e-planet **23** (2): 129-147 (December 2025)

Advancing sustainable agriculture through CRISPR/ Cas-mediated crop improvement

T.R. DAS[1*], P. CHAND[2] AND A. SINGH[3]

[1]*Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi-110012, India*

[2]*Department of Genetics and Plant Breeding, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, 250110, India*

[3]*Department of Genetics and Plant Breeding, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand-263145, India*

[*]*trdas.iari@gmail.com*

Date of receipt: 09.10. 2025 Date of acceptance: 07.12.25

ABSTRACT

CRISPR/Cas is a precise and efficient genome-editing technology that has revolutionized crop improvement by enabling the development of crops that are more productive, resilient, and nutritionally enhanced. As global agriculture faces pressures from climate change, pests, diseases, land degradation, and rising food demand, CRISPR/Cas-based approaches offer unprecedented opportunities for building a sustainable and resilient food system. Compared to traditional breeding and earlier genetic modification techniques, CRISPR/Cas is faster, highly accurate, and capable of achieving desired traits in less time. It enhances crop resistance to both abiotic and biotic stresses, improves nutrient uptake, mitigates the effects of heavy metals, and supports plant growth under challenging conditions such as drought, salinity, and heat. Beyond stress resilience, CRISPR/Cas is being used to improve nutritional quality, including iron- and zinc-enriched grains, low-gluten wheat for celiac-sensitive individuals, and oilseeds with healthier fatty acid profiles. These improvements not only benefit farmers and consumers but also reduce the environmental footprint of agriculture by decreasing the need for fertilizers, pesticides, and irrigation, thereby conserving water and lowering carbon emissions. Despite these advantages, several challenges remain, including potential off-target genetic edits, difficulties in delivering CRISPR components to certain species, and varying ethical and regulatory frameworks across countries. Addressing these issues requires transparent communication, public engagement, and inclusive policies to ensure equitable access, particularly for smallholder farmers and developing regions. The future of CRISPR/Cas is further strengthened by integration with emerging technologies such as artificial intelligence, machine learning, and speed breeding. Together, these innovations herald a new era of sustainable agriculture and serve as a cornerstone for global food security, enabling the development of high-yielding, climate-resilient, and nutrient-rich varieties that contribute to multiple Sustainable Development Goals (SDGs).

Key words: Artificial intelligence, CRISPR, crop improvement, gene editing, genomics

INTRODUCTION

A potent molecular tool for many biological research fields where it is helpful to target or alter particular DNA sequences is the clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 genome engineering technology (Hsu et al*.,* 2014; Sander and Joung, 2014; Cox et al*.,* 2015; Wright et al., 2016). The Cas9 nuclease, which was initially developed from the CRISPR-Cas bacterial adaptive immune system (Barrangou et al*.,*

2007), can locate and cleave a target DNA using a guide RNA (Gasiunas et al*.,* 2012). In eukaryotic cells, the Cas9 nuclease has been designed to modify target DNA at specific locations (Mali et al*.,* 2013). But like its predecessors in genome editing, zinc finger nucleases and transcriptional activator-like effector nucleases, Cas9 is known to bind and cleave at off-target sites in the context of large eukaryotic genomes (Fu et al*.,* 2013). Therefore, there has been a lot of interest in the field in efforts to quantify, comprehend, and enhance Cas9 specificity. Although earlier review articles should be consulted for background information and understanding on the development and applications of Cas9 genome editing but in last five years, novel mutant lines with improved desirable traits have been produced in large part by CRISPR-based genome-editing techniques for crop genome modification. The ability to simultaneously improve multiple traits directly in elite lines is one of the biggest benefits of using genome editing techniques for crop breeding. This option speeds up the development of commercial products, which is typically impractical when using conventional breeding (Gao et al*.,* 2020). These strategies, commonly known as multiplex editing, involve the simultaneous modification of several loci, which is particularly important for the improvement of characteristics controlled by QTLs (Quantitative Trait Loci) (Rodríguez-Leal et al*.,* 2017). Therefore, the CRISPR system was a significant advancement in genome editing technology, particularly as it confers target specificity without relying on the drawn-out and expensive process of protein modification. The technology swiftly spread throughout labs worldwide since, generally speaking, reprogramming the system to edit other targets hinges merely on the exchange of guide RNA molecules. Since then, a number of organisms and crop plants have benefited from the increased efficiency of CRISPR/Cas genome editing technology (Li et al., 2020).

CRISPR - A REVOLUTION IN GENOME EDITING

The story of CRISPR begins with a bacterium. Japanese researchers that looked into the genome of the bacterium *E. coli* announced the first finding of CRISPR sequences in 1987. They discovered five identical DNA fragments that were repeated and divided by identically sized non-repetitive DNA sequences. Since these DNA repetitions could not be explained at the time, they were regarded as a curiosity. Nevertheless, these repetitive DNA sequences were consistently observed by researchers looking at the genomes of additional bacterial species. These species included gut-dwelling bacteria and those utilized to produce cheese and yoghurt. Since then, it has been discovered that CRISPR sequences are present in over half of all bacterial species (Makarova et al., 2015). The puzzle was further compounded by the discovery that these regular DNA repetitions usually co-occur with a common set of genes known as CAS genes. A group of Dutch microbiologists named the DNA region containing these repeats "CRISPR" in 2002. "CRISPR" stands for "clustered regularly interspaced short palindromic repeats," and the associated genes were dubbed "CAS" genes, or CRISPR-associated genes (Jansen et al*.,* 2002). The proteins that the CAS genes encode act as molecular scissors that can cut DNA, as was soon discovered. The DNA sequences between the repeats are nearly identical to the genetic material of viruses that infect bacteria (Mojica et al., 2005; Pourcel et al*.,* 2005). The idea that CRISPR-Cas was a mechanism to defend bacteria against bacteriophages was then put forth. The bacterium employs Cas proteins in conjunction with DNA sequences it has collected from invasive viruses to identify and cut the attacking viruses' DNA. Researchers initially experimentally showed that CRISPR-Cas is an efficient component of the bacterial immune system in 2007 using the yogurt-making bacterium *Streptococcus thermophilus*. Bacteria that are repeatedly exposed to a virus eventually become resistant to it. The presence of viral DNA pieces in the bacteria's CRISPR region coexists with this resistance. The resistance vanished right away once the researchers extracted the viral segments from the CRISPR area. Over time, other CRISPR-Cas systems have been discovered, and while they differ in certain ways, the process is always the same. The bacterium's CRISPR library contains DNA fragments from which RNA is read. After then, these CRISPR RNA fragments start looking for viral genes. The viral DNA is then cut by the Cas protein, which is directed by the CRISPR RNA sequence. Thus, the

accumulation of virus DNA pieces functions as a sort of memory which makes it possible for the bacteria to identify and combat the virus the next time it hits (Jinek et al*.,* 2012). In 2012, Jennifer Doudna and Emmanuelle Charpentier demonstrated that it was possible to reprogram the CRISPR-Cas complex, marking a significant advancement in the use of CRISPR Cas as a technology for editing the genomes of microbes, plants, and animals. It is possible to make the complicated cut at any chosen position in the genome by altering the CRISPR RNA molecules sequence. It is important to make sure that the CRISPR RNA sequence corresponds to the DNA sequence where the cut is to be done (Gasiunas et al*.,* 2012). Numerous articles soon followed, in which the technique was used to various crops and for various objectives that demonstrated the great adaptability of CRISPR-Cas technology in crop improvement.

MECHANISM OF CRISPR-CAS TECHNOLOGY

According to Cong et al. (2013), the CRISPR-associated protein-9 (Cas9) and a guide RNA (sgRNA) make up the CRISPR/Cas9 system. The transactivating crRNA and the protospacer-matching crRNA, which are essential for CRISPR action, are combined to form the sgRNA. The 20 nucleotides at the 5'end of sgRNA/Cas9 target the desired gene in the genome. The target site is located directly upstream of the Protospacer Adjacent Motif (PAM) sequence (NGG for SpCas9 from *Streptococcus pyogenes*) and varies depending on the species of bacterium. A little DNA region called the PAM is situated downstream of the cleavage point. Large DNA endonuclease SpCas9 produces blunt-ended double strand breaks (DSBs) by cleaving particular genomic areas. Two routes for Cas-9 protein-induced DSB repair were discovered by Liu et al. (2019). Non-homologous end joining (NHEJ) and homology-directed repair (HDR) are the two categories of repair methods. At first, Cas9 from *Streptococcus pyogenes* was used in the majority of genome editing investigations. But thanks to recent developments, several improved forms of the wild-type Cas9 have been created (Tycko et al., 2016). There are more Cas types being researched for genome editing besides Cas9. For this reason, Cas3, Cas12, and Cas13 are also being studied. The recommended technique for genome editing is Cas12a.

