Journal of Soil Science and Plant Nutrition
https://doi.org/10.1007/s42729-024-01778-x

REVIEW

# CRISPR-Cas9 System Mediated Genome Editing Technology: An Ultimate Tool to Enhance Abiotic Stress in Crop Plants

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Received: 30 January 2024 / Accepted: 9 April 2024


**Abstract**
The drastic rise in the human population globally might uplift the issue of food scarcity in the coming few decades. This problem could affect the agricultural sector entirely, and to set targets for uplift, major issues like climate change and environmental stresses should be fixed for possible high crop production. To develop highly productive and resistant varieties using old traditional methods is now a waste of time, and fast practices like the use of genome editing tools are required. Among all the technological tools, CRISPR-Cas9 is the most precise, productive, and quickest system, with extensive usage to resist biotic and abiotic stresses. This technique has direct or indirect influence over quantitative genes to withstand abiotic shocks. More than 20 crops have been modified using CRISPR-Cas tools to withstand stresses and improve yield. Researchers are using CRISPR/Cas-based genome editing to improve staple crops for biotic and abiotic stress resistance and improved nutritional quality.Irrespective of rules regarding genetically modified organisms, CRISPR/Cas9 insert genes through agro-infiltration, viral infection, or preassembled Cas9 protein-sgRNA ribonucleoprotein transformation in crops without transgenic impression. Certain undesirable genes that result in starch degradation and maltose amassing were deleted by using CRISPR to reduce cold sensitivity. Precise noxious ion and metal removal from roots and their effective counterbalancing in protoplast notions to distant structures could also be managed through gene editing tools. Spindly gene knockout creates stress-tolerant (drought and salt) plants. Researchers can make cost-effective use of CRISPR technology in multiple sectors. The global population needs to be fed as climate change has severely affected food security, which could be overcome in the future through advancements in CRIPSR technology.

**Keywords** CRISPR/Cas9 · Cold stress · Drought stress · Heat stress · Heavy metal stress · Herbicide stress · Gene knockout

## 1 Introduction

With the continuous rise of the world population (60%), there will be a scarcity of food, and climate change has adversely affected it (Zafar et al. 2020), and that has put more pressure on the agriculture sector (Suweisa et al. 2015). Besides abiotic stresses (salinity, drought, toxicity of heavy metals, and hot temperatures) and climate change, humans (harmful activities) have also had adverse effects on cultivated regions (Zafar et al. 2020). Both types of stresses (biotic and abiotic) have minimized (70%) food production (Ahmad et al. 2020). Therefore, more advanced and resistant crops should be developed against such stresses to overcome food shortages (Driedonks et al. 2016) and enhanced up to 70–85% as an estimated increase of 9.7 billion could occur in the population by 2050 (Zaidi et al. 2016). Traditional actions take more time to develop resistant crops (Adeyinka et al. 2023). So, it is needed to adopt such genetic tools (i.e., genome editing) for crop improvement and to overcome stresses (Driedonks et al. 2016). Besides accepting GM crops (genetically modified, stress-resistant and nutritive) in certain pockets of the world (Shukla et al. 2018), it has developed several crops known as golden rice and Bt-cotton that represent the gene revolution (Napier et al. 2019).

Several tools and the three main divisions of CRISPR (meganucleases, zinc finger nucleases (ZFNs), transcription

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Published online: 24 April 2024

activator like nucleases (TALENs), CRISPR-Cas9, and clustered regularly interspaced short palindromic repeats) have been developed for gene editing (deletion, insertion, or changing a particular pattern of DNA), especially in plants (Kumar et al. 2023) or the *4OMT2* gene knocking out in *Papaver somniferum* (regulating the biosynthetic pathway of benzylisoquinoline alkaloids) (Alagoz et al. 2016). Among them, CRISPR-Cas9 (productive, quickest, and most precise) has wide usage (acquired from the natural resistance system found in bacteria against virus attacks), which identifies and cleaves the corresponding DNA pattern along with CAS enzymes (Chen et al. 2019a, b). This technique has direct or indirect influence over quantitative genes to withstand abiotic shocks (Mushtaq et al. 2018; Hossain et al. 2022). The approach of foreign DNA will enhance social acceptance rates. Commercially, tomatoes with more γ-aminobutyric acid than typical ones are available in Japan (edited by CRISPR-Cas9) (Waltz 2022). This technology enhanced the activities of stress tolerant genes and increased production (Zafar et al. 2020).

In this review, we summarize most potential applications of the CRISPR/Cas9-mediated genome editing approach in crop plants aimed at managing abiotic stresses such as drought, salinity, cold, heavy metal, and heat resistance in sustainable agriculture, and discuss the advantages, and limitations.

## 2 Background History of CRISPR/Cas9

Prokaryotes genomes (bacterial and archaeal) have 40–90% CRISPR to survive against attackers through defence mechanisms (Gajardo et al. 2023). In mid-1980, editing of genes was initiated, and several crops (wheat, rice, cotton, maize, potato, tomato, and vice versa) were developed (Nascimento et al. 2023). Beside the extended period taken by zinc finger nucleases (ZFNs) and termed TAL effector nucleases (TALENs) to target DNA binding proteins (Ceasar et al. 2016), TALENs, ZFNs, and CRISPR-Cas9 are very fruitful against numerous stresses (Nongpiur et al. 2016). Addition and removal of a particular pattern happen by the NHEJ (non-homologous end-joining) repair pathway (Fig. 1), where double strand breaks (DSBs) form from cleavage by order-particular nucleases after pattern detection and are remoulded by non-homologous end joining (NHEJ) and HR (homologous recombination) (Belhaj et al. 2015). Restrictions of DNA binding proteins (ZFNs and TALENs) were identified due to CRISPR's beginning (Kumar et al. 2023). There are two components of the CRISPR-Cas9 structure (namely CRISPR RNAs and Cas9 proteins). GM crop production and plant function could be described by CRISPR/Cas9 (Tripathi et al. 2020).

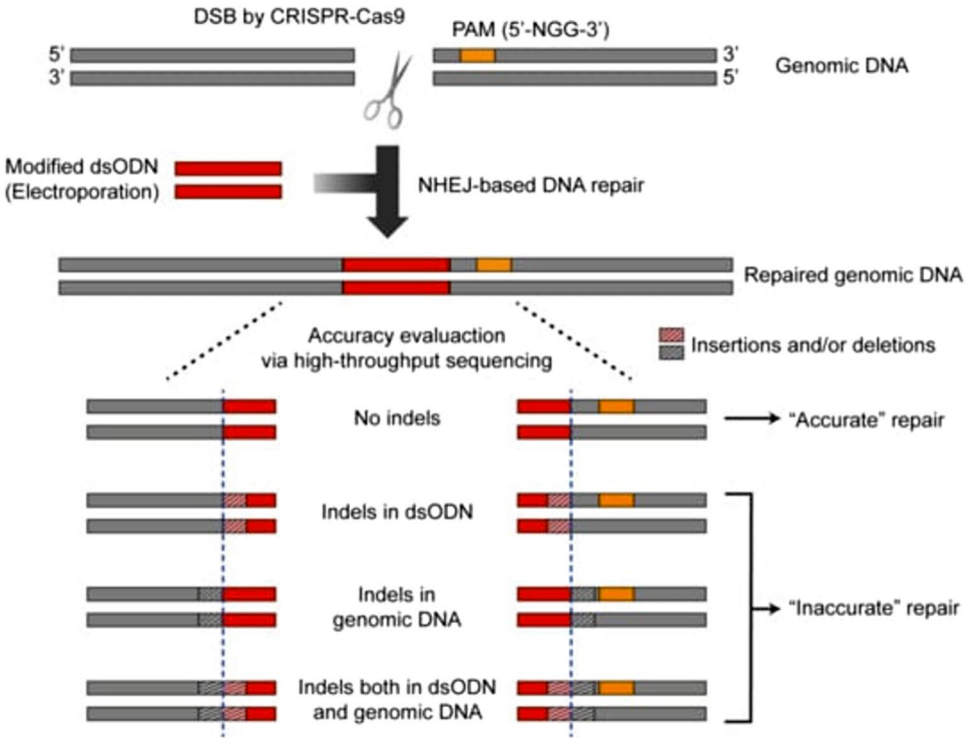


**Fig. 1** Procedure of CRISPR-Cas9 (driven by RNA) producing DSB in the required pattern of DNA and its suitable renovation paths (HDR and NHEJ). Double strand breaks (DSBs), protospacer adjacent motif (PAM), non-homologous end joining (NHEJ), homology-directed repair (HDR), non-homologous double-stranded oligodeoxynucleotide (dsODN)

## 3 CRISPR-Cas9 Tool: A RNA-Directed Nuclease for Genes Editing

Functions and the beginning of details about CRISPR/Cas9's modular nature for genome editing have been provided in several reviews (Zhong et al. 2024). Target DNA (through Watson–Crick base pairing) could be detected by Cas9 nuclease-mediated cleavage (sgRNA) (Zhong et al. 2024), and its pattern (5′-N) could be altered through Cas9 nuclease. sgRNAs (20–22 nucleotides; small size) are oligonucleotides, which represents their high effectiveness without any issues, and the presence of protospacer adjacent motif (PAM), NGG, or NAG site immediately 3′ is necessary for a targeted spot. So, CRISPR-Cas9 has a highly advanced role in plant biology due to its efficiency in targeting various genes.

## 4 Components of CRISPR Gene Editing System

Small guide RNA (sgRNA) and Cas9 nuclease are the two key constituents of the CRISPR-Cas system represented in Fig. 2.

### 4.1 sgRNA (Guide RNA)

It consists of two RNA parts along the Cas9 genes known as small CRISPR RNA (crRNA) (orders non-coding RNA constituents) and trans-activating CRISPR RNA (tracrRNA). Targeting pattern and cleaved position are detected by crRNA, while their arrangements are supported by tracrRNA (artificially bonded), and sgRNA has more efficiency than the other two due to further advancements. While minimizing off target influences, truRNA (transcribed guide RNAs) has a great role in Cas9 upgrading (Fu et al. 2014). A synthetic gene (Ribozyme RGR) develops the required gRNA through self-catalyzing cleavage (RNA molecules formed by ribozyme patterns) (Gao and Zhao 2014). As this synthetic gene is easily defined by the promoter, it provides quick details about sudden changes and gene editing, while various targets could be achieved by polycistronic tRNA-gRNA (composed of tRNA-gRNA with various spacer patterns) (Xie et al. 2015).

### 4.2 Cas9 Nuclease

sgRNA has two smaller RNAs (crRNA and tracrRNA) bent together to efficiently target outsider DNA through Cas9 (Li et al. 2024). Through two pathways, non-homologous end joining (NHEJ) and homology-directed repair (HDR), CRISPR-Cas9 shows immunity in cells (Gaj et al. 2013) and is helpful in gene alteration at a particular point. DSB fusion occurs through NHEJ (either removing or adding nucleotides) (Cong et al. 2013). Undeveloped proteins denature (not workable) as a result of frame shift changes (if editing of nucleotides is not divisible by 3). DSBs (double stranded breaks) are formed by the Cas9 protein (cut DNA), which further forms two sections (α-helical identifying lobe known as REC and a NUC called the nuclease lobe) with target DNA and sgRNA along the CTD (C-terminal domain), HNH, and RuvC spheres. There are 3-α helix domain (Hel-I, Hel-II, and Hel-III) in the REC section. Cas-9 particular binds (which consist of PAM points) are defined by CTD. HNH nuclease has ββα-metal fold carrying a 1-metal ion cleavage methodology, while RNase H and HNH nuclease are alike and possess 2-metal ion catalytic procedures. Thus,

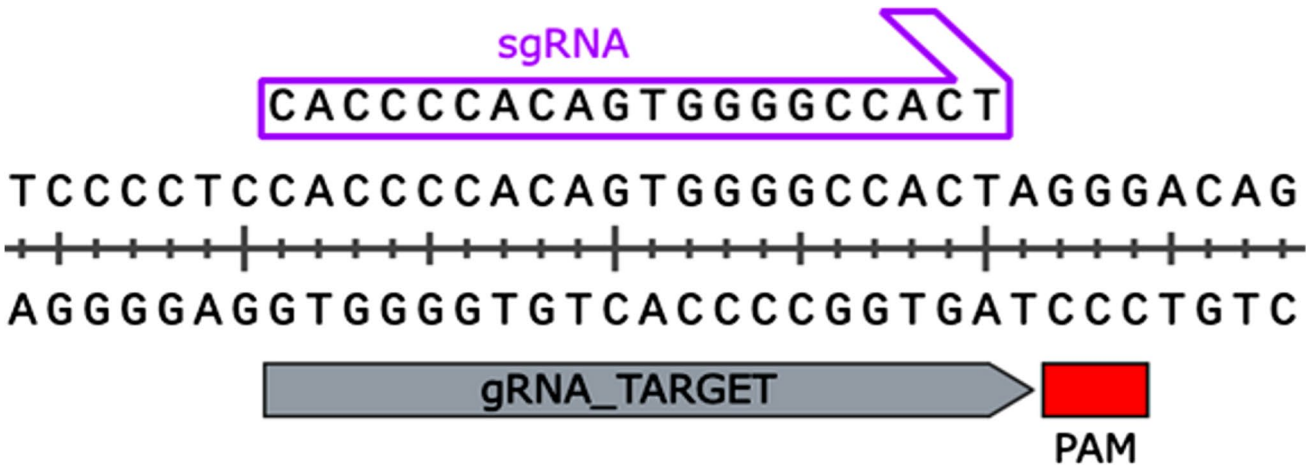


**Fig. 2** DNA sequence. The gRNA Target (grey box) next to it there is the PAM (GGG, N can be A/T /G orC) and then the single guide RNA (sgRNA) as a blue arrow. Protospacer adjacent motif (PAM)

the innate Cas9/sgRNA approach is used in crops for gene editing and different gene identification techniques (Li et al. 2018a, b).

## 5 Concepts and Mechanisms of CRISPR-Cas9 Genome Editing Technology

Islam (2019) describes all aspects of CRISPR. Gene patterning in *Escherichia coli* (alkaline phosphatase isozyme) causing amino peptidase transformation was done by Japanese scientists (in the late 1980s), who observed an irregular downward gene replication pattern. The CRISPR name was highly accepted by researchers due to its abbreviation, which openly defines recurrence features (Jansen et al. 2002), and the association of CRISPR was shown with conserved CRISPR (CRISPR-associated genes; Cas), while its absence was observed in species without CRISPR units. These genes (cas1–cas4) were found scattered in CRISPR loci, showing CRISPR's beginning (Jansen et al. 2002). Similarly, relative gene inquiry (archaea and bacteria) also noted these genes (encoding nucleases and helicases) presence, which represents their role in DNA metabolism (Makarova et al. 2002). Such proteins, known as RAMPs (Makarova et al. 2002), have repairing abilities connected with CRISPR arrangement (Haft et al. 2005).

Due to compatibility between host and foreign DNA, and also due to RNAi (eukaryotic RNA interference), CRISPR can split DNA and survive against contagion (through the RNA guide method) (Makarova et al. 2006). Cas9 nuclease work under the type I CRISPR locus (*Escherichia coli*) shifts to minute crRNAs with sole parts (Brouns et al. 2008). DNA is directed by Cas nuclease in *Staphylococcus epidermidis* rather than RNA, as CRISPR restricts plasmid conjugation (Marraffini and Sontheimer 2008). PAM (pattern motif at CRISPR end) (Mojica et al. 2009) directed Cas9-facilitated inference (Deveau et al. 2008). In *Pyrococcus furiosus,* target RNA has a role in splitting (via type III-B CRISPR-Cas inquiry) (Hale et al. 2009, 2012). Actual details about CRISPR were found in 2010. The CRISPR-Cas9 system in S. thermophilus causes DNA double-strand breaks (DSBs) on the PAM (3-bp location of DNA), which results in the appearance of new microbial strains (Garneau et al. 2010).

*Streptococcus pyogenes* extra-minute RNA (tracrRNA) was also seen to create settled crRNA (through CRISPR interference) (Deltcheva et al. 2011). CRISPR-Cas loci (*S. thermophilus*) were inserted in *E. coli* to successfully create an immune response (Sapranauskas et al. 2011). During 2012, CRISPR was described as being copied into RNA following splitting into Cas protein-led dsDNA due to RNA (Gasiunas et al. 2012). CrRNA binding along with tracrRNA led to sgRNA formation and DNA cleavage (Gasiunas et al. 2012). Strategies of CRISPR-Cas9 have been efficiently utilized in many valuable agronomical crop plants (Fig. 3).

Three stages of CRISPR-Cas9 (type II) methodology observed in bacteria towards protection have been displayed in Fig. 4.

### 5.1 Adaptation or Spacer Acquisition

Adaptation is composed of protospacer assortment and spacer assimilation (analyzing new recurrence). Nowrousian et al. (2010). The needed pattern is 5′-NGG-3′ (kinds I and II methods).

