–	–	Ma et al. (2018)
	BSR-k1	CRISPR/Cas9	Bacterial blight		–	–	–	Zhou et al. (2018)
	eIF4G	CRISPR/Cas9	Rice tungro spherical virus (RTSV)	*Ta*U6	36–86.6%	–	IR64	Macovei et al. (2018)

Cereals	Targeted gene	Genome-editing technique	Molecular function related to disease	Promotor used	Efficiency/ mutations	Type of editing	Cultivar	References
	USTA ustiloxin and UvSLT2 MAP kinase	CRISPR/Cas9	False smut	U6	–	Knockout	–	Liang et al. (2018)
	ALB1, SDI1 and *RSY1*	CRISPR/Cas9 (RNP)	Rice blast fungus	–	–	–	CO-39	Foster et al. (2018)
	SWEET11, *SWEET13* and *SWEET14*	CRISPR/Cas9	Bacterial blight	*Zm*Ubi	–	5 mutations in promoter region	Kitaake, IR64 and Ciherang-Sub1	Oliva et al. (2019)
	TMS5, Pi21, and *Xa13*	CRISPR/Cas9	Bacterial blight	–	47%	Homozygous frame-shift mutations	Pinzhan	Li et al. (2019)
Wheat	*MLO*	CRISPR/Cas9	Powdery mildew	*Ta*U6	28.5%	Knock-out	Kenong199	Shan et al. (2013)
	MLO-A1, *MLO-B1* and *MLO-D1*	TALENs and CRISPR/Cas9	Powdery mildew	*Ta*U6	5.6%	Gene disruption	Kenong199	Wang et al. (2014)
	ABCC6	CRISPR/Cas9	Fusarium head blight	*Ta*U6	6.6–13%	Knock-out	Fielder	Cui (2017)
	LTP9.4	CRISPR/Cas9	Fusarium head blight	*Ta*U6	0–11.9%	Knock-out	Fielder	Cui (2017)
	NFXL1	CRISPR/Cas9	Fusarium head blight	*Ta*U6	0–42.2%	Knock-out	Fielder	Cui (2017)
	EDR1	CRISPR/Cas9	Powdery mildew	*Ta*U6	5 mutants	Knock-out	Bread wheat KN199	Zhang et al. (2017)

(continued)

Table 3.2 (continued)

Cereals	Targeted gene	Genome-editing technique	Molecular function related to disease	Promotor used	Efficiency/ mutations	Type of editing	Cultivar	References
	Lpx-1/MLO	CRISPR/Cas9	Fusarium head blight, Powdery mildew	*Ta*U6	22 mutants	Knock-out	Bobwhite	Wang et al. (2018b)
	ALA and *ACC*	CRISPR/Cas9	Herbicide resistance	*Ta*U6	33–75%	Base editing	PI653509	Zhang et al. (2019b)
Maize	*glossy2* (*gl2*) locus	TALEN	Glossy phenotype	CaMV 35S	10%	Small bp deletions	Hi-II, B104	Char et al. (2015)
	bW2 and *bE1*	CRISPR/Cas9	Corn smut	U6 snRNA	70–100%	Gene disruption	–	Schuster et al. (2016)
	NLB 18	CRISPR/Cas9	Northern Leaf Blight	–	–	–	–	USDA (2017)
	DsRed	CRISPR/Cas9	Wheat dwarf virus	*Zm*UBI	Comparatively more efficient	Mutation		Kis et al. (2019)
	GA20ox3	CRISPR/Cas9	Transgene-Free Semidwarf Maize	*Ta*U3	–	Base editing	Inbred line Cal	Zhang et al. (2020)

2.2 DNA-Free Genome Engineering

DNA based and non-DNA based CRISPR/Cas approaches have been implemented in recent scenarios. DNA-based approach usually involves *Agrobacterium*-based transformation, whereas non-DNA-based approach involves PEG-mediated protoplast fusion method. In the classical CRISPR/Cas method, T-plasmid is constructed along with the required sgRNA and Cas9/Cpf1/Cas variant coding sequence. During transformation, RNA and Cas sequences get incorporated into the host genome. Guide RNA and Cas9 get translated inside the host cell and in vivo gRNA-Cas9 RNP (ribonucleic protein complex) is formed. As the target DNA sequence is detected, double-strand DNA breaks are induced and mutations are generated by the cell's internal DNA repair mechanisms. Here, the CRISPR/Cas complex is continuously getting expressed and actively produces desired mutations in host cells.

DNA-free CRISPR/Cas9 approach is getting wider acceptance, as it can create safer and ethically accepted GMO products. In one approach, in vitro-synthesized Cas protein and in vitro-translated gRNA are used to generate the RNP complex in vitro and delivered inside the host cell protoplast using the PEG-mediated fusion method. Since the complex is already formed in a tube, it is active and once inside the cells, it detects the target sequence and induces dsDNA breaks/nicks. The cellular repair mechanisms lead to the generation of mutations in the host genome at desired target sequences and do not add any foreign DNA in the host genome. With time, the CRISPR/Cas9 complex gets degraded inside the cell and their availability is decreased over time. A suitable screening process is required to identify stable cells for further growth (Metje-Sprink et al. 2019). Other DNA-free approaches include momentarily expressed CRISPR/Cas9 plasmid DNA (TECCDNA), and CRISPR/Cas9 in vitro transcripts (IVTs). All these techniques do not allow integration of foreign DNA into the host genomic DNA and hence, they diminish off-target effects. These techniques are comparatively faster and less costly than the CRISPR/Cas cassettes already available in the global market. The most suitable methods for the delivery of these CRISPR cargos are the polyethylene glycol (PEG), electroporation, biolistic bombardment, or cationic lipid-based method (Zhang et al. 2016; Yin et al. 2017).

The most advanced CRISPR/Cas variant in the market is CRISPR base editors which create single-nucleotide changes at the target loci. The dsDNA breaks or template DNA sequences are not required here. This approach for the generation of a single-nucleotide mutant is widely used in monocot and dicot plants. This variant utilizes dCas9 (dead/inactive cas9), nCas9 (Cas9 nickase), and adenine or cytidine deaminase enzymes. Deaminases convert cytosine to thymine via uracil and adenine to guanine via inosine (Monsur et al. 2020). They are more advanced, effective, and efficient in editing. *Sp*Cas9, Sacas9, *Sp*VQR-Cas9, *Sp*EQR-Cas9, *Sa*KKH-Cas9, and *Sp*VRER-Cas9 are a few variants which are available in the market having differential specificity toward PAM (protospacer-adjacent motif). They provide product purity at a high level and have low off-target editing efficiency.

3 Role of Genome Editing Tools in Biotic Stress Management

The factors eliciting biological stress in plants are a major threat to cereal crops causing substantial yield losses annually on a global scale (Langner et al. 2018). Cereal crops are attacked by numerous pathogens and pests, including myriad bacteria, virus, fungal entities, insects, and parasites (Rahman et al. 2019; Singh et al. 2019; Mehta et al. 2019b). These biotic stressors generally harm the host plant by directly or indirectly depriving its host of its vital nutrients and become a major cause of pre- and post-harvest crop damage. The native defense mechanisms of plants protecting against biotic stresses are diverse and dynamic, which are genetically governed by resistance genes encoded within the plant genome (Diaz 2018). Numerous methods such as traditional breeding, molecular breeding, and genetic modulation tools have been deployed with the ultimate goal of enhanced food security by enabling the crops to combat pathogenic infections. In recent years, gene-editing technology has appeared to be an opportunistic strategy that serves to improve resistance in crops toward biotic stress-inducing agents through targeted gene manipulation. Numerous pioneer studies reporting the application of gene-editing tools to improve resistance in various cereal crops towards bacterial and fungal diseases are presented in Table 3.2.