Makarova et al. (2011) identified three types of CRISPR/Cas. Bacteria and archaea have type I CRISPR/Cas systems, identified by the unique signature of the Cas protein. Cas3 attaches to DNA using its endonuclease activity. Microbes use type II CRISPR/Cas arrays. The system includes Cas1, Cas2, Cas4/Csn2, and Cas9. Archaea and some bacteria use type III CRISPR/Cas to find DNA and RNA. Cas6, Cas10, and RAMP are present. Cas10 protein cleaves DNA after crRNA digestion. The creation of a single chimeric molecule, known as sgRNA (single guide RNA) or gRNA (guide RNA), that combines the crRNA and tracrRNA simplifies the eukaryotic genome editing platform (Koonin et al*.,* 2017). Accordingly, the tracrRNA, which has a secondary structure in the form of three hairpins required for the Cas enzyme's recognition as well as a hairpin structure to halt its transcription, is fused to the sgRNA molecule, which contains the crRNA sequence with its spacer complementary to the target DNA sequence (protospacer) (Jinek et al*.,* 2012). Almost any particular sequence of interest in the genome can be recognized by this streamlined two-component method, provided that it is located next to a PAM site. The Cas9 enzyme first uses its recognition lobe (REC) to identify the sgRNA. After forming, the Cas9-sgRNA complex looks for a PAM site on the DNA molecule, which the Cas9 Rec lobe also recognizes. The complementary target DNA and the sgRNA can then link with 20-24 nucleotides when Cas9 opens the DNA molecule directly upstream of the PAM. When there are more than three non-complementary nucleotides in a DNA site, the Cas9-sgRNA complex typically cannot identify it. It also cannot identify and cleave target DNA that has any non-complementary nucleotides in the 10-12 nucleotides close to the PAM site. The complementary and non-complementary strands of DNA are only cleaved by the HNH and RuvC Cas9 nuclease activity domains after full pairing, more precisely in the third nucleotide upstream of the PAM site. Therefore, the conserved PAM sequence in the target DNA and the pairing of its protospacer region with the spacer region (mostly the seed region) of the sgRNA dictate the Cas9-binding selectivity to the target DNA. Lastly, the organism being edited recruits DNA repair mechanisms as a result of the double-

strand cleavage. The repair system can generally proceed in one of two ways: either by homologous recombination (HR) or non-homologous ends joining (NHEJ). HR repair is guided by homology, enabling the high-fidelity insertion of sequences of interest in the edited region, whereas NHEJ repair typically results in minor insertions and/or deletions (indels) surrounding the cleavage site (Chen et al*.,* 2019; Anzalone et al*.,* 2020).

Homology-directed repair is highly accurate using a homologous DNA template. HDR operates in the late S and G_2 phases of the cell cycle, requiring multiple donor DNA templates containing the target DNA sequence. Gene editing involves transferring DNA from a donor to the target genome at the predicted double-strand break location (Liu et al*.,* 2019; Yang et al*.,* 2020). Non-homologous end-joining is a process that connects DNA fragments without the need for external homologous DNA, which helps speed up DSB repairs. CRISPR/Cas is a superior method for genome modification compared to previous approaches in terms of ease, stability, and success rates. CRISPR/Cas replaced ZFN and TALEN as the preferred method for genome editing due to its superior features. CRISPR/Cas system's genome targeting abilities are being actively studied in different crops for improvement of different desirable characters.

APPLICATION OF CRISPR TECHNOLOGY IN CROP IMPROVEMENT

Enhancing crop yield

Improving yield in a short period of time is essential to meet growing global food demand which can be fulfilled by CRISPR/Cas technology as it can enhance yield attributing traits by targeting specific genes and pathways. Recently, the CRISPR/Cas9 technique was employed to boost yields in maize and rice through loss-of-function mutations of the Kernel Row Number 2 (KRN2) genes ZmKRN2 and OsKRN2, respectively, without impacting other agronomic parameters. These findings implied that these KRN2 orthologs might be the focus of crop development initiatives (Chen et al., 2022). Enhancing photosynthetic efficiency by targeting the OsSXK1 gene in rice, has improved photosynthetic rates and increased grain yield (Zheng et al., 2021). By using CRISPR to decouple the length and quantity of panicles per plant, Ideal Plant Architecture 1 (IPA1) was eliminated. This prevented IPA1 from being expressed in both the roots and the panicles, increasing the yields of rice grains (Song, 2022). The CRISPR-mediated editing of ZmRAVL also reduced ZmUPA1 and ZmUPA2 expression, which increased maize yields at different planting densities (Wei, 2022). While DUO-B1 CRISPR modification in wheat had no influence on other agronomic parameters, it enhanced the formation of extra spikelet and grain yields (Wang, 2022). CRISPR/Cas9 was used to create single and double mutants of the pectin methylesterase genes Ovule Pectin Modifier 1 and 2 (HvOPM1 and HvOPM2) in barley (*Hordeum vulgare*) (Yang, 2023). The mutants' phenotypes revealed an increase in ovule, ovarian, and grain size, which may result in increased plant yield overall. Some other achievements are given in Table 1.

Table 1. CRISPR/Cas technology in Enhancing crop yield

Crops	Targeted genes	Applications	References
Rice	OsGS2/GRF4	Increase seed size and yield	Wang et al., 2022
	RDD	Suppress miR166 recognition and influences photosynthesis	Iwamoto, 2022
	OsCKX,	Enhance growth by hormone regulation	Zheng et al., 2023
	DEP1	Development of semi- dwarf plants with lodging resistance and higher grain yield	Zhang et al., 2023a
	OsNAS2	Increase zinc uptake and plant yield	Ludwig et al., 2024
	OsAPL	Increase yield in rice by improvement in nutrient transport	Zhang et al.,2024
Maize	RZ2MS9	Enhance growth	Figueredo et al., 2023
Barley	GW2.1	Reduce seed setting and yield	Kis et al., 2024

Improving abiotic and biotic stress resistant

One of the main causes of low productivity in India is the lack of suitable genotypes having resistance to different biotic and abiotic stresses with high yielding capacity (Das et al. 2021a). In order to assure sustainable food supply, agricultural output must expand by 70% to 80% by 2050. In present changing climatic conditions, crops face greater challenges due to various abiotic stresses, that is, drought, salinity, heavy metal, high radiation and extreme temperature (Veerala et al. 2024). Only 10% of cropland is designated as stress-free, indicating that most crops are exposed to one or more environmental pressures. To solve these problems, plant genome editing tools have been developed as an alternative for crop improvement. These technologies involve mobilization of genes or their regulatory elements in the genome of interest to generate stress resistant abiotic crops. By altering the plant genome in a more precise and focused manner, genome editing methods enable the development of crops with desired characteristics, such increased resilience compared to pre-environmental modifications. Through the induction of stress tolerance traits, CRISPR has the potential to enhance plants and enable precise change of the targeted genome (Table 2).

Table 2. CRISPR/Cas technology in enhancing abiotic stress resistant

Crops	Targeted genes	Applications	References
Rice	OsDEP1, OsROCs	Enhanced heat resistance	Malzahn et al., 2019
	OsGER4	Enhanced heat resistance	Nguyen et al., 2023
	OsPUB7	Enhanced drought resistance	Kim et al., 2023
	OsTPP3	Improved salt resistance	Ye et al., 2023
	OsLCD	Generated low cadmium	Chen et al., 2023
	K5.2	Increased Ca accumulation	Wang et al., 2024a
	OsNIP3	Reduced arsenic accumulation	Xu et al., 2024
	OsMYB84	Modulated copper uptake and transport	Ding et al., 2024
	OsCOP1,	Improved UV protection	Hu et al., 2024
	OsCAT2	Alleviates the oxidative stress by scavenging ROS	Shen et al., 2024
Wheat	TaIPK1	Improved iron and zinc accumulation	Ibrahim et al., 2022
	TaPGK	Enhanced cold resistance	Zhang et al., 2023b
	TaRR12	Enhanced drought resistance	Li et al., 2024a
	TaHKT1;5	Improved salt resistance	Wang et al., 2024b
Maize	ZmG6PDH1	Enhanced cold resistance	Li et al., 2023a
	ZmHSPs	Enhanced heat resistance	Li et al., 2024b
Barley	HvGSK1.1	Enhanced salinity tolerance	Kloc et al., 2024
Soybean	GmHsp90A2	Enhanced heat resistance	Jianing et al., 2022
Potato	VInv	Enhanced cold resistance	Yasmeen et al., 2022

Drought stress

The largest threat to global food security is drought stress, which is the primary factor behind the catastrophic drop in agricultural output and productivity (Joshi et al*.,* 2020). AITRs are a recently identified family of transcription factors that regulate feedback and are essential for ABA signaling. Osakabe et al.(2015) found that drought resistance was provided via a CRISPR/Cas9-created mutation in the Arabidopsis OST2 gene. Zhao et al*.* (2016) discovered that *Arabidopsis* plants' ability to withstand drought was enhanced by CRISPR/Cas9-mediated deletion of the miR169a gene. By expressing the AVP1 gene, CRISPR/Cas9 enhanced *Arabidopsis*' resistance to drought (Park et al., 2017). In order to enable drought tolerance in *Arabidopsis thaliana*, Nuñez et al. (2021) used CRISPR/Cas9 to mute the TRE1 gene. Many recent developments in drought stress adaptation and resistance can be attributed to CRISPR-Cas9 technology (Table 1).

Cold stress

Several cell compartments and metabolic pathways are involved in plants' ability to withstand cold stress (Hannah et al*.,* 2006). During the seedling stage, cold stress can have a detrimental effect on seed germination and emergence. By attaching to the promoter of the amylase gene and combining with OsJAZ9 under cold stress, OsMYB30 is a factor that lowers cold tolerance. This complex improves sensitivity to cold by decreasing the expression of the amylase gene, which leads to the buildup of maltose and the breakdown of starch (Lv et al*.,* 2017).