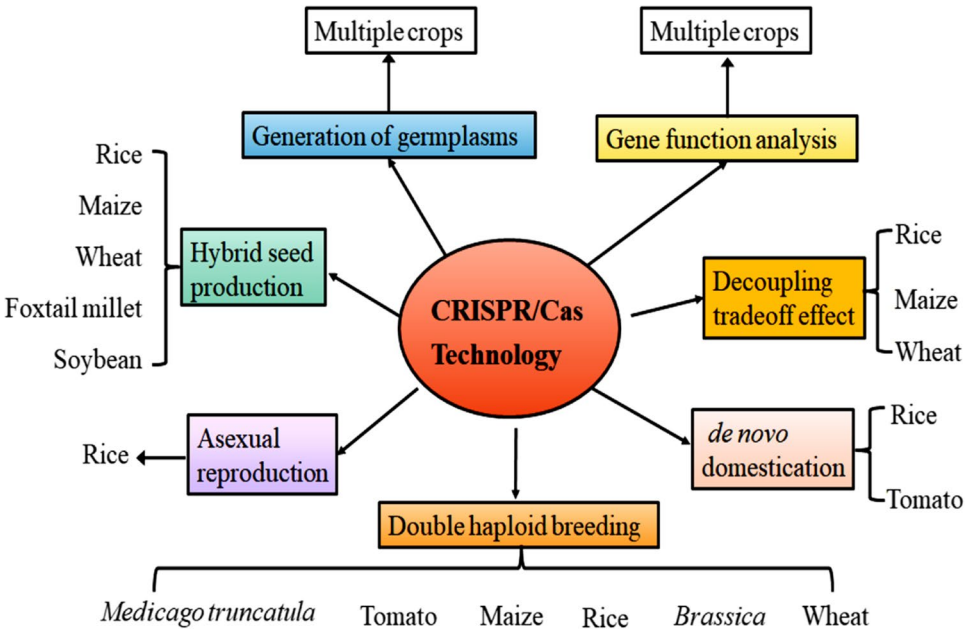


**Fig. 3** Strategies of CRISPR-Cas9 have been efficiently utilized in many valuable agronomical crop plants

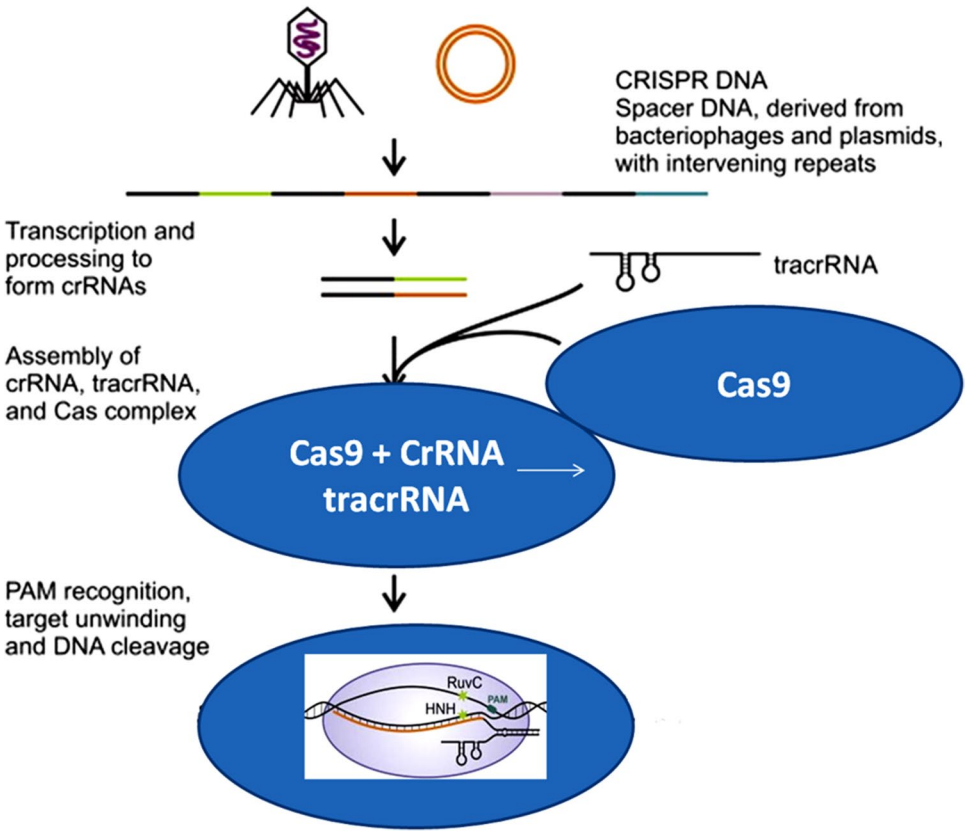


**Fig. 4** The CRISPR-associated (Cas) system in bacteria plays a natural role in the clustered regularly interspaced short palindromic repeats (CRISPR) system. Protospacer adjacent motif (PAM), small CRISPR RNA (crRNA), trans-activating CRISPR RNA (tracrRNA)

### 5.2 Handling crRNA and Maturation or Biogenesis

Biogenesis (pre-crRNA processing with various Cas proteins) of transcript tracrRNA led to mature crRNA from premature forms and crRNA-tracrRNA duplex making (Ma et al. 2016). CRISPR-associated complexes are formed by Cas proteins fusing crRNA to attach to the required DNA.

### 5.3 Interference

Immunity against outside outbreaks became active when cRNA led the Cas enzyme complex to detect a corresponding pattern. So, inhibition of self-targeting CRISPR occurs with PAM pattern detection (via the crRNA-tracrRNA duplex). As a result of the duplex, Cas9 nuclease fused and split, leading to DSB formation. There should be proper pattern balance (between target and spacer) along the PAM pattern. The Cas9/sgRNA complex, after recognition, attaches to target DNA (resembling sgRNA). DSB (due to 3 bp upward PAM) form which gets recovered by host restoration methodology, otherwise useless genes due to NHEJ and HDR.

## 6 CRISPR-Cas Based Gene Mutagenesis

Several hardens, like trait complexity and useful gene categorization, have limited the development of crops against abiotic stresses (Scheben et al. 2017), such as < 1% useful gene identification in maize (Andorf et al. 2015). Certain genes responsible for indicating, transcription elements, membrane shielding, and secondary metabolites have been studied, while numerous traits (such as post translational and transcriptional elements, protein-DNA, and protein–protein connections) are not described, which are needed to understand while developing crops. Two methods, random T-DNA mutations and confined cut of genes pointing could be used to adjust abiotic stress-resistance genes (Hussain et al. 2018), but they require more time and labor. Using PAM and sgRNA (target whole genome), CRISPR is working to edit gene patterns (single or multiple) to produce mutations (Sander and Joung 2014), while sgRNA detects plant appearances (via cloning and sequencing) against abiotic stress (used as a binary vector) (Sedeek et al. 2019). Various CRISPR-Cas9 benefits (pointed and biallelic changes, fast gene finding, and gene multiplexing) have been observed over other mutations (chemical and T-DNA). Abiotic stress

resistance using various variants (Cas9 and Cas12a) could be created (Zetsche et al. 2015), especially in transgene-free edited plants using CRISPR/Cas9. CRISPR/Cas9 (after mutation) could be removed through segregation. dCas9 or Cas3 could be used as a result of pleiotropic effects (constant change), and such effects will be minimized while using Cas13a and Cas13b (RNA editing). Climate hardy crops (such as rice) could be developed through CRISPR/Cas (Lu and Zhu 2017).

## 7 Role of CRISPR in Crop Improvement and Growth

Yield declined (42%), especially due to biotic stress (15% global food decline) rather than other factors (Islam et al. 2016). 24 crops have been modified using CRISPR-Cas tools to withstand stresses (Ricroch et al. 2017) and enhance yield (Bhowmik et al. 2019). Numerous gene modifications at once are done by CRISPR-Cas technology (Molla et al. 2020), with various methods to use in crops (Islam et al. 2020), while also solely developing species. Several crops that have developed resistance against abiotic stress through this technique are presented in Fig. 5.

Three genes (brassinosteroid insensitive 1, jasmonate-zim-domain protein 1, and gibberellic acid insensitive) were modified by Feng et al. (2013), while Mao et al. (2013) modified magnesium-chelates subunit I (albinism-related). Similarly, such genes were modified at various points (multiplex CRISPR-Cas9) (Lowder et al. 2017). *PDS3*, *AtFLS2*, *CYCD3*, *RACK1*, and several other genes were also edited (Li et al. 2013). Changes could be different due to various factors (fusing power of sgRNA and chromatic assembly). Germline-causing changes were identified through several methods of floral dip mode of transformation (using tissue-definite agents and terminators) (Wang et al. 2015), such as 50 regulatory patterns worked in such genes (*SPOROCYTELESS*, *DD45*, and *LAT52*) development (Mao et al. 2016). Under high temperatures, a 5 bend (body tissues) and 100 bend (germ tissues) editing rate has been observed (LeBlanc et al. 2018). Production in rice has been improved to make it resistant against various stresses (Islam 2019) by modifying genes (phytoene desaturase (9%), betain aldehyde dehydrogenase, and *OsMPK5),* which was considered a great achievement (Jagodzik et al. 2018). The efficiency of the CRISPS tool was recognized by DSB production in crop cells through sgRNA practice (Miao et al. 2013). Oxygenase and *LAZY* genes have also been edited.

Polyploidy plants represent efficient multiplex gene modification (Wang et al. 2018), which in wheat (ethylene responsive element 3 and dehydration responsive element fusing protein 2) (Kim et al. 2018), led to unique offspring development. Hexaploid wheat also represents gene editing (via appropriate practice) (Bhowmik et al. 2018). Phytoene desaturase gene knockout was done efficiently (40.7%–52.0%) in watermelon (Tian et al. 2017a, b). Furthermore, the *ALS* gene was modified to withstand herbicide toxicity. *GmFT2* (the flowering time-responsible gene) was edited, leading to late flowering irrespective of day length (Cai et al. 2018). *GmFEI2* and *GmSHR* were edited for hairy root system improvement (Cai et al. 2015).

CRISPR-Cas9 is playing a transformative role in enhancing the nutritional value of crops. This application is particularly important given the global nutrition challenges, such as micronutrient deficiencies. CRISPR-Cas9 can be utilized to increase the levels of essential vitamins, minerals, and other beneficial compounds in crops, thereby improving their nutritional profile (Molla et al. 2020). One notable example is biofortification, where crops are genetically engineered to accumulate higher levels of micronutrients (Bhowmik et al. 2019). For instance, CRISPR-Cas9 has been used to increase the iron content in rice, addressing iron deficiency, which is

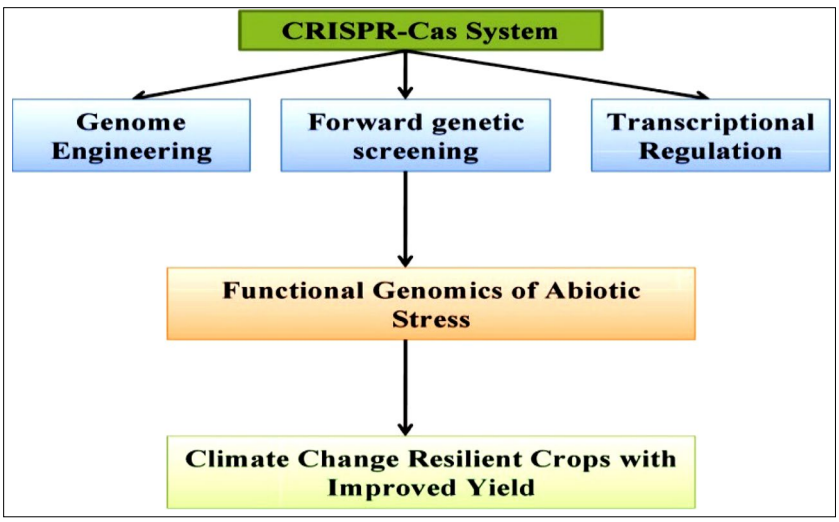


**Fig. 5** Studying multiple aspects through CRISPR-Cas9 to develop stress tolerant crops

a major global health issue. Similarly, the technology can be employed to enhance the levels of vitamins, such as vitamin A, in crops like rice (Golden Rice) and banana, which is crucial for preventing vitamin A deficiency related diseases. In addition to increasing micronutrients, CRISPR-Cas9 can also be used to modify the composition of macronutrients to improve the overall nutritional quality of crops (Caiet al. 2015). This includes increasing the content of essential amino acids, unsaturated fatty acids, and dietary fibers, as well as reducing anti-nutritional factors or allergens. For example, using CRISPR-Cas9 to reduce gluten in wheat can be beneficial for individuals with gluten intolerance or celiac disease (Jouanin et al. 2020).

## 8 Uses of CRISPR/Cas9 for Advancing Crop Genomics

Due to the simplicity of CRISPR-Cas9 (accurate) and its effectiveness, it has been widely used in genetic editing and breeding of crops (exclusive cultivars and successions), rather than traditional breeding procedures. According to Bortesi and Fischer (2015), it plays a significant role in pyramid breeding that employs simultaneous multiplex editing, such as gene elimination by NHEJ.. In addition to CRISPR, other technologies (epigenetic modification and gene expression regulation) play a role in gene editing in agriculture to improve production and resistance to pest infestations.. Irrespective of rules regarding genetically modified organisms, CRISPR/Cas9 inserts genes (by agro-infiltration, viral infection, or preassembled Cas9 protein-sgRNA ribonucleoprotein transformation) in crops without transgenic impression (Woo et al. 2015). Figure 6 shows the CRISPR-Cas9 mechanism for editing crop genes.

## 9 Gene Editing Through CRISPR/Cas9 Influences Production and Plant Survival Against Abiotic Stresses

Crop yield declines as a result of numerous abiotic stresses. Table 1 represents the number of genes activated during abiotic stress along with plant alteration approaches to cope with abiotic stresses. More than 20 crops have been modified against stresses through the CRISPR-Cas system (Ricroch et al. 2017). For example, AB sensitivity in *Arabidopsis* was minimized through the addition of ABA-induced transcription repressors (AITR; adjust feedback of ABA), while declining *AITR genes became* nonfunctional (Tian et al. 2017a, b), leading to survival against abiotic stresses (drought and salinity) (Chen et al. 2021), while being less responsive (salinity) in the case of *AITR5* gene overexpression (Chen et al. 2021). Furthermore, *AITR6* also enhanced tolerance (Chen et al. 2021). Similarly, knocking out 3 genes (*aitr2*, *aitr5*, and aitr6) led to quintuple mutants resulting in enhanced resistivity towards stress (Bhattacharya et al. 2021).

### 9.1 Drought Stress

Plants get sensitive to stress as a result of unbalanced hormone levels, declining antioxidant actions, and higher ROS assembly (Abd El Mageed et al. 2023). Metabolic and signaling molecules build up, such as genes sensitive to low water along with transcription elements, resulting in enhanced plant survival against drought (Santosh Kumar et al. 2020). *AREB1* overexpression makes plants resistant, while its removal makes plants sensitive to drought (Singh and Laxmi 2015). *SlLBD40* (lateral organ boundaries domain transcription factor) knockout helps tomato plants against drought stress tolerance (Liu et al. 2020a, b). Furthermore, *ARGOS8* gene increment declines ethylene activity, making *Zea mays* resistant to drought, leading to high grain yields (Hirai et al. 2007). *WRKY3* and *WRKY4* genes help in drought resistance, while WRKY transcription factors help against both stresses (Li et al. 2021a, b).

According to Chen et al. (2021), tomatoes are more resistant to water deficiency when the tomato Auxin Response Factor (*SlARF4*) gene is knocked down. By converting chromatin to a relaxed state, Arabidopsis histone acetyltransferase 1 (*AtHAT1*) encourages the activation of gene expression. By fusing the CRISPR/dCas9 gene with the Histone Acetyl Transferase (*AtHAT*) gene, Arabidopsis was shown to have improved resistance to drought stress (Roca Paixão et al. 2019). Negative regulators of drought tolerance include the genes for drought-induced SINA protein 1 (*OsDIS1*), drought and salt-tolerant protein 1 (*OsDST*), and ring finger protein 1 (*OsSRFP1*). Reducing the expression of these genes related to drought enhanced the levels of antioxidant enzymes, lowered $H_2O_2$ concentrations, and strengthened rice plants' resistance to drought stress (Santosh Kumar et al. 2020).

Plants' responses to dehydration and ABA signaling are controlled by the Enhanced Response 1 (*ERA1*) protein gene. Ogata et al. (2020) found that altering the *OsERA1* gene in rice resulted in greater tolerance to drought stress. The mutant plants displayed increased sensitivity to ABA and stomatal closure under drought stress conditions.

