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Genome editing: A sustainable approach to improving crop productivity and mitigating biotic and abiotic stresses in response to climate change

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Abstract
Mitigation of the environmental stresses and increased crop production is an important target to address the rising needs of a growing global population and ensure food security. The conventional breeding methods for improvement in crop production such as random mutagenesis or gene recombination appears to be tedious and less effective. Thereby, the recent genomic approach such as targeted gene editing through CRISPR/Cas9 has been identified to be a viable strategy for developing crops with better adaptability to hazardous environmental conditions. CRISPR/Cas9 based genome editing provides higher specificity and unearth increased approach for high resolution gene editing as compared to conventional approach. However, despite its effectiveness, social acceptance of genome editing remains a challenge. In this study, the potential application of CRISPR/Cas9 for developing crop plants with improved adaptation to diverse environmental stress conditions has been discussed.

Keywords: CRISPR/Cas9, stress, climate change, genome editing

Introduction

In recent days, the assessment of plant responses at the genetic level to surrounding environmental challenges has been the primary focus of crop breeders (Podevin *et al*., 2013) [27]. Identification and molecular understanding of the genes associated with the response to environmental stresses has been widely studied and reported (Abdelrahmana *et al*., 2018) [2]. Through the conventional genome editing based plant breeding approach, the introduction of genes associated with key agronomic traits for generation of high-yielding varieties has been successful in producing superior crop plants (Flint-Garcia, 2013; Abdelrahman *et al*., 2015, 2017) [9]. But, the usage of these superior cultivars has significantly reduced the genetic variability of significant crop plants, increasing their vulnerability to biotic and abiotic stress factor under variable climatic situations (Flint-Garcia, 2013) [9]. Thereby, in accordance to vulnerability of crops, a high throughput, gene-specific endonucleases-based techniques, such as Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR/Cas9), Zinc-finger nucleases (ZFNs) and Transcription Activator-Like Effector Nucleases (TALENs) may be applied to create crop varieties that are resilient to climate change, have high yield potential, and can tolerate various stresses. These targeted genomic processes have been extensively employed in genome editing to deliberately introduce precise alterations to the DNA sequence of crop plants (Abdallah *et al*., 2015; Zhu *et al*., 2017) [1, 47]. These genome editing approaches are particularly useful for crops with complex genomes that are difficult to breed using traditional methods (Abdelrahmana *et al*., 2018) [2].

In comparison to traditional plant breeding, a time-consuming, labour-intensive process and other tools like ZFN and TALEN, the CRISPR technology is more time and money efficient, more accurate, and has the ability to edit numerous areas of the targeted site at once. CRISPR allows for precise modification at nucleotide level in the genome, accurate insertion of new genes and the replacement or modification of existing genes with ones from wild progenitors. This approach has been reported in several studies and proved to be a more effective and targeted way of introducing desirable character into crop species (Abdallah *et al*., 2015; Zhu *et al*., 2017; Kamburova *et al*., 2017; Abdelrahmana *et al*., 2018) [1, 47, 16, 2].

