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Review Article

Rice grain yield and quality improvement via CRISPR/Cas9 system: an updated review

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Abstract

Rice (*Oryza sativa* L.) is an important staple food crop worldwide. To meet the growing nutritional requirements of the increasing population in the face of climate change, qualitative and quantitative traits of rice need to be improved. During recent years, genome editing has played a great role in the development of superior varieties of grain crops. Genome editing and speed breeding have improved the accuracy and pace of rice breeding. New breeding technologies including genome editing have been established in rice, expanding the potential for crop improvement. Over a decade, site-directed mutagenesis tools like Zinc Finger Nucleases (ZFN), Transcriptional activator-like Effector Nucleases (TALENs), and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) System were used and have played a great role in rice yield and quality enhancement. In addition, most recently other genome editing techniques like prime editing and base editors have also been used for efficient genome editing in rice. Since rice is an excellent model system for functional studies due to its small genome and close synthetic relationships with other cereal crops, new genome-editing technologies continue to be developed for use in rice. Genomic alteration employing genome editing technologies (GETs) like CRISPR/Cas9 for reverse genetics has opened new avenues in agricultural sciences such as rice yield and grain quality improvement. Currently, CRISPR/Cas9 technology is widely used by researchers for genome editing to achieve the desired biological objectives, because of its simple targeting, easy-to-design, cost-effective, and versatile tool for precise and efficient plant genome editing. Over the past few years many genes related to rice grain quality and yield enhancement have been successfully edited via CRISPR/Cas9 technology method to cater to the growing demand for food worldwide. The effectiveness of these methods is being verified by the researchers and crop scientists worldwide. In this review we focus on genome-editing tools for rice improvement to address the progress made and provide examples of genome editing in rice. We also discuss safety concerns and methods for obtaining transgene-free crops.

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Keywords: base editing; CRISPR/Cas-9; genome editing; grain quality improvement; mega-nucleases; transgene free

Introduction

To address major issues including the growing population, environmental changes, and food scarcity, rice (*Oryza sativa* L.) varieties must be developed with higher yields and tolerance to environmental stress (Clarke and Zhang, 2013). Different strategies have been used to increase rice grain quality and yields. For example, although conventional breeding methods have improved, there have only been small increases in crop yields in recent years (Ansari *et al*., 2017; Ahmad *et al*., 2021). Genome engineering has also been accomplished in rice but engineered crops have not reached end-users mainly due to public acceptance and political issues. Ultimately, genome engineering technologies offer expanded potential for crop improvement because they allow specific alterations of DNA sequences to be performed *in vivo*. Numerous techniques have been developed, from conventional *Agrobacterium*-mediated gene transfer, site-specific mutagenesis to newer and targeted techniques like the ZFN (Zinc Finger Nucleases), TALEN (Transcription activator-like effector nucleases) and CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats) system, base editing and prime editing. Over the past several years, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) with CRISPR-associated protein Cas9 (CRISPR/Cas9) has transformed the field of genome engineering and emerges as novel system for genome editing (Ahmad *et al*., 2021). This technique is versatile and simple compared to other genome-editing tools such as zinc finger nucleases and transcriptional activator like effector nucleases (TALENs) (Cong *et al*., 2013; Miao *et al*., 2013; Ma *et al*., 2015). CRISPR/Cas9 is economical, easy to use, highly accurate and effective even when performing multiplex genome editing (Wang *et al*., 2017b). Multiplex genome editing is important because it allows multiple genes to be manipulated at several genomic locations. CRISPR/Cas9 targets specific genes and creates double-stranded breaks (DSBs) at the desired site. These DSBs are then repaired by the host's repair machinery, which performs homology directed repair (HDR) or non-homologous end joining (NHEJ) to produce genomic alterations, gene knockouts and gene insertions. In NHEJ, the repair is performed by the host without the need for donor template, whereas in HDR, a donor template is required to repair the DSB. The frequency of HDR is lower than NHEJ, making the use of HDR in plants very challenging (Puchta, 2004). CRISPR/Cas9 has multiple uses and is a rapidly growing area in the field of plant genome editing, especially in rice. Rice is an excellent model system for studying functional genomics due to its small genome size and close synthetic relationships with other cereal crops (Tabassum *et al*., 2021; Riaz *et al*., 2022). The current review focuses on different genome editing strategies for improving rice yield and quality and their potential for addressing current challenges. We also focus on the safety concerns of rice genome editing and methods for obtaining transgene-free crops.