### 9.2 Salinity Stress

Saline land will be increased in the future (up to 2025), which changes the plant physiology and morphology, leading to adverse changes (leaves shedding and spotting before their time, and severe ion disruption in cells) on plant growth

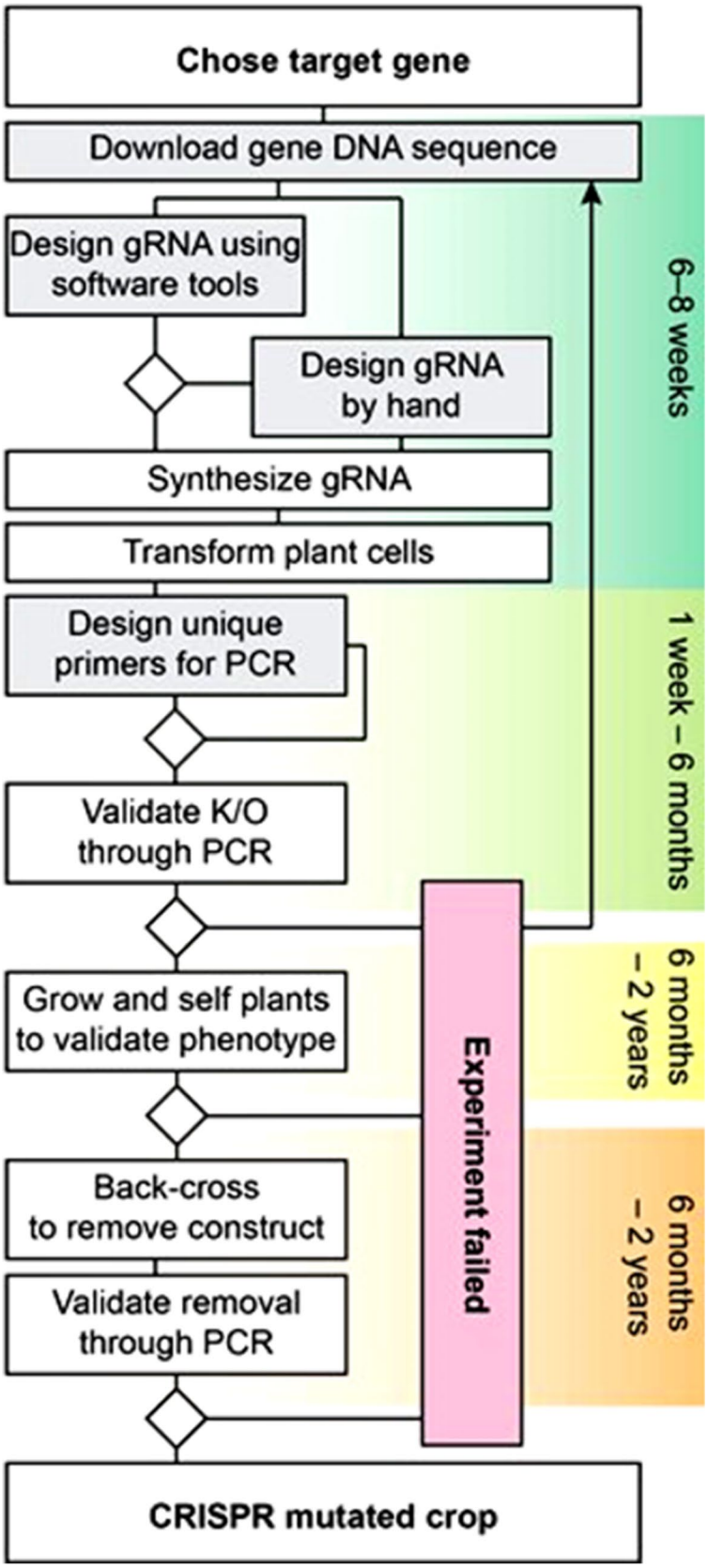


**Fig. 6** Diagram of a typical knockout editing experiment in a crop plant, with associated timeline

**Table 1** CRISPR/Cas induced resistance against abiotic stress

| Stress tolerance | Plant specie | Marked gene | Method of transfer | Reference |
|---|---|---|---|---|
| Herbicide resistance | *Zea mays* | *MS26* | Biolistic-mediated | Svitashev et al. (2015) |
| | *Triticum aestivum* | *TaALS* | Biolistic-mediated | Zhang et al. (2019a, b, c) |
| | *Zea mays* | *ZmALS1, ZmALS2* | PEG-mediated protoplast transformation | Li et al. (2020) |
| | *Oryza sativa* | *ALS* | Agro-bacterium mediated | Sun et al. (2016) |
| | *Oryza sativa* | *PPO1*<br>*HPPD* | PEG-mediated protoplast transformation | Lu et al. (2021) |
| | *Oryza sativa* | *OsTubA2* | Agro-bacterium mediated | Liu et al. (2021) |
| | *Triticum aestivum* | *TaALS-P174* | particle bombardment transformation | Zhang et al. (2019a, b, c) |
| | *Oryza sativa* | *OsPUT1/2/3* | Agro-bacterium mediated | Lyu et al. (2022) |
| | *Oryza sativa* | *OsTB1* | Agro-bacterium mediated | Butt et al. (2020) |
| | *Saccharum officinarum* | *SoALS* | biolistic gene transfer | Oz et al. (2021) |
| | *Solanum lycopersicum* | *SlEPSPS* | Agro-bacterium mediated | Yang et al. (2022) |
| | *Solanum lycopersicum* | *SlALS1, SlALS2* | Agro-bacterium mediated | Yang et al. (2022) |
| | *Brassica napus* | *ALS* | Agro-bacterium mediated | Cheng et al. (2021) |
| | *Citrullus lanatus* | *ALS* | Agro-bacterium mediated | Tian et al. (2018) |
| Cold tolerance | *Arabidopsis thaliana* | *UGT79-B2, and B3* | Agro-bacterium mediated | Li et al. (2017a, b) |
| | *Oryza sativa* | *OsPRP1* | Agro-bacterium mediated | Nawaz et al. (2019) |
| | *Solanum lycopersicum* | *SICBF1* | Agro-bacterium mediated | Li et al. (2018a, b) |
| | *Oryza sativa* | *OsAnn3* | Agro-bacterium mediated | Zafar et al. (2020) |
| | *Oryza sativa* | *OsMYB30* | Agro-bacterium mediated | Zeng et al. (2020) |
| Salt tolerance | *Oryza sativa* | *OsFLN2* | Agro-bacterium mediated | Chen et al. (2020) |
| | *Triticum aestivum* | *TaHAG1* | Agro-bacterium mediated | Zheng et al. (2021) |
| | *Solanum lycopersicum* | *SlARF4* | Agro-bacterium mediated | Bouzroud et al. (2020) |
| | *Solanum lycopersicum* | *SlHyPRP1* | Agro-bacterium mediated | Tran et al. (2021) |
| | *Oryza sativa* | *OsRR22* | Agro-bacterium mediated | Zhang et al. (2019a, b, c) |
| | *Oryza sativa* | *OsDST* | Agro-bacterium mediated | Santosh Kumar et al. (2020) |
| | *Oryza sativa* | *OsbHLH024* | Agro-bacterium mediated | Alam et al. (2022) |
| | *Oryza sativa* | *OsGTγ-2* | Agro-bacterium mediated | Liu et al. (2020a, b) |
| | *Oryza sativa* | *OsmiR535* | Agro-bacterium mediated | Yue et al. (2020) |
| | *Triticum aestivum* | *TaHAG1* | Agro-bacterium mediated | Vlˇcko and Ohnoutková (2020) |
| | *Hordeum vulgare* | *ITPK* | Agro-bacterium mediated | Santosh Kumar et al. (2020) |
| | *Oryza sativa* | *OsRR22* | Agro-bacterium mediated | Han et al. (2022) |
| | *Arabidopsis thaliana* | *AtACQOS* | Agro-bacterium mediated | Kim et al. (2021) |
| | *Medicago Truncatula* | *MtHEN1* | Agro-bacterium mediated | Curtin et al. (2018) |
| Heavy metal tolerance | *Oryza sativa* | *OsATX1* | Agro-bacterium mediated | Zhang et al. (2021) |
| | *Arabidopsis thaliana* | *Atoxp1* | Agro-bacterium mediated | Baeg et al. (2021) |
| | *Oryza sativa* | *OsHAK1* | Agro-bacterium mediated | Nieves-Cordones et al. (2017) |
| | *Oryza sativa* | *OsNRAMP5* | Agro-bacterium mediated | Tang et al. (2017a, b) |
| | *Oryza sativa* | *OsNRAMP1* | Agro-bacterium mediated | Chu et al. (2022) |
| | *Oryza sativa* | *OsNRAMP1* | Agro-bacterium mediated | Chang et al. (2020) |
| | *Oryza sativa* | *OsPRX2* | Agro-bacterium mediated | Mao et al. (2019) |

**Table 1** (continued)

| Stress tolerance | Plant specie | Marked gene | Method of transfer | Reference |
|---|---|---|---|---|
| Heat tolerance | *Oryza sativa* | *OsPDS* | Gene gum | Nandy et al. (2019) |
| | *Solanum lycopersicum* | *SLAGL6* | Agro-bacterium mediated | Klap et al. (2017) |
| | *Gossypium hirsutum* | *GhPGF, GhCLA1* | Agro-bacterium mediated | Li et al. (2021a, b) |
| | *Lactuca sativa* | *LsNCED4* | Agro-bacterium mediated | Bertier et al. (2018) |
| | *Oryza sativa* | *OsHSA1* | Agro-bacterium mediated | Qiu et al. (2018) |
| | *Oryza sativa* | *OsNAC006* | PEG-mediated | Wang et al. (2020) |
| | *Oryza sativa* | *OsPYL1/4/6* | Agro-bacterium mediated | Miao et al. (2018) |
| | *Solanum lycopersicum* | *SlCPK28* | Agro-bacterium mediated | Hu et al. (2021) |
| | *Solanum lycopersicum* | *SlMAPK3* | Agro-bacterium mediated | Yu et al. (2019) |
| | *Zea mays* | *ZmTMS5 gene* | Particle bombardment | Li et al. (2017a, b) |
| Drought tolerance | *Oryza sativa* | *OsDST* | Agro-bacterium mediated | Kim et al. (2014) |
| | *Triticum aestivum* | *TaDREB2, TaERF3* | Agro-bacterium mediated | Kim et al. (2018) |
| | *Solanum lycopersicum* | *SlMAPK3* | Agro-bacterium mediated | Wang et al. (2017) |
| | *Zea mays* | *ARGOS8* | Agro-bacterium mediated | Shi et al. (2017) |
| | *Arabidopsis thaliana* | *AVP1* | PEG-mediated transformation | Park et al. (2017) |
| | *Oryza sativa* | *OsSAPK2* | Agro-bacterium mediated | Lou et al. (2017) |
| | *Solanum lycopersicum* | *SlNPR1* | Agro-bacterium mediated | Li et al (2019) |
| | *Brassica napus* | *BnaA6.RGA* | Agro-bacterium mediated | Wu et al. (2020) |
| | *Cicer arietinum* | *At4CL, AtRVE7* | PEG-mediated | Badhan et al. (2021) |
| | *Oryza sativa* | *OsERA1* | Agro-bacterium mediated | Ogata et al. (2020) |
| | *Oryza sativa* | *OsPUB67* | Agro-bacterium mediated | Qin et al. (2020) |
| | *Solanum lycopersicum* | *SlARF4* | Agro-bacterium mediated | Chen et al. (2021) |
| | *Glycine max* | *GmMYB118* | Agro-bacterium mediated | Du et al. (2018) |
| | *Glycine max* | *GmHdz4* | Agro-bacterium mediated | Zhong et al. (2022) |
| | *Triticum aestivum* | *Sal1* | Agro-bacterium mediated | Mohr et al. (2022) |
| | *Triticum aestivum* | *TaSal1* | Agro-bacterium mediated | Abdallah et al. (2022) |

and development (Dawood et al. 2022). Knocking out genes (*AtWRKY3* and *AtWRKY4*) in *A. thaliana* causes more ion escape along with lower antioxidant actions, creating salinity sensitivity (Chen et al. 2021), while acquired osmotolerancegenes make plants saline tolerant (Kim et al. 2021).The GT-1 element's regulatory role in rice led to the CRISPR/Cas9-mediated editing of the *OsRAV2* gene, which demonstrated salt stress tolerance (Duan et al. 2016). Rice that was exposed to salt stress was resistant to CRISPR mutants that had lost the ability to function of *SnRK2* and the osmotic stress/ABA-activated protein kinases *SAPK-1* and *SAPK-2* genes (Lou et al. 2017).

Other studies have developed CRISPR-mutants in rice to develop plants that can withstand salt stress by knocking out *OsDST* (Santosh Kumar et al. 2020), *OsNAC45* (Zhang et al. 2020), *AGO2* (ARGONAUTE2) (Yin et al. 2020), *OsRR22* (rice type-B response regulator) (Zhang et al. 2019a, b, c), and *OsBBS1* (bilateral blade senescenc1) (Zeng et al. 2018). Salt tolerance was increased in wheat plants with *TaHAG1* gene knockout mutants produced by CRISPR/Cas9 (Zheng et al. 2021). Alam et al. (2022) produced rice mutant plants with altered DNA by focusing on the *OsbHLH024* gene in order to investigate its function in conditions of salt stress. The osbhlh024 mutants had a single nucleotide base deleted, and when they were exposed to salt stress, their total chlorophyll content and shoot biomass increased significantly. Alfatih et al. (2020) reported that *OsPQT3* knockout mutants in rice showed increased resistance to oxidative stress and salt stress, as well as elevated expression of *OsGPX1, OsAPX1, and OsSOD1*. Additionally, Mo et al. (2020) found that the loss of SLR1 functioned to promote mesocotyl and root growth, particularly in dark and salt stress conditions.

### 9.3 Heat Stress

Due to global warming, high temperatures are another limiting factor in crop reduction (Mohamed and Abdel-Hamid 2013). Using advanced tools, several genes (heat

stress-responsive) have been detected to minimize intricate molecular system activities (such as the appearance of high heat sensing genes, indicator transduction, and metabolite assembly). Several mechanisms (heat shock proteins, heat shock transcription factors) enhance the pathway of signals beginning at high temperatures and decontaminate reactive oxygen species (Mohamed et al. 2019). CRISPR is a more powerful tool than others to create resistance against stresses (Duan et al. 2016). Irrespective of high temperature, *SlAGAMOUS-LIKE 6* increases fruit set in tomatoes (Klap et al. 2017).

*SlMAPK3* is a member of the mitogen-activated protein kinase family and plays a role in several environmental stress responses in tomatoes. Following genome editing with CRISPR/Cas9, slmapk3 mutants displayed increased thermotolerance in comparison to wild-type plants, suggesting that *slmapk3* functions as a negative regulator of thermotolerance (Yu et al. 2019).

In the tomato apoplastic region ROS production is positively regulated by BRZ1, which also contributes to heat tolerance. This has been confirmed by bzr1 mutants based on CRISPR/Cas9, which demonstrated reduced $H_2O_2$ generation in apoplasts and decreased heat tolerance as measured by Respiratory Burst Oxidase Homolog 1 (*RBOH1*) (Yin et al. 2018).

Tomatoes that were created as CRISPR/Cas-mediated *HSA1* (heat-stress sensitive albino 1) mutants were more sensitive to heat stress than plants of the wild type (Qiu et al. 2018). Li et al. (2017a, b) reported that thermosensitive male-sterile plants in maize were enhanced by CRISPR mutants of the thermosensitive genic malesterile 5 (*TMS5*) gene. In lettuce, knockouts of *NCED4*, a crucial regulatory enzyme in the production of ABA, allowed the seeds to germinate at a higher temperature. Consequently, in regions of production where temperatures are high, *LsNCED4* mutants may have commercial value (Bertier et al. 2018).

### 9.4 Cold Stress

Metabolic trails and cell tissues get highly injured under cold temperatures (Jha and Mohamed 2023). Plants (interspecific or inter-generic hybrids) have been modified against cold tolerance using traditional procedures. Certain negative genes (OsMYB30) that result in starch degradation and maltose amassing) were deleted by using CRISPR (Mo et al. 2020) to reduce cold sensitivity (Lv et al. 2017). Three genes (*OsPIN5b*, *GS3*, and *OsMYB30*) were also modified at once to cope with cold (Zeng et al. 2020). Annexin genes (*OsAnn3 and OsAnn5*) help to cope with cold at the seedling phase (Li et al. 2022).

To combat climate change and guarantee future food security, CRISPR/Cas9 is an alluring and approachable method for creating non-transgenic genome-edited crop plants (Mo et al. 2020). To improve the rice plant's resistance to cold, editing is directed to eliminate a few negative regulator transcription factors. A transcription factor called *OsMYB30* has a deleterious effect on cold tolerance by binding to the β-amylase gene promoter. According to Lv et al. (2017), *OsMYB30* forms a complex with *OsJAZ9* during cold stress, which suppresses the expression of the β-amylase gene and affects starch breakdown and maltose buildup. This could potentially lead to an increase in cold sensitivity.

*OsPIN5b, GS3*, and *OsMYB30* were three genes that underwent simultaneous mutations by CRISPR/Cas9-based genome editing, exhibiting increased yield and resistance to cold stress (Zeng et al. 2020). Plant annexins are engaged in protecting plants from environmental stressors and controlling how they develop. At the seedling stage, the rice annexin genes *OsAnn3* and *OsAnn5* are positive regulators of cold stress resistance. Sensitivity to cold treatments was the outcome of knocking out *OsAnn3* and *OsAnn5* (Shen et al. 2017).

Low temperature (4 °C) was observed to boost *SlNPR1* expression and protein concentration in tomatoes. CRISPR/Cas9-mediated genome editing of *SlNPR1* induced the symptoms of chilling injury in tomato plants that were substantiated by the accumulation of hydrogen peroxide ($H_2O_2$), superoxide anion ($O_2-$), and malonic dialdehyde (MDA), as well as reduction in proline content, antioxidant enzymatic activities, and soluble protein content (Shu et al. 2023). In strawberries, overexpression of the *FvICE1* gene led to phenotypic and physiological tolerance to cold and drought. On the other hand, strawberries with CRISPR/cas9-mediated gene editing displayed reduced resistance to cold and drought. According to Han et al. (2023), these findings implied that *FvICE1* acted as a positive regulator of cold and drought.

### 9.5 Metal Stress

Linkage and gathering of heavy metals with oxidative stress led to cell damage, which could be minimized by crops using several techniques (Abu-Shahba et al. 2022). Such mechanisms are driven by multiple genes (Hasanuzzaman et al. 2019). Genes (OXP1 and γ-glutamylcyclotransferase) that build up high levels of glutathione create resistance to stress using CRISPR-Cas9. Cd resistance in plants (Arabidopsis) rather than WT Col0 was observed under oxp1/CRISPR mutant usage (Baeg et al. 2021).