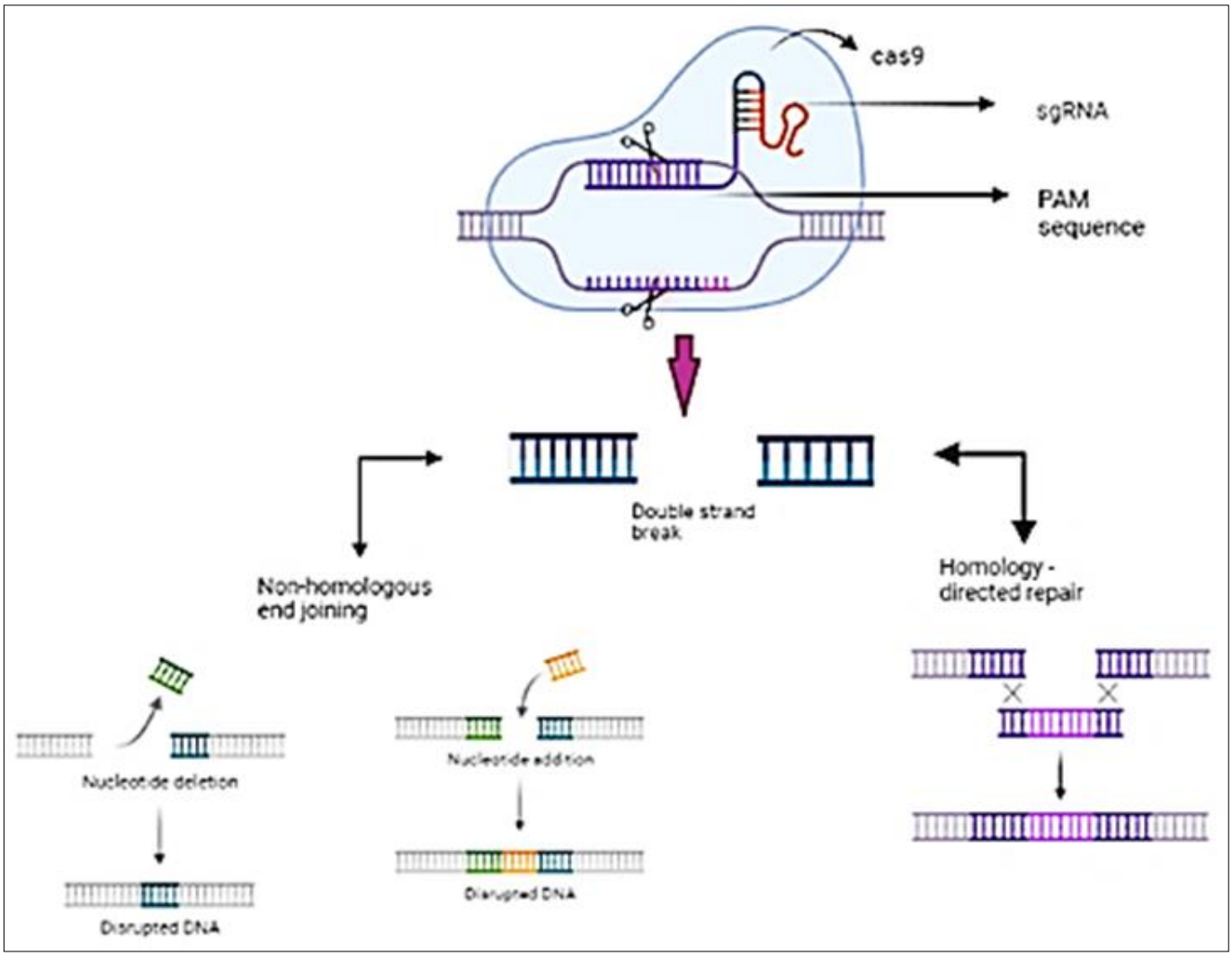

Fig 1: Method of CRISPR/Cas6 system using sgRNA induce non-homologous end joining and homologous recombination method.

Rapid development of novel crop cultivars is possible with precise genome editing techniques and with a minimal chance of unexpected consequences. The CRISPR/Cas9 technique is an immune based mechanism observed mainly in naturally existing bacteria such as *Corynebacterium glutamicum*. This mechanism of CRISPR uses the DNA fragments from invading viruses to create CRISPR array. These arrays are then translated into segments of RNA known as crRNA and tracrRNA, which combine to form a structure of two RNAs. The target site is then directed by Cas9 nuclease, to the structure gene, allowing for precise DNA editing (Wiedenheft *et al*., 2012) [43]. To efficiently target specific genomic locations, the natural dual tracrRNA is modified to create a single-guided RNA chimaera (sgRNA). This modification involves changing the crRNA structure and introducing Watson-Crick base pairing occurs at the 5' end of the sgRNA, which allows it to bind with guided Cas9 nucleases. This binding, results in cleavage at a specific spot and recognition to the targeted DNA that has the adjacent protospacer motif (PAM) (Tsai *et al*., 2015; Abdelrahmana *et al*., 2018) [37, 2]. Two natural DNA repair mechanisms, namely non-homologous end-joining (NHEJ) and homologous recombination (HR), to rectify damaged DNA are employed in cellular processes. This repair process can result in the emergence of new plant with undesired allele removed or the addition of desirable traits (Fig. 1).