Genome Engineering and Editing Methods

Currently, numerous genome-editing tools and techniques have been adopted to overcome the problems arising in plants to compensate for the increased demand for food in the future (Zhang *et al*., 2017). Gene-editing techniques, such as engineered endonucleases/mega-nucleases (EMNs), zinc-finger nucleases (ZFNs), TAL effector nucleases (TALENs), prime editing, base editing and clustered regularly interspaced short palindromic repeats (CRISPR), are important tools in plant research, as they allow the remodeling of future crops (Ahmad *et al*., 2021). Among endonucleases, mega-nucleases are characterized by the presence of a broad recognition site of about 12–40 bp. Because of their specific nature and long recognition site, these enzymes are regarded as the most precise restriction enzymes (Mishra *et al*., 2018). Therefore, mega-nucleases

are also known as homing endonucleases. Repair in double-stranded breaks (DSBs) occurs via non-homologous end joining (NHEJ), which is functionally responsible for knocking out genes in tobacco and *Arabidopsis* plants (Townsend *et al*., 2009: Zhang *et al*., 2010). However, it is difficult to remodify mega-nucleases together with other genome-targeting techniques, because DNA-binding domains are often intermingled with the catalytic domain of mega-nucleases and cannot be detached from one another (Puchta *et al*., 2005). The outcomes of previous research demonstrated gene editing in plants by incorporating modified mega-nucleases in *Arabidopsis*, cotton, and maize. However, additional efforts are needed to improve this approach, as the manipulation of mega-nucleases seems to be difficult. Hence, researchers focused on other more efficient, accurate, and simpler methods of gene editing, such as ZFNs, TALENs, and CRISPR.

Zinc-finger nucleases (ZFNs) are one of the most efficient and effective tools for genome editing by targeting DSBs (Durai *et al*., 2005). ZFNs were the first truly targeting protein reagents to revolutionize the genome manipulation area of research. ZFNs are binding domains for DNA that recognize three base pairs at the target site (Rai *et al*., 2005). ZFNs have been commonly used for targeted genome modification in different plant species, such as Arabidopsis thaliana (Arabidopsis), Nicotiana tabacum (tobacco), rice and maize (Osakabe *et al*., 2010; Li *et al*., 2012). In rice, ZNF targeted mutagenesis was done for gene IPK1 responsible for the production of Phytic acid that is found in high quality in the rice kernel (Cassandri *et al*., 2017).

Another site-driven mutagenesis genome-editing system, TALENs, was defined first in plant pathogenic bacteria (Xanthomonas) and is based on a concept similar to that of ZFNs. TALENs target one nucleotide at the target site (instead of three), thus rendering TALENs precise (Boch *et al*., 2009). TALENs were successfully used for genome editing in angiosperms and bryophytes (Li *et al*., 2012; Kopischke *et al*., 2017). TALENs were also used for enhancing the fragrance of rice by targeting the *OsBADH2* gene responsible for Betaine aldehyde dehydrogenase (Mishra *et al*., 2018). TALENs activate or recognize unique transcription factors in plant promotors through a set of tandem repeats that is the base of its working. Both ZFN and TALENs had the same strategy of cutting and knocking off the genes but they also produced undesirable results. Non-specific mutations and off-target gene mutations were major concerns (Abdelrahman and Zhao, 2020) (Table 1).

Table 1. Comparison of EMNs, ZFNs, TALENs, CRISPR/Cas9, base editing and prime editing

Functions	EMNs	ZFNs	TALENS	CRISPR/CAS9	Base Editing	Prime Editing
DNA binding determinant	Mega nucleases	Zinc finger protein	Transcription activator like effector	Cr-RNA/sgRNA	dCas/nCas	nCas9/pegRNA
Endonuclease	Mega nucleases	*FokI*	*FokI*	*Cas9*	*dCas*	*pegRNA*
Recognition	Protein-DNA	Protein-DNA	Protein-DNA	RNA-DNA	RNA-DNA-Protein	RNA-DNA-Protein
Mutation rate	High	Medium	Medium	Low	High	Very High
Off-target effects	High	High	Low	Variable	Low	Very Low
Length of target sequence (bp)	14-40	18-36	30-40	20-22	4-6	8-16
Cost effectiveness	No	No	Moderate	High	Very High	Very High
Mode of action	Information strand directs conversion(s) within the target region	Double-strand breaks in the target DNA	Double-strand breaks in the target DNA	Double-strand breaks or single-strand nicks in the target DNA	Double-strand breaks or single-strand nicks in the target DNA	No Double strand or single strand nicks break in the target DNA
Target recognition efficiency	Low	High	High	High	Very High	Very High
Multiplexing	Not Possible	Difficult	Difficult	Easy	Easy	Not Yet Tested

Methylation sensitivity	High	High	High	Low	-	-
References	Kim *et al*., 2014	Ain *et al*., 2015; Gao *et al*., 2016	Ain *et al*., 2015; Gao *et al*., 2016	Ain *et al*., 2015; Gao *et al*., 2016	Komor *et al*., 2016; Kim *et al*., 2017; Zafra *et al*., 2018	Anzalone *et al*., 2019