For instance, the γ-glutamylcyclotransferase loss-of-function mutant exhibited protective features against heavy metal toxicity, indicating that the increased glutathione (GSH) accumulation in the loss-of-function mutants of OXP1 and γ-glutamylcyclotransferase demonstrates heavy metal and xenobiotic detoxification (Paulose et al. 2013).

Understanding the role of a possible target, *OsPRX2*, for enhanced potassium deficit tolerance is another use of CRISPR-Cas-based editing in rice. Under $K^+$ limitation, *OsPRX2* has been shown to decrease ROS generation. It was discovered that stomatal closure and $K^+$ deficit tolerance increase when *OsPRX2* is overexpressed. Moreover, under the $K^+$-deficiency tolerance, *OsPRX2* deletion results in severe abnormalities in the phenotypic of leaves and stomatal opening (Mao et al. 2019).

In rice, the transporters *OsNramp1, OsNramp5*, and *OsCd1* help the roots absorb cadmium from the soil. According to Chen et al. (2019a, b), *OsHMA3* is responsible for sequestering cadmium within the root vacuole and adversely regulating xylem loading, while *OsLCT1* is involved in cadmium transport to the grains. Reducing the amount of cadmium in grain crops has been partially achieved by genome editing techniques that manipulate the expression of these transporter genes. *OsNramp5* and *OsLCT1* CRISPR/Cas9-based mutations led to an acceptable cadmium level in rice (Wang et al. 2017). The primary route for $Cs^+$ absorption and translocation in rice is the Cs + -permeable OsHAK1 transporter. Utilizing the CRISPR-Cas technology, transgenic plants devoid of *OsHAK1* function were created in order to reduce the amount of radioactive caesium (Cs) that rice plants in Fukusima soil polluted with 137 $Cs^+$ absorbed. According to Nieves-Cordones et al. (2017), the OsHAK1 knock-out plants showed remarkably lower levels of 137 $Cs^+$ in their roots as well as lower radioactive caesium contents.

### 9.6 Herbicide Stress

Herbicide application is one of the best tools among others to minimize its population (Gage et al. 2019). While CRISPR-Cas9 edited genes to protect plants against weeds (Toda and Okamoto 2020), this led to a higher yield than crops grown by traditional procedures (Dong et al. 2021). Genes (acetyl-coenzyme A carboxylase and *acetolactate synthase*) editing (base) occurs against aryloxyphenoxy propionate-, sulfonylurea-, and imidazolinone-type herbicides using CRISPR in wheat (Zhang et al. 2020).

Tomato, soybean, rice, wheat, corn, watermelon, oilseed rape, tobacco, potato, and Arabidopsis are a few examples of crops that have been modified for ALS gene-based herbicide resistance (Dong et al. 2021). All things considered, genome editing provides a potent tool for creating crops resistant to herbicides by altering important genes like ALS. Through CRISPR-based editing of the *OsALS1* gene, a novel herbicide tolerance phenotype has been inserted into *Oryza sativa* (Kuang et al. 2020).

According to research by Lu et al. (2021), large-scale genomic duplication or inversion can be designed in rice by CRISPR/Cas9 mediated genome editing, resulting in the development of new genes and characteristics. They demonstrated that leaves exhibited high transcript accumulation of *CP12* and *Ubiquitin2* genes, and that edited plants with homozygous structural variation alleles had 10 times higher expression levels of *HPPD* and *PPO1*, which led to herbicide resistance in field trials without compromising yield or other agronomically significant traits.

Herbicide-tolerant crop plants can be produced through genome editing using CRISPR/Cas9. Herbicide resistance was imparted in *Solanum lycopersicum* cv. Micro-Tom by CRISPR/Cas9-based targeted mutagenesis of three genes: *EPSPS* (5-Enolpyruvylshikimate-3-phosphate synthase), *ALS* (acetolactate synthase), and *pds* (phytoene desaturase) (Yang et al. 2022). These characteristics of herbicide tolerance present a potentially effective strategy for controlling weeds. The building of herbicide-resistant genes in crop plants could thus be precisely advanced by the CRISPR-based genome-editing technique.

### 9.7 Anoxia/Hypoxia Stress

Irrespective of oxygen usage by plants, its free form minimizes its yield and plants activate transcriptional factors along with initiating signals to cope stress (Zahra et al. 2021). ROS gathering (in rice shoots) awakens certain assemblies such as *Lysigenous aerenchyma* formed by Respiratory Burst Oxidase Homolog (Colmer and Pedersen 2008), and inside wheat and maize roots against stress tolerance (Yamauchi et al. 2017) due to prompted ethylene using CRISPR (Yamauchi et al. 2017).

## 10 Targeting the Structural and Regulatory Genes Enhancing Abiotic Stress Tolerance

Stress-related genes (especially structural genes) have a key role in stress detection. Several mechanisms (regulation of gene appearance, shielding from pathogens, and nitrogen fixation by ROS) have been described (Ribeiro et al. 2017). While several aberrations (male infertility, lower photosynthesis causing cell demise, and less production) occur due to overproduction of ROS due to stress (abiotic and oxidative) (Zafar et al. 2019), cell redox stability should be maintained through regular ROS production and scavenging checks (Abu-Shahba et al. 2022). Moreover, numerous *T* genes (coding antioxidant enzymes) are involved in foraging against stress survival (Abu-Shahba et al. 2022). While oxidative stress increases programmed cell death along with antioxidant decline through *S* genes (disturbs hormonal homeostasis), plant stress is sensitive (Zhao et al. 2017). *SPCP2* and *TaCP* increase stress tolerance in crops (such as wheat and sweet potato), while

others (*Oryza sativa* stress-related RING finger protein 1; negative regulator) increase $H_2O_2$ and minimizes antioxidant functions (Fang et al. 2015). Furthermore, tolerance is produced by *OsSRFP1* knockdown (antioxidants get clearly adjusted and $H_2O_2$ disorders) (Fang et al. 2015).

Another gene class known as regulatory (giving code to transcription elements, kinases, and phosphatases) also initiates signals and manifestations of genes. Lower ROS-scavenging capacity and increased proline biosynthesis are caused by *Arabidopsis S genes* (*NAM-ATAF1/2 and CUC2 TF*), where *ANAC069* binds to the core motif pattern in the promoter region (He et al. 2017). Similarly, its knockdown led to tolerance against stresses (salt and osmotic) (He et al. 2017). Table 2 shows several genes edited using the CRISPR-Cas tool in multiple crops.

## 11 CRISPR-Based Targeted Gene Knockout for Abiotic Stress Tolerance

Multiple genes could be edited using CRISPR (rather than new gene detection using genome checking) to develop stress-resistant species (Abdelrahman et al. 2018). For example, spindly gene (SPY-3) knockouts create stress tolerance (less water and high salt conditions). Arabidopsis, while MODD and TaDREB2 work depressingly (Kim et al. 2018). MicroRNAs (20–22-nucleotide noncoding RNAs target transcription factors) should be revised using CRISPR for resistive crop development (Farhat et al. 2019), especially fine-tuning the appearance of miRNA (Tang and Chu 2017).

## 12 CRISPR/Cas9 Uses in Considering Abiotic Stress Tolerance

The role of CRISPR/Cas9 (gene editing) has been observed in various species (Lei et al. 2014), using improved sgRNAs (for gene detection) (Montague et al. 2014). Practices (for targeted mutagenesis) (Shan et al. 2014), along with required paths and accessories (Xing et al. 2014), accessible through AddGene (a volunteer plasmid storehouse) (https://www.addgene.org/crispr/plant/), helpful in producing tolerant crops against abiotic stress, have been mentioned. Various genes and the collaboration of various mechanisms (indicating, adjusting, and metabolic ways) led to abiotic stress (Mickelbart et al. 2015).

Complete genome replication mechanisms along gene segment efficient severance have been undertaken by plants, which could be resolved through CRISPR-Cas9 (sgRNAs; targeting various genes), because sole gene editing is not enough for the required results (Zhou et al. 2014). Gene marking through HDR (another method) has a role in stress reactions; using such practices, useful genes could be fired and various genes could be interpreted (for abiotic stress). Plants could be developed in the presence of high native germplasm and gene deviations while becoming difficult due to the absence of the mentioned components, which could be resolved by gene editing (using sgRNA for multiple gene pointing) on an advanced genetic level. The role of CRISPR in humans (vigorous gene assortment) has also been recorded (Wang et al. 2014).

Discovery of other Cas9 kinds will enhance CRISPR dominancy, such as dCas9 (using CRISPRi), which could interrupt genes workability (Qi et al. 2013), and improve

**Table 2** Gene editing occurred using CRISPR-Cas technology

| Abiotic stress | Mode of action | Target gene | References |
|---|---|---|---|
| Cold tolerance | | | |
| *Oryza sativa* | Knock out | OsAnn3 | Shen et al. (2017) |
| *Solanum lycopersicum* | Knock out | S1CBF1 | Li et al. (2018a, b) |
| Salinity tolerance | | | |
| *Oryza sativa* | Knock out | OsNAC041 | Bo et al. (2019) |
| | | OsRR22 | Zhang et al. (2019a, b, c) |
| | | OsOTS1 | Zhang et al. (2019a, b, c) |
| | | SAPK1 and SAPK2 | Lou et al. (2017) |
| Other stresses | | | |
| *Oryza sativa* | Knock-out | OsNramp5 | Tang et al. (2017a, b) |
| *Solanum tuberosum Oryza sativa* | Gene knock in | StALS1 | Shin et al. (2017) |
| | Knock out | OsHAK1 | Nieves-Cordones et al. (2017) |
| Drought tolerance | | | |
| *Oryza sativa* | Knock out | OsSAPK2 | Lou et al. (2017) |
| | | OsNAC14 | Shim et al. (2018) |
| *Solanum lycopersicum* | Knock out | SIMPAK3 | Wang et al. (2017) |
| | | SINPR1 | Li et al. (2019) |

transcriptional subjugation (due to KRAB/SID binding with dCas9) (Konermann et al. 2013), which led to considerable improved mutagenesis (Cho et al. 2014). It also plays a role in other activities (methylation, chromatin positions, controlling or polishing genes). Cas9 rearrangement is also helpful in accurate editing such as TALENS production (using CIB1 and CRY2; light inducible domains) (Konermann et al. 2013).

Specific genes have been improved through various sgRNAs (Perez-Pinera et al. 2013). The efficiency of pointed gene transcription has been controlled by CRISPR (activator or repressor) (Piatek et al. 2015). Non-required results could be skipped using uncertain promoters (initiative of Cas9 and sgRNA appearance). AP2/ERF (transcriptive gene) and *ABA-INSENSITIVE4* produce sudden changes (using ZFNs in combination with heat shock protein led to the required results) (Osakabe et al. 2010). Homozygous biallelic mutants could be characterized by CRISPR tools (Kim et al. 2014), along with homozygous transgenic plant production (the quickest approach led to stress-tolerant species production) (Feng et al. 2013; Zhou et al. 2014). Using nucleases (via transgenic procedures) is helpful in improving plant production (known as non-GM) (Marton et al. 2010). Figure 7 represents the role of CRISPR for improved crop production against abiotic stress tolerance.

## 13 Proofs of Multiple Genes Modification in Various Crops Through CRISPR-Cas9

Table 3 shows a particular gene pattern assessment using the CRISPR-Cas9 tool. Various studies have clarified CRISPR-Cas's role in tolerant crop development towards abiotic stress. SAPK2 (ABA-activated protein kinase 2) features were explained by avoiding mutants (produced by CRISPR) having a high role in abiotic stress (Lou et al. 2017). *slmapk3* mutants were produced (through CRISPR) by pointing MAPKs while comparing remote and transgenic tomatoes (towards stress), where higher symptoms (wilting, more membrane injury, and hydrogen peroxide, with fewer antioxidant enzyme actions)

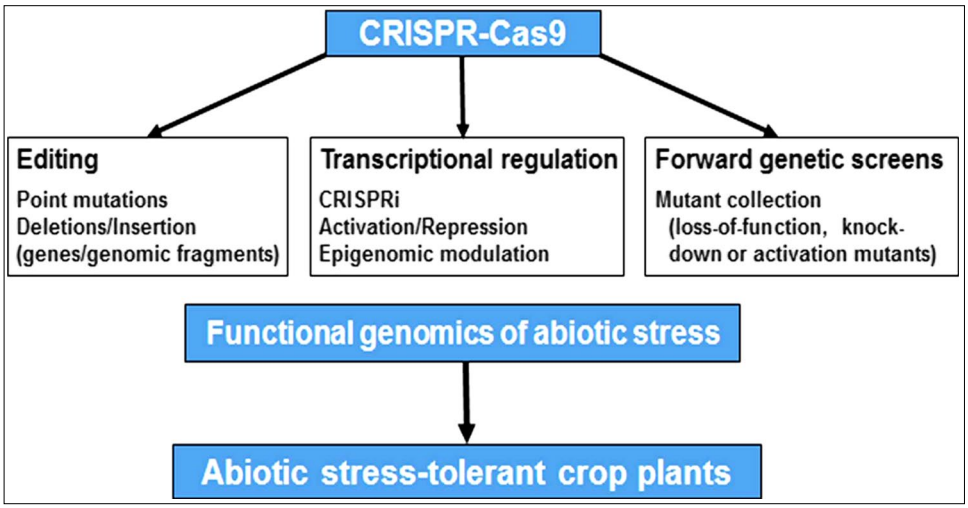


**Fig. 7** Action of the CRISPR-Cas9 method against stress survival using efficient genomics

**Table 3** Overall assessment among the particular genome editing elements

| Attributes | CRISPR/Cas9 | Zinc-finger nucleases (ZFNs) | Transcription activator-like effector nucleases (TALENs) |
|---|---|---|---|
| Target specificity determined by | CrRNA or SgRNA | Zinc finger proteins | Transcription activator like effector protein |
| Target length | 20bp | 18-36bp | 30-40bp |
| Type of interaction | RNA-DNA | Protein-DNA | Protein-DNA |
| Target site | End with PAM sequences like NGG or NAG | Rich in G content | Start with T and end with A |
| Nuclease used | Cas9 | *Fok*I | *Fok*I |
| Cost | Inexpensive | Expensive | Expensive |
| Activity location | Nucleus | Nucleus | Nucleus |
| Target identification efficiency | High | Low | High |
| Type of modifications | Permanent and heritable | Permanent and heritable | Permanent and heritable |

were noted in transgenic tomatoes than wild-type tomatoes. Similarly, CRISPR-Cas9 developed ARGOS8 variants for higher grain production under water scarcity (Shi et al. 2017). Furthermore, ARGOS8 variants (addition of the GOS2 promoter to the 5'-untranslated section of such a gene) having high transcripts in relation to the native allele, as noted in all maize tissues, led to higher yields and improved crop production under abiotic stress.

## 14 Confrontation and Prospects in Developing Stress Tolerant Crops Through Moderating Genes Using CRISPR-Cas Tools

Various factors (less effective entrant genes, significant alteration, and plant restoration tools) limit the role of CRISPR-Cas in abiotic-tolerant crop species, which could be overcome by several methods (detecting new genes and accurate plant tissue culture procedures). With the passage of time, more advanced genetic tools (for improved drought-tolerant crop production) have been used (Mickelbart et al. 2015). Multiple mechanisms (depicting dictation elements and molecular division of indicating paths) are responsible for the discovery of new genes (Licausi et al. 2013). Through CRISPR-Cas9, various stress-tolerant genes have been identified in multiple crop species (Grover et al. 2013), using multiple tools (i.e., promoter recognition to initiate and prompt Cas9 along diverse bright correspondents, and assortment pointers) (Bhowmik et al. 2018). For traits (responsible for abiotic stress tolerance), detection, workability, and categorization along gRNAs could be done using microspore-based gene editing systems rather than typical body cell-based gene editing (Bhowmik et al. 2018).

Besides being a useful and effective tool (CRISPR), it has some issues, such as off-target editing (unnecessary genes edited along essential genes) through an effective blend of NGG PAM and definite gRNA (Zhang et al. 2015), while NAG PAM also has a fusion with Cas9 (Hsu et al. 2013). The involvement of various nucleases makes CRISPR unable to cut the whole necessary pattern (due to regulated PAM). Similarly, there is a lack of suitable procedures to provide lots of giver patterns at the DSB point using HDR-mediated accurate editing. CRISPR is a friendly tool to develop crops that are more tolerant (more yielding, more climate-hardy) against abiotic stress to resolve food decline.

## 15 Modifying Genes to Cope with Abiotic Stress Via CRISPR-Cas9 System

### 15.1 Stress Signal Transduction

In order to withstand abiotic stresses, plants react to various outsider incentives, such as transportation by the cytosolic kinase domain, as a result of signals detected by multiple membrane-localized protein receptors (at the intracellular level) (Osakabe et al. 2013). Numerous (600–1000) RLKs (stress-inducible receptor-like kinases) with multiple actions have been found in the growth and design of crop species (*A. thaliana* and *O. sativa*), while also detecting inner and outer incitements (connecting hormonal and ecological signaling) through various procedures. For example, ABA signaling has certain RLKs (CRK36, PRK1, GHR1, PERK4, ABA, and osmotic-stress-inducible receptor-like cytosolic kinase 1) in *Arabidopsis* during waterless situations (Hua et al. 2012). Hormone incentives to cope with abiotic stress are also described by HKs (histidine kinases) (Nongpiur et al. 2012), where phytohormone incentives are carried by their multiple members (phytohormones like cytokinin and ethylene) (Pham et al. 2012). HKs and RLKs downstream detect stress signals and activate multiple essential hormones in response, where MAPK (signaling pathways) make species tolerant to stress, such as NPK1 (Nicotiana-Protein-Kinase1) in maize towards low temperature stress.