3.1 Resistance Against Fungal Diseases

Shan et al. 2013 was the first to report the successful application CRISPR/Cas9 technology to enhance resistance against *Blumeria graminis* F. sp. *tritici* which causes powdery mildew disease in wheat plants leading to significant yield losses. CRISPR/Cas9-mediated knock-out of *TaMLO* (MILDEW-RESISTANCE LOCUS) in wheat, imparted disease resistance, wherein 28.5% mutational frequency was observed in *TaMLO* for protoplasts (Shan et al. 2013). Further, Wang and his team demonstrated the use of CRISPR/Cas9 and TALENs systems for the concurrent expurgation of three homoalleles of *TaMLO* gene in hexaploid bread wheat and reported similar mutation frequency (5.6%) by both the editing methods (Wang et al. 2014). More recently, the feasibility of CRISPR/Cas9 in wheat for achieving fungal resistance was confirmed by Zhang and his colleagues, who targeted the *TaEDR1* gene which negatively regulates powdery mildew resistance (Zhang et al. 2017). They were able to simultaneously knock-down the three homologs of *EDR1* generating *Taedr1* wheat lines tolerant to mildew-induced cell death. Similarly, a group of researchers targeted lipoxygenase genes (*TaLpx1* and *TaLox2*) for enhancing wheat resistance to fusarium, the causative agent of one of the most destructive fungal diseases. Lipoxygenases stimulate jasmonic acid-mediated defense mechanism in plants by hydrolyzing polyunsaturated fatty acids and initiating oxylipin biosynthesis. *TaLpx-1* gene silencing has been reported to render wheat plant resistant towards *Fusarium graminearum* (Nalam et al. 2015). The mutation frequency

of 9% and 45% was achieved for *TaLpx1* and *TaLox2* genes, respectively (Shan et al. 2014; Wang et al. 2018b). Hexaploid wheat plants carrying mutated *TaLOX2* were developed with a frequency of 9.5%, accounting for 44.7% homozygous mutants (Zhang et al. 2016). Similarly, three genes earlier reported to be linked with disease susceptibility (*TaNFXL1, TaABCC6*) and resistance (*TansLTP9.4*) toward *fusarium* head blight (FHB) (Ouellet et al. 2013; Balcerzak et al. 2016) were also targeted in wheat to confer resistance against FHB (Cui 2017) (Table 3.3).

Fungal blast has been associated with extensive losses in rice yields worldwide. Therefore, various techniques are being explored to develop blast-resistant cultivars using advanced gene-editing techniques. In an attempt to develop resistance in japonica rice, Wang et al. performed CRISPR/Cas9-targeted knockout of *OsERF922,* a negative regulator of fungal blast resistance (Wang et al. 2016). Additionally, Cas9/sgRNAs-mediated multiplex targeting of two or three sites within the *OsERF922* gene was also shown to increase mutagenic frequency (Wang et al. 2016). Agronomic traits of the mutant lines were significantly consistent with those of wild-type plants, indicating no negative effect on plant growth and sustainability. Likewise, blast resistance in rice plants was achieved through the disruption of genes *OsERF922* and *OsSEC3A* using the CRISPR/Cas9 system (Ma et al. 2018). *OsSEC3A* mutation was found to be associated with enhanced resistance against *Magnaporthe oryzae*, elevated concentrations of salicylic acid, as well as upregulation of defense responsive genes and salicylic acid synthesis (Ma et al. 2018). CRISPR/Cas9-based knock-out of a stress-responsive gene "mitogen-activated Protein Kinase5" (*Os*MPK5) using three sgRNAs, reportedly enhanced fungal as well as bacterial disease resistance in rice plants by showing constitutive expression of pathogenesis-related (PR) genes. The mutation frequency ranging from 3% to 8% was observed in Nipponbare rice protoplasts (Xie and Yang 2013). DuPont Pioneer is exploring the CRISPR/Cas9-mediated approach to generate improved disease-resistant maize cultivar by targeting the NLB-sensitive allele.

The development of multiplex genome-editing methods further increases the application of CRISPR system in cereal crops to confront more challenging attributes encompassing multiple genes by utilizing a single CRISPR construct (Wang et al. 2018a). A study adapting CRISPR/Cas9 technology to disrupt bE1 and bW2 genes in *Ustilaginoidea maydis* with efficiency of 70% and 100% in progeny from a single transformant provided proof of concept for developing resistance against corn smut. In more recent reports, the use of CRISPR-based multiplexed genome-editing method has been attempted in hexaploid wheat by employing three sgRNA to target three genes viz. *TaGW2, TaLpx-1,* and *TaMLO* to achieve resistance toward FHB and powdery mildew (Wang et al. 2018b). Further, Liang et al. successfully demonstrated an approach which employed CRISPR/Cas9 for efficient gene replacement or editing in *Ustilaginoidea virens* for imparting resistance against rice false smut, one of the major fungal diseases of rice (Liang et al. 2018). Additionally, a novel co-editing and counter-selection strategy presented by Foster and his colleagues allows precise editing in fungal strains to generate completely isogenic lines with no foreign DNA. The study demonstrated rapid plasmid-free CRISPR/Cas9-mediated editing in *Magnaporthe oryzae* with improved precision and speed of

Table 3.3 Review of literature summarizing the reports of genome editing for enhancing tolerance to abiotic stresses

Cereals	Targeted gene	Genome-editing technique	Molecular function	Promotor used	Delivery method	Efficiency/ mutations/ modified plants/ HR	Type of editing	Cultivar	Effect	References
Wheat	*LOX2*	CRISPR/ Cas9	Carotenoid biosynthesis	*TaU6*	Biolistic mediated	55–70% mutation rate efficiency	Base Editing	Bobwhite	Improved Carotene content	Zong et al. (2017)
	AHAS	ZFN	Role in branched amino acid formation	–	Particle bombardment	1.2% gain of function, 2.9% loss of function	Insertion and replacement	Bobwhite MBP26RH	Resistance against herbicide	Ran et al. (2018)
	EPSPS	CRISPR/ Cas9	Role in shikimate biosynthesis pathway	*TaU6*	PEG mediated transformation	0–20% Mean indels of which 8.5% were large insertions	Insertions	WT	Resistance against Glyphosate herbicide	Arndell et al. (2019)
	IPK1	ZFNs	Phytate formation (Intracellular signaling)	–	Cell-penetrating peptide-mediated transfection	–	Deletion	Bread wheat	Removal of anti-nutritional phytate, mineral accumulation against abiotic stress (Fe, Zn)	Bilichak et al. (2020)
Rice	*LG1*	ZFNs	Metal binding (Zinc)	–	Transfection	39%	Deletion, Insertions	Wild type	Improves seed shattering, metal binding	Gao et al. (2010)
	PDS, IPK1A, IPK, MRP4	TALENs	Phytic acid biosynthetic pathway genes	35S promoter	PEG/ Agrobacterium-mediated	9.1% mutation rate efficiency	Deletion 2–17 bp	Hi-II	Recovery from various biotic stresses	Liang et al. (2014)