Extensive investigation in this field led to the development of new genome-editing tools, such as CRISPR/Cas9 and CRISPR/Cpf1 (Bortesi *et al*., 2015; Zhang *et al*., 2020). Initially, these techniques were developed in prokaryotes, because there were no efficient genome-editing techniques for eukaryotes at specific sites. However, at the advent of eukaryotic genome editing, the CRISPR technology has revolutionized our ability to generate specific changes in crops (Butt *et al*., 2020). The CRISPR system requires only the guide RNA sequence to be changed for each DNA target site. Under different circumstances, the usage and modification of CRISPR technology are quite simple and efficient (Puchta *et al*., 2017; Ahmar *et al*., 2020). For more than a decade the scientists never tested its potential as a tool to modify genome, its first use came after 2012 when it was employed as a tool to modify genes in animals and fungi (Miao *et a*l., 2013). The first use of the CRISPR/Cas9 system emerged in rice and wheat species (Shan *et a*l., 2014).

As compared to ZFN and TALENS which are more expensive and often result in off-target mutations, the CRISPR/Cas9 system is easy to build and simplest to operate. In the engineered system, programing Cas9 to target a speci*fi*c genome sequence only requires the introduction of a 20-bp spacer sequence in the sgRNA. Therefore, CRISPR/Cas9 is much simpler and easier to manipulate than the previously available methods ZFNs and TALENs, and most importantly, it has a much higher ef*fi*ciency in producing targeted mutations. Multiple sgRNAs with different target sequences can be easily designed for simultaneous multiplex editing. CRISPR's technical simplicity and efficiency made it a true breakthrough in genome editing, allowing non-specialized laboratories to use it routinely. Since its *fi*rst report for plant gene editing in 2013, CRISPR/Cas9 has been used in a large number of species, including rice, wheat, tobacco, Arabidopsis, sorghum, tomato, maize, potato, poplar, soybean, barley, moss, Brassica oleracea, sweet orange, apple, liverwort, grape, lettuce, cotton, Lotus japonicus, dandelion, *fl*ax, petunia, citrus, watermelon and mushroom (Jiang *et al*., 2013; Li *et al*., 2013; Mao *et al*., 2013; Nekrasov *et al*., 2013; Shan *et al*., 2013a; Brooks *et al*., 2014; Feng *et al*., 2014; Jia and Wang, 2014; Sugano *et al*., 2014; Fan *et al*., 2015; Lawrenson *et al*., 2015; Li *et al*., 2015; Svitashev *et al*., 2015; Wang *et al*., 2015b; Woo *et al*., 2015; Iaffaldano *et al*., 2016; Lopez-Obando *et al*., 2016; Nishitani *et al*., 2016; Ren *et al*., 2016; Sauer *et al*., 2016; Waltz, 2016; Wang *et al*., 2016b; Zhang *et al*., 2016a; Chen *et al*., 2017; Jia *et al*., 2017; Tian *et al*., 2017).

Unlike other DNA editing tools mentioned earlier, base editing is a newer method that makes irreversible changes in DNA bases using Cas9 protein variants (Rees and Liu, 2018; Monsur *et al*., 2022). DNA base editors comprise a catalytically disabled nuclease fused to a nucleobase deaminase enzyme and, in some cases, a DNA glycosylase inhibitor. RNA base editors achieve analogous changes using components that target RNA (Kuang *et al*., 2020). Base editors directly convert one base or base pair into another, enabling the efficient installation of point mutations in non-dividing cells without generating excess undesired editing by products (Hua *et al*., 2019). Base editing was performed in rice by Zong *et al*. (2017). The authors targeted *OsCDC48* (which regulates senescence and cell death) to determine whether this system could be used to create nucleotide substitutions in rice. The target regions were successfully substituted, with a mutation frequency of 43.48%. There were no off-target mutations and no indels in the target region (Zong *et al*., 2017). In another study, a C-to-T substitution was created in *OsALS* to confer herbicide resistance in rice. Analanine-to-valine substitution at position 96 provided the rice plants with resistance to the herbicide imazamox (Shimatani *et al*., 2017).

Although the CRISPR-cas9 system and other genome editing systems has provided a great way to introduce mutations and precisely introduce desired traits, it has some limitations. Especially in the case of crop plants, the limitations are low HDR availability and NHEJ. There is a need for more options to enhance crop plant characters (Tang *et al*., 2020). Prime editing is the option here which provides a chance to introduce all 12 types of mutations and is less complex as compared to other systems. The system engineered by fusing a mutated M-MLV-RT (Moloney murine leukemia virus reverse transcriptase) to the C terminus of a catalytically impaired Cas9 (H840A) (Cas9 nickase, nCas9), and programmed with a prime editing guide RNA (pegRNA) composed of a single chimeric guide RNA (sgRNA) targeting the specific site, a primer-binding site (PBS), and a reverse transcription (RT) template encoding the desired edit (Xu *et al*., 2020). Recently, several attempts have been made to establish an efficient prime editing system in rice and achieved herbicide-tolerant varieties (Li *et al*., 2020; Lin *et al*., 2020; Xu *et al*., 2020). It is noteworthy that the mutation efficiency of prime editing is similar to that of the base editing system; however, the specificity was much higher than that of previously discussed Genome Engineering Technologies. The prime editing system is at the foundation stage, and further developments and applications for crop improvement programs will take place over time.