### 15.2 Transcription Elements

Dehydration-responsive element-binding-factors (DREB1A, DREB2A, and DREB1B) attach to cis-acting components (situated at the promoter point of the gene causing tolerance) to control gene action. The competence of DREB could be enhanced via CRIPSR/Cas9 to cope with abiotic stress. Abscisic acid (ABA) abating makes bZIP (leucine-zipper) indestructible when withstanding stress. Furthermore, ABA signaling in *A. thaliana* is controlled by multiple components (AREB1, AREB2, and ABF3) during waterless conditions, which could be advanced through CRISPR usage (Yoshida et al. 2010).

### 15.3 Phytohormones Metabolic Pathway

Phytohormones (less quantity in cells) have a great role in various actions of plants (adaption, regulation of growth and development, nutrition distribution, and conversion of bases) towards different conditions (Fahad et al. 2015). They are very important signaling molecules for plants (sessile organisms), which control certain responses (molecular and physiological). Waterless conditions become ineffective in the presence of *Phaseolus vulgaris* PvNCED1 in transgenic tobacco (Qin and Zeevaart 2002). Similarly high rice grain yield in the presence of ABA3/LOS5 (during drought situations) and also less leaf ageing in the presence of more cytokinin (Ma 2008), followed by lower leaf ageing due to flooding in *Arabidopsis* due to isopentenyl transferase (IPT) (Huynh et al. 2005).

## 16 Role of CRISPR-Cas System in Developing Climate Smart Crops

Finding specialty and editing any target gene pattern of plants to create target mutations based on DNA or RNA could be easily and efficiently done by CRISPR-Cas9 technology, but it needs to be improved to increase plants heritability. Where unnecessary genes could get targeted, this led to stress responses in the case of Cas9, which could be lessened using appropriate sgRNAs (which only edit target genes), such as CRISPR-P usage in various plant species (Jain 2015). Cpf1 (in mammalian cells) causes mutagenesis (Mahfouz 2017) by working (multiple cleavage at a pointed site) in the presence of crRNA (using T-rich PAM with a RuvC-like nuclease area rather than HNH) and leading to DNA cleavage a little far from PAM. Cpf1 is target-specific and more efficient in plants than animals (Tang et al. 2017a, b).

## 17 Advantages of Genome Editing Over Other Conventional and Transgenic Techniques

Specific features from one line into another are inserted using a typical breeding cross (repeated), while mutation breeding causes arbitrary changes that take 8–12 years. Similarly, transgenic breeding (unsystematic assimilation of distant DNA in the genome) also transmits beneficial genes within 2–5 years without continual backcrossing. Furthermore, gene editing causes less alteration in DNA (which looks natural) for developed features than external DNA assimilation for resilient crop development in less time. Gene editing only edits pointed gene sites effectively (Song et al. 2016) and with precise DNA editing (Barrangou 2015).

## 18 Limitations

Although the CRISPR/Cas systems exhibit powerful ability in crop genetic improvement, some limitations still need to be overcome in this field. SpCas9 requires a 50-NGG-30PAM immediately adjacent to the 20 nt DNA target sequence because it can only recognize NGG PAM sites, which limits its effectiveness. Although this restriction is vital, the goal is to turn off the gene through selective mutagenesis in any situation (Fig. 8). *Agrobacterium tumefaciens*-mediated transformation is necessary to use the CRISPR/Cas system in plants, however it is a time-consuming, expensive, and difficult process. Target gene options are extremely constrained. On the one hand, shutting down a gene by itself cannot confer resistance because the role of resistance genes is redundant. On the other hand, PAM limits the knockout of resistance genes, thus sequences near PAM must be chosen. The plant genome may experience random off-target changes due to CRISPR/Cas. The inability to properly isolate Cas proteins and produce transgene-free

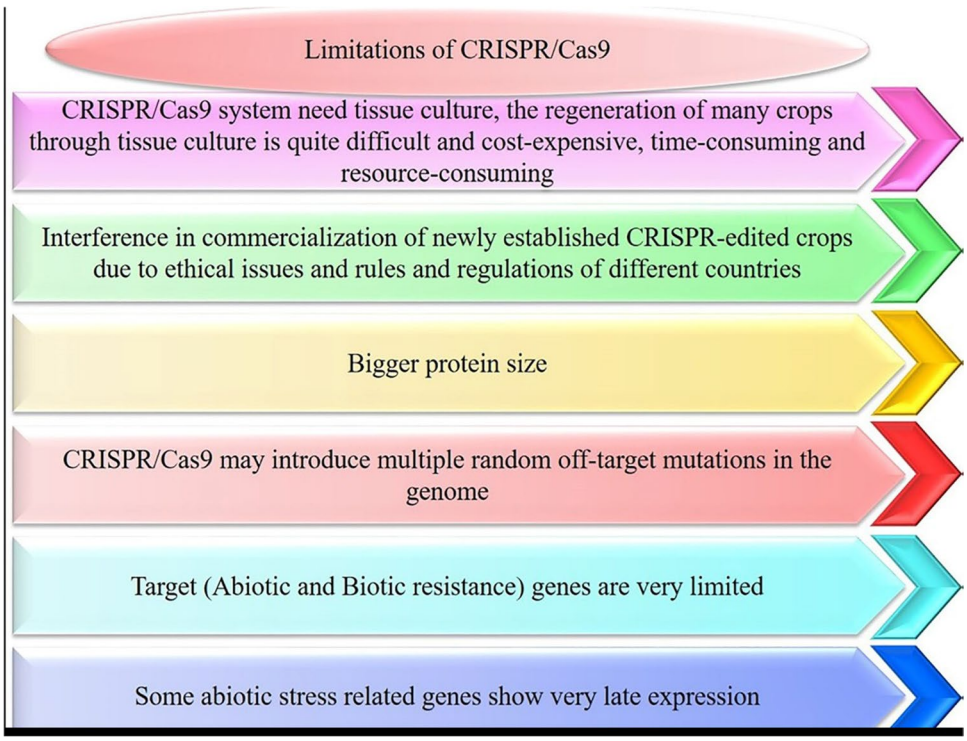


**Fig. 8** Limitations of the current CRISPR/Cas system

crops has hampered the commercialization of CRISPR-edited crops (Wang et al. 2022). Currently, homologous donor sequence transformation into plant cells has a low efficiency and the homologous recombination route (knock-in/gene replacement) has a lower efficiency, which reduces the difficulty and efficiency of knock-in. Therefore, there is still much work to be done before CRISPR/Cas-mediated homologous recombination in plants can effectively knock in genes (Wang et al. 2022).

Varietal usage has been recorded for CRISPR-Cas9, and currently CRISPRi (balancing RNAi) is doing gene quieting (Qi et al. 2013). CRISPR works better when paired with other tools, such as ease of feature analysis along molecular crossing (which occurs through genetic alteration at the allelic level) and wrong gene correction (Zaidi et al. 2016). It also lowers the herbicide (chemical) application on plants through gene editing (Xu et al. 2015). CRISPR should be used with care for the required results (Zaidi et al. 2016), due to its large size and the lack of unnecessary PAM site points caused by RNA misguidance (Zaidi et al. 2020). Viruses (with speedy replication) do not get affected by CRISPR. Various states don't allow CRISPR created crops due to social problems (Eş et al. 2019).

## 19 Conclusions

CRISPR-mediated editing has the potential to improve crops with abiotic stress tolerance and improved nutritional content. The CRISPR-based genome editing tool is considered one of the most powerful technologies for improving agriculture to feed the rapidly growing population. It can develop genome-edited crop varieties with no foreign-gene integration like those created through conventional breeding. Gene editing is a very powerful method rather than other traditional breeding procedures due to the accurate and minute alteration production in plant DNA (without analyzing external DNA) for crop improvement, high yield, resistance to stress, and vice versa. Climate-hardy crops could be obtained in less time than 12–15 years (without repeated crossing). CRISPR only targets specific points, not whole genome patterns, resulting in accurate mutagenesis.

CRISPR has made crops tolerant to abiotic stress by editing genes irrespective of their availability, which will be a powerful tool to be applied on a commercial level. This technology should be used in the agriculture sector for better results, although its limitations are there.. In the future, researchers will find CRISPR technology more profitable to use in multiple sectors. The global population needs to be fed as climate change has severely affected food security, which could be overcome in the future through advancements in CRIPSR technology.

**Author contributions** Heba I. Mohamed, Ayesha Khan, and Abdul Basit: designed the conception of the review, conducted the literature research, and wrote the manuscript; Heba I. Mohamed, Ayesha Khan, andAbdul Basit: read the first draft of the article; Heba I. Mohamed, Ayesha Khan, and Abdul Basit:, critically revised the manuscript. All authors read and approved the final version of the manuscript.

**Funding** Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB). No fund.

**Data Availability** All data are present in the manuscript.

## Declarations

**Conflict of interest** The authors declare no competing interests.



## References

Abd El Mageed TA, Semida W, Hemida KA, Gyushi MAH, Rady MM, Abdelkhalik A, Merah O, Brestic M, Mohamed HI, El Sabagh A, Abdelhamid MT (2023) Glutathione-mediated changes in productivity, photosynthetic efficiency, osmolytes, and antioxidant capacity of common beans (*Phaseolus vulgaris*) grown under water deficit. PeerJ 11:e15343. https://doi.org/10.7717/peerj.15343

Abdallah NA, Elsharawy H, Abulela HA, Thilmony R, Abdelhadi AA, Elarabi NI (2022) Multiplex CRISPR/Cas9-mediated genome editing to address drought tolerance in wheat. GM Crops Food. https://doi.org/10.1080/21645698.2022.2120313

Abdelrahman M, Al-Sadi AM, Pour-Aboughadareh A, Burritt DJ, Tran L-SP (2018) Genome editing using CRISPR/Cas9–targeted mutagenesis: an opportunity for yield improvements of crop plants grown under environmental stresses. Plant Physiol Biochem 131:31–36. https://doi.org/10.1016/j.plaphy.2018.03.012

Abu-Shahba MS, Mansour MM, Mohamed HI, Sofy MR (2022) Effect of biosorptive removal of cadmium ions from hydroponic solution containing indigenous garlic peel and mercerized garlic peel on lettuce productivity. Sci Horti 293:110727. https://doi.org/10.1016/j.scienta.2021.110727

Adeyinka OS, Tabassum B, Koloko BL, Ogungbe IV (2023) Enhancing the quality of staple food crops through CRISPR/Cas-mediated site-directed mutagenesis. Planta 257:78. https://doi.org/10.1007/s00425-023-04110-6

Ahmad S, Wei X, Sheng Z, Hu P, Tang S (2020) CRISPR/Cas9 for development of disease resistance in plants: recent progress, limitations and future prospects. Brief Funct Geno 19:26–39. https://doi.org/10.1093/bfgp/elz041

Alagoz Y, Gurkok T, Zhang B, Unver T (2016) Manipulating the biosynthesis of bioactive compound alkaloids for next-generation metabolic engineering in opium poppy using CRISPR-Cas 9 genome editing technology. Sci Rep 6:30910. https://doi.org/10.1038/srep30910

Alam MS, Kong J, Tao R, Ahmed T, Alamin M, Alotaibi SS, Abdelsalam NR, Xu JH (2022) CRISPR/Cas9 mediated knockout of the OsbHLH024 transcription factor improves salt stress resistance in rice (*Oryza sativa* L.). Plants 11:1184. https://doi.org/10.3390/plants11091184

Alfatih A, Wu J, Jan SU, Zhang ZS, Xia JQ, Xiang CB (2020) Loss of rice PARAQUAT TOLERANCE 3 confers enhanced resistance to abiotic stresses and increases grain yield in field. Plant Cell Environ 43:2743–2754. https://doi.org/10.1101/2020.02.22.961151

Andorf CM, Cannon EK, Portwood JL, Gardiner JM, Harper LC, Schaeffer ML, Braun BL, Campbell DA, Vinnakota AG, Sribalusu VV (2015) Maize GDB update: new tools, data and interface for the maize model organism database. Nucleic Acids Res 44:D1195–D1201. https://doi.org/10.1093/nar/gkv1007

Badhan S, Ball AS, Mantri N (2021) First report of CRISPR/Cas9 mediated DNA-free editing of 4CL and RVE7 genes in chickpea protoplasts. Int J Mol Sci 22:396. https://doi.org/10.3390/ijms22010396

Baeg GJ, Kim SH, Choi DM, Tripathi S, Han YJ, Kim J (2021) CRISPR/Cas9-mediated mutation of 5-oxoprolinase gene confers resistance to sulfonamide compounds in Arabidopsis. Plant Biotechnol Rep 15:753–764. https://doi.org/10.1007/s11816-021-00718-w

Barrangou R (2015) Diversity of CRISPR-Cas immune systems and molecular machines. Genome Biol 16:247. https://doi.org/10.1186/s13059-015-0816-9

Belhaj K, Chaparro-Garcia A, Kamoun S, Patron NJ, Nekrasov V (2015) Editing plant genomes with CRISPR/Cas9. Curr Opin Biotechnol 32:76–84. https://doi.org/10.1016/j.copbio.2014.11.007

Bertier LD, Ron M, Huo H, Bradford KJ, Britt AB, Michelmore RW (2018) High-resolution analysis of the efficiency, heritability, and editing outcomes of CRISPR/Cas9-induced modifications of NCED4 in lettuce (*Lactuca sativa*). G3: Genes Genomes Genet 8:1513–1521. https://doi.org/10.1534/g3.117.300396

Bhattacharya A, Parkhi V, Char B (2021) Genome editing for crop improvement: a perspective from India. In Vitro Cell Dev Biol Plant 57:565–573. https://doi.org/10.1007/s11627-021-10184-2

Bhowmik P, Ellison E, Polley B, Bollina V, Kulkarni M, Ghanbarnia K et al (2018) Targeted mutagenesis in wheat microspores using CRISPR/Cas9. Sci Rep 8:6502. https://doi.org/10.1038/s41598-018-24690-8

Bhowmik P, Hassan MM, Molla K, Rahman M, Islam MT (2019) Application of CRISPR-Cas genome editing tools for the improvement of plant abiotic stress tolerance. Approaches for enhancing abiotic stress tolerance in plants. CRC Press, New York, pp 459–472. https://doi.org/10.1201/9781351104722-26

Bo W, Zhaohui Z, Huanhuan Z, Xia W, Binglin L, Lijia Y, Xiangyan H, Deshui Y, Xuelian Z, Chunguo W et al (2019) Targeted mutagenesis of NAC transcription factor gene, OsNAC041, leading to salt sensitivity in rice. Rice Sci 26:98–108. https://doi.org/10.1016/j.rsci.2018.12.005

Bortesi L, Fischer R (2015) The CRISPR/Cas9 system for plant genome editing and beyond. Biotechnol Adv 33:41–52. https://doi.org/10.1016/j.biotechadv.2014.12.006

Bouzroud S, Gasparini K, Hu G, Barbosa MAM, Rosa BL, Fahr M, Bendaou N, Bouzayen M, Zsögön A, Smouni A et al (2020) Down regulation and loss of Auxin response factor 4 function using CRISPR/Cas9 alters plant growth, stomatal function and improves tomato tolerance to salinity and osmotic stress. Genes 11:272. https://doi.org/10.3390/genes11030272

Brouns SJ, Jore MM, Lundgren M, Westra ER, Slijkhuis RJ, Snijders AP, Dickman MJ, Makarova KS, Koonin EV, van der Oost J (2008) Small CRISPR RNAs guide antiviral defense in prokaryotes. Science 321:960–964. https://doi.org/10.1126/science.1159689

Butt H, Rao GS, Sedeek K, Aman R, Kamel R, Mahfouz M (2020) Engineering herbicide resistance via prime editing in rice. Plant Biotechnol J 18:2370. https://doi.org/10.1111/pbi.13399

Cai Y, Chen L, Liu X, Sun S, Wu C, Jiang B et al (2015) CRISPR/Cas9-mediated genome editing in soybean hairy roots. PLoS ONE 10:e0136064. https://doi.org/10.1371/journal.pone.0136064

Cai Y, Chen L, Liu X, Guo C, Sun S, Wu C et al (2018) CRISPR/Cas9-mediated targeted mutagenesis of GmFT2a delays flowering time in soybean. Plant Biotechnol J 16:176–185. https://doi.org/10.1111/pbi.12758

Ceasar SA, Rajan V, Prykhozhij SV, Berman JN, Ignacimuthu S (2016) Insert, remove or replace: a highly advanced genome editing system using CRISPR/Cas9. Biochim Biophys Acta 1863:2333–2344. https://doi.org/10.1016/j.bbamcr.2016.06.009

Chang JD, Huang S, Yamaji N, ZhangW MJF, Zhao FJ (2020) OsNRAMP1 transporter contributes to cadmium and manganese uptake in rice. Plant Cell Environ 43:2476–2491. https://doi.org/10.1111/pce.13843

Chen K, Wang Y, Zhang R, Zhang H, Gao C (2019a) CRISPR/Cas genome editing and precision plant breeding in agriculture. Annual Rev Plant Biol 70:667–697. https://doi.org/10.1146/annurev-arplant-050718-100049

Chen J, Zou W, Meng L, Fan X, Xu G, Ye G (2019b) Advances in the uptake and transport mechanisms and QTLs mapping of cadmium in rice. Int J Mol Sci 20:3417. https://doi.org/10.3390/ijms20143417

Chen G, Hu J, Dong L, Zeng D, Guo L, Zhang G, Zhu L, Qian Q (2020) The tolerance of salinity in rice requires the presence of a functional copy of FLN2. Biomolecules 10:17. https://doi.org/10.3390/biom10010017