Cereals	Targeted gene	Genome-editing technique	Molecular function	Promotor used	Delivery method	Efficiency/ mutations/ modified plants/ HR	Type of editing	Cultivar	Effect	References
	ALS2	CRISPR Cas9/ gRNA	Target for sulfonylurea and imidazolinone herbicides	UBI or MDI promoter	Agrobacterium-mediated	2.23% target mutation	Point mutation P165S	Hi -II	Recovery from Herbicide sensitivity	Svitashev et al. (2015)
	AOX1a, AOX1b, AOX1c,BEL	CRISPR sgRNA	Multiple abiotic stress regulators	U6	–	35.3%	Knockout	Wild type	Multiple abiotic stresses regulation	Xu et al. (2015)
	CENH3	CRISPR/ Cas9	Centromere segregation during cell division	*ZmU3*	Agrobacterium-mediated	55–70% mutation rate efficiency	Base Editing	Inbred line Zong31	Improvement in cellular division process	Zong et al. (2017)
	SAPK2	CRISPR/ Cas9	Drought response	SAPK2 promoter	PEG mediated	81–100% (germination), 6.5 to 9.1% (Water stress survival),36–38% (stomatal closure), 36–38% (stomatal opening)	Deletion mutants	*O. sativa* L. *japonica*	Improved drought tolerance, ROS detoxification	Lou et al. (2017)
	Ann3	CRISPR/ Cas9	Cold response	–	Agrobacterium mediates	8.3, 5.5, 55.5% survival	Knockout	Wildtype	Cold stress tolerance, lesser electrical conductivity	Shen et al. (2017)

(continued)

Table 3.3 (continued)

Cereals	Targeted gene	Genome-editing technique	Molecular function	Promotor used	Delivery method	Efficiency/ mutations/ modified plants/ HR	Type of editing	Cultivar	Effect	References
	CDC48	CRISPR/ Cas9	Prevention of senescence and plant death	*OsU3*	Agrobacterium-mediated	43.48%	Base Editing	*Japonica* rice variety Nipponbare	Prevention of senescence	Zong et al. (2017)
	NRT1.1B	CRISPR/ Cas9	High yield and early maturation	*OsU3*	Agrobacterium-mediated	43.48%	Base Editing	*Japonica* rice variety Nipponbare	Increased grain yield	Zong et al. (2017)
	RR22	CRISPR/ Cas9	Salinity tolerance	pUbi	Agrobacterium-mediated	64.3%	Deletion, Insertion, substitution	Japonica rice WPB106	Agronomic traits and Salt Tolerance	Zhang et al. (2019a)
	AOC	CRISPR/ Cas9	Jasmonic acid synthesis pathway involvement	–	Agrobacterium-mediated	–	Deletion, Insertion, substitution	Oryza sativa japonica Kitaake	Efficient coordination with environment	Nguyen et al. (2020)
Barley	*ENGase*	CRISPR/ Cas9	Production of GN1 type FNGs (Free N Glycans)	UBI promoter	Agrobacterium-mediated	78%	Indels and deletions	Golden promise	Increased abiotic tolerance	Kapusi et al. (2017)
	PDS1	CRISPR/ Cas9	Phytoene desaturate gene	CaMV 35S promoter	Agrobacterium-mediated	–	Gene editing	Carotenoid biosynthesis	Reduction in off target mutations	Raitskin et al. (2019)

genetic manipulation which are likely to be applicable to a range of fungal species (Foster et al. 2018).

3.2 Resistance Against Bacterial Diseases

Bacterial diseases such as bacterial leaf blight (BLB) and bacterial leaf streak (BLS), caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) and *Xanthomonas oryzae* pv. *oryzicola* (Xoc), respectively, are two of the most devastating diseases of rice (Verdier et al. 2012; Mehta et al. 2019b). Moreover, the advent of novel virulent pathotypes of *Xanthomonas oryzae* (Xoo) (Gonzalez et al. 2007; Mehta et al. 2019b) has rendered conventional breeding programs and resistant cultivars ineffective and further intensifying the utilization of advanced gene-editing tools to combat such diseases. The transcription activator-like effectors (TALEs) are key determinants of *Xanthomonas* pathogenicity since the activation of host target genes by TALEs is associated with susceptibility and/ or resistance in rice (Bogdanove et al. 2010; Bogdanove and Voytas 2011; Boch et al. 2014). This provides the opportunity to target several TALEs to enhance resistance towards Xoo and Xoc diseases. The sucrose-efflux transporter (SWEET) gene family in rice is the best-studied class of TALE virulence targets which include *OsSWEET11*, *OsSWEET13*, and *OsSWEET14* genes. The activation of *OsSWEET14* gene by TAL effectors *AvrXa7* or *PthXo3* of Xoo is known to facilitate the export of sugars from plant cell to pathogen which requires it for growth and virulence (Antony et al. 2010). The TALEN-based system was implemented for disrupting the gene associated with bacterial blight defense *OsSWEET14* (*Os11N3*) to confer resistance in rice lines toward *AvrXa7*- and *PthXo3*-based Xoo strains in rice lines (Li et al. 2012). Thereafter, some investigations employing TALEN and CRISPR/Cas9-targeting susceptible genes have been conducted to impart resistance toward blight disease in rice (Jiang et al. 2013; Hutin et al. 2015; Blanvillain-Baufum et al. 2017; Cai et al. 2017). In an attempt to demonstrate the CRISPR/Cas9-mediated targeted gene alterations, Jiang et al. 2013 designed CRISPR/Cas9-sgRNA constructs capable of inducing site-specific disruption in the promoter sequence of bacterial blight susceptibility genes, *OsSWEET14* and *OsSWEET11* for enhanced resistance (Jiang et al. 2013). CRISPR/Cas9 system was also deployed to construct *OsSWEET13* null mutant in *indica* rice, IR24 to prevent TAL effector gene *pthXo2*-mediated neutralization, thereby improving the resistance toward bacterial blight disease (Zhou et al. 2015). Genetic modification through TALEN of *EBEtal7* binding site in promoter sequence of *Os09g29100* gene by removal of its Tal7-binding sequence can reduce the severity of bacterial disease through *avrXa7-Xa7* defense in rice (Cai et al. 2017). Some other TAL effectors such as *AvrXa7*, *TalC*, and *Tal5* contributing to Xoo susceptibility in rice can also be exploited to target *OsSWEET14* to improve blight resistance (Blanvillain-Baufum et al. 2017). The potential of rice disease resistance genes *Xa10-Ni* or *Xa23-Ni* to impart broad-spectrum resistance to *Xanthomonas oryzae* pv. *Oryzae* has also been explored through TALEN and CRISPR/Cas9 technology (Wang et al. 2017).

Recently, a Japonica rice cultivar with an improved resistance to *Xanthomonas oryzae* pv. *oryzae* was developed by CRISPR/Cas9-based gene mutagenesis of *Os8N3/OsSWEET11*. The mutant lines were observed to possess equivalent agronomic characteristics such as plant height, leaf length/width, the number/length of panicles, and pollen development (Kim et al. 2019). Furthermore, a research group engineered a broad-spectrum resistance to bacterial blight in rice through CRISPR–Cas9 gene editing. They systematically targeted multiple sites in *SWEET* promoters to confer resistance in Kitaake, and elite varieties IR64 and Ciherang-Sub1 exhibiting normal agronomic features (Oliva et al. 2019). A multitude of such studies ascertain the prospects of advanced genome-editing strategies for engineering important cereal cultivars with reduced susceptibility to bacterial diseases. In 2019, Oliva et al. showed the role of CRISPR/Cas9 in curing bacterial blight using genes *SWEET11, 13,* and *14* in rice cultivar Kitaake. They used ZmUbi promoter and mutations in promoter regions were observed.