The CRISPR/Cas9 technique is a revolutionary tool in genetic manipulation that is used to precisely edit the genetic content of living organisms. It consists of two primary constituents *i.e.*, CRISPR and the Cas9 protein (Rahman *et al*., 2022) [29]. The primary function utilized in CRISPR/Cas9 system is to perform gene knockouts. This involves disrupting or inactivating a specific gene by introducing targeted mutations into its DNA sequence (Lopez *et al*., 2015) [22]. The Cas9 protein, with guidance from a single guide RNA (sgRNA), attaches to the targeted gene's DNA sequence and induces a precise double-strand break (DSB) at a specific site (Rahman *et al*., 2022) [29]. When the cell repairs this break, errors can occur, leading to insertions or deletions (indels) in the gene's sequence (Rahman *et al*., 2022) [29]. These indels can cause frameshift mutations that result in the production of non-functional or truncated proteins, effectively knocking out the gene's function. Multiplex genome editing: Another significance of the CRISPR/Cas9 system involves its capacity to perform multiplex genome editing (Rahman *et al*., 2022) [29]. Designing multiple sgRNAs, each specific to a different gene, researchers can introduce multiple DSBs in various genomic locations. The repair process can then lead to simultaneous modifications in multiple genes, enabling the study of gene interactions and complex genetic networks. The CRISPR/Cas9 system can be utilized to modulate gene expression by either repressing or activating target genes,

thereby providing control over the activity of specific genes (Lopez *et al*., 2015) [22]. Instead of introducing mutations, researchers can modify the Cas9 protein to act as a transcriptional repressor or activator. This modified Cas9, called dCas9, is unable to induce DSBs but can still bind to specific DNA sequences guided by sgRNAs. When dCas9 is fused with a repressor domain, it can block the transcription of a targeted gene, effectively silencing its expression (Lopez *et al*., 2015) [22]. On the other hand, when dCas9 is fused with an activator domain, it can enhance the transcription of a target gene, leading to increased gene expression. Various options provided by the CRISPR/Cas9 system offer researchers tremendous flexibility and precision in genetic manipulation (Zhang *et al*., 2020). They enable the study of gene function, the investigation of complex biological processes, and the potential development of therapeutic interventions by precisely modifying and controlling genes within living organisms.

Implementation of CRISPR/Cas9 to enhance abiotic stress tolerance in crop plants

The use of genetically engineered technology, which allows for the manipulation of gene expression, is a crucial tool that contribute to a plant's resistance to abiotic stress (Abdelrahmana *et al*., 2018) [2]. Various findings have been reported the successful use of CRISPR technology to enhance the drought resistance of plants. Insufficient water availability and unfavourable temperatures during any stage of crop growth can have adverse impacts on the overall development and productivity of plants. The intensity and duration of the stress, along with the growth stage of the plant, have a significant impact on the extent of these detrimental effects. These stressors lead to diverse alterations in the morphology, physiology, biochemistry, and molecular mechanisms of the affected plants. For example, researchers have demonstrated that by down-regulating specific regulatory genes like *DERF1*, *MSH1*, *PMS3*, *MYB5*, and *SPP* using CRISPR/Cas9, rice plants can be made more drought-tolerant (Zhang *et al*., 2014). Similarly, in *Arabidopsis*, the use of CRISPR/Cas9 to target the *OST2* structural gene has been found to enhance the plant's drought tolerance (Rahman *et al*., 2022) [29]. In a study conducted by Wang *et al*. (2017), CRISPR/Cas9 technology was employed to disrupt the *SlMAPK3* gene in tomato plants, aiming to gain deeper insights into the gene's involvement in drought tolerance. Through a comparison of the phenotype and gene expression patterns between the *SlMAPK3*-knockout tomato plants and the wild-type plants under drought stress, it was discovered that the *SlMAPK3* gene has a significant influence on the regulation of genes involved in the drought response pathway in tomato plants. This finding, consistent with the research conducted by Li *et al*. (2017), underscores the potential of CRISPR/Cas9 technology for functional analysis of plant genes and identification of novel targets for crop improvement under abiotic stress conditions.