Chen S, Zhang N, Zhou G, Hussain S, Ahmed S, Tian H, Wang S (2021) Knockout of the entire family of AITR genes in Arabidopsis leads to enhanced drought and salinity tolerance without fitness costs. BMC Plant Biol 21:137. https://doi.org/10.1186/s12870-021-02907-9

Cheng H, Hao M, Ding B, Mei D, Wang W, Wang H, Zhou R, Liu J, Li C, Hu Q (2021) Base editing with high efficiency in allotetraploid oilseed rape by A3A-PBE system. Plant Biotechnol J 19:87–97. https://doi.org/10.1111/pbi.13444

Cho SW, Kim S, Kim Y, Kweon J, Kim HS, Bae S et al (2014) Analysis of off-target effects of CRISPR/Cas-derived RNA-guided endonucleases and nickases. Genome Res 24:132–141. https://doi.org/10.1101/gr.162339.113

Chu C, Huang R, Liu L, Tang G, Xiao J, Yoo H, Yuan M (2022) The rice heavy-metal transporter OsNRAMP1 regulates disease resistance by modulating ROS homoeostasis. Plant Cell Environ 45:1109–1126. https://doi.org/10.1111/pce.14263

Colmer TD, Pedersen O (2008) Oxygen dynamics in submerged rice (*Oryza sativa*). New Phytol 178:326–334. https://doi.org/10.1111/j.1469-37.2007.02364.x

Cong Á, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, Zhang F (2013) Multiplex genome engineering using CRISPR/Cas systems. Science 339:819. https://doi.org/10.1126/science.1231143

Curtin SJ, Xiong Y, Michno JM, Campbell BW, StecČermák AOT et al (2018) CRISPR/Cas9 and TALENs generate heritable mutations for genes involved in small RNA processing of glycine max and

Medicago truncatula. Plant Biotechnol J 16:1125–1137. https://doi.org/10.1111/pbi.12857

Dawood MF, Sofy MR, Mohamed HI, Sofy AR, Abdel-Kader HA (2022) Hydrogen sulfide modulates salinity stress in common bean plants by maintaining osmolytes and regulating nitric oxide levels and antioxidant enzyme expression. J Soil Sci Plant Nutr 22:3708–3726. https://doi.org/10.1007/s42729-022-00921-w

Deltcheva E, Chylinski K, Sharma CM, Gonzales K, Chao Y, Pirzada ZA, Eckert MR, Vogel J, Charpentier E (2011) CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III. Nature 471:602–607. https://doi.org/10.1038/nature09886

Deveau H, Barrangou R, Garneau JE, Labonte J, Fremaux C, Boyaval P, Romero DA, Horvath P, Moineau S (2008) Phage response to CRISPR-encoded resistance in *Streptococcus thermophilus*. J Bacteriol 190:1390–1400. https://doi.org/10.1128/JB.01412-07

Dong H, Huang Y, Wang K (2021) The development of herbicide resistance crop plants using Crispr/Cas9-mediated gene editing. Genes 12:912. https://doi.org/10.3390/genes12060912

Driedonks N, Rieu I, Vriezen WH (2016) Breeding for plant heat tolerance at vegetative and reproductive stages. Plant Reprod 29:67–79. https://doi.org/10.1007/s00497-016-0275-9

Du YT, Zhao MJ, Wang CT, Gao Y, Wang YX, Liu YW et al (2018) Identification and characterization of GmMYB118 responses to drought and salt stress. BMC Plant Biol 18:320. https://doi.org/10.1186/s12870-018-1551-7

Duan YB, Li J, Qin RY, Xu RF, Li H, Yang YC, Ma H, Li L, Wei PC, Yang JB (2016) Identification of a regulatory element responsible for salt induction of rice OsRAV2 through ex situ and in situ promoter analysis. Plant Mol Biol 90:49–62. https://doi.org/10.1007/s11103-015-0393-z

Eş I, Gavahian M, Marti-Quijal FJ, Lorenzo JM, Mousavi Khaneghah A, Tsatsanis C, Kampranis SC, Barba FJ (2019) The application of the CRISPR-Cas9 genome editing machinery in food and agricultural science: current status, future perspectives, and associated challenges. Biotechnol Adv 37:410–421. https://doi.org/10.1016/j.biotechadv.2019.02.006

Fahad S, Hussain S, Bano A, Saud S, Hassan S, Shan D, Khan FA, Khan F, Chen Y, Wu C, Tabassum MA, Chun MX, Afzal M, Jan A, Jan MT, Huang J (2015) Potential role of phytohormones and plant growth-promoting rhizobacteria in abiotic stresses: consequences for changing environment. Environ Sci Pollut Res 22:4907–4921. https://doi.org/10.1007/s11356-014-3754-2

Fang H, Meng Q, Xu J, Tang H, Tang S, Zhang H, Huang J (2015) Knock-down of stress inducible OsSRFP1 encoding an E3 ubiquitin ligase with transcriptional activation activity confers abiotic stress tolerance through enhancing antioxidant protection in rice. Plant Mol Biol 87:441–458. https://doi.org/10.1007/s11103-015-0294-1

Farhat S, Jain N, Singh N, Sreevathsa R, Das PK, Rai R, Yadav S, Kumar P, Sarkar A, Jain A (2019) CRISPR-Cas9 directed genome engineering for enhancing salt stress tolerance in rice. Seminars Cell Develop Biol 96:91–99. https://doi.org/10.1016/j.semcdb.2019.05.003

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 23:1229–1232. https://doi.org/10.1038/cr.2013.114

Fu Y, Sander JD, Reyon D, Cascio VM, Joung JK (2014) Improving CRISPR-Cas nuclease specificity using truncated guide RNAs. Nat Biotechnol 32:279–284. https://doi.org/10.1038/nbt.2808

Gage KL, Krausz RF, Walters SA (2019) Emerging challenges for weed management in herbicide-resistant crops. Agriculture 9:126. https://doi.org/10.3390/agronomy14010126

Gaj T, Gersbach CA, Barbas CF (2013) ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol. 31:397–405. https://doi.org/10.1016/j.tibtech.2013.04.004

Gajardo HA, Gómez-Espinoza O, Boscariol Ferreira P, Carrer H, Bravo LA (2023) The potential of CRISPR/Cas technology to enhance crop performance on adverse soil conditions. Plants 12:1892. https://doi.org/10.3390/plants12091892

Gao Y, Zhao Y (2014) Specific and heritable gene editing in Arabidopsis. Proc Natl Acad Sci USA 111:4357–4358. https://doi.org/10.1073/pnas.1402295111

Garneau JE, Dupuis ME, Villion M, Romero DA, Barrangou R, Boyaval P, Horvath P, Magadan AH, Moineau S (2010) The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. Nature 468:67–71. https://doi.org/10.1038/nature09523

Gasiunas G, Barrangou R, Horvath P, Siksnys V (2012) Cas9-crRNA ribonucleo protein complex mediates specific DNA cleavage for adaptive immunity in bacteria. Proc Natl Acad Sci USA 109:E2579–E2586. https://doi.org/10.1073/pnas.1208507109

Grover A, Mittal D, Negi M, Lavania D (2013) Generating high temperature tolerant transgenic plants: achievements and challenges. Plant Sci 205–206:38–47. https://doi.org/10.1016/j.plantsci.2013.01.005

Haft DH, Selengut J, Mongodin EF, Nelson KE (2005) A guild of 45 CRISPR-associated (Cas) protein families and multiple CRISPR/Cas subtypes exist in prokaryotic genomes. PLoS Comput Biol 1:e60. https://doi.org/10.1371/journal.pcbi.0010060

Hale CR, Zhao P, Olson S, Duff MO, Graveley BR, Wells L, Terns RM, Terns MP (2009) RNA-guided RNA cleavage by a CRISPR RNA-Cas protein complex. Cell 139:945–956. https://doi.org/10.1016/j.cell.2009.07.040

Hale CR, Majumdar S, Elmore J, Pfister N, Compton M, Olson S, Resch AM, Glover CV, Graveley BR, Terns RM, Terns MP (2012) Essential features and rational design of CRISPR RNAs that function with the Cas RAMP module complex to cleave RNAs. Mol Cell 45:292–302. https://doi.org/10.1016/j.molcel.2011.10.023

Han X, Chen Z, Li P, Xu H, Liu K, Zha W, Li S, Chen J, Yang G, Huang J, You A (2022) Development of novel rice germplasm for salt-tolerance at seedling stage using CRISPR-Cas9. Sustainability 14:2621. https://doi.org/10.3390/su14052621

Han J, Li X, Li W, Yang Q, Li Z, Cheng Z et al (2023) Isolation and preliminary functional analysis of FvICE1, involved in cold and drought tolerance in *Fragaria vesca* through overexpression and CRISPR/Cas9 technologies. Plant Physiol Biochem 196:270–280. https://doi.org/10.1016/j.plaphy.2023.01.048

Hasanuzzaman M, Alhaithloul HAS, Parvin K, Bhuyan MHMB, Tanveer M, Mohsin SM, Nahar K, Soliman MH, Mahmud JA, Fujita M (2019) Polyamine Action under Metal/Metalloid Stress: Regulation of Biosynthesis, Metabolism, and Molecular Interactions. Int J Mol Sci 20(13):3215. https://doi.org/10.3390/ijms20133

He L, Shi X, Wang Y, Guo Y, Yang K, Wang Y (2017) *Arabidopsis* ANAC069 binds to C[A/G]CG[T/G] sequences to negatively regulate salt and osmotic stress tolerance. Plant Mol Biol 93:369–387. https://doi.org/10.1007/s11103-016-0567-3

Hirai MY, Sugiyama K, Sawada Y, Tohge T, Obayashi T, Suzuki A, Araki R, Sakurai N, Suzuki H, Aoki K et al (2007) Omics-based identification of Arabidopsis Myb transcription factors regulating aliphatic glucosinolate biosynthesis. Proc Natl Acad Sci USA 104:6478–6483. https://doi.org/10.1073/pnas.0611629104

Hossain A, Rahman MME, Ali S, Islam T, Syed MA, Syed T, Zafar SA, Behera L, Skalicky M, Brestic M, Islam T (2022) CRISPR-Cas9-mediated genome editing technology for abiotic stress tolerance in crop plant. Plant Perspect Glob Clim Chang 331–354. https://doi.org/10.1016/B978-0-323-85665-2.00008-X

Hsu PD, Scott DA, Weinstein JA, Ran FA, Konermann S, Agarwala V et al (2013) DNA targeting specificity of RNA-guided Cas9

nucleases. Nature Biotechnol 31:827. https://doi.org/10.1038/nbt.2647
Hu Z, Li J, Ding S, Cheng F, Li X, Jiang Y et al (2021) The protein kinase CPK28 phosphorylates ascorbate peroxidase and enhances thermotolerance in tomato. Plant Physiol 186:1302–1317. https://doi.org/10.1093/PLPHYS/KIAB120
Hua D, Wang C, He J, Liao H, Duan Y, Zhu Z, Guo Y, Chen Z, Gong Z (2012) A plasma membrane receptor kinase, GHR1, mediates abscisic acid- and hydrogen peroxide-regulated stomatal movement in Arabidopsis. Plant Cell 24:2546–2561. https://doi.org/10.1105/tpc.112.100107
Hussain B, Lucas SJ, Budak H (2018) CRISPR/Cas9 in plants: at play in the genome and at work for crop improvement. Briefings Funct Geno 17:319–328. https://doi.org/10.1093/bfgp/ely016
Huynh N, Vantoai T, Streeter J, Banowetz G (2005) Regulation of flooding tolerance of SAG12:ipt Arabidopsis plants by cytokinin. J Exp Bot 56:1397–1407. https://doi.org/10.1093/jxb/eri141
Islam T (2019) CRISPR-Cas technology in modifying food crops. Cabi Rev. https://doi.org/10.1079/PAVSNNR201914050
Islam MA, Rony SA, Rahman MB, Cinar MU, Villena J, Uddin MJ, Kitazawa H (2020) Improvement of Disease Resistance in Livestock: Application of Immunogenomics and CRISPR/Cas9 Technology. Animals 10(12):2236. https://doi.org/10.3390/ani10122236
Islam MT, Croll D, Gladieux P, Soanes DM, Persoons A, Bhattacharjee P et al (2016) Emergence of wheat blast in Bangladesh was caused by a south American lineage of *Magnaporthe oryzae*. BMC Biol 14:84. https://doi.org/10.1186/s12915-016-0309-7
Jagodzik P, Tajdel-Zielinska M, Ciesla A et al (2018) Mitogen-activated protein kinase cascades in plant hormone signaling. Front Plant Sci 64:301–341. https://doi.org/10.3389/fpls.2018.01387
Jain M (2015) Function genomics of abiotic stress tolerance in plants: a CRISPR approach. Front Plant Sci 6:375. https://doi.org/10.3389/fpls.2015.00375
Jansen R, Embden JD, Gaastra W, Schouls LM (2002) Identification of genes that are associated with DNA repeats in prokaryotes. Mol Microbiol 43:1565–1575. https://doi.org/10.1046/j.1365-2958.2002.02839.x
Jha Y, Mohamed HI (2023) Inoculation with *Lysinibacillus fusiformis* strain YJ4 and *Lysinibacillus sphaericus* strain YJ5 alleviates the effects of cold stress in maize plants. Gesunde Pflanz 75:77–95. https://doi.org/10.1007/s10343-022-00666-7
Jouanin A, Gilissen LJ, Schaart JG, Leigh FJ, Cockram J, Wallington EJ, Boyd LA, van den Broeck HC, van der Meer IM, America AH, Visser RG (2020) CRISPR/Cas9 gene editing of gluten in wheat to reduce gluten content and exposure—Reviewing methods to screen for coeliac safety. Front Nutrit 7:51. https://doi.org/10.3389/fnut.2020.00051
Kim S, Kim D, Cho SW, Kim J, Kim JS (2014) Highly efficient RNA-guided genome editing in human cells via delivery of purified Cas9 ribonucleoproteins. Genome Res 24:1012–1019. https://doi.org/10.1101/gr.171322.113
Kim D, Alptekin B, Budak H (2018) CRISPR/Cas9 Genome Editing in Wheat. Funct Integ Genom 18:265. https://doi.org/10.1007/s10142-017-0572-x
Kim ST, Choi M, Bae SJ, Kim JS (2021) The functional association of Acqos/Victr with salt stress resistance in *Arabidopsis Thaliana* was confirmed by Crispr-mediated mutagenesis. Int J Mol Sci 22. https://doi.org/10.3390/ijms222111389
Klap C, Yeshayahou E, Bolger AM, Arazi T, Gupta SK, Shabtai S, Usadel B, Salts Y, Barg R (2017) Tomato facultative parthenocarpy results from SlAGAMOUS-LIKE 6 loss of function. Plant Biotechnol J 15:634–637. https://doi.org/10.1111/pbi.12662
Konermann S, Brigham MD, Trevino AE, Hsu PD, Heidenreich M, Cong L et al (2013) Optical control of mammalian endogenous transcription and epigenetic states. Nature 500:472–476. https://doi.org/10.1038/nature12466
Kuang Y, Li S, Ren B, Yan F, Spetz C, Li X et al (2020) Base-Editing-Mediated artificial evolution of OsALS1 in planta to develop novel herbicide-tolerant rice germplasms. Mol Plant 13:565–572. https://doi.org/10.1016/j.molp.2020.01.010
Kumar M, Prusty MR, Pandey MK, Singh PK, Bohra A, Guo B, Varshney RK (2023) Application of CRISPR/Cas9-mediated gene editing for abiotic stress management in crop plants. Front Plant Sci 14:1157678. https://doi.org/10.3389/fpls.2023.1157678
LeBlanc C, Zhang F, Mendez J, Lozano Y, Chatpar K, Irish VF et al (2018) Increased efficiency of targeted mutagenesis by CRISPR/Cas9 in plants using heat stress. Plant J 93:377–386. https://doi.org/10.1111/tpj.13782
Lei Y, Lu L, Liu HY, Li S, Xing F, Chen LL (2014) CRISPR-P:aweb tool for synthetic single-guide RNA design of CRISPR-system in plants. Mol Plant 7:1494–1496. https://doi.org/10.1093/mp/ssu044
Li J, Norville JE, Aach J, McCormack M, Zhang D, Bush J, Church GM, Sheen J (2013) Multiplex and homologous recombination–mediated genome editing in Arabidopsis and Nicotiana benthamiana using guide RNA and Cas9. Nature Biotechnol 31(8):688-691. https://doi.org/10.1038/nbt.2654
Li P, Li YJ, Zhang FJ, Zhang GZ, Jiang XY, Yu HM, Hou BK (2017a) The Arabidopsis UDP-glycosyltransferases UGT79B2 and UGT79B3, contribute to cold, salt and drought stress tolerance via modulating anthocyanin accumulation. Plant J 89:85–103. https://doi.org/10.1111/tpj.13324
Li J, Zhang H, Si X, Tian Y, Chen K, Liu J et al (2017b) Generation of thermosensitive male-sterile maize by targeted knockout of the ZmTMS5 gene. J Genet Genom 44:465–468. https://doi.org/10.1016/j.jgg.2017.02.002
Li R, Zhang L, Wang L, Chen L, Zhao R, Sheng J, Shen L (2018a) Reduction of tomato-plant chilling tolerance by CRISPR–Cas9-mediated SlCBF1 mutagenesis. J Agri Food Chem 66:9042–9051. https://doi.org/10.1021/acs.jafc.8b02177
Li X, Wang Y, Chen S, Tian H, Fu D, Zhu B, Luo Y, Zhu H (2018b) Lycopene is enriched in tomato fruit by CRISPR/Cas9-mediated multiplex genome editing. Front Plant Sci 9:559. https://doi.org/10.3389/fpls.2018.00559
Li R, Liu C, Zhao R, Wang L, Chen L, Yu W, Zhang S, Sheng J, Shen L (2019) CRISPR/Cas9-Mediated SlNPR1 mutagenesis reduces tomato plant drought tolerance. BMC Plant Biol 19:38. https://doi.org/10.1186/s12870-018-1627-4
Li YM, Zhu JJ, Wu H, Liu CL, Huang CL, Lan JH, Zhao YM, Xie CX (2020) Precise base editing of non-allelic acetolactate synthase genes confers sulfonylurea herbicide resistance in maize. Crop J 8:449–456. https://doi.org/10.1016/j.cj.2019.10.001
Li P, Li X, Jiang M (2021a) CRISPR/Cas9-mediated mutagenesis of WRKY3 and WRKY4 function decreases salt and Me-JA stress tolerance in *Arabidopsis Thaliana*. Mol Biol Rep 48. https://doi.org/10.1007/s11033-021-06541-4
Li B, Liang S, Alariqi M, Wang F, Wang G, Wang Q et al (2021b) The application of temperature sensitivity CRISPR/LbCpf1 (LbCas12a) mediated genome editing in allotetraploid cotton (*G. hirsutum*) and creation of nontransgenic, gossypolfree cotton. Plant Biotechnol J 19:221. https://doi.org/10.1111/pbi.13470
Li X, Xu S, Fuhrmann-Aoyagi MB, Yuan S, Iwama T, Kobayashi M, Miura K (2022) CRISPR/Cas9 technique for temperature, drought, and salinity stress responses. Curr Issues Mol Biol 44:2664–2682. https://doi.org/10.3390/cimb44060182
Li J, Kong D, Ke Y, Zeng W, Miki D (2024) Application of multiple sgRNAs boosts efficiency of CRISPR/Cas9-mediated gene targeting in Arabidopsis. BMC Biol 22:6. https://doi.org/10.1186/s12915-024-01810-7