3.3 Resistance to Viral Diseases

Rice tungro disease (RTD) is caused by the concurrence of two different viruses, namely rice tungro spherical virus (RTSV) and rice tungro bacilliform virus (RTBV), which severely affect rice production across tropical Asia (Macovei et al. 2018; Mehta et al. 2019b). Macovei and his team attempted to engineer resistance in RTSV-susceptible IR4 rice through CRISPR/Cas9-mediated changes in the *eIF4G* gene (Macovei et al. 2018), which is known to control RTSV resistance. These resistant plants carrying mutant *eIF4G* alleles can further be exploited as a source for additional RTSV-resistant rice varieties. Kis et al. (2019) showed the role of DsRED protein for reporting purposes by indicating that the role of CRISPR/Cas9 under ZmUbi promoter via the *Agrobacterium*-mediated transfection method led to resistance against wheat dwarf virus.

4 Engineered Abiotic Stress Tolerance

Plants experience unfavorable environmental conditions very often called abiotic stress, for example, excess of sunlight, excess CO_2, decreased availability of soil minerals, decreased availability of water or excess of water, very extreme temperatures (hyperthermia or hypothermia), presence of toxic ions in the soil, etc. (Compant et al. 2010; Hirayama and Shinozaki 2010; Lal et al. 2018). These abiotic stresses influence various attributes of the plant affecting development and output (Rejeb et al. 2014; Lal et al. 2018). Abiotic stress is responsible for up to 50% losses in crop yields (Rodziewicz et al. 2014; Sharma et al. 2020). According to IPCC (intergovernmental panel on climate change), plants experience abiotic stress due to ever-changing climatic circumstances (Mittler 2006). which result in huge loss of food

security and environmental sustainability in developing countries (Andy 2016). This has attracted the attention of researchers to develop advanced adaptation strategies for plants under stress and make them adaptable under changing environmental conditions (Wheeler and Von Braun 2013). The major challenge is to identify how these plants respond to different stresses by activating different pathways and switching on/off of responsible genes (Wallace et al. 2003; Andy 2016; Anamika et al. 2019). The capability of plants to overcome or to develop resilience against all these factors solely depends upon photosynthesis in addition to various other physiological or genetic processes. A well-known feature of plants to combat abiotic stresses is the involvement of several genes, and activation and deactivation of several interlinked molecular pathways. Abruptly changing abiotic stresses such as temperature, air humidity, or light evokes multiple intracellular processes at the molecular, biochemical, cellular, and physiological levels (Vahisalu et al. 2010; Mittler et al. 2012; Vainonen and Kangasjärvi 2015; Suzuki et al. 2015; Dietz 2015; Hulsmans et al. 2016; Pommerrenig et al. 2018). CRISPR/Cas9 is used extensively for inducing site-specific mutations in many grass plant species such as rice, sorghum, wheat, and switchgrass.

4.1 Drought Resistance

Drought stress is one of the outcomes of climate change that has an adverse impact on crop growth, and yield by affecting biochemical and physiological processes (Husen et al. 2014, 2017; Getnet et al. 2015; Embiale et al. 2016; Siddiqi and Husen 2017, 2019). It is due to unavailability of water to plants which may cause shoot biomass reduction and grain yield losses. Yield losses are maximum due to water stress as compared to other stresses (Farooq et al. 2009). Water depletion in the rooting area causes increased vapor pressure deficit, which multiplies drought stress (Ahanger et al. 2014). This stress leads to increased crop yield losses when compared to other abiotic stresses. Thus, the intensity and duration of drought stress coupled with other environmental factors play a key role in determining crop yields. The reductions in yields depends on plant type, growth stage, severity, and longevity of the drought conditions. Lou et al. (2017) showed amelioration of drought stress by employing CRISPR/Cas9 approach on *SAPK* gene under *SAPK* promoter via PEG-mediated transformation. ROS detoxification was observed in the mutant plants.

4.2 Cold and Heat Resistance Stress

Every crop requires optimal temperature for its optimal growth. Below optimum temperatures result in cold stress while temperatures above optimal temperature results in heat stress. The temperature of 0–10 °C results in chilling stress which is very common in temperate and subtropical species such as cereals. Cold shocks in

early reproduction stages lead to flower abortion, pollen and ovule infertility, as well as low seed sets in cereals which hamper metabolic rates and hence, grain yields (Thakur et al. 2010). The low-temperature shock causes enormous changes in membrane permeability, free proline content, and malonic dialdehyde (MDA) (Nesterova et al. 2019). Under frost conditions, photosynthesis gets hampered due to low temperature and internal injuries occur, resulting in ROS production (Sharma et al. 2020). Different physiological processes are greatly affected due to temperatures above the ambient temperature such as rate of photosynthesis and respiration, production of ROS, etc. Since flowering has low threshold value, it gets affected the most at high-temperature stress and thus, there is less seed formation and loss in grain yield (Prasad et al. 2017). *Ann3* gene was edited by CRISPR/Cas9 for curing cold response using the *Agrobacterium*-mediated approach where survival of knockout plants was observed. There was an improvement in cold stress tolerance as well as reduced electrical conductivity as observed by Shen et al. (2017).

4.3 Salinity and Submergence Stress

High salt concentration above a certain threshold concentration is considered as salt stress. Soil salinity is one of the most damaging abiotic stresses. This stress damages plant crops and yield loss has been reported in several investigations (Husen et al. 2016, 2018, 2019; Hussein et al. 2017; Siddiqi and Husen 2017, 2019). It has been reported that 7% of total cultivable land area and 20% from irrigated arable land are stressed due to excessive salt concentrations (Li et al. 2014), thus leading to decrease in crop yields via overpowering the crop performance due to non-availability of nutrients to plants. India has a 6.74 million ha area under salt stress. Submergence stress is due to waterlogging, Water-deficient conditions result in drought and excess of water results in waterlogging which further affects biological processes in crop and results in yield loss. During the early flowering, crop metabolism is very sensitive. Waterlogging gives rise to several fungal diseases, and under these conditions, requirement of metabolic energy shoots up. Production of metabolic energy decreases due to anaerobic respiration and growth of the crop is arrested. *RR22* gene was mutated using the CRISPR/Cas9 approach for salinity tolerance using the *Agrobacterium*-mediated transformation method. Similarly, the successful application of CRISPR resulted in improved agronomic traits as well as salt tolerance (Zhang et al. 2019a, b).

4.4 Adaptation Stress

Plants face different kinds of biotic or abiotic stress, and each of them has their genetic makeup to fight against these stresses and come up with a solution for adaptation in the changing environment. “Survival of the fittest” or the natural law of

selection is applicable everywhere regarding the survival of the fittest. Adaptation stress is the stress faced by different crops to adjust themselves according to the evolving environmental conditions. Physiological changes, molecular changes, or biochemical changes are constitutive processes undergoing within cellular structures and switching ON/OFF of different genes allows plants to survive in the evolving environmental conditions. Zhang et al. (2020) recently published a study where CRISPR/Cas9-based base editing in maize inbred line resulted in transgene-free semi-dwarf maize, which is more efficient in withstanding environmental stress.