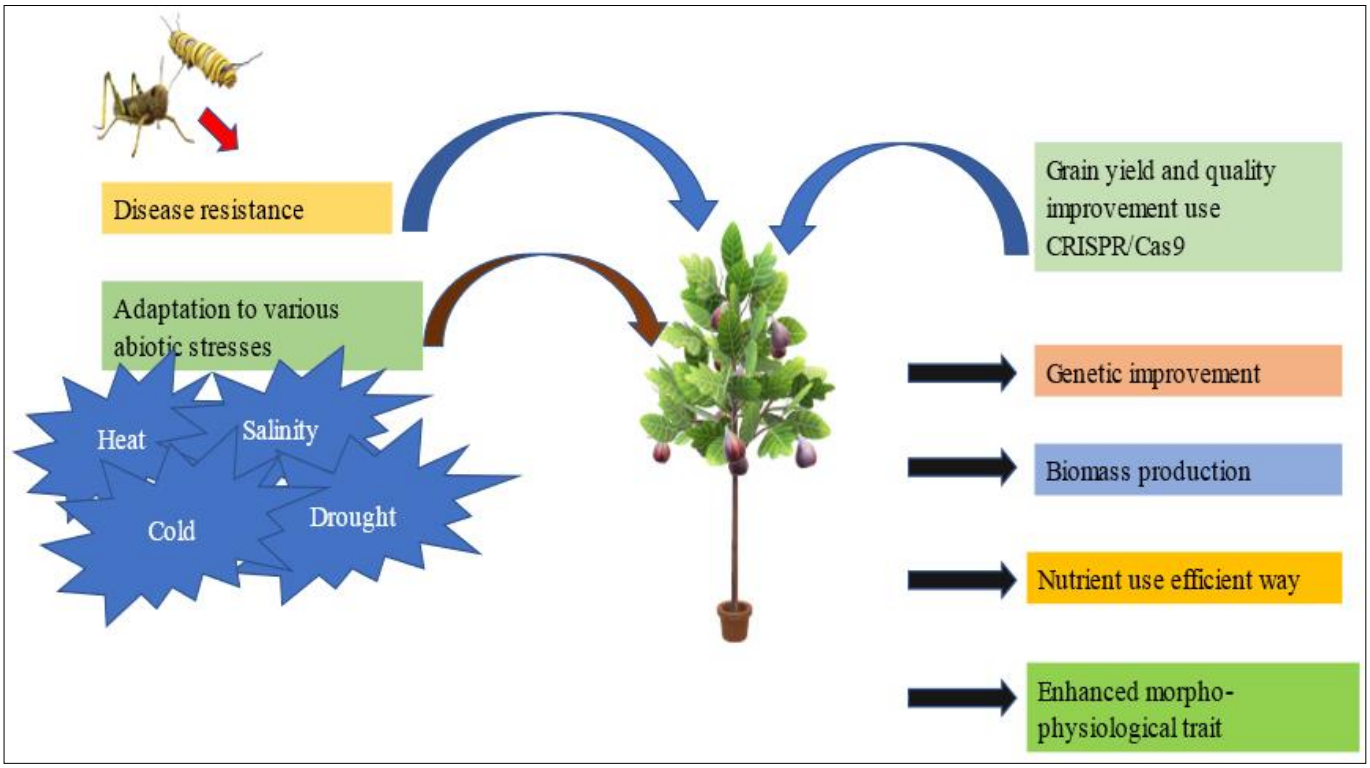

Fig 2: Representation of various abiotic and biotic stress on plant and improvement through CRISPR/Cas9 system

In addition to drought stress, the usage of CRISPR genome editing mechanism has also been studied in analysis of different other abiotic stress tolerance process. Salinization of soil or salt stress due to excessive salt content is another environmental factor that significantly reduces soil fertility and impede crop cultivation (Rahneshan *et al*., 2018; Shrivastava *et al*., 2015) [30, 33]. Salt stress induces osmotic stress and nutritional imbalances in plants, leading to negative impacts on their morphology, biochemistry, and biomass (Ali *et al*., 2017) [3]. Under salt stress conditions, genome editing and genetic engineering techniques have been utilized to specifically target genes associated with ion transport. This enables the regulation of osmotic adjustment mechanisms, aiding in the plant's ability to cope with salt