Licausi F, Ohme-Takagi M, Perata P (2013) APETALA2/ethylene responsive factor (AP2/ERF) transcription factors: mediators of stress responses and developmental programs. New Phytol 199:639–649. https://doi.org/10.1111/nph.12291

Liu L, Zhang J, Xu J, Li Y, Guo L, Wang Z, Zhang X, Zhao B, Guo YD, Zhang N (2020a) CRISPR/Cas9 targeted mutagenesis of SlLBD40, a lateral organ boundaries domain transcription factor, enhances drought tolerance in tomato. Plant Sci 301:110683. https://doi.org/10.1016/j.plantsci.2020.110683

Liu X, Wu D, Shan T, Xu S, Qin R, Li H, Negm M, Wu D, Li J (2020b) The trihelix transcription factor OsGTγ-2 is involved adaption to salt stress in rice. Plant Mol Biol 103:545–560. https://doi.org/10.1007/s11103-020-01010-1

Liu L, Kuang Y, Yan F, Li S, Ren B, Gosavi G, Spetz C, Li X, Wang X, Zhou X et al (2021) Developing a novel artificial rice germplasm for dinitroaniline herbicide resistance by base editing of OsTubA2. Plant Biotechnol J 19:5–7. https://doi.org/10.1111/pbi.13430

Lou D, Wang H, Liang G, Yu D (2017) OsSAPK2 confers abscisic acid sensitivity and tolerance to drought stress in rice. Front Plant Sci 8:1371. https://doi.org/10.3389/fpls.2017.00993

Lowder L, Malzahn A, Qi Y (2017) Rapid construction of multiplexed CRISPR-cas9 systems for plant genome editing. Methods Mol Biol 1578:291–307. https://doi.org/10.1007/978-1-4939-6859-6_25

Lu Y, Zhu JK (2017) Precise editing of a target base in the rice genome using a modified CRISPR/Cas9 system. Mol Plant 10:523–525. https://doi.org/10.1016/j.molp.2016.11.013

Lu Y, Wang J, Chen B, Mo S, Lian L, Luo Y et al (2021) A donor-DNA-free CRISPR/Cas-based approach to gene knock-up in rice. Nat Plants 7:1445–1452. https://doi.org/10.1038/s41477-021-01019-4

Lv Y, Yang M, Hu D, Yang Z, Ma S, Li X et al (2017) The OsMYb30 transcription factor suppresses cold tolerance by interacting with a JAZ protein and suppressing β-amylase expression1. Plant Physiol 173:1475–1491. https://doi.org/10.1104/pp.16.01725

Lyu YS, Cao LM, Huang WQ, Liu JX, Lu HP (2022) Disruption of three polyamine uptake transporter genes in rice byCRISPR/Cas9 gene editing confers tolerance to herbicide paraquat. aBIOTECH 3:140–145. https://doi.org/10.1007/s42994-022-00075-4

Ma QH (2008) Genetic engineering of cytokinins and their application to agriculture. Crit Rev Biotechnol 28:213–232. https://doi.org/10.1080/07388550802262205

Ma X, Zhu Q, Chen Y, Liu YG (2016) CRISPR/Cas9 platforms for genome editing in plants: developments and applications. Mol Plant 9:961–974. https://doi.org/10.1016/j.molp.2016.04.009

Mahfouz MM (2017) The efficient tool CRISPR-Cpf1. Nature Plants 3:17028. https://doi.org/10.1038/nplants.2017.28

Makarova KS, Aravind L, Grishi NV, Rogozin IB, Koonin EV (2002) A DNA repair system specific for thermophilic archaea and bacteria predicted by genomic context analysis. Nucleic Acids Res 30:482–496. https://doi.org/10.1093/nar/30.2.482

Makarova KS, Grishin NV, Shabalina SA, Wolf YI, Koonin EV (2006) A putative RNA-interference based immune system in prokaryotes: computational analysis of the predicted enzymatic machinery, functional analogies with eukaryotic RNAi, and hypothetical mechanisms of action. Biol Direct 1:7. https://doi.org/10.1186/1745-6150-1-7

Mao Y, Zhang H, Xu N, Zhang B, Gou F, Zhu JK (2013) Application of the CRISPR-Cas system for efficient genome engineering in plants. Mol Plant 6:2008–2011. https://doi.org/10.1093/mp/sst121

Mao Y, Zhang Z, Feng Z, Wei P, Zhang H, Botella JR et al (2016) Development of germ-line-specific CRISPRCas9 systems to improve the production of heritable gene modifications in Arabidopsis. Plant Biotechnol J 14:519–532. https://doi.org/10.1111/pbi.12468

Mao Y, Botella JR, Liu Y, Zhu JK (2019) Gene editing in plants: progress and challenges. Natl Sci Rev 6:421–437. https://doi.org/10.1093/nsr/nwz005

Marraffini LA, Sontheimer EJ (2008) CRISPR interference limits horizontal gene transfer in staphylococci by targeting DNA. Science 322:1843–1845. https://doi.org/10.1126/science.1165771

Marton I, Zuker A, Shklarman E, Zeevi V, Tovkach A, Roffe S et al (2010) Non transgenic genome modification in plant cells. Plant Physiol 154:1079–1087. https://doi.org/10.1104/pp.110.164806

Miao J, Guo D, Zhang J et al (2013) Targeted mutagenesis in rice using CRISPR-Cas system. Cell Res 23:1233–1236. https://doi.org/10.1038/cr.2013.123

Miao C, Xiao L, Hua K, Zou C, Zhao Y, Bressan RA et al (2018) Mutations in a subfamily of abscisic acid recepto genes promote rice growth and productivity. Proc Natl Acad Sci USA 115:6058–6063. https://doi.org/10.1073/pnas.1804774115

Mickelbart MV, Hasegawa PM, Bailey-Serres J (2015) Genetic mechanisms of abiotic stress tolerance that translate to crop yield stability. Nature Rev Gene 16:237–251. https://doi.org/10.1038/nrg390

Mo W, Tang W, Du Y, Jing Y, Bu Q, Lin R (2020) PHYTOCHROME-INTERACTING FACTOR-LIKE14 and SLENDER RICE1 interaction controls seedling growth under salt stress. Plant Physiol 184:506–517. https://doi.org/10.1104/PP.20.00024

Mohamed HI, Abdel-Hamid AM (2013) Molecular and biochemical studies for heat tolerance on four cotton genotypes. Romanian Biotechnol Lett 18:8823–8831

Mohamed HI, Ashry NA, Ghonaim MM (2019) Physiological and biochemical effects of heat shock stress and determination of molecular markers related to heat tolerance in maize hybrids. Gesunde Pflanz 71:213–222. https://doi.org/10.1007/s10343-019-00467-5

Mohr T, Horstman J, Gu YQ, Elarabi NI, Abdallah NA, Thilmony R (2022) CRISPR-Cas9 gene editing of the Sal1 gene family in wheat. Plants 11:2259. https://doi.org/10.3390/plants11172259

Mojica FJ, Diez-Villasenor C, Garcia-Martinez J, Almendros C (2009) Short motif sequences determine the targets of the prokaryotic CRISPR defence system. Microbiol 155:733–740. https://doi.org/10.1099/mic.0.023960-0

Molla KA, Karmakar S, Islam MT (2020) Wide horizon of CRISPR-Cas-derived technologies for basic biology, agriculture, and medicine. In: Islam MT, Bhowmik PK, Molla KA (eds) CRISPR-Cas methods. Springer Science + Business Media, New York, NY, pp 1–24. https://doi.org/10.1007/978-1-0716-0616-2_1

Montague TG, Cruz JM, Gagnon JA, Church GM, Valen E (2014) CHOPCHOP: a CRISPR/Cas9 and TALEN web tool for genome editing. Nucl Acids Res 42:W401–W407. https://doi.org/10.1093/nar/gku410

Mushtaq M, Bhat JA, Mir ZA, Sakina A, Ali S, Singh AK, Tyagi A, Salgotra RK, Dar AA, Bhat R (2018) CRISPR/Cas approach: a new way of looking at plant-abiotic interactions. J Plant Physiol 224–225:156–162. https://doi.org/10.1016/j.jplph.2018.04.001

Nandy S, Pathak B, Zhao S, Srivastava V (2019) Heat-shock-inducible CRISPR/Cas9 system generates heritable mutations in rice. Plant Direct 3:e00145. https://doi.org/10.1002/pld3.145

Napier JA, Haslam RP, Tsalavouta M, Sayanova O (2019) The challenges of delivering genetically modified crops with nutritional enhancement traits. Nat Plants 5:563–567. https://doi.org/10.1038/s41477-019-0430-z

Nascimento FD, Rocha AD, Soares JM, Mascarenhas MS, Ferreira MD, Morais Lino LS, Ramos AP, Diniz LE, Mendes TA, Ferreira CF, Santos-Serejo JA (2023) Gene editing for plant resistance to abiotic factors: a systematic review. Plants 12:305. https://doi.org/10.3390/plants12020305

Nawaz G, Han Y, Usman B, Liu F, Qin B, Li R (2019) Knockout of OsPRP1, a gene encoding proline-rich protein, confers enhanced cold sensitivity in rice (*Oryza Sativa* L.) at the seedling stage. Biotech 9:254. https://doi.org/10.1007/s13205-019-1787-4

Nieves-Cordones M, Mohamed S, Tanoi K, Kobayashi NI, Takagi K, Vernet A, Guiderdoni E, Périn C, Sentenac H, Véry AA (2017) Production of low-Cs+ rice plants by inactivation of the K+ transporter Os HAK 1 with the CRISPR-Cas system. Plant J 92:43–56. https://doi.org/10.1111/tpj.13632

Nongpiur R, Soni P, Karan R, Singla-Pareek SL, Pareek A (2012) Histidine kinases in plants: cross talk between hormone and stress responses. Plant Signal Behav 7:1230–1237. https://doi.org/10.4161/psb.21516

Nongpiur RC, Singla-Pareek SL, Pareek A (2016) Genomics approaches for improving salinity stress tolerance in crop plants. Curr Genomics 17:343–357. https://doi.org/10.2174/1389202917666160331202517

Nowrousian M, Stajich JE, Chu M, Engh I, Espagne E, Halliday K, E, Halliday K et al. (2010) De novo Assembly of a 40 Mb Eukaryotic Genome from Short Sequence Reads: Sordaria macrospora, a Model Organism for Fungal Morphogenesis. PLoS Genet 6(4):e1000891. https://doi.org/10.1371/journal.pgen.1000891

Ogata T, Ishizaki T, Fujita M, Fujita Y (2020) CRISPR/Cas9-targeted mutagenesis of OsERA1 confers enhanced responses to abscisic acid and drought stress and increased primary root growth under nonstressed conditions in rice. PLoS ONE 15:e0243376. https://doi.org/10.1371/journal.pone.0243376

Osakabe Y, Mizuno S, Tanaka H, Maruyama K, Osakabe K, Todaka D, Fujita Y, Kobayashi M, Shinozaki K, Yamaguchi-Shinozaki K (2010) Overproduction of the membrane-bound receptor-like protein kinase 1, RPK1, enhances abiotic stress tolerance in Arabidopsis. J Biol Chem 285:9190–9201. https://doi.org/10.1074/jbc.M109.051938

Osakabe Y, Yamaguchi-Shinozaki K, Shinozaki K, Tran LSP (2013) Sensing the environment: key roles of membrane-localized kinases in plant perception and response to abiotic stress. J Exp Bot 64:445–458. https://doi.org/10.1093/jxb/ers354

Oz MT, Altpeter A, Karan R, Merotto A, Altpeter F (2021) CRISPR/Cas9-mediated multi-allelic gene targeting in sugarcane confers herbicide tolerance. Front Genome Edit 3:673566. https://doi.org/10.3389/fgeed.2021.673566

Park JJ, Dempewolf E, Zhang WZ, Wang ZY (2017) RNA-guided transcriptional activation via CRISPR/dCas9 mimics overexpression phenotypes in Arabidopsis. PLoS ONE 12:e0179410. https://doi.org/10.1371/journal.pone.0179410

Paulose B, Chhikara S, Coomey J, Jung HI, Vatamaniuk O, Dhankher OP (2013) A γ-glutamyl cyclotransferase protects arabidopsis plants from heavy metal toxicity by recycling glutamate to maintain glutathione homeostasis. Plant Cell 11:4580–4595. https://doi.org/10.1105/tpc.113.111815

Perez-Pinera P, Kocak DD, Vockley CM, Adler AF, Kabadi AM, Polstein LR et al (2013) RNA-guided gene activation by CRISPR-Cas9- based transcription factors. Nat Methods 10:973–976. https://doi.org/10.1038/nmeth.2600

Pham J, Liu J, Bennett MH, Mansfield JW, Desikan R (2012) Arabidopsis histidine kinase 5 regulates salt sensitivity and resistance against bacterial and fungal infection. New Phytol 194:168–180. https://doi.org/10.1111/j.1469-8137.2011.04033.x

Piatek A, Ali Z, Baazim H, Li L, Abulfaraj A, Al-Shareef S et al (2015) RNA-guided transcriptional regulation in planta via synthetic Cas9-based transcription factors. Plant Bio Technol J 13:578–589. https://doi.org/10.1111/pbi.12284

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 152:1173–1183. https://doi.org/10.1016/j.cell.2013.02.022

Qin X, Zeevaart JA (2002) Overexpression of a 9-cis-epoxycarotenoid dioxygenase gene in *Nicotiana plumbaginifolia* increases abscisic acid and phaseic acid levels and enhances drought tolerance. Plant Physiol 128:544–551. https://doi.org/10.1104/pp.010663

Qin Q, Wang Y, Huang L, Du F, Zhao X, Li Z et al (2020) A U-box E3 ubiquitin ligase OsPUB67 is positively involved in drought tolerance in rice. Plant Mol Biol 102:89–107. https://doi.org/10.1007/s11103-019-00933-8

Qiu Z, Kang S, He L, Zhao J, Zhang S, Hu J et al (2018) The newly identified heat-stress sensitive albino 1 gene affects chloroplast development in rice. Plant Sci 267:168–179. https://doi.org/10.1016/j.plantsci.2017.11.015

Ribeiro CW, Korbes AP, Garighan JA, Jardim-Messeder D, Carvalho FEL, Sousa RHV, Caverzan A, Teixeira FK, Silveira JAG, Margis-Pinheiro M (2017) Rice peroxisomal ascorbate peroxidase knockdown affects ROS signaling and triggers early leaf senescence. Plant Sci 263:55–65. https://doi.org/10.1016/j.plantsci.2017.07.009

Ricroch A, Clairand P, Harwood W (2017) Use of CRISPR systems in plant genome editing: toward new opportunities in agriculture. Emerg Top Life Sci 1:169–182. https://doi.org/10.1042/etls20170085

Roca Paixão JF, Gillet FX, Ribeiro TP, Bournaud C, Lourenço-Tessutti IT, Noriega DD et al (2019) Improved drought stress tolerance in arabidopsis by CRISPR/dCas9 fusion with a histone AcetylTransferase. Sci Rep 9:8080. https://doi.org/10.1038/s41598-019-44571-y

Sander JD, Joung JK (2014) CRISPR-Cas systems for editing, regulating and targeting genomes. Nat Biotechnol 32:347. https://doi.org/10.1038/nbt.2842

Santosh Kumar VV, Verma RK, Yadav SK, Yadav P, Watts A, Rao MV, Chinnusamy V (2020) CRISPR-Cas9 mediated genome editing of drought and salt tolerance (OsDST) gene in indica mega rice cultivar MTU1010. Physiol Mol Biol of Plants 26:1099–1110. https://doi.org/10.1007/s12298-020-00819-w