5 Conclusion

Cereal crops are a prime component of the human diet accounting for a significant proportion of nutrition consumed worldwide. The trends of the progressively growing population and predicted climate changes are expected to boost the global crop demand. Genome engineering techniques are powerful tools that are likely to contribute significantly to the redressal of these anticipated challenges. Advanced gene engineering techniques such as CRISPR/Cas9 have superseded the limitations of conventional genomics-based breeding approaches for crop improvement by eliminating the obstacle of genotypic limitation. Moreover, genome-editing methods surmount the imprecision associated with the use of markers by engineering innovative alternatives affecting yield and stress tolerance. Genome engineering facilitates the production of cereal crops with superior agronomical traits such as better yield, enhanced resistance to biological as well as non-biological stress, by targeting a suite of genes controlling these factors. The applications of genome-editing techniques have broadened in the field of cereal research, as it permits the biofortification of cereals in terms of favoring human health. The cereals of the future can now be developed with a specified composition and quality and offering desired nutritional performance and end-use applicability in foodstuffs. With the ability of genomic rearrangement combined with its high potential to simultaneously edit multiple genes associated with plant characteristics and the regulatory elements, the modern gene-editing tools enable crop improvement by targeting complex traits. This multiplexing approach further allows a useful combination of edits to stack multiple traits in a new variety. These techniques enable the production of transgene-free crop varieties. However, the regulation of genome-edited cereals and public acceptance puts significant constraints in the commercialization of these crops. Therefore, these issues must be addressed to accurately differentiate between transgenic and non-transgenic genome-edited crops because, unlike transgenic varieties, genetically engineered crops can be indistinguishable from crops formed by conservative breeding methods. The challenges associated with off-target alterations and changes in cleavage effectiveness remain to be overcome for establishing efficient genome-editing methods for crop improvement. The systematic analysis of target sites using efficient genomics, the upgraded delivery systems, and the availability of high throughput screening methods needs to be taken into account to modify

essential cereal crops. Further, the capability of the CRISPR/Cas9 system to deploy multiple gRNAs and the availability of NGS-next-generation sequencing technologies will provide adequate data for the comparison of gene-editing systems in a diverse range of crop species. The progressive research being carried to develop and improve gene-editing methods is expected to bring a revolution by addressing the agricultural issues related to yield, quality, and biotic/abiotic stress management. Overall, the genome engineering system offers numerous opportunities to improve cereal crops by overcoming the antagonistic effects of climate change and may support global food security.

References

Ahanger MA, Tyagi SR, Wani MR, Ahmad P (2014) Drought tolerance: role of organic osmolytes, growth regulators, and mineral nutrients. In: Physiological mechanisms and adaptation strategies in plants under changing environment. Springer, New York, pp 25–55

Ali Z, Abulfaraj A, Idris A, Ali S, Tashkandi M, Mahfouz MM (2015) CRISPR/Cas9-mediated viral interference in plants. Genome Biol 16:1–11

Anamika, Mehta S, Singh B, Patra A, Islam MA (2019) Databases: a weapon from the arsenal of bioinformatics for plant abiotic stress research. In: Recent approaches in omics for plant resilience to climate change. Springer, Cham, pp 135–169

Andolfo G, Iovieno P, Frusciante L, Ercolano MR (2016) Genome-editing technologies for enhancing plant disease resistance. Front Plant Sci 7:1813

Andy P (2016) Abiotic stress tolerance in plants. Plant Sci 7:1–9

Antony G, Zhou J, 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 22:3864–3876

Arndell T, Sharma N, Langridge P, Baumann U, Watson-Haigh NS, Whitford R (2019) gRNA validation for wheat genome editing with the CRISPR-Cas9 system. BMC Biotechnol 19:1–2

Arora L, Narula A (2017) Gene editing and crop improvement using CRISPR-Cas9 system. Front Plant Sci 28:1932

Balcerzak M, Leung W, Ouellet T (2016) Two disease susceptibility genes for FHB in wheat. In: Proceedings of the 8th Canadian workshop on fusarium head blight. Ottawa, Canada 39

Bernstein E, Caudy AA, Hammond SM, Hannon GJ (2001) Role for a bidentate ribonuclease in the initiation step of RNA interference. Nature 409:363–366

Bilichak A, Sastry-Dent L, Sriram S, Simpson M, Samuel P, Webb S, Jiang F, Eudes F (2020) Genome editing in wheat microspores and haploid embryos mediated by delivery of ZFN proteins and cell-penetrating peptide complexes. Plant Biotechnol J 18:1307–1316

Blanvillain-Baufumé S, Reschke M, Solé M, Auguy F, Doucoure H, Szurek B, Meynard D, Portefaix M, Cunnac S, Guiderdoni E, Boch J (2017) Targeted promoter editing for rice resistance to Xanthomonas oryzae pv. oryzae reveals differential activities for SWEET 14-inducing TAL effectors. Plant Biotechnol J 15:306–317

Boch J, Bonas U, Lahaye T (2014) TAL effectors–pathogen strategies and plant resistance engineering. New Phytol 204:823–832

Bogdanove AJ, Voytas DF (2011) TAL effectors: customizable proteins for DNA targeting. Science 333:1843–1846

Bogdanove AJ, Schornack S, Lahaye T (2010) TAL effectors: finding plant genes for disease and defense. Curr Opin Plant Biol 13:394–401

Borrelli VM, Brambilla V, Rogowsky P, Marocco A, Lanubile A (2018) The enhancement of plant disease resistance using CRISPR/Cas9 technology. Front Plant Sci 9:1245

Cai L, Cao Y, Xu Z, Ma W, Zakria M, Zou L, Cheng Z, Chen G (2017) A transcription activator-like effector Tal7 of *Xanthomonas oryzae pv. oryzicola* activates rice gene Os09g29100 to suppress rice immunity. Sci Rep 7:1–3

Char SN, Unger-Wallace E, Frame B, Briggs SA, Main M, Spalding MH, Vollbrecht E, Wang K, Yang B (2015) Heritable site-specific mutagenesis using TALEN s in maize. Plant Biotechnol J 13:1002–1010

Christian M, Cermak T, Doyle EL, Schmidt C, Zhang F, Hummel A, Bogdanove AJ, Voytas DF (2010) Targeting DNA double-strand breaks with TAL effector nucleases. Genetics 186:757–761

Compant S, Van Der Heijden MG, Sessitsch A (2010) Climate change effects on beneficial plant–microorganism interactions. FEMS Microbiol Ecol 73:197–214

Cui X (2017) Targeted gene editing using CRISPR/Cas9 in a wheat protoplast system (Doctoral dissertation, Université d'Ottawa/University of Ottawa)

Devkota S (2018) The road less travelled: strategies to enhance the frequency of homology-directed repair (HDR) for increased efficiency of CRISPR/Cas-mediated transgenesis. BMB Rep 51:437–443

Diaz I (2018) Plant defense genes against biotic stresses. Int J Mol Sci 19:1–5

Dietz KJ (2015) Efficient high light acclimation involves rapid processes at multiple mechanistic levels. J Exp Bot 66:2401–2414

Dominguez AA, Lim WA, Qi LS (2016) Beyond editing: repurposing CRISPR–Cas9 for precision genome regulation and interrogation. Nat Rev Mol Cell Biol 17:5–15

Durai S, Mani M, Kandavelou K, Wu J, Porteus MH, Chandrasegaran S (2005) Zinc finger nucleases: custom-designed molecular scissors for genome engineering of plant and mammalian cells. Nucleic Acids Res 33:5978–5990

Embiale A, Hussein A, Husen A, Sahile S, Mohammed K (2016) Differential sensitivity of *Pisum sativum* L. cultivars to water-deficit stress: changes in growth, water status, chlorophyll fluorescence and gas exchange attributes. J Agron 15:45–57

Farooq M, Wahid A, Kobayashi N, Fujita DB, Basra SM (2009) Plant drought stress: effects, mechanisms and management. In: Sustainable agriculture. Springer, Dordrecht, pp 153–188