stress (Volkov *et al*., 2015) [41]. Several studies have demonstrated the positive effects of manipulating certain genes using these tools (Yue *et al*., 2012). For example, overexpression of the *SOS1* gene in Arabidopsis and the *HvHKT2;1* gene in barley has been shown to enhance salinity tolerance by increasing the translocation of sodium ions (Kim *et al*., 2021). Similarly, editing the *OsRR22* gene in rice has resulted in increased salinity tolerance. Additionally, the use of CRISPR/Cas9 technology has been employed to create mutants of the *OsPQT3* gene in rice, leading to a high degree of salinity tolerance (Zhang *et al*., 2019; Liang *et al*., 2022) [46, 19]. To explored the role of *OsmiR535*, a microRNA, in salt stress tolerance in rice. Knocking out the *OsmiR535* gene using CRISPR/Cas9 has been suggested as a strategy to improve salinity tolerance in rice (Zhang *et al*., 2019) [46]. Specifically, a homozygous five-base pair deletion in the coding sequence of *OsmiR535* has been identified as a potential target for enhancing salinity tolerance (Yue *et al*., 2020). In tomato plants, the negative regulator of salt stress, Hybrid Proline-Rich Protein 1 (*HyPRP1*) gene, has been manipulated using CRISPR/Cas9 to improve salinity tolerance (Tran *et al*., 2021). Knocking down the negative-response domain(s) of the *SlHyPRP1* gene has shown to enhance salinity tolerance at both seedling and vegetative stages in tomato plants. Another gene, *ACQOS*, has been investigated in *Arabidopsis* using CRISPR/Cas9 technology. The induction of small insertion/deletion mutations in ACQOS has suggested its direct linkage with salt stress resistance in *Arabidopsis* (Kim *et al*., 2021). Overall, the reports mentioned highlights the major applications of CRISPR/Cas9 in manipulating specific genes engaged in the reaction and adaptability to salt-induced stress and offering promising strategies for improving crop resilience to salinity.

Heat stress another significant abiotic stress factor that occurs when the temperature in an environment rises 10-15℃ over the temperature needed for regular growth of the plant (Harvey, 2018) [11]. Plants when exposed to temperatures above their optimal range, undergoes physiological changes that negatively impact their developement and productivity (Ali *et al*., 2022). The negative effects of heat stress include reduced photosynthesis, decreased water-use efficiency, impaired nutrient uptake, and altered hormone signalling and increased generation of ROS (Haider *et al*., 2022; Dos Santos *et al*., 2022) [12, 8]. It has been reported that the knockout of the heat-sensitive albino1 (*OsHSA1*) gene in rice leads to increased sensitivity to heat stress, as well as a faster greening phenotype (Qiu *et al*., 2018) [28]. This shown that the *OsHSA1* gene is crucial for the growth and defence of chloroplasts against heat stress. While rice is in various growth stages, the gene produces a protein like fructokinase that aids in protecting and developing the chloroplasts (Qiu *et al*., 2018) [28]. Research has demonstrated that the gene *HSA1* plays crucial roles in the early stages of chloroplast development and also functions as a protective mechanism for chloroplasts during heat stress in later stages (Zaman *et al*., 2019) [45].

High temperatures highly impact the development of pollen grains, leading to decreased mitochondrial activity and the elimination of respiratory substrates (Alsamir *et al*., 2021) [4]. Aux/IAA9 (IAA9) is a gene that is required in regulating plant growth and development by controlling the response to the plant hormone auxin. In tomato plants, IAA9 is specifically involved in fruit development and plays a role in preventing parthenocarpy, which is the development of fruit without pollination (Ueta *et al*., 2017) [39]. *AUX/IAA9* Mutant develop through CRISPER/Cas9 plants showed Fruit development occur without the need for fertilization, and these developmental traits passed down to future generations through genetic mutations (Ueta *et al*.,2017) [39]. Parthenocarpy, which refers to the development of fruits without the need for fertilization, is a valuable trait to generate seedless fruits. This is because it allows for fruit development independent of fertilization, resulting in high-quality fruits with high demand (Rahman *et al*., 2022) [29]. In the process of screening a population of tomato plants that were exposed to heat stress and treated with ethyl-methane sulfonate (EMS) mutation, a mutant variety was identified. This mutant exhibited the desirable characteristic of producing seedless fruits of excellent quality (Rahman *et al*., 2022) [29]. Kashojiya *et al*. (2022) [17] reported the ultimate utilization of CRISPR/Cas9 system to precisely target and edit the *DELLA* gene under heat stress, resulting in increased parthenocarpy and potentially higher yields. *DELLA*, a hormone, exerts an inhibitory effect on the signalling pathway of gibberellin. Similarly, this technique can also be used to enhance associated species of Solanaceae family, like peppers and eggplants. (Livne *et al*., 2015; Tabassum *et al*., 2021) [21, 35].