Sapranauskas R, Gasiunas G, Fremaux C, Barrangou R, Horvath P, Siksnys V (2011) The Streptococcus thermophiles CRISPR/Cas system provides immunity in *Escherichia coli*. Nucleic Acids Res 39(21):9275–9282. https://doi.org/10.1093/nar/gkr606

Scheben A, Wolter F, Batley J, Puchta H, Edwards D (2017) Towards CRISPR/Cas crops–bringing together genomics and genome editing. New Phytol 216:682–698. https://doi.org/10.1111/nph.14702

Sedeek KE, Mahas A, Mahfouz M (2019) Plant genome engineering for targeted improvement of crop traits. Front Plant Sci 10:114. https://doi.org/10.3389/fpls.2019.00114

Shan Q, WangY LJ, Gao C (2014) Genome editing in rice and wheat using the CRISPR/Cas system. Nat Protoc 9:2395–2410. https://doi.org/10.1038/nprot.2014.157

Shen C, Que Z, Xia Y, Tang N, Li D, He R et al (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. https://doi.org/10.1007/s12374-016-0400-1

Shi J, Gao H, Wang H, Lafitte HR, Archibald RL, Yang M, Hakimi SM, Mo H, Habben JE (2017) ARGOS8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. Plant Biotechnol J 15:207–216. https://doi.org/10.1111/pbi.12603

Shim JS, Oh N, Chung PJ, Kim YS, Choi YD, Kim JK (2018) Overexpression of OsNAC14 improves drought tolerance in rice. Front Plant Sci 9:310. https://doi.org/10.3389/fpls.2018.00310

Shin J, Jiang F, Liu JJ, Bray NL, Rauch BJ, Baik SH, Nogales E, Bondy-Denomy J, Corn JE, Doudna JA (2017) Disabling Cas9 by an anti-CRISPR DNA mimic. Sci Adv 3:e1701620. https://doi.org/10.1126/sciadv.1701620

Shu P, Li Y, Xiang L, Sheng J, Shen L (2023) SlNPR1 modulates chilling stress resistance in tomato plant by alleviating oxidative damage and affecting the synthesis of ferulic acid. Sci Hortic 307:111486. https://doi.org/10.1016/j.scienta.2022.111486

Shukla M, Al-Busaidi KT, Trivedi M, Tiwari RK (2018) Status of research, regulations and challenges for genetically modified crops in India. GM Crops Food 9:173–188. https://doi.org/10.1080/21645698.2018.1529518

Singh D, Laxmi A (2015) Transcriptional regulation of drought response: a tortuous network of transcriptional factors. Front Plant Sci 6:895. https://doi.org/10.3389/fpls.2015.00895

Song G, Jia M, Chen K, Kong X, Khattak B, Xie C, Li A, Mao L (2016) CRISPR/Cas9: a powerful tool for crop genome editing. Crop J 4:75–82. https://doi.org/10.1016/j.cj.2015.12.002

Sun YW, Zhang X, Wu CY, He YB, Ma YZ, Hou H, Guo XP, Du WM, Zhao YD, Xia LQ (2016) Engineering herbicide resistant rice plants through CRISPR/Cas9-mediated homologous recombination of acetolactate synthase. Mol Plant 9:628–631. https://doi.org/10.1016/j.molp.2016.01.001

Suweisa S, Carrb JA, Maritana A, Rinaldoc A, D'Odoricob P (2015) Correction for Suweis *et al*. Resilience and reactivity of global food security. Proc Natl Acad Sci USA 112:E4811. https://doi.org/10.1073/pnas.1512971112

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. https://doi.org/10.1104/pp.15.00793

Tang J, Chu C (2017) MicroRNAs in crop improvement: fine-tuners for complex traits. Nat Plants 3:17077. https://doi.org/10.1038/nplants.2017.77

Tang L, Mao B, Li Y, Lv Q, Zhang L, Chen C, He H, Wang W, Zeng X, Shao Y, Pan Y (2017a) Knockout of OsNramp5 using the CRISPR/Cas9 system produces low Cd-accumulating indica rice without compromising yield. Sci Repo 7:14438. https://doi.org/10.1038/s41598-017-14832-9

Tang X, Lowder LG, Zhang T, Malzahn AA, Zheng X, Voytas DF, Zhong Z, Chen Y, Ren Q, Li Q, Kirkland ER (2017b) A CRISPR– Cpf1 system for efficient genome editing and transcriptional repression in plants. Nat Plants 3:17018. https://doi.org/10.1038/nplants.2017.18

Tian H, Chen S, Yang W, Wang T, Zheng K, Wang Y, Cheng Y, Zhang N, Liu S, Li D et al (2017a) A novel family of transcription factors conserved in angiosperms is required for ABA signaling. Plant Cell Environ 40:2958–2971. https://doi.org/10.1111/pce.13058

Tian S, Jiang L, Gao Q, Zhang J, Zong M, Zhang H et al (2017b) Efficient CRISPR/Cas9-based gene knockout in watermelon. Plant Cell Rep 36:399–406. https://doi.org/10.1007/s00299-016-2089-5

Tian S, Jiang L, Cui X, Zhang J, Guo S, Li M, Zhang H, Ren Y, Gong G, Zong M et al (2018) Engineering herbicide-resistant watermelon variety through CRISPR/Cas9-mediated base-editing. Plant Cell Rep 37:1353–1356. https://doi.org/10.1007/s00299-018-2299-0

Toda E, Okamoto T (2020) CRISPR/Cas9-based genome editing using rice zygotes. Curr Protoc Plant Biol 5:e20111. https://doi.org/10.1002/cppb.20111

Tran MT, Doan DTH, Kim J, Song YJ, Sung YW, Das S, Kim EJ, Son GH, Kim SH, Van Vu T et al (2021) CRISPR/Cas9- based precise excision of SlHyPRP1 domain(s) to obtain salt stress-tolerant tomato. Plant Cell Rep 40:999–1011. https://doi.org/10.1007/s00299-020-02622-z

Tripathi L, Ntui VO, Tripathi JN (2020) CRISPR/Cas9-based genome editing of banana for disease resistance. Curr Opin Plant Biol 56:118–126. https://doi.org/10.1016/j.pbi.2020.05.003

Vlˇcko T, Ohnoutková L (2020) Allelic variants of CRISPR/Cas9 induced mutation in an inositol trisphosphate 5/6 kinase gene manifest different phenotypes in barley. Plants 9:195. https://doi.org/10.3390/plants9020195

Waltz E (2022) GABA-enriched tomato is first CRISPR-edited food to enter market. Nat Biotechnol 40:9–11. https://doi.org/10.1038/d41587-021-00026-2

Wang T, Wei JJ, Sabatini DM, Lander ES (2014) Genetic screens in human cells using the CRISPR-Cas9 system. Science 343:80–84. https://doi.org/10.1126/science.1246981

Wang ZP, Xing HL, Dong L, Zhang HY, Han CY, Wang XC et al (2015) Egg cell-specific promoter-controlled CRISPR/Cas9 efficiently generates homozygous mutants for multiple target genes in Arabidopsis in a single generation. Genome Biol 16:144. https://doi.org/10.1186/s13059-015-0715-0

Wang L, Chen L, Li R, Zhao R, Yang M, Sheng J et al (2017) Reduced drought tolerance by CRISPR/Cas9-mediated SlMAPK3 mutagenesis in tomato plants. J Agric Food Chem 65:8674–8682. https://doi.org/10.1021/acs.jafc.7b02745

Wang W, Pan Q, He F, Akhunova A, Chao S, Trick H et al (2018) Transgenerational CRISPR-Cas9 activity facilitates multiplex gene editing in allopolyploid wheat. CRISPR J 1:65–74. https://doi.org/10.1089/crispr.2017.0010

Wang B, Zhong Z, Wang X, Han X, Yu D, Wang C et al (2020) Knockout of the OsNAC006 transcription factor causes drought and heat sensitivity in rice. Int J Mol Sci 21:2288. https://doi.org/10.3390/ijms21072288

Wang Y, Zafar N, Ali Q, Manghwar H, Wang G, Yu L, Ding X, Ding F, Hong N, Wang G et al (2022) RISPR/Cas genome editing technologies for plant improvement against biotic and abiotic stresses: advances, limitations, and future perspectives. Cells 11:3928. https://doi.org/10.3390/cells11233928

Woo JW, Kim J, Kwon SI, Corvalan C, Cho SW, Kim H, Kim SG, Kim ST, Choe S, Kim JS (2015) DNA-free genome editing in plants with preassembled CRISPR-Cas9 ribonucleoproteins. Nat Biotechnol 33:1162–1164. https://doi.org/10.1038/nbt.3389

Wu J, Yan G, Duan Z, Wang Z, Kang C, Guo L et al (2020) Roles of the brassica napus DELLA protein BnaA6.RGA, in modulating drought tolerance by interacting with the ABA signaling component BnaA10.ABF2. Front Plant Sci 11:577. https://doi.org/10.3389/fpls.2020.00577

Xie K, Minkenberg B, Yang Y (2015) Boosting CRISPR/Cas9 multiplex editing capability with the endogenous tRNA-processing system. Proc Nat Acad Sci 112:3570–3575. https://doi.org/10.1073/pnas.1420294112

Xing HL, Dong L, Wang ZP, Zhang HY, Han CY, Liu B et al (2014) A CRISPR/Cas9 toolkit for multiplex genome editing in plants. BMC Plant Biol 14:327. https://doi.org/10.1186/s12870-014-0327-y

Xu RF, Li H, Qin RY, Li J, Qiu CH, Yang YC, Ma H, Li L, Wei PC, Yang JB (2015) Generation of inheritable and "transgene clean" targeted genome-modified rice in later generations using the CRISPR/Cas9 system. Sci Rep 5:11491. https://doi.org/10.1038/srep11491

Yamauchi T, Yoshioka M, Fukazawa A, Mori H, Nishizawa NK, Tsutsumi N, Nakazono M (2017) An NADPH oxidase RBOH functions in rice roots during lysigenous aerenchyma formation under oxygen-deficient conditions. Plant Cell 29:775–790. https://doi.org/10.1105/tpc.16.00976

Yang SH, Kim E, Park H, Koo Y (2022) Selection of the high efficient sgRNA for CRISPR-Cas9 to edit herbicide related genes, PDS, ALS, And EPSPS in tomato. Appl Biol Chem 65:13. https://doi.org/10.1186/s13765-022-00679-w

Yin Y, Qin K, Song X, Zhang Q, Zhou Y, Xia X et al (2018) BZR1 transcription factor regulates heat stress tolerance through FERONIA receptor-like kinase-mediated reactive oxygen species

signaling in tomato. Plant Cell Physiol 59:2239–2254. https://doi.org/10.1093/pcp/pcy146

Yin W, Xiao Y, Niu M, Meng W, Li L, Zhang X et al (2020) ARGONAUTE2 enhances grain length and salt tolerance by activating BIG GRAIN3 to modulate cytokinin distribution in rice. Plant Cell 32:2292–2306. https://doi.org/10.1105/tpc.19.00542

Yoshida T, Fujita Y, Sayama H, Kidokoro S, Maruyama K, Mizoi J, Shinozaki K, Yamaguchi-Shinozaki K (2010) AREB1, AREB2, and ABF3 are master transcription factors that cooperatively regulate ABRE-dependent ABA signaling involved in drought stress tolerance and require ABA for full activation. Plant J 61:672–685. https://doi.org/10.1111/j.1365-313X.2009.04092.x

Yu W, Wang L, Zhao R, Sheng J, Zhang S, Li R et al (2019) Knockout of SlMAPK3 enhances tolerance to heat stress involving ROS homeostasis in tomato plants. BMC Plant Biol 19:354. https://doi.org/10.1186/s12870-019-1939-z

Yue E, Cao H, Liu B (2020) OsmiR535, a potential genetic editing target for drought and salinity stress tolerance in *Oryza sativa*. Plants 9:1337. https://doi.org/10.3390/plants9101337

Zafar SA, Patil S, Uzair M, Fang J, Zhao J, Yuan S, Uzair M, Luo Q, Shi J, Schreiber L, Li X (2019) DEGENERATED PANICLE AND PARTIAL STERILITY 1 (DPS1) encodes a CBS domain containing protein required for anther cuticle and panicle development in rice. New Phytol 225:356–375. https://doi.org/10.1111/nph.16133

Zafar SA, Zaidi SSA, Gaba Y, Singla-Pareek SL, Dhankher OP, Li X, Mansoor S, Pareek A (2020) Engineering abiotic stress tolerance *via* CRISPR/ Cas-mediated genome editing. J Exp Bot 71:470–479. https://doi.org/10.1093/jxb/erz476

Zahra N, Hafeez MB, Shaukat K, Wahid A, Hussain S, Naseer R, Farooq M (2021) Hypoxia and anoxia stress: plant responses and tolerance mechanisms. J Agron Crop Sci 207:249–284. https://doi.org/10.1111/jac.12471

Zaidi SSA, Tashkandi M, Mansoor S, Mahfouz MM (2016) Engineering plant immunity: using CRISPR/Cas9 to generate virus resistance. Front Plant Sci 7:1673. https://doi.org/10.3389/fpls.2016.01673

Zaidi SSA, Mahas A, Vanderschuren H, Mahfouz MM (2020) Engineering crops of the future: CRISPR approaches to develop climate-resilient and disease-resistant plants. Genome Biol 21:289. https://doi.org/10.1186/s13059-020-02204-y

Zeng DD, Yang CC, Qin R, Alamin M, Yue EK, Jin XL et al (2018) A guanine insert in OsBBS1 leads to early leaf senescence and salt stress sensitivity in rice (*Oryza sativa* l.). Plant Cell Rep 37:933–946. https://doi.org/10.1007/s00299-018-2280-y

Zeng Y, Wen J, Zhao W, Wang Q, Huang W (2020) Rational improvement of rice yield and cold tolerance by editing the three genes OsPIN5b, GS3, and OsMYB30 with the CRISPR–Cas9 system. Front Plant Sci 10:1663. https://doi.org/10.3389/fpls.2019.01663

Zetsche B, Gootenberg JS, Abudayyeh OO, Slaymaker IM, Makarova KS, Essletzbichler P, Volz SE, Joung J, Van Der Oost J, Regev A (2015) Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. Cell 163:759–771. https://doi.org/10.1016/j.cell.2015.09.038

Zhang XH, Tee LY, Wang XG, Huang QS, Yang SH (2015) Off-target effects in CRISPR/Cas9-mediated genome engineering. Mol Therapy-Nucleic Acids 40:e264. https://doi.org/10.1038/mtna.2015.37

Zhang C, Srivastava AK, Sadanandom A (2019a) Targeted mutagenesis of the SUMO protease, overly tolerant to salt1 in rice through CRISPR/Cas9-mediated genome editing reveals a major role of this SUMO protease in salt tolerance. BioRxiv 2019:555706. https://doi.org/10.1101/555706

Zhang A, Liu Y, Wang F, Li T, Chen Z, Kong D, Bi J, Zhang F, Luo X, Wang J et al (2019b) Enhanced rice salinity tolerance via CRISPR/Cas9-targeted mutagenesis of the OsRR22 gene. Mol Breed 39:47. https://doi.org/10.1007/s11032-019-0954-y

Zhang R, Liu JX, Chai ZZ, Chen S, Bai Y, Zong Y, Chen KL, Li JY, Jiang LJ, Gao CX (2019c) Generation of herbicide tolerance traits and a new selectable marker in wheat using base editing. Nat Plants 5:480–485. https://doi.org/10.1038/s41477-019-0405-0

Zhang X, Long Y, Huang J, Xia J (2020) OsNAC45 is involved in ABA response and salt tolerance in rice. Rice 13:79. https://doi.org/10.1186/s12284-020-00440-1

Zhang Y, Iaffaldano B, Qi Y (2021) CRISPR Ribonucleoprotein-mediated genetic engineering in plants. Plant Comm 2:100168. https://doi.org/10.1016/j.xplc.2021.100168

Zhao J, Zhao L, Zhang M, Zafar SA, Fang J, Li M, Zhang W, Li X (2017) Arabidopsis E3 ubiquitin ligases PUB22 and PUB23 negatively regulate drought tolerance by targeting ABA receptor PYL9 for degradation. Int J Mol Sci 18:1841. https://doi.org/10.3390/ijms18091841

Zheng M, Lin J, Liu X, Chu W, Li J, Gao Y et al (2021) Histone acetyltransferase TaHAG1 acts as a crucial regulator to strengthen salt tolerance of hexaploid wheat. Plant Physiol 186:1951–1969. https://doi.org/10.1093/plphys/kiab187

Zhong X, Hong W, Shu Y, Li J, Liu L, Chen X, Islam F, Zhou W, Tang G (2022) CRISPR/Cas9 mediated gene-editing of GmHdz4 transcription factor enhances drought tolerance in soybean (*Glycine max* [L.] Merr.). Front Plant Sci 13:988505. https://doi.org/10.3389/fpls.2022.988505

Zhong X, Hu L, Tang G (2024) The application of genome editing technologies in soybean (*Glycine max* L.) for abiotic stress tolerance. In: Chen JT, Ahmar S (eds) Plant genome editing technologies. Interdisciplinary Biotechnological Advances. Springer, Singapore. https://doi.org/10.1007/978-981-99-9338-3_8

Zhou H, Liu B, Weeks DP, Spalding MH, Yang B (2014) Large chromosomal deletions and heritable small genetic changes induced by CRISPR/Cas9inrice. Nucl Acids Res 42:10903–10914. https://doi.org/10.1093/nar/gku806