Salinity stress

Salt stress in plants leads to changes in gene expression and signaling pathways, resulting in physiological and morphological alterations. Salinity stress causes necrosis, premature death of old leaves, and disruption of ions in cells (Julkowska and Testerink, 2015). Using CRISPR/Cas9 technology, the OsRR22 gene which controls signaling and cytokinin metabolism in plants-was modified to increase rice's resistance to salt stress. The cytokinin pathway is hampered by OsRR22 loss of function, which increases plants' resilience to salt stress (Takagi et al*.,* 2015). Under salt stress, rice OsRR22 knockouts performed better (Zhang et al*.,* 2019). CRISPR/Cas9 was used to knock out OsmiR535 in rice, which improved its tolerance to salt stress. A 5bp deletion in the OsmiR535 coding region was found to potentially enhance rice's salt tolerance (Yue et al*.,* 2020).

Heavy metals stress

Heavy metal stress harms plant productivity in agriculture (Jha and Bohra, 2016). Heavy metals such as cadmium (Cd), arsenic (As), and lead (Pb) promote the generation of hydroxyl radicals (OH), superoxide radicals, and hydrogen peroxide (H2O2), causing oxidative stress. Loss-of-function mutants of γ-glutamyl cyclotransferase and OXP1 have shown defensive properties against heavy metal toxicity in plant molecular biology. This highlights their potential in detoxifying heavy metals and xenobiotics by increasing glutathione accumulation (Paulose et al., 2013). Long-term use of rice tainted with cadmium can result in chronic illnesses like cancer and renal failure. Thus, scientists face a problem in developing low-heavy-metal rice in places contaminated with cadmium (Bertin and Averbeck, 2006). CRISPR/Cas9 mutants can help plants deal with heavy metal stress. In rice, Cadmium absorption by roots involves transporters OsNramp1, OsNramp5, and OsCd1. OsHMA3 helps store Cadmium in the root vacuole and reduces its movement through the xylem, while OsLCT1 transports Cadmium to the grains (Chen et al., 2019). CRISPR/Cas9 was used to edit OsNramp5 and OsLCT1 genes, reducing Cadmium levels in rice grains (Tang et al*.,* 2017). OsARM1 is a regulator of arsenic-associated transporter genes in rice. It is involved in phloem expression in vascular bundles at basal and upper nodes. OsARM1 knock-out using CRISPR improves arsenic tolerance, while overexpression worsens sensitivity to arsenic (Wang et al., 2017).

UV radiation stress

CRISPR/Cas technology can boost the plant's protective mechanisms against UV damage,

by enabling precise genetic modifications that enhance tolerance to DNA damage, oxidative stress, and impaired photosynthesis. The OsCOP1 gene has demonstrated potential in improving UV tolerance in rice by this technology, enhancing their resistance to UV-B radiation (Hu et al., 2024).

Oxidative stress

Oxidative stress results from the accumulation of reactive oxygen species (ROS) under various stress conditions. CRISPR/Cas technology can enhance oxidative stress tolerance in crops by targeting genes that involved in reactive oxygen species (ROS) under various stress conditions. Utilizing this technology in editing OsCAT3 gene, which is crucial for detoxifying superoxide radicals and hydrogen peroxide, can enhance rice's ability to mitigate oxidative damage (Jiang et al., 2023). This technology can offer a comprehensive approach to improving oxidative stress resilience in crops by modifying different antioxidant genes.

Biotic stresses such as pathogens, pests, and parasitic weeds severely affect crop productivity and global food security by reducing yield and quality. The pesticides which are deliberately used in agricultural practices for protection of the crops from diseases and pests are highly toxic thus create potential risk to the environment and human health (Das and Baisakh, 2022). Traditional breeding for resistance is often time-consuming and limited by genetic variability. CRISPR/Cas genome editing offers a revolutionary approach to precisely modify susceptibility(S) genes, enhance innate immunity, and develop durable resistance against multiple biotic stresses in many crops (Table 3 and 4).

Table 3. CRISPR/Cas technology in improving disease resistant

Crops	Targeted genes	Applications	References
Rice	Pi21, OsSULTR3;6	Resistance to rice blast	Yang et al., 2023
	OsPUB9	Resistance to bacterial leaf blight	Kim et al., 2024
	OsCPR5.1	Yellow mottle virus resistance	Arra et al., 2024
Wheat	TaCIPK14	Resistance to stripe rust	He et al., 2023
Maize	ZmAGO18b	Resistance to southern leaf blight	Dai et al., 2023
	MCMV	Reduced viral infections	Lei et al., 2023
	Zmpdrp1	Reduced robust virus	Xie et al., 2024
Barley	BnHva22c	Reduced fungal pathogen, susceptibility	Ye et al., 2024
Soybean	Glyma05g29080	Resistance to white mold	Zhang et al., 2022a
	GmUGT	Resistance against leaf-chewing Insects	Zhang et al., 2022b
Potato	SlDCL2b	Spindle tuber viroid resistance	Tiwari et al., 2022
	PVY, PVS, PVX, PLRV	Reduced viral infections	Zhan et al., 2023
	StNRL1	resistance to late blight	Norouzi et al., 2024
Cassava	MeRPPL1	Resistance to geminivirus	Ramulifho and Rey, 2024

Table 4. CRISPR/Cas technology in developing pest resistant

Crops	Targeted genes	Applications	References
Rice	OsWRKY71, Bph15	Resistance against brown plant hopper	Li et al., 2023b
	OsHPP04	Resistance to rice root-knot nematode	Huang et al., 2023
Maize	Cry1F	Improved pest resistance	Kumari et al., 2024
Soybean	GmUGT	Resistance against leaf-chewing Insects	Zhang et al., 2022b
	GmSNAP11, α-SNAP	Resistance to soybean cyst nematode	Shaibu et al., 2022; Usovsky et al., 2023

Viruses stress

CRISPR technology has shown targeting and degrading the RNA genomes of RNA viruses, preventing their replication within the host plant (Sarkar et al., 2024). This approach has been effectively demonstrated in crops like wheat and rice, CRISPR/Cas9 has been employed to knock out susceptibility genes such as TaPDIL5 or OsDjA2 and OsERF that facilitate viral infection, thus providing broad-spectrum virus resistance (Távora et al., 2022), The utility of CRISPR technology not only highlights for crop trait improvement but also as a powerful tool for dissecting gene functions in plant-pathogen interactions.

Bacteria stress

CRISPR technology involves targeting bacterial virulence genes and enhancing the plant's immune response by disrupting key genes in bacterial pathogens. Editing the FERONI and SlWak1 depend on FLS gene in rice and wheat, which encodes a receptor involved in pathogen recognition, has improved the plants' ability to detect and respond to bacterial infections, thereby enhancing resistance (Huang et al., 2020; Zhang et al., 2020).

Fungi stress

Fungal diseases are a major concern for crop health and yield Loss. CRISPR/Cas technology is a new hope for this problem and has been applied in various fungal species, including *Fusarium* and *Botrytis*, which are responsible for significant agricultural losses. Using CRISPR/Cas to knock out susceptibility genes that fungi exploit in wheat and soybean, which has been shown resistance to powdery mildew by making the plants less susceptible to fungal infections (Li et al., 2022; Bui et al., 2023).

Pests stress

Now a days pest infestation problem in crops is a major concern as it not only reduce the yield but also increases the environmental pollution due to use of pesticides for their manage. CRISPR/Cas technology can enhance resistance to pests in crop plants by knocking out susceptibility genes. For example, editing the ABC transporter gene in soybean has been shown to confer resistance to bollworms by disrupting the insect's ability to digest plant tissues (Amezian et al., 2024). Also another strategy is to enhancethe expression of genes involved in the biosynthesis of phenolic compounds that has been shown to reduce insect preference in crops. This technology has also been used to modify genes encoding insecticidal proteins, such as Cry proteins, VIP proteins improving pest resistance in crops (Dubovskiy et al., 2024). CRISPR/Cas technology can develop nematode resistance in crop plants by targeting genes that facilitate nematode infection and reproduction which not only reduce yield losses but also save the environment by reducing the use of chemical nematicides.

Parasitic plants

Parasitic plants, such as *Striga* and *Orobanche*, attach to the host crops and extract water and nutrients as a resultcrop yields reduce significantly. CRISPR/Cas technology can enhance resistance to parasitic plants by targeting genes involved in host parasite interactions and develop defense mechanism by modifying specific signaling pathways. Editing the LGS1 gene in sorghum by this technology developed resistance to *Striga* by disrupting the production of strigolactones, which are essential for *Striga* seed germination and attachment (Makaza et al., 2023).

Improving crop quality

A major factor in determining the market value of crops has been crop quality. Generally speaking, both internal and external characteristics influence crop quality. Physical and aesthetic qualities including size, color, texture, and scent are examples of external quality features. The internal quality factors, on the other hand, consist of bioactive substances like carotenoids, lycopene, γ-amino butyric acid, flavonoids, and others, as well as nutrients like protein, carbohydrate, fats, etc. Crop quality enhancement using CRISPR/Cas9 concentrated on the fruit's texture, nutritional value, edible quality, and physical beauty (Table 5). Food products that are nutritious and healthful

are becoming more and more popular. In order to meet the needs of this expanding industry, researchers have been urged to develop new items. Numerous nutrients found in fruits and vegetables have anti-oxidant, anti-inflammatory, and anti-cancer properties. Breeding initiatives have been put in place to bio-fortify a variety of nutrients, such as the amounts of iron, zinc, γ-aminobutyric acid (GABA), and carotenoid in different crops. It has been attempted to use gene editing for biofortification in order to provide high-quality nutrients to satiate the "hidden hunger."