Feng Z, Zhang B, Ding W, Liu X, Yang DL, Wei P, Cao F, Zhu S, Zhang F, Mao Y, Zhu JK (2013) Efficient genome editing in plants using a CRISPR/Cas system. Cell Res 23(10):1229–1232

Foster AJ, Martin-Urdiroz M, Yan X, Wright HS, Soanes DM, Talbot NJ (2018) CRISPR-Cas9 ribonucleoprotein-mediated co-editing and counterselection in the rice blast fungus. Sci Rep 8:14355

Gao H, Smith J, Yang M, Jones S, Djukanovic V, Nicholson MG, West A, Bidney D, Falco SC, Jantz D, Lyznik LA (2010) Heritable targeted mutagenesis in maize using a designed endonuclease. Plant J 61:176–187

Getnet Z, Husen A, Fetene M, Yemata G (2015) Growth, water status, physiological, biochemical and yield response of stay green sorghum {*Sorghum bicolor* (L.) Moench} varieties - a field trial under drought-prone area in Amhara regional state, Ethiopia. J Agron 14:188–202

Gilbert LA, Larson MH, Morsut L, Liu Z, Brar GA, Torres SE, Stern-Ginossar N, Brandman O, Whitehead EH, Doudna JA, Lim WA (2013) CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. Cell 154:442–451

Gonzalez C, Szurek B, Manceau C, Mathieu T, Séré Y, Verdier V (2007) Molecular and pathotypic characterization of new Xanthomonas oryzae strains from West Africa. Mol Plant-Microbe Interact 20:534–546

Griffiths BS, Caul S, Thompson J, Birch AN, Scrimgeour C, Andersen MN, Cortet J, Messean A, Sausse C, Lacroix B, Krogh PH (2005) A comparison of soil microbial community structure, protozoa and nematodes in field plots of conventional and genetically modified maize expressing the Bacillus thuringiens is CryIAb toxin. Plant Soil 275:135–146

Hirayama T, Shinozaki K (2010) Research on plant abiotic stress responses in the post-genome era: past, present and future. Plant J 61:1041–1052

Huang CH, Shen CR, Li H, Sung LY, Wu MY, Hu YC (2016) CRISPR interference (CRISPRi) for gene regulation and succinate production in cyanobacterium *S. elongatus* PCC 7942. Micro Cell Fact 15:19

Hulsmans S, Rodriguez M, De Coninck B, Rolland F (2016) The SnRK1 energy sensor in plant biotic interactions. Trends Plant Sci 21:648–661

Husen A, Iqbal M, Aref IM (2014) Growth, water status and leaf characteristics of *Brassica carinata* under drought and rehydration conditions. Braz J Bot 37:217–227

Hutin M, Sabot F, Ghesquiere 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 84:694–703

Husen A, Iqbal M, Aref IM (2016) IAA-induced alteration in growth and photosynthesis of pea (*Pisum sativum* L.) plants grown under salt stress. J Environ Biol 37:421–429

Husen A, Iqbal M, Aref IM (2017) Plant growth and foliar characteristics of faba bean (*Vicia faba* L.) as affected by indole-acetic acid under water-sufficient and water-deficient conditions. J Environ Biol 38:179–186

Husen A, Iqbal M, Sohrab SS, Ansari MKA (2018) Salicylic acid alleviates salinity caused damage to foliar functions, plant growth and antioxidant system in Ethiopian mustard (*Brassica carinata* a. Br.). Agric Food Secur 7:44

Husen A, Iqbal M, Khanum N, Aref IM, Sohrab SS, Meshresa G (2019) Modulation of salt-stress tolerance of Niger (*Guizotia abyssinica*), an oilseed plant, by application of salicylic acid. J Environ Biol 40:94–104

Hussein M, Embiale A, Husen A, Aref IM, Iqbal M (2017) Salinity-induced modulation of plant growth and photosynthetic parameters in faba bean *(Vicia faba*) cultivars. Pak J Bot 49:867–877

ISAAA (2020) ISAAA. [Online]. [12 July 2020]. Available from: http://www.isaaa.org/mailchimp/

Jain M (2015) Function genomics of abiotic stress tolerance in plants: a CRISPR approach. Front Plant Sci 6:5

Jiang W, Zhou H, Bi H, Fromm M, Yang B, Weeks DP (2013) Demonstration of CRISPR/Cas9/sgRNA-mediated targeted gene modification in Arabidopsis, tobacco, sorghum and rice. Nucleic Acids Res 41:188

Kapusi E, Corcuera-Gómez M, Melnik S, Stoger E (2017) Heritable genomic fragment deletions and small indels in the putative ENGase gene induced by CRISPR/Cas9 in barley. Front Plant Sci 8:540

Kim YA, Moon H, Park CJ (2019) CRISPR/Cas9-targeted mutagenesis of Os8N3 in rice to confer resistance to *Xanthomonas oryzae* pv. *oryzae*. Rice 12:1–13

Kis A, Hamar É, Tholt G, Bán R, Havelda Z (2019) Creating highly efficient resistance against wheat dwarf virus in barley by employing CRISPR/Cas9 system. Plant Biotechnol J 17:1004–1006

Kumar S, Muthusamy SK, Mishra CN, Gupta V, Venkatesh K (2018) Importance of genomic selection in crop improvement and future prospects. In: Advanced molecular plant breeding: meeting the challenge of food security. CRC, Boca Raton

Lal SK, Kumar S, Sheri V, Mehta S, Varakumar P, Ram B, Borphukan B, James D, Fartyal D, Reddy MK (2018) Seed priming: an emerging technology to impart abiotic stress tolerance in crop plants. In: Advances in seed priming. Springer, Singapore, pp 41–50

Langner T, Kamoun S, Belhaj K (2018) CRISPR crops: plant genome editing toward disease resistance. Annu Rev Phytopathol 56:479–512

Li T, Liu B, Spalding MH, Weeks DP, Yang B (2012) High-efficiency TALEN-based gene editing produces disease-resistant rice. Nat Biotechnol 30:390

Li J, Pu L, Han M, Zhu M, Zhang R, Xiang Y (2014) Soil salinization research in China: advances and prospects. J Geogr Sci 24:943–960

Li P, Li YJ, Zhang FJ, Zhang GZ, Jiang XY, Yu HM, Hou BK (2017) The Arabidopsis UDP-glycosyltransferases UGT79B2 and UGT79B3, contribute to cold, salt and drought stress tolerance via modulating anthocyanin accumulation. Plant J 89:85–103