By targeting specific genes that regulate important agronomic traits, such as fruit size, disease resistance, and stress tolerance, CRISPR/Cas9 can help to create new varieties with improved characteristics that are better suited to specific growing conditions and market demands (Wang, C *et al*.,2018) [18]. These investigations highlight the need of comprehending the genetic pathways underlying plant responses to heat stress. By identifying key genes and proteins involved in protecting plants from heat stress, researchers may be able to create fresh methods for enhancing crop output and resilience under difficult environmental situations. (Liao *et al*., 2019) [20].

Extreme cold temperatures also observed in various ecological regions worldwide, in addition to heat stress also highly impacts the expansion of plants (Carol *et al*., 2013). These low temperatures are categorized into stresses *i.e.*, freezing stress, which is below 0°C, and chilling stress, which ranges from 0°C to 15°C (Shi, Y.; Ding *et al*., 2018; Guo *et al*., 2018) [18]. When plants are kept under excessive cold temperatures, their growth can be hindered due to mechanical damage and disruption of metabolic processes (Yadav *et al*., 2010; Rahman *et al*., 2022; Adams *et al*., 2016; Muller *et al*., 2014; Rahman *et al*., 2022) [29]. Low temperatures can cause harm to crop species, leading to negative effects on their growth, productivity, and survival (Sanghera, *et al*., 2011; Rahman *et al*., 2022) [29]. To combat this, Proline-rich proteins (PRPs), which are associated with development and capacity of tolerance in plants, controls biological processes (Rahman *et al*., 2022) [29]. In rice, a gene called *OsMYB30*, which responds to cold, was deactivated using CRISPR/Cas9 technology (Zeng, Y *et al*., 2020; Rahman *et al*., 2022) [48, 29]. The resulting plants showed better tolerance to cold compared to normal rice. Similarly, in Arabidopsis, a study using CRISPR/Cas9 was conducted to study the role of three genes (*CBF1*, *CBF2*, and *CBF3*) in cold adaptation. Mutants lacking all three genes were found to be highly sensitive to chill stress in comparison to normal plants (Zhao *et al*., 2016; Navada *et*

al., 2020) [44, 24]. Plants can enhance their freezing tolerance in response to prolonged exposure to cold temperatures by increasing the upregulation of *CBF* genes. This results in the creation of CBF proteins, which in turn trigger the transcription of additional genes that are responsive to cold, assisting the plant in adjusting to the cold conditions (Zhao *et al*.,2016) [44]. Overall, CRISPR technology has the potential in significantly improving the crop resilience to abiotic stresses, ultimately leading to increased yields and improved food security (Table 1).

Table 1: Application of CRISPR/Cas9 based on specific gene-targeted approach for abiotic stress tolerance in crop plants

Crop plants	Genome editing technology used	Targeted gene	Function	Types of mutation to understand the function of gene	Reference
Tomato	CRISPR/Cas9	SlMAPK3	Drought tolerance	Gene knockout	Wang *et al*., 2017
Rice	CRISPR/Cas9	DERF1, PMS3, MSH1, MYB5	Drought tolerance	Gene knockout	Zhang *et al*., 2014
Arabidopsis thaliana	CRISPR/Cas9	OST2	Drought resistance	Gene knock-in	Osakabe *et al*., 2016; Rahman *et al*., 2022 [29]
Rice	CRISPR/Cas9	OsHSA1	Heat tolerance	Gene knockout	Qiu *et al*., 2018 [28]
Tomato	CRISPR/Cas9	IAA9	Preventing parthenocarpy	Gene knock-in	Ueta *et al*., 2017 [39]
Rice	CRISPR/Cas9	OsMYB30	Cold responsive	Gene knockout	Zeng *et al*., 2020; [48] Rahman *et al*., 2022 [29]
Arabidopsis thaliana	CRISPR/Cas9	CBF1, CBF2, and CBF3	Cold tolerance	Gene knockout	Zhao *et al*., 2016 [44]