Table 5. Crop quality and nutritional improvement using CRISPR technology

Crop	Targeted Gene	Associated Traits	References
Rice	CrtI, PSY	High Beta carotene	Dong et al., 2020
	OsNramp5	Low Cd accumulations	Tang et al., 2017
	OsPLDα1	Low phytic content	Khan et al., 2019
	SIGAD2. SIGAD3	High GABA content	Nonaka et al., 2017
	OsAUX5, OsWRKY78	Control grain essential amino acid accumulation	Shi et al., 2023
Wheat	TalPK1	increase iron and zinc content	Ibrahim et al., 2022
	TaPDI	Accumulate storage protein.	Hu et al., 2022
Barley	HGGT, HPT	Increase vitamin E biosynthesis	Zeng et al., 2020
Soybean,	GmFAD2	Increase fatty acid	Zhou et al., 2023
Potato	StSBE1, StSBE2	High amylose content	Tuncel et al., 2019
Rapeseed	BnTT8	High oil production and GPC	Zhai et al., 2020
Sweet Potato	IbGBSSI, IbSBEII	High amylose content	Wang et al., 2019
Cassava	CYP79D1	Lower levels of cyanide	Juma et al., 2022
Grape	IdnDH	Low tartaric acid	Ren et al, 2016

Increasing γ-amino butyric acid content

A non-protein amino acid inhibitory neurotransmitter, GABA helps regulate blood pressure and reduce anxiety (Nuss, 2015). Thus, the food business has turned its attention to creating new foods that are high in GABA. One important enzyme that catalyzes the conversion of glutamate to GABA is glutamate decarboxylase (GAD). GAD activity is negatively regulated by its C-terminal autoinhibitory domain. Using CRISPR/Cas9, the C-terminal has been entirely removed to enhance the amount of GABA. Mutant tomatoes showed a seven-fold increase in GABA accumulation (Nonaka et al*.,* 2017).

Increasing carotenoid content

Carotenoids have been implicated in the prevention of eye-related diseases and antioxidant processes. However, carotenoids must be consumed through diet because humans are unable to produce them. Furthermore, phytoene and lycopene lower the risk of cardiovascular disease and cancer. In the past, scientists used traditional genetic engineering to produce β-carotene in rice while simultaneously introducing the *CrtI* and *PSY* genes. However, despite a stringent GM regulatory environment, such genetically modified (GM) golden rice caused widespread alarm. Since golden rice might not contain enough β-carotene to completely cure vitamin A deficiency, and because planting and eating golden rice could result in allergies or antibiotic resistance, many anti-GMO activists maintain that this effort is overly optimistic. Rice, tomato, and banana carotenoid biofortification has been achieved by the use of CRISPR/Cas9-mediated genome editing. Because there is no external gene integration in host genomes, those generated by this method show promise in evading a GM regulatory

regime. In general, carotenoid biofortification was accomplished using two types of techniques. First, the carotenoid biosynthesis pathway is subjected to carbon flux due to the overexpression of phytoene synthase genes caused by CRISPR/Cas9-mediated knock-in. In this way, a caroteno genesis cassette with the genes *CrtI* and *PSY* has been incorporated into the rice target site, producing marker-free gene-edited mutants with a dry weight of 7.9 µg/g β-carotene (Dong et al., 2020).

Increasing fatty acid composition

Olive oil is rich in monounsaturated fatty acids (MUFA), such as oleic acid (18:1). Diets high in oleic acid are beneficial to the cardiovascular system. Trans- and saturated fatty acids are frequently cited as "unhealthy" fats and associated with heart disease (Briggs et al., 2017). Soybean oil, the most often produced and consumed edible oil, has just 20% oleic acid, which is significantly less than that of olive oil (65-85%).

Eliminating anti-nutrients

Phytic acid cannot be metabolized by humans because they lack the appropriate degradation enzymes. Because phytic acid can interact with minerals and proteins to create complexes, consuming large amounts of phytic acid will limit the absorption of these nutrients (Oatway et al., 2001). CRISPR/Cas9 has been used to knock out an ITPK gene that code for an enzyme that catalyzes the penultimate step of phytate production, thereby lowering the amount of phytic acid in rapeseeds. Without affecting plant performance, the ITPK mutants showed a 35% decrease in phytic acid (Sashidhar et al., 2020).

Biological nitrogen fixation

The production of major crops like rice, maize, and wheat steadily increased between the 1930s and the late 1960s as a result of the development of high-yielding cereal varieties, chemical fertilizers, new irrigation and cultivation techniques, and modern management techniques. For plants to absorb atmospheric nitrogen gas, it must be converted into organic nitrogen. More than 50% of the global nitrogen fixation (estimated at 413 million tons in 2010) comes from biological nitrogen fixation (BNF), an environmentally friendly alternative to industrial nitrogen fixation by the energy-intensive Haber-Bosch process, which currently produces 120 million tons of nitrogen fertilizer annually (Fowler et al., 2013). *Rhizobium rhizogenes* (formerly *Agrobacterium rhizogenes*) mediated hairy root transformation, which was the initial application of CRISPR/Cas9 for soybean genome modification. The GmU6-10 and GmU6-16g-1 promoters were found to be more effective than the AtU6-26 promoter in transgenic hairy roots when the effectiveness of genome editing using sgRNAs driven by different endogenous and exogenous RNA polymerase III (pol III) promoters was assessed.However, when employing the soybean U6 promoter, the TALENs' mutation efficiency was significantly lower than that of the Cas9/sgRNA method. More encouragingly, homozygous T1 plants with the anticipated precise DNA knock-in were achieved after Cas9-sgRNA and donor DNA fragments were effectively transformed into soybean utilizing the particle bombardment approach (Li et al, 2015). The sgRNA component of the Cas9/sgRNA complex plays a major role in determining the specificity and effectiveness of CRISPR-mediated genomic alterations. Designing suitable sgRNAs that reduce the possibility of generating DSBs in off-target locations in the genome is therefore crucial. CRISPR-PLANT and CRISPR-P are two example online platforms that are frequently utilized for sgRNA design in legumes (Xie et al., 2014).

CHALLENGES OF CRISPR TECHNOLOGY IN CROP IMPROVEMENT

Although CRISPR/Cas technology has rapidly evolved from its discovery to become one of the most versatile genome-editing tools with a revolutionary potential but a number of scientific, technical, regulatory, and ethical challenges still constrain its wide spread use in crop improvement. Understanding these challenges is essential to fully harness CRISPR's potential for sustainable agriculture and global food security.

Scientific and technical challenges

One of the most fundamental challenges in CRISPR/Cas-mediated crop improvement is off-target editing, where unintended genetic modifications occur at sites similar to the intended target. Such off-target effects may lead to unpredictable phenotypes or even compromise plant health and productivity. Although the use of high-fidelity Cas variants such as SpCas9-HF1, eSpCas9, and Cas12a has improved precision, the complete elimination of off-target mutations remains difficult, especially in polyploid crops like wheat that have complex genomes (Schaart *et al*. 2021). Moreover, the efficiency of the CRISPR system can vary widely depending on factors such as target sequence, GC content, and chromatin accessibility, which influence Cas binding and cleavage efficiency.Another significant issue involves delivery of CRISPR components into plant cells which are often species-specific, labor-intensive, and have low transformation efficiencies in many recalcitrant crops like legumes, sugarcane, and woody perennials In addition, gene redundancy in polyploid species poses a challenge because multiple copies of the same gene may mask the effects of editing a single locus, necessitating multiplexed editing strategies. Grain yield is a polygenic controlled complex character with low to moderate heritability owing to environmental effects and also greatly influenced by many interrelated component traits, which are also mostly polygenic (Das and Baisakh, 2019; Prusti and Das, 2020). Also other traits such as drought and salt tolerance, nutrient use efficiency etcare governed by complex polygenic networks, making single-gene edits insufficient for major improvements

Regulatory and policy issues

One of the most contentious challenges surrounding CRISPR/Cas technology lies in the regulatory and policy frameworks that govern its use. Different countries have adopted divergent stances toward genome-edited crops, creating uncertainty for global trade and innovation due to lack of uniform global policy which harmonization impedes international research collaboration and commercialization of gene-edited varieties.Additionally, biosafety evaluation and traceability present practical difficulties. Unlike transgenic crops, which contain identifiable foreign DNA sequences, CRISPR-induced edits are often indistinguishable from natural mutations, making detection and labeling challenging (Schaart et al., 2021).

Ethical and socio-economic concerns

Many consumers still conflate gene editing with traditional genetic modification (GMOs), leading to skepticism and resistance. Transparent communication from researchers and policymakers, effective public engagement, ethical education, and open dialogue about the benefits and risks of CRISPR-edited crops are essential for building trust and ensuring responsible innovation.

Future pathways to overcome challenges

Overcoming these challenges requires a multi-pronged and globally coordinated approach. Technically, the development of next-generation CRISPR systems such as base editors, prime editors, and RNA-targeting Cas variants (Cas13, Cas14) promises to minimize off-target effectsand expand the editing toolkit (Zhang et al., 2023c). Improvement in DNA-free editing techniques—using RNP complexes or transient expression systems—can help produce transgene-free crops, simplifying regulatory approval. On the policy front, harmonizing international regulations and establishing science-based risk assessment protocols will be critical to promoting global acceptance. Strengthening public communication and ethics education can bridge the gap between scientific innovation and societal trust. Capacity building in developing countries is equally vital. Investment in local genomic resources, bioinformatics infrastructure, and public-sector breeding programs can ensure that CRISPR technology benefits smallholder farmers and contributes to sustainable development. Collaboration between universities, research institutes, and international agencies will further accelerate progress while maintaining transparency and safety.