Li S, Shen L, Hu P, Liu Q, Zhu X, Qian Q, Wang K, Wang Y (2019) Developing disease-resistant thermosensitive male sterile rice by multiplex gene editing. J Integr Plant Biol 61:1201–1205
Liang Z, Zhang K, Chen K, Gao C (2014) Targeted mutagenesis in *Zea mays* using TALENs and the CRISPR/Cas system. J Genet Genomics 41:63–68
Liang Y, Han Y, Wang C, Jiang C, Xu J-R (2018) Targeted deletion of the USTA and UvSLT2 genes efficiently in Ustilaginoidea virens with the CRISPR-Cas9 system. Front Plant Sci 9:1–11
Liu W, Yuan JS, Stewart CN Jr (2013) Advanced genetic tools for plant biotechnology. Nat Rev Genet 14:781
Lo A, Qi L (2017) Genetic and epigenetic control of gene expression by CRISPR–Cas systems. F1000Res 6:F1000 Faculty Rev-747
Lou D, Wang H, Liang G, Yu D (2017) OsSAPK2 confers abscisic acid sensitivity and tolerance to drought stress in rice. Front Plant Sci 8:993
Ma J, Chen J, Wang M, Ren Y, Wang S, Lei C, Cheng Z (2018) Disruption of OsSEC3A increases the content of salicylic acid and induces plant defense responses in rice. J Exp Bot 69:1051–1064
Macovei A, Sevilla NR, Cantos C, Jonson GB, Slamet-Loedin I, Čermák T, 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 16:1918–1927
Mahfouz MM (2017) Genome editing: the efficient tool CRISPR–Cpf1. Nat Plants 3:1–2
Mehta S, James D, Reddy MK (2019a) Omics technologies for abiotic stress tolerance in plants: current status and prospects. In: Recent approaches in omics for plant resilience to climate change. Springer, Cham, pp 1–34
Mehta S, Singh B, Dhakate P, Rahman M, Islam MA (2019b) Rice, marker-assisted breeding, and disease resistance. In: Disease resistance in crop plants. Springer, Cham, pp 83–111
Mehta S, Lal SK, Sahu KP, Venkatapuram AK, Kumar M, Sheri V, Varakumar P, Vishwakarma C, Yadav R, Jameel MR, Ali M, Achary VMM, Reddy MK (2020) CRISPR/Cas9-edited rice: a new frontier for sustainable agriculture. In: New frontiers in stress management for durable agriculture. Springer, Singapore, pp 427–458
Metje-Sprink J, Menz J, Modrzejewski D, Sprink T (2019) DNA-free genome editing: past, present and future. Front Plant Sci 9:1957
Miller JC, Holmes MC, Wang J, Guschin DY, Lee YL, Rupniewski I, Beausejour CM, Waite AJ, Wang NS, Kim KA, Gregory PD (2007) An improved zinc-finger nuclease architecture for highly specific genome editing. Nat Biotechnol 25:778–785
Mittler R (2006) Abiotic stress, the field environment and stress combination. Trends Plant Sci 11:15–19
Mittler R, Finka A, Goloubinoff P (2012) How do plants feel the heat? Trends Biochem Sci 37:118–125
Monsur MB, Shao G, Lv Y, Ahmad S, Wei X, Hu P, Tang S (2020) Base editing: the ever expanding Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) tool kit for precise genome editing in plants. Genes 11:1–15
Montagne L, Raimondo A, Delobel B, Duban-Bedu B, Noblet FS, Dechaume A, Bersten DC, Meyre D, Whitelaw ML, Froguel P, Bonnefond A (2014) Identification of two novel loss-of-function SIM1 mutations in two overweight children with developmental delay. Obesity 22:2621–2624
Nalam VJ, Alam S, Keereetaweep J, Venables B, Burdan D, Lee H, Shah J (2015) Facilitation of Fusarium graminearum infection by 9-lipoxygenases in Arabidopsis and wheat. Mol Plant-Microbe Interact 28:1142–1152
Nesterova N, Pareniuk O, Illienko V, Ruban Y, Shavanova K, Shpyrka N (2019) Physiological reactions in cereals family *Avena Sativa L.* and *Avena Nuda L* caused by low-temperature stress factors. In: 2019 IEEE 39th international conference on electronics and nanotechnology (ELNANO). IEEE, pp 502–506
Nguyen TH, Mai HTT, Moukouanga D, Lebrun M, Bellafiore S, Champion A (2020) CRISPR/Cas9-mediated gene editing of the Jasmonate biosynthesis OsAOC gene in rice. In: Champion

A, Laplaze L (eds) Jasmonate in plant biology. Methods in molecular biology, vol 2085, pp 199–209
Oliva R, Ji C, Atienza-Grande G, Huguet-Tapia JC, Perez-Quintero A, Li T, Auguy F (2019) Broad-spectrum resistance to bacterial blight in rice using genome editing. Nat Biotechnol 37:1344–1350
Osakabe Y, Osakabe K (2017) Genome editing to improve abiotic stress responses in plants. In: Progress in molecular biology and translational science, vol 149. Academic Press, Walthem, pp 99–109
Ouellet T, Balcerzak M, Rocheleau H, Wang L, Wojcik P, Dzwinel W (2013) Comparison of fusarium head blight-resistant and-susceptible wheat using global expression profiling. In: Proceedings of the 7th international Triticeae symposium, Chengdu
Piatek A, Ali Z, Baazim H, Li L, Abulfaraj A, Al-Shareef S, Aouida M, Mahfouz MM (2015) RNA-guided transcriptional regulation in planta via synthetic dC as9-based transcription factors. Plant Biotechnol J 13:578–589
Pommerrenig B, Ludewig F, Cvetkovic J, Trentmann O, Klemens PA, Neuhaus HE (2018) In concert: orchestrated changes in carbohydrate homeostasis are critical for plant abiotic stress tolerance. Plant Cell Physiol 59:1290–1299
Prasad PV, Bheemanahalli R, Jagadish SK (2017) Field crops and the fear of heat stress—opportunities, challenges and future directions. Field Crops Res 200:114–121
Qi LS, Larson MH, Gilbert LA, Doudna JA, Weissman JS, Arkin AP, Lim WA (2013) Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. Cell 15:1173–1183
Rahman M, Sultana S, Nath D, Kalita S, Chakravarty D, Mehta S, Wani SH, Islam MA (2019) Molecular breeding approaches for disease resistance in sugarcane. In: Disease resistance in crop plants. Springer, Cham, pp 131–155
Raitskin O, Schudoma C, West A, Patron NJ (2019) Comparison ofefficiency and specificity of CRISPR-associated (Cas) nucleases in plants: an expanded toolkit for precision genome engineering. PLoS One 14:e0211598
Ran Y, Patron N, Kay P, Wong D, Buchanan M, Cao YY, Sawbridge T, Davies JP, Mason J, Webb SR, Spangenberg G (2018) Zinc finger nuclease-mediated precision genome editing of an endogenous gene in hexaploid bread wheat (*Triticum aestivum L.*) using a DNA repair template. Plant Biotechnol J 16:2088–2101
Rejeb IB, Pastor V, Mauch-Mani B (2014) Plant responses to simultaneous biotic and abiotic stress: molecular mechanisms. Plan Theory 3:458–475
Rodziewicz P, Swarcewicz B, Chmielewska K, Wojakowska A, Stobiecki M (2014) Influence of abiotic stresses on plant proteome and metabolome changes. Acta Physiol Plant 36:1–19
Sander JD, Joung JK (2014) CRISPR/Cas systems for editing, regulating and targeting genomes. Nat Biotechnol 32:347–355
Sasano Y, Nagasawa K, Kaboli S, Sugiyama M, Harashima S (2016) CRISPR-PCS: a powerful new approach to inducing multiple chromosomes splitting in *Saccharomyces cerevisiae*. Sci Rep 6:1–11
Schuster M, Schweizer G, Reissmann S, Kahmann R (2016) Genome editing in Ustilago maydis using the CRISPR-Cas system. Fungal Genet Biol 89:3–9
Shan Q, Wang Y, Li J, Zhang Y, Chen K, Liang Z, Gao C (2013) Targeted genome modification of crop plants using a CRISPR/Cas system. Nat Biotechnol 31:686–688
Shan Q, Wang Y, Li J, Gao C (2014) Genome editing in rice and wheat using the CRISPR/Cas system. Nat Protoc 9:2395–2410
Sharma P, Sharma MMM, Patra A, Vashisth M, Mehta S, Singh B, Tiwari M, Pandey V (2020) The role of key transcription factors for cold tolerance in plants. In: Transcription factors for abiotic stress tolerance in plants. Academic Press, London, pp 123–152
Shen C, Que Z, Xia Y, Tang N, Li D, He R, Cao M (2017) Knock out of the annexin gene OsAnn3 via CRISPR/Cas9-mediated genome editing decreased cold tolerance in rice. J Plant Biol 60:539–547