Utilization of CRISPR/Cas9 for development of disease resistant plants

To cope up with sufficient food supply against the rising global population and meet food security worldwide, a consistent and long-lasting growth for high production of crops is crucial for sustainability in agriculture. Uneven food distribution around the world is because of the range of factors such as economic, social, and environmental issues. The occurrence of fungal, bacterial, and viral pathogens constitutes biotic stress that negatively affects crop production. The constant emergence of new and more potent pathogens further complicates the efforts to control them. For instance, in 1998, a highly aggressive strain of *Puccinia graminis*, known as Ug99, emerged in Uganda and caused up to 90% yield losses in vulnerable wheat varieties (Abdelrahman *et al*., 2018) [2]. Similar to this, over a million trees in the Middle East died as a result of the advent of Phytoplasma that induced the deadly witches' broom disease in acid lime trees between the 1970s and 1990s. These examples highlight the devastating effects of biotic stresses on crops and the urgent need to find ways to prevent and control them (Nuñez-Muñoz *et al*., 2021) [25].

CRISPR/Cas9 technique was employed to make changes to the regulating region of two different variants of the canker susceptibility gene known as *CsLOB1* in *Citrus paradisi*. This was done with the aim of altering the function of the gene and potentially increasing the resistance of the grapefruit to canker disease. Specifically, the mutations induced in the effector binding elements (EBEs) that are responsible for regulating the activity of the *CsLOB1* transcription factor which results in disruption and frame shift of *CsLOB1* coding region (Jia *et al*., 2017) [15]. *DLOB9* and *DLOB10* mutant lines had a high mutation rate as a result, which frameshift variation in the *CsLOB1* coding area. This disruption led to a disease resistance against *Xanthomonas citri* subsp. citri has improved, thereby making the mutant grapefruit more resistant to this disease (Jia *et al*., in 2017; Abdelrahman *et al*., 2018) [15, 2]. An alternative approach for enhancing the inherent resistance of crop plants involves modifying the synthesis or signaling pathways of phytohormones. (Wang *et al*.,2016; Shukla *et al*.,2017). For example, the successfully rice plants with enhanced immunity against the rice blast virus was developed using the CRISPR/Cas9 system technology. In this using CRISPR technology the ethylene-responsive factor922 (*OsERF922*) gene was deactivated which led to enhanced immunity (Wang *et al*., 2016). A recent finding using the CRISPR/Cas9 technology, two mutant alleles known as *wrky75-c1* and *wrky75-c2* were produced in the *Arabidopsis wrky75* plant (Guo *et al*., 2017). These mutant alleles were generated by inducing a single-base insertion and a four-base deletion, which resulted in the creation of an early stop codon, respectively (Guo *et al*., 2017; Abdelrahman *et al*., 2018). According to study, plants of wrky*75* mutant lines succumbed to infection with *Pseudomonas syringe* pv tomato, that showed decreased levels of salicylic acid (SA), with down-regulation of the SA induction-deficient 2 (SID2) gene, and increase in higher disease severity as compared to the wild-type plants (Guo *et al*.,2017; Abdelrahman *et al*.,2018). In another study, it was found that Arabidopsis plants overexpressing the *WRKY75* gene using the 35S promoter had higher levels of SA and up-regulated SID2 expression compared to wild type (WT) plants (Guo *et al*., 2017)As a result, these plants had improved resistance to *Pseudomonas syringe*, a plant pathogen, compared to WT plants. This finding indicates that WRKY75 modulates salicylic levels to enhance tolerance to *P. syringe* in Arabidopsis. (Guo *et al*., 2017). The wrky75 gene was changed using the CRISPR/Cas9 technique, and the mutant plants that were produced aged their leaves more slowly than the wild type. On the other hand, plants overexpressing wrky75 showed an earlier onset of leaf aging, which is an important characteristic for prolonging the period of grain filling during adverse environmental effect (Abdelrahman *et al*., 2017; Guo *et al*., 2017).

Hence a potent genetic engineering technology CRISPR/Cas9 now has been applied to enhance their tolerant capacity, by targeting specific genes that control susceptibility to pathogens (Table 2).