FUTURE PROSPECTS AND SDG OBJECTIVES

The future prospects of CRISPR/Cas technology in crop improvement are exceptionally promising, offering transformative potential for agriculture, food security, and environmental sustainability.CRISPR's capacity to precisely modify genes by cutting and pasting at specified locations is one of its biggest benefits. This accuracy makes it possible to alter several genes at once, transport proteins to specific genes to adjust their activity, and create completely new genetic pathways.Advances in genome editing, such as base editing and prime editing, offer unprecedented precision in modifying plant genomes, allowing for the enhancement of traits like drought tolerance, disease resistance, and nutritional conten (Chen et al., 2024). The capacity to edit multiple genes simultaneously through multiplexed CRISPR/Cas systems also opens the door to developing crops with stacked traits, such as drought and heat tolerance coupled with improved nutritional profiles, thereby addressing multiple challenges in a single breeding cycle. CRISPR/Cas technology can be used to develop edible vaccines by engineering plants which are consumed as food. The edible vaccine has the potential to solve the problem of bioterrorism by immunizing against a wide range of different dreaded viruses and can save the earth from any future epidemic and pandemic (Das et al. 2021b). In future the integration of CRISPR/Cas with other technologies such as speed breeding, high-throughput phenotyping, and artificial intelligence-driven predictive models can enhance the efficiency of gene editing processes, enabling the rapid development of climate-smart crops tailored to specific environments. Furthermore, CRISPR/Cas applications are increasingly being explored to improve nutrient use efficiency, nitrogen fixation, and water-use efficiency, which can significantly reduce reliance on chemical fertilizers and irrigation, mitigating greenhouse gas emissions and environmental degradation.

The integration of CRISPR/Cas technology into sustainable agriculture is closely aligned with several United Nations Sustainable Development Goals (SDGs), particularly SDG-2 (Zero Hunger), SDG-12 (Responsible Consumption and Production), SDG-13 (Climate Action), and SDG-15 (Life on Land). By enabling the development of high-yielding, nutrient-dense, and climate-resilient crops, CRISPR/Cas contributes directly to food security and nutritional adequacy in both developing and developed countries. Crops engineered for enhanced micronutrient content, biofortification, or reduced allergens can combat malnutrition and improve public health, directly supporting SDG-2. Additionally, CRISPR / Cas- mediated improvements in resource-use efficiency contribute to SDG-12 by promoting sustainable production practices, reducing chemical inputs, and lowering the environmental impact of agriculture. Climate-resilient crops generated through CRISPR/Cas address SDG-13 by helping agricultural systems adapt to changing climatic conditions, ensuring stable food production despite increasing droughts, heat waves, and erratic rainfall patterns. Moreover, by preserving wild relatives and landrace varieties while improving cultivated crops, CRISPR/Cas supports biodiversity conservation, aligning with SDG-15. The technology can also facilitate precision breeding strategies that reduce the need for land expansion, helping conserve forests and natural habitats while sustaining agricultural output.

The future of CRISPR/Cas technology will depend not only on technical advancements but also on regulatory harmonization, ethical governance, and public acceptance. As the scientific community continues to demonstrate the safety, efficacy, and benefits of genome-edited crops, policies are gradually evolving to accommodate non-transgenic CRISPR/Cas products without imposing undue barriers. Public engagement, transparent communication, and capacity-building programs are critical to ensuring equitable access to this technology, particularly for smallholder farmers and resource-limited regions. The integration of CRISPR/Cas with digital agriculture, remote sensing and data-driven decision-making will further enhance its impact, enabling precision-targeted interventions that maximize productivity while minimizing environmental harm. In the long term, CRISPR/Cas technology holds the potential

to transform global agriculture into a system that is not only productive and resilient but also ecologically sustainable and socially inclusive. By aligning its applications with the SDGs, CRISPR Cas can serve as a cornerstone of a new green revolution, enabling humanity to feed a growing population, protect natural resources, and build a more sustainable and equitable future.

CONCLUSION

The CRISPR/Cas technology has emerged as one of the most powerful tools in modern agricultural science, reshaping the future of crop improvement and sustainable food production. It offers an efficient pathway to develop crops that are not only higher yielding but also more resilient to climate stress, pests, and diseases with promising quality and nutritive value. The ability to edit specific genes without introducing foreign DNA also makes CRISPR-based crops more acceptable to the public and regulators compared to older genetically modified organisms (GMOs). This distinction is essential for promoting wider acceptance of genome-edited crops and enabling their safe and responsible use in agriculture. Furthermore, the integration of CRISPR/Cas with bioinformatics, speed breeding, artificial intelligence, and high-throughput phenotyping are leading to a new era of precision agriculture, where crop development is guided by data and molecular insight rather than trial and error. Ultimately, CRISPR/Cas represents more than a scientific tool. It embodies a new philosophy of agriculture grounded in sustainability, innovation, and responsibility. If applied ethically and inclusively, it has the potential to secure food for future generations while preserving natural ecosystems.

ACKNOWLEDGEMENT

The authors are grateful to Dr. T. Mohapatra, Chairperson, Protection of Plant Varieties and Farmers' Rights Authority (PPVFRA), and former Secretary (DARE) & Director General (ICAR), Govt. of India, New Delhi, for his constant encouragement and inspiration.

REFERENCES

Amezian, D., Nauen, R., Van Leeuwen, T. 2024. The role of ATP-binding cassette transporters in arthropod pesticide toxicity and resistance. *Curr. Opin. Insect Sci.* **63**: 101200.

Anzalone, A.V., Randolph, P.B., Davis, J.R., Sousa, A.A., Koblan, L.W., Levy, J.M., Chen, P.J., Wilson, C., Newby, G.A., Raguram, A., Liu, D.R. 2019. Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature* **576**(7786): 149-157.

Arra, Y., Auguy, F., Stiebner, M., Cherón, S., Wudick, M.M., Miras, M., et al. 2024. Rice Yellow Mottle Virus resistance by genome editing of the Oryza sativa L. ssp. japonica nucleoporin gene OsCPR5.1 but not OsCPR5.2. *Plant Biotechnol. J.* **22**(5): 1299-1311.

Barrangou, R., Fremaux, C., Deveau, H., Richards, M., Boyaval, P., Moineau, S., Romero, D.A., Horvath, P. 2007. CRISPR provides acquired resistance against viruses in prokaryotes. *Science* **315**(5819): 1709-1712.

Bertin, G., Averbeck, D. 2006. Cadmium: Cellular effects, modifications of biomolecules, modulation of DNA repair and genotoxic consequences. *Biochimie* **88**(11): 1549-1559.

Briggs, M.A., Petersen, K.S., Kris-Etherton, P.M. 2017. Saturated fatty acids and cardiovascular disease: Replacements for saturated fat to reduce cardiovascular risk. *Healthcare* **5**: 29.

Bui, T.P., Le, H., Ta, D.T., Nguyen, C.X., Le, N.T., Tran, T.T., et al. 2023. Enhancing powdery mildew resistance in soybean by targeted mutation of MLO genes using CRISPR/Cas9. *BMC Plant Biol.* **23**: 533.

Chen, F., Chen, L., Yan, Z., Xu, J., Feng, L., He, N., et al. 2024. Recent advances of CRISPR-based genome editing for enhancing staple crops. *Front. Plant Sci.* **15**: 1478398.

Chen, H., Ye, R., Liang, Y., Zhang, S., Liu, X., Sun, C., et al. 2023. Generation of low cadmium rice germplasms via knockout of OsLCD using CRISPR/Cas9. *J. Environ. Sci.* **126**: 138-152.

Chen, K., Wang, Y., Zhang, R., Zhang, H., Gao, C. 2019. CRISPR/Cas genome editing and precision plant breeding in agriculture. *Ann. Rev. Plant Biol.* **70**(1): 667-697.

Chen, W. 2022. Convergent selection of a WD40 protein that enhances grain yield in maize and rice. *Science* **375**(6587): eabg7985.

Cong, L., Ran, F.A., Cox, D., Lin, S., Barretto, R., Habib, N., et al. 2013. Multiplex genome engineering using CRISPR/Cas systems. *Science* **339**(6121): 819-823.

Cox, D.B., Platt, R.J., Zhang, F. 2015. Therapeutic genome editing: Prospects and challenges. *Nat. Med.* **21**: 121-131.

Dai, Z., Yang, Q., Chen, D., Li, B., Que, J., Hu, L., et al. 2023. ZmAGO18b negatively regulates maize resistance against southern leaf blight. *Theor. Appl. Genet.* **136**: 158.

Das, T.R., Pradhan, S.K., Singh, O.N. 2021a. Marker-assisted selection for bacterial blight resistance in rice. *e-planet* **19**(2): 99-103.

Das, T.R., Samantarai, R.K., Panda, P.K. 2021b. Prospects of plant-based edible vaccines in combating COVID-19 and other viral pandemics. *e-planet* **19**(1): 1-18.

Das, T.R., Baisakh, B. 2019. Selection indices and discriminant function analysis for grain yield in greengram. *e-planet* **17**(1): 13-21.

Das, T.R., Baisakh, B. 2022. RNAi mediated gene silencing in crop improvement. *e-planet* **20**(2): 91-104.