Shi J, Gao H, Wang H, Lafitte HR, Archibald RL, Yang M, Hakimi SM, Mo H, Habben JE (2017) ARGOS 8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. Plant Biotechnol J 15:207–216

Siddiqi KS, Husen A (2017) Plant response to strigolactones: current developments and emerging trends. App Soil Ecol 120:247–253

Siddiqi KS, Husen A (2019) Plant response to jasmonates: current developments and their role in changing environment. Bull Nat Res Cent 43:153

Sindhu M, Kumar A, Yadav H, Chaudhary D, Jaiwal R, Jaiwal PK (2019) Current advances and future directions in genetic enhancement of a climate resilient food cereal crop, cowpea (*Vigna unguiculata L. Walp.*). Plant Cell Tissue Organ Cult 139:429–453

Singh B, Mehta S, Tiwari M, Bhatia S (2018) Legume breeding for fungal resistance: a lesson to learn. In: Molecular approaches for plant improvement. Kalpaz Publication, New Delhi, pp 159–180

Singh B, Mehta S, Aggarwal SK, Tiwari M, Bhuyan SI, Bhatia S, Islam MA (2019) Barley, disease resistance, and molecular breeding approaches. In: Disease resistance in crop plants. Springer, Cham, pp 261–299

Stella S, Alcón P, Montoya G (2017) Structure of the Cpf1 endonuclease R-loop complex after target DNA cleavage. Nature 546:559–563

Suzuki N, Devireddy AR, Inupakutika MA, Baxter A, Miller G, Song L, Shulaev E, Azad RK, Shulaev V, Mittler R (2015) Ultra-fast alterations in mRNA levels uncover multiple players in light stress acclimation in plants. Plant J 84:760–772

Svitashev S, Young JK, Schwartz C, Gao H, Falco SC, Cigan AM (2015) Targeted mutagenesis, precise gene editing, and site-specific gene insertion in maize using Cas9 and guide RNA. Plant Physiol 169:931–945

Tang X, Lowder LG, Zhang T, Malzahn AA, Zheng X, Voytas DF, Zhong Z, Chen Y, Ren Q, Li Q, Kirkland ER (2017) A CRISPR–Cpf1 system for efficient genome editing and transcriptional repression in plants. Nat Plants 3:1–5

Thakur P, Kumar S, Malik JA, Berger JD, Nayyar H (2010) Cold stress effects on reproductive development in grain crops: an overview. Environ Exp Bot 67:429–443

USDA (2017). https://www.aphis.usda.gov/biotechnology/downloads/reg_loi/17-076-01_air_inquiry_a1_cbidel.pdf

Vahisalu T, Puzõrjova I, Brosché M, Valk E, Lepiku M, Moldau H, Pechter P, Wang YS, Lindgren O, Salojärvi J, Loog M (2010) Ozone-triggered rapid stomatal response involves the production of reactive oxygen species, and is controlled by SLAC1 and OST1. Plant J 62:442–453

Vainonen JP, Kangasjärvi J (2015) Plant signalling in acute ozone exposure. Plant Cell Environ 38:240–252

Verdier V, Cruz CV, Leach JE (2012) Controlling rice bacterial blight in Africa: needs and prospects. J Biotechnol 159:320–328

Wallace JS, Acreman MC, Sullivan CA (2003) The sharing of water between society and ecosystems: from conflict to catchment–based co–management. Philos T R Soc B: Biol Sci 358:2011–2026

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

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

Wang J, Tian D, Gu K, Yang X, Wang L, Zeng X, Yin Z (2017) Induction of Xa10-like genes in rice cultivar Nipponbare confers disease resistance to rice bacterial blight. Mol Plant Microbe Interact 30:466–477

Wang M, Wang S, Liang Z, Shi W, Gao C, Xia G (2018a) From genetic stock to genome editing: gene exploitation in wheat. Trends Biotechnol 36:160–172

Wang W, Pan Q, He F, Akhunova A, Chao S, Trick H, Akhunov E (2018b) Transgenerational CRISPR/Cas9 activity facilitates multiplex gene editing in allopolyploid wheat. CRISPR J 1:65–74

Wei C, Liu J, Yu Z, Zhang B, Gao G, Jiao R (2013) TALEN or Cas9–rapid, efficient and specific choices for genome modifications. J Genet Genomics 40:281–289

Wheeler T, Von Braun J (2013) Climate change impacts on global food security. Science 341:508–513

Wu X, Kriz AJ, Sharp PA (2014) Target specificity of the CRISPR/Cas9 system. Quant Biol 2:59–70

Xie K, Yang Y (2013) RNA-guided genome editing in plants using a CRISPR–Cas system. Mol Plant 6:1975–1983

Xing HL, Dong L, Wang ZP, Zhang HY, Han CY, Liu B, Wang XC, Chen QJ (2014) A CRISPR/Cas9 toolkit for multiplex genome editing in plants. BMC Plant Biol 14:327

Xu H, Xiao T, Chen CH, Li W, Meyer CA, Wu Q, Wu D, Cong L, Zhang F, Liu JS, Brown M, Liu XS (2015) Sequence determinants of improved CRISPR sgRNA design. Genome Res 25:1147–1157

Yin K, Gao C, Qiu JL (2017) Progress and prospects in plant genome editing. Nat Plants 3:1–6

Zetsche B, Gootenberg JS, Abudayyeh OO, Slaymaker IM, Makarova KS, Essletzbichler P, Volz SE, Joung J, Van Der Oost J, Regev A, Koonin EV (2015) Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR/Cas system. Cell 163:759–771

Zhang Y, Liang Z, Zong Y, Wang Y, Liu J, Chen K, Gao C (2016) Efficient and transgene-free genome editing in wheat through transient expression of CRISPR/Cas9 DNA or RNA. Nat Commun 7:1–8

Zhang Y, Bai Y, Wu G, Zou S, Chen Y, Gao C, Tang D (2017) Simultaneous modification of three homoeologs of Ta EDR 1 by genome editing enhances powdery mildew resistance in wheat. Plant J 91:714–724

Zhang A, Liu Y, Wang F, Li T, Chen Z, Kong D, Bi J, Zhang F, Luo X, Wang J, Tang J (2019a) Enhanced rice salinity tolerance via CRISPR/Cas9-targeted mutagenesis of the OsRR22 gene. Mol Breed 39:47

Zhang R, Liu J, Chai Z, Chen S, Bai Y, Zong Y, Chen K, Li J, Jiang L, Gao C (2019b) Generation of herbicide tolerance traits and a new selectable marker in wheat using base editing. Nat Plants 5:480–485

Zhang J, Zhang X, Chen R, Yang L, Fan K, Liu Y, Wang G, Ren Z, Liu Y (2020) Generation of transgene-free semi dwarf maize plants by gene editing of Gibberellin-Oxidase20-3 using CRISPR/Cas9. Front Plant Sci 11:1–9

Zhou J, Peng Z, Long J, Sosso D, Liu B, Eom JS, White FF (2015) Gene targeting by the TAL effector PthXo2 reveals cryptic resistance gene for bacterial blight of rice. Plant J 82:632–643

Zhou X, Liao H, Chern M, Yin J, Chen Y, Wang J, Zhu X, Chen Z, Yuan C, Zhao W, Wang J (2018) Loss of function of a rice TPR-domain RNA-binding protein confers broad-spectrum disease resistance. PNAS 115:3174–3179

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