Table 2: An overview on recent CRISPR/Cas9 technological uses for plant disease resistance

Pathogen	Targeted crop plant name	Targeted site/ gene	Used technology	Function	Reference
Puccinia graminis	Wheat (Black rust)	Stem of wheat	CRISPR/Cas9	Yield loss	Pretorius *et al*., 2000; Abdelrahmana *et al*., 2018
Xanthomonascitri	*Citrus paradisi*	Canker susceptibility lateral organ boundaries (CsLOB1)	CRISPR/Cas9	Disruption of *CsLOB1* site to become resistance (*Xanthomonas citri*)	Jia *et al*., 2017; Abdelrahmana *et al*., 2018
Magnaportheoryza	*Oryza sativa*	Ethylene-responsive factor922 (OsERF922)	CRISPR/Cas9	Enhanced immunity of rice plant by disrupting *OsERF922* gene	Wang *et al*., 2016
Pseudomonas syringae	*Arabidopsis*	WRKY75	CRISPR/Cas9	*WRKY75* mutant line reduce level of SA acid increase disease severity with *Pseudomonas syringae*	Guo *et al*., 2017; Abdelrahmana *et al*., 2018 [2]

Enhancing plant tolerance to climate change using CRISPR

In the recent scenario, the phenotypic development with the potential for the production of crop plants are negatively impacted by changes in the global environment, such as climate change and environmental pollution. To address this issue, new phytoremediation methods are being developed that can withstand the condition of climate change and generate resilience to face global environmental challenges. Nowadays, the gene-editing technique CRISPR/ Cas9 has been employed extensively for heavy metal phytoremediation, which lessens the negative impacts of environmental pollutants on different agricultural plants (Venegas *et al*., 2021). This method has shown great potential for phytoremediation, and several plants, including *Thlaspi caerulescens* (which accumulates Ni, Zn, and Cd), *Arabidopsis halleri* (which accumulates Zn and Cd), *Hirschfeldia incana* (which controls Pb), *Brassica juncea* and *Pteris vittata* have been sequenced as phytoremediators (Basharatet *et al*., 2018) For example, in indica rice, a gene responsible for transporting metals called *OsNRAMP5* was modified using the CRISPR-Cas9 genome editing technology. This modification resulted in a decrease in the accumulation of the toxic heavy metal cadmium, while maintaining the crop yield (Tang *et al*., 2017; Rahman *et al*., 2022) [29]. The ability of plants to tolerate, detoxify, and absorb heavy metals has been demonstrated to be improved by the application of CRISPR/ Cas9 genome editing, which supports phytoremediation (Rahman *et al*., 2022) [29]. Researchers have observed that overexpressing genes such as *MT1*, *MT2*, and *MTA1*, which encode metallothioneins, can increase the potential of Arabidopsis and tobacco plants to accumulate copper, zinc, and cadmium (Sebastian *et al*., 2019; Rahman *et al*., 2022) [39, 29]. The ability of Arabidopsis to withstand high amounts of copper and cadmium was enhanced by the insertion of the *BcMT1* and *BcMT2* metallothionein genes from *B. campestris* (Lv *et al*., 2013) [23]. The utilization of CRISPR technology has now opened up the possibility of using phytoremediation techniques in other plants like maize and poplar, which were previously not studied because of the complexity of their genomes (Saxena *et al*., 2020) [31].

Conclusion and future prospective

Humans have been practicing plant breeding since ancient times to generate high yielding crops for food security. It has been a crucial and challenging task to meet the growing demand for food. Modern genetic engineering technologies, particularly CRISPR/Cas9, offer several advantages over traditional breeding methods in overcoming compatibility issues that may arise. This technique has been effectively used to improve crop tolerance against environmental stresses. However, to avoid off-target mutations, it is important to carefully select the on-target site in the genome. The limited availability of genomic sequence information in many crop species hinders the implementation of CRISPR/Cas9 in agriculture. While CRISPR/Cas9 technology has shown the potential to create crops that can better withstand stress, further research is necessary to develop crops that can withstand stress under field conditions. The CRISPR/Cas9 system has also shown potential in producing new varieties of staple crops that are more tolerant to stress (Abdelrahmana *et al*., 2018) [2]. Regulator difficulties and public acceptance of genetically altered crops, however, continue to be important problems that require attention.

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