Ding, J., Ji, C., Yu, L., Wang, C., Ding, G., Wang, S., et al. 2024. OsMYB84 modulates copper uptake and yield in rice. *Crop J.* **12**(2): 456-469.

Dong, O.X., Yu, S., Jain, R., Zhang, N., Duong, P.Q., Butler, C., et al. 2020. Marker-free carotenoid-enriched rice generated through targeted gene insertion using CRISPR/Cas9. *Nat. Commun.* **11**(1): 1-10.

Dubovskiy, I.M., Grizanova, E.V., Gerasimova, S.V. 2024. Plant recombinant gene technology for pest control. In: Gene Editing in Plants. Springer, pp. 17-72.

Figueredo, E.F., Cruz, T.A.D., Almeida, J.R.D., Batista, B.D., Marcon, J., Andrade, P.A.M.D., et al. 2023. The key role of indole-3-acetic acid biosynthesis by *Bacillus thuringiensis* RZ2MS9 in promoting maize growth revealed by the ipdC gene knockout mediated by the CRISPR-Cas9 system. *Microbiol. Res.* **266**: 127218.

Fowler, D., Coyle, M., Skiba, U. 2013. The global nitrogen cycle in the twenty-first century. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **368**(1621): 20130164.

Fu, Y., Foden, J.A., Khayter, C., Maeder, M.L., Reyon, D., Joung, J.K., Sander, J.D. 2013. High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. *Nat. Biotechnol.* **31**(9): 822-826.

Gao, H., Mutti, J., Young, J.K., Yang, M., Schroder, M., Lenderts, B., et al. 2020. Complex trait loci in maize enabled by CRISPR-Cas9 mediated gene insertion. *Front. Plant Sci.* **11**: 1-14.

Gasiunas, G., Barrangou, R., Horvath, P., Siksnys, V. 2012. Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. *Proc. Natl. Acad. Sci. USA* **109**(39): E2579-E2586.

Hannah, M.A., Wiese, D., Freund, S., Fiehn, O., Heyer, A.G., Hincha, D.K. 2006. Natural genetic variation of freezing tolerance in *Arabidopsis*. *Plant Physiol.* **142**(1): 98-112.

He, F., Wang, C., Sun, H., Tian, S., Zhao, G., Liu, C., et al. 2023. Simultaneous editing of three homoeologues confers broad-spectrum resistance to stripe rust in wheat. *Plant Biotechnol. J.* **21**(2): 354-368.

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

Hu, J., Yu, M., Chang, Y., Tang, H., Wang, W., Du, L., et al. 2022. Functional analysis of TaPDI genes on storage protein accumulation by CRISPR/Cas9 edited wheat mutants. *Int. J. Biol. Macromol.* **196**: 131-143.

Hu, S., Chen, Y., Qian, C., Ren, H., Liang, X., Tao, W., et al. 2024. Nuclear accumulation of rice UV-B photoreceptors is UV-B- and OsCOP1-independent for UV-B responses. *Nat. Commun.* **15**: 6396.

Huang, Q., Lin, B., Cao, Y., Zhang, Y., Song, H., Huang, C., et al. 2023. CRISPR/Cas9-mediated mutagenesis of the susceptibility gene OsHPP04 in rice confers enhanced resistance to rice root-knot nematode. *Front. Plant Sci.* **14**: 1134653.

Huang, Y.-Y., Liu, X.-X., Xie, Y., Lin, X.-Y., Hu, Z.-J., Wang, H., et al. 2020. Identification of FERONIA-like receptor genes involved in rice-*Magnaporthe oryzae* interaction. *Phytopathol. Res.* **2**: 14.

Ibrahim, S., Saleem, B., Rehman, N., Zafar, S.A., Naeem, M.K., Khan, M.R. 2022. CRISPR/Cas9 mediated disruption of Inositol Pentakisphosphate 2-Kinase 1 (TaIPK1) reduces phytic acid and improves

iron and zinc accumulation in wheat grains. *J. Adv. Res.* **37**: 33-41.

Iwamoto, M. 2022. In-frame editing of transcription factor gene RDD1 to suppress miR166 recognition influences nutrient uptake, photosynthesis, and grain quality in rice. *Sci. Rep.* **12**: 10795.

Jansen, R., Embden, J.D., Gaastra, W., Schouls, L.M. 2002. Identification of genes that are associated with DNA repeats in prokaryotes. *Mol. Microbiol.* **43**(6): 1565-1575.

Jha, U.C., Bohra, A. 2016. Genomics enabled breeding approaches for improving cadmium stress tolerance in plants. *Euphytica* **208**(1): 1-31.

Jiang, W., Ye, Q., Wu, Z., Zhang, Q., Wang, L., Liu, J., et al. 2023. Analysis of CAT gene family and functional identification of OsCAT3 in rice. *Genes* **14**: 138.

Jianing, G., Yuhong, G., Yijun, G., Rasheed, A., Qian, Z., Zhiming, X., et al. 2022. Improvement of heat stress tolerance in soybean (*Glycine max* L.) by using conventional and molecular tools. *Front. Plant Sci.* **13**: 993189.

Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A., Charpentier, E. 2012. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* **337**(6096): 816-821.

Joshi, R.K., Bharat, S.S., Mishra, R. 2020. Engineering drought tolerance in plants through CRISPR/Cas genome editing. *3 Biotech* **10**: 400.

Julkowska, M.M., Testerink, C. 2015. Tuning plant signaling and growth to survive salt. *Trends Plant Sci.* **20**(9): 586-594.

Juma, B.S., Mukami, A., Mweu, C., Ngugi, M.P., Mbinda, W. 2022. Targeted mutagenesis of the CYP79D1 gene via CRISPR/Cas9-mediated genome editing results in lower levels of cyanide in cassava. *Front. Plant Sci.* **13**: 1009860.

Khan, M.S.S., Basnet, R., Islam, S.A., Shu, Q. 2019. Mutational analysis of OsPLDα1 reveals its involvement in phytic acid biosynthesis in rice grains. *J. Agric. Food Chem.* **67**(41): 11436-11443.

Kim, M.-S., Ko, S.-R., Jung, Y.J., Kang, K.-K., Lee, Y.-J., Cho, Y.-G. 2023. Knockout mutants of OsPUB7 generated using CRISPR/Cas9 revealed abiotic stress tolerance in rice. *Int. J. Mol. Sci.* **24**: 5338.

Kim, M.-S., Le, V.T., Jung, Y.J., Kang, K.-K., Cho, Y.-G. 2024. OsPUB9 gene edited by CRISPR/Cas9 enhanced resistance to bacterial leaf blight in rice (*Oryza sativa* L.). *Int. J. Mol. Sci.* **25**: 7145.

Kis, A., Polgári, D., Dalmadi, Á., Ahmad, I., Rakszegi, M., Sági, L., et al. 2024. Targeted mutations in the GW2.1 gene modulate grain traits and induce yield loss in barley. *Plant Sci.* **340**: 111968.

Kloc, Y., Dmochowska-Boguta, M., Żebrowska-Różańska, P., Łaczmański, Ł., Nadolska-Orczyk, A., Orczyk, W. 2024. HvGSK1.1 controls salt tolerance and yield through the brassinosteroid signaling pathway in barley. *Int. J. Mol. Sci.* **25**: 998.

Koonin, E.V., Makarova, K.S., Zhang, F. 2017. Diversity, classification and evolution of CRISPR-Cas systems. *Curr. Opin. Microbiol.* **37**: 67-78.

Kumari, R., Saha, T., Kumar, P., Singh, A.K. 2024. CRISPR/Cas9-mediated genome editing technique to control fall armyworm (*Spodoptera frugiperda*) in crop plants with special reference to maize. *Physiol. Mol. Biol. Plants* **30**(7): 1161-1173.

Lei, R., Kuang, R., Peng, X., Jiao, Z., Zhao, Z., Cong, H., et al. 2023. Portable rapid detection of maize chlorotic mottle virus using RT-RAA/CRISPR-Cas12a based lateral flow assay. *Front. Plant Sci.* **14**: 1088544.

Li, Z., Liu, Z.B., Xing, A. 2015. Cas9-guide RNA directed genome editing in soybean. *Plant Physiol.* **169**(2): 960-970.

Li, J., Li, H., Chen, J., Yan, L., Xia, L. 2020. Toward precision genome editing in crop plants. *Mol. Plant* **3**(6): 811-813.

Li, S., Lin, D., Zhang, Y., Deng, M., Chen, Y., Lv, B., et al. 2022. Genome-edited powdery mildew resistance in wheat without growth penalties. *Nature* **602**(7897): 455-460.

Li, S., Zhang, Y., Liu, Y., Zhang, P., Wang, X., Chen, B., et al. 2024a. The E3 ligase TaGW2 mediates transcription factor TaARR12 degradation to promote drought resistance in wheat. *Plant Cell* **36**(3): 605-625.

Li, X., Cai, Q., Yu, T., Li, S., Li, S., Li, Y., et al. 2023a. ZmG6PDH1 in glucose-6-phosphate dehydrogenase family enhances cold stress tolerance in maize. *Front. Plant Sci.* **14**: 1116237.

Li, X., Zhang, J., Shangguan, X., Yin, J., Zhu, L., Hu, J., et al. 2023b. Knockout of OsWRKY71 impairs Bph15-mediated resistance against brown plant hopper in rice. *Front. Plant Sci.* **14**: 1260526.

Li, Z., Li, Z., Ji, Y., Wang, C., Wang, S., Shi, Y., et al. 2024b. The heat shock factor 20-HSF4-cellulose synthase A2 module regulates heat stress tolerance in maize. *Plant Cell* **36**(7): 2652-2667.

Liu, M., Rehman, S., Tang, X., Gu, K., Fan, Q., Chen, D., Ma, W. 2019. Methodologies for improving HDR efficiency. *Front. Genet.* **9**: 691.

Ludwig, Y., Dueñas, C., Arcillas, E., Macalalad-Cabral, R.J., Kohli, A., Reinke, R., et al. 2024. CRISPR-mediated promoter editing of a cis-regulatory element of OsNAS2 increases Zn uptake/translocation and plant yield in rice. *Front. Genome Editing* **5**: 1308228.

Lv, Y., Yang, M., Hu, D., Yang, Z., Ma, S., Li, X., Xiong, L. 2017. The OsMYB30 transcription factor suppresses cold tolerance by interacting with a JAZ protein and suppressing β-amylase expression. *Plant Physiol.* **173**(2): 1475-1491.

Makarova, K.S., Koonin, E.V. 2015. Annotation and classification of CRISPR-Cas systems. *Methods Mol. Biol.* **1311**: 47-75.

Makaza, W., En-Nahli, Y., Amri, M. 2023. Harnessing plant resistance against *Striga* spp. parasitism in major cereal crops for enhanced crop production and food security in Sub-Saharan Africa: a review. *Food Secur.* **15**: 1127-1149.

Mali, P., Yang, L., Esvelt, K.M., Aach, J., Guell, M., DiCarlo, J.E., Norville, J.E., Church, G.M. 2013. RNA-guided human genome engineering via Cas9. *Science* **339**(6121): 823-826.

Malzahn, A.A., Tang, X., Lee, K., Ren, Q., Sretenovic, S., Zhang, Y., et al. 2019. Application of CRISPR-Cas12a temperature sensitivity for improved genome editing in rice, maize, and *Arabidopsis*. *BMC Biol.* **17**: 9.

Mojica, F.J., Díez-Villaseñor, C., García-Martínez, J., Soria, E. 2005. Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. *J. Mol. Evol.* **60**(2): 174-182.

Nguyen, T.T., Pham, D.T., Nguyen, N.H., Do, P.T., To, H.T.M. 2023. The germin-like protein gene OsGER4 is involved in heat stress response in rice root development. *Funct. Integr. Genomics* **23**: 271.

Nonaka, S., Arai, C., Takayama, M., Matsukura, C., Ezura, H. 2017. Efficient increase of ɣ-aminobutyric acid (GABA) content in tomato fruits by targeted mutagenesis. *Sci. Rep.* **7**: 7057.

Norouzi, M., Nazarain, F.F., Ismaili, A., Ahmadvand, R., Poormazaheri, H. 2024. CRISPR/Cas StNRL1 gene knockout increases resistance to late blight and susceptibility to early blight in potato. *Front. Plant Sci.* **14**: 1278127.

Nuñez, J.K., Chen, J., Pommier, G.C., Cogan, J.Z., Replogle, J.M., et al. 2021. Genome-wide programmable transcriptional memory by CRISPR-based epigenome editing. *Cell* **184**(9): 2503-2519.e17.

Nuss, P. 2015. Anxiety disorders and GABA neurotransmission: a disturbance of modulation. *Neuropsychiatr. Dis. Treat.* **11**: 165.

Oatway, L., Vasanthan, T., Helm, J.H. 2001. Phytic acid. *Food Rev. Int.* **17**(4): 419-431.

Osakabe, Y., Osakabe, K. 2015. Genome editing with engineered nucleases in plants. *Plant Cell Physiol.* **56**(3): 389-400.

Park, S.Y., Yang, D., Ha, S.H., Lee, S.Y. 2018. Metabolic engineering of microorganisms for the production of natural compounds. *Adv. Biosyst.* **2**: 1700190.

Paulose, B., Chhikara, S., Coomey, J., Jung, H., Vatamaniuk, O., Dhankher, O.P. 2013. A γ-glutamyl cyclotransferase protects *Arabidopsis* plants from heavy metal toxicity by recycling glutamate to maintain glutathione homeostasis. *Plant Cell* **25**(11): 4580-4595.

Pourcel, C., Salvignol, G., Vergnaud, G. 2005. CRISPR elements in *Yersinia pestis* acquire new repeats by preferential uptake of bacteriophage DNA, and provide additional tools for evolutionary studies. *Microbiology* **151**(Pt 3): 653-663.

Prusti, A.M., Das, T.R. 2020. Variability and correlation studies of yield and yield attributing characters in finger millet [*Eleusine coracana* (L.) Gaertn.]. *e-planet* **18**(2): 115-119.

Ramulifho, E., Rey, C. 2024. A coiled-coil nucleotide-binding domain leucine-rich repeat receptor gene MeRPPL1 plays a role in the replication of a geminivirus in cassava. *Viruses* **16**: 941.

Ren, C., Liu, X., Zhang, Z., Wang, Y., Duan, W., Li, S., Liang, Z. 2016. CRISPR/Cas9-mediated efficient targeted mutagenesis in Chardonnay (*Vitis vinifera* L.). *Sci. Rep.* **6**: 1-9.

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

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

Sarkar, J., Jyoti, T.P., Sahana, S., Bhattacharya, A., Chandel, S., Singh, R. 2024. CRISPR-Cas13d in plant biology: an insight. *Plant Biotechnol.* **18**: 301-311.

Sashidhar, N., Harloff, H.J., Potgieter, L., Jung, C. 2020. Gene editing of three BnITPK genes in tetraploid oilseed rape leads to significant reduction of phytic acid in seeds. *Plant Biotechnol. J.* **18**(11): 2241-2250.

Schaart, J.G., van de Wiel, C.C.M., Smulders, M.J.M. 2021. Genome editing of polyploid crops: prospects, achievements and bottlenecks. *Transgenic Res.* **30**(4): 337-351.

Shaibu, A.S., Zhang, S., Ma, J., Feng, Y., Huai, Y., Qi, J., et al. 2022. The GmSNAP11 contributes to resistance to soybean cyst nematode race 4 in *Glycine max*. *Front. Plant Sci.* **13**: 939763.

Shen, Y., Ye, Q., Wu, Z., Jiang, W., Wang, L., Zhang, Q., et al. 2024. Functional characterization of OsCAT2 gene in rice that regulates ROS scavenging and plant growth and development. *Plant Growth Regul.* **103**: 165-175.

Shi, Y., Zhang, Y., Sun, Y., Xie, Z., Luo, Y., Long, Q., et al. 2023. Natural variations of OsAUX5, a target gene of OsWRKY78, control the neutral essential amino acid content in rice grains. *Mol. Plant* **16**: 322-336.

Song, X. 2022. Targeting a gene regulatory element enhances rice grain yield by decoupling panicle number and size. *Nat. Biotechnol.* **40**(9): 1403-1411.

Takagi, H., Oli, M.T., Abe, A., Yoshida, K., Uemura, A., Yaegashi, H., Obara, T., et al. 2015. MutMap accelerates breeding of a salt-tolerant rice cultivar. *Nat. Biotechnol.* **33**(5): 445-449.

Tang, L., Mao, B., Li, Y., Lv, Q., Zhang, L., Chen, C., He, H., et al. 2017. Knockout of OsNramp5 using the CRISPR/Cas9 system produces low Cd-accumulating indica rice without compromising yield. *Sci. Rep.* **7**(1): 14438.

Távora, F.T.P.K., Meunier, A.C., Vernet, A., Portefaix, M., Milazzo, J., Adreit, H., et al. 2022. CRISPR/Cas9-targeted knockout of rice susceptibility genes OsDjA2 and OsERF104 reveals alternative sources of resistance to *Pyricularia oryzae*. *Rice Sci.* **29**: 535-544.

Tiwari, J.K., A, J., Tuteja, N., Khurana, S.M.P. 2022. Genome editing (CRISPR-Cas)-mediated virus resistance in potato (*Solanum tuberosum* L.). *Mol. Biol. Rep.* **49**(12): 12109-12119.

Tuncel, A., Corbin, K.R., Ahn-Jarvis, J., Harris, S., Hawkins, E., Smedley, M.A., Harwood, W., Warren, F.J., Patron, N.J., Smith, A.M. 2019. Cas9-mediated mutagenesis of potato starch-branching enzymes generates a range of tuber starch phenotypes. *Plant Biotechnol. J.* **17**(12): 2259-2271.

Tycko, J., Myer, V.E., Hsu, P.D. 2016. Methods for optimizing CRISPR-Cas9 genome editing specificity. *Mol. Cell* **63**(3): 355-370.

Usovsky, M., Gamage, V.A., Meinhardt, C.G., Dietz, N., Triller, M., Basnet, P., et al. 2023. Loss-of-function of a-SNAP gene confers resistance to soybean cyst nematode. *Nat. Commun.* **14**: 7629.

Veerala, P., Chand, P., Das, T.R., Gangwar, L.K. 2024. Prediction and validation of putative candidate genes underlying drought-tolerant QTLs through in silico and RT-PCR approaches in rice (*Oryza sativa*). *Plant Breed.* **143**(5): 675-694.

Wang, F.-Z., Chen, M.-X., Yu, L.-J., Xie, L.-J., Yuan, L.-B., Qi, H., Xiao, M., Guo, W., Chen, Z., Yi, K., Zhang, J., Qiu, R., Shu, W., Xiao, S., Chen, Q.-F. 2017. OsARM1, an R2R3 MYB transcription factor, is involved in regulation of the response to arsenic stress in rice. *Front. Plant Sci.* **8**: 1868.

Wang, H., Wu, Y., Zhang, Y., Yang, J., Fan, W., Zhang, H., Zhao, S., Yuan, L., Zhang, P. 2019. CRISPR/Cas9-based mutagenesis of starch biosynthetic genes in sweet potato (*Ipomoea batatas*) for the improvement of starch quality. *Int. J. Mol. Sci.* **20**(19): 4702.

Wang, M., Cheng, J., Wu, J., Chen, J., Liu, D., Wang, C., et al. 2024b. Variation in TaSPL6-D confers salinity tolerance in bread wheat by activating TaHKT1;5-D while preserving yield-related traits. *Nat. Genet.* **56**(6): 1257-1269.

Wang, P., Yamaji, N., Mitani-Ueno, N., Ge, J., Ma, J.F. 2024a. Knockout of a rice K5.2 gene increases Ca accumulation in the grain. *J. Integr. Plant Biol.* **66**(2): 252-264.

Wang, W., Wang, W., Pan, Y., Tan, C., Li, H., Chen, Y., et al. 2022. A new gain-of-function OsGS2/GRF4 allele generated by CRISPR/Cas9 genome editing increases rice grain size and yield. *Crop J.* **10**(4): 1207-1212.

Wang, Y. 2022. Improving bread wheat yield through modulating an unselected AP2/ERF gene. *Nat. Plants* **8**(8): 930-939.

Wei, S. 2022. A transcriptional regulator that boosts grain yields and shortens the growth duration of rice. *Science* **377**: eabi8455.

Wright, A.V., Nuñez, J.K., Doudna, J.A. 2016. Biology and applications of CRISPR systems: harnessing nature's toolbox for genome engineering. *Cell* **164**(1-2): 29-44.

Xie, K., Zhang, J., Yang, Y. 2014. Genome-wide prediction of highly specific guide RNA spacers for CRISPR-Cas9-mediated genome editing in model plants and major crops. *Mol. Plant* **7**(5): 923-926.

Xie, J., Fei, X., Yan, Q., Jiang, T., Li, Z., Chen, H., et al. 2024. The C4 photosynthesis bifunctional enzymes, PDRPs, of maize are co-opted to cytoplasmic viral replication complexes to promote infection of a prevalent potyvirus sugarcane mosaic virus. *Plant Biotechnol. J.* **22**(7): 1812-1832.

Xu, X., Sun, S.-K., Zhang, W., Tang, Z., Zhao, F.-J. 2024. Editing silicon transporter genes to reduce arsenic accumulation in rice. *Environ. Sci. Technol.* **58**(4): 1976-1985.

Yang, H., Ren, S., Yu, S., Pan, H., Li, T., Ge, S., Zhang, J., Xia, N. 2020. Methods favoring homology-directed repair choice in response to CRISPR/Cas9-induced double-strand breaks. *Int. J. Mol. Sci.* **21**(18): 1-20.

Yang, J., Fang, Y., Wu, H., Zhao, N., Guo, X., Mackon, E., et al. 2023. Improvement of resistance to rice blast and bacterial leaf streak by CRISPR/Cas9-mediated mutagenesis of Pi21 and OsSULTR3;6 in rice (*Oryza sativa* L.). *Front. Plant Sci.* **14**: 1209384.

Yang, X. 2023. Ovule cell wall composition is a maternal determinant of grain size in barley. *New Phytol.* **237**: 2136-2147.

Yasmeen, A., Shakoor, S., Azam, S., Bakhsh, A., Shahid, N., Latif, A., et al. 2022. CRISPR/Cas-mediated knockdown of vacuolar invertase gene expression lowers the cold-induced sweetening in potatoes. *Planta* **256**: 107.

Ye, N., Wang, Y., Yu, H., Qin, Z., Zhang, J., Duan, M., et al. 2023. Abscisic acid enhances trehalose content via OsTPP3 to improve salt tolerance in rice seedlings. *Plants* **12**: 2665.

Ye, W., Hossain, R., Pröbsting, M., Ali, A.A.M., Han, L., Miao, Y., et al. 2024. Knockout of BnHva22c reduces the susceptibility of *Brassica napus* to infection with the fungal pathogen *Verticillium longisporum*. *Crop J.* **12**(2): 503-514.

Yue, E., Cao, H., Liu, B. 2020. OsmiR535, a potential genetic editing target for drought and salinity stress tolerance in *Oryza sativa*. *Plants* **9**(10): Article 10.

Zeng, Z., Han, N., Liu, C., Buerte, B., Zhou, C., Chen, J., et al. 2020. Functional dissection of HGGT and HPT in barley vitamin E biosynthesis via CRISPR/Cas9-enabled genome editing. *Ann. Bot.* **126**(5): 929-942.

Zhai, Y., Yu, K., Cai, S., Hu, L., Amoo, O., Xu, L., Yang, Y., Ma, B., Jiao, C., Zhang, C. 2020. Targeted mutagenesis of BnTT8 homologs controls yellow seed coat development for effective oil production in *Brassica napus* L. *Plant Biotechnol. J.* **18(5)**: 1153-1168.

Zhan, X., Liu, W., Nie, B., Zhang, F., Zhang, J. 2023. Cas13d-mediated multiplex RNA targeting confers a broad-spectrum resistance against RNA viruses in potato. *Commun. Biol.* **6**: 855.

Zhang, A., Liu, Y., Wang, F., Li, T., Chen, Z., Kong, D., Bi, J., Zhang, F., Luo, X., Wang, J., Tang, J., Yu, X., Liu, G., Luo, L. 2019. Enhanced rice salinity tolerance via CRISPR/Cas9-targeted mutagenesis of the OsRR22 gene. *Mol. Breed.* **39**(3): 47.

Zhang, A., Wang, F., Kong, D., Hou, D., Huang, L., Bi, J., et al. 2023a. Mutation of DEP1 facilitates the improvement of plant architecture in Xian rice (*Oryza sativa*). *Plant Breed.* **142**: 338-344.

Zhang, H., Liu, M., Yin, K., Liu, H., Liu, J., Yan, Z. 2024. A novel OsHB5-OsAPL-OsMADS27/OsWRKY102 regulatory module regulates grain size in rice. *J. Plant Physiol.* **295**: 154210.

Zhang, J., Zhang, H., Li, S., Li, J., Yan, L., Xia, L. 2021. Increasing yield potential through manipulation of an ARE1 ortholog related to nitrogen use efficiency in wheat by CRISPR/Cas9. *J. Integr. Plant Biol.* **63**(9): 1649-1663.

Zhang, L., He, W., Fu, R., Wang, S., Chen, Y., Xu, H. 2023c. Guide-specific loss of efficiency and off-target reduction with Cas9 variants. *Nucleic Acids Res.* **51**(18): 9880-9893.

Zhang, N., Pombo, M.A., Rosli, H.G., Martin, G.B. 2020. Tomato wall-associated kinase SlWak1 depends on Fls2/Fls3 to promote apoplastic immune responses to *Pseudomonas syringae*. *Plant Physiol.* **183**(4): 1869-1882.

Zhang, N., Wang, S., Zhao, S., Chen, D., Tian, H., Li, J., et al. 2023b. Global crotonylatome and GWAS

revealed a TaSRT1-TaPGK model regulating wheat cold tolerance through mediating pyruvate. *Sci. Adv.* **9**: eadg1012.

Zhang, Y., Blahut-Beatty, L., Zheng, S., Clough, S.J., Simmonds, D.H. 2022a. The role of a soybean 14-3-3 gene (Glyma05g29080) on white mold resistance and nodulation investigations using CRISPR-Cas9 editing and RNA silencing. *Mol. Plant-Microbe Interact.* **36**(3): 159-164.

Zhang, Y., Guo, W., Chen, L., Shen, X., Yang, H., Fang, Y., et al. 2022b. CRISPR/Cas9-mediated targeted mutagenesis of GmUGT enhanced soybean resistance against leaf-chewing insects through flavonoids biosynthesis. *Front. Plant Sci.* **13**: 802716.

Zhao, Y., Zhang, C., Liu, W., Gao, W., Liu, C., Song, G., Li, W.X., Mao, L., Chen, B., Xu, Y., Li, X., Xie, C. 2016. An alternative strategy for targeted gene replacement in plants using a dual-sgRNA/Cas9 design. *Sci. Rep.* **6**: 1-11.

Zheng, S., Ye, C., Lu, J., Liufu, J., Lin, L., Dong, Z., et al. 2021. Improving the rice photosynthetic efficiency and yield by editing OsHXK1 via CRISPR/Cas9 system. *Int. J. Mol. Sci.* **22**: 9594.

Zheng, X., Zhang, S., Liang, Y., Zhang, R., Liu, L., Qin, P., et al. 2023. Loss-of-function mutants of OsCKX gene family based on CRISPR-Cas systems revealed their diversified roles in rice. *Plant Genome* **16**: e20283.

Zhou, J., Li, Z., Li, Y., Zhao, Q., Luan, X., Wang, L., et al. 2023. Effects of different gene editing modes of CRISPR/Cas9 on soybean fatty acid anabolic metabolism based on GmFAD2 family. *Int. J. Mol. Sci.* **24**: 4769.