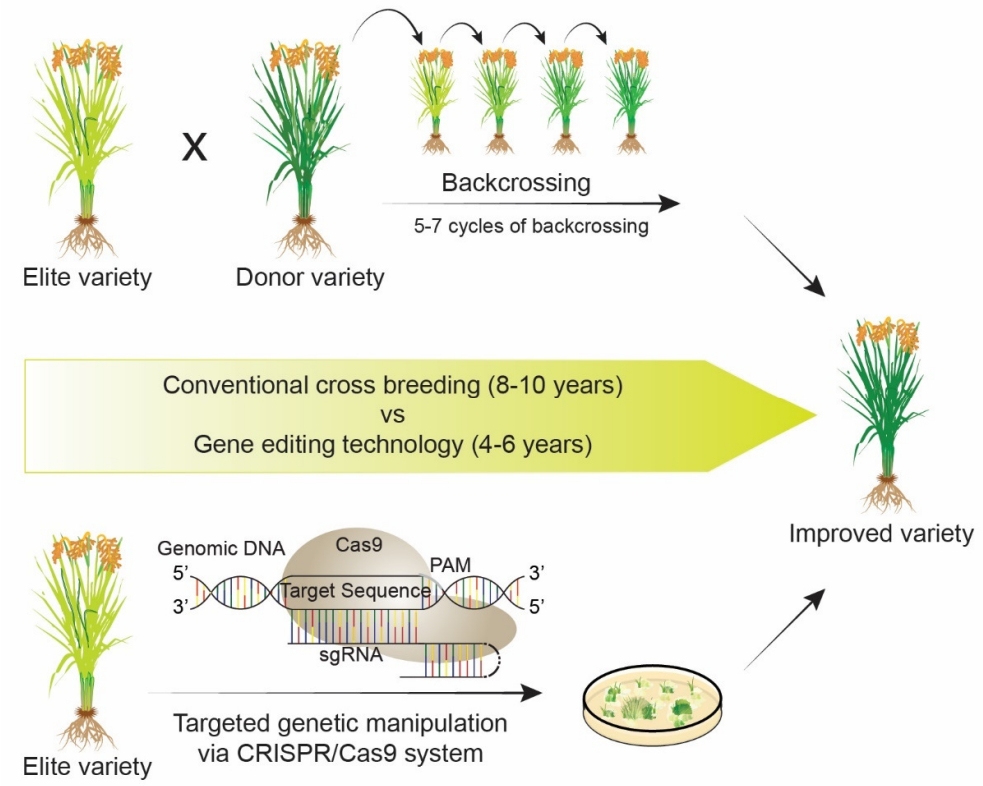

Figure 1. Application of CRISPR/Cas9 system for developing elite variety with desirable improved traits in rice and its comparison with conventional crop breeding technique

Applications of gene editing (GE) system in yield and quality improvement of rice

The main characteristics that are determinative of rice yield are grain size and weight. Both of these are multigene traits and there are more than 400 genes that are related to Quantitative trait loci (QTL) but fewer genes have been identified responsible for the grain weight and number. Thus there is a dearth of data available in this regard (Azizi *et al*., 2019). For rice quality and yield, enhancement scientists have employed conventional methods of breeding that is not only slower but often are not efficient.

There has been a little progress in terms of yield increase using the ZFN, and TALENs but the CRISPR-Cas9 system has been explored extensively in this regard (Cui *et al*., 2020). It has been employed for multigene manipulation. Photosynthesis manipulation is an ideal candidate in this regard. Rice is a C3 plant which is an inefficient method of fixing atmospheric CO_2. The quest to convert C3 to the C4 cycle is most promising, as it can increase the yield by 40-50% but the research has been limited (Kohli *et al*., 2020). Rice yield has been improved by knocking out genes including *GS3*, *DEP1*, *GS5*, *GW2*, *Gn1a*, and *TGW6* that are known to be negative regulators of grain size and number and grain weight (Zeng *et al*., 2020).

Grain quality and quantity is a large subject that includes diverse subjects such as grain shape, cooking qualities, starch content, and nutritional qualities, and shelf life, grain size, and kernel shape. As rice is a food of half of the population of the world (Lv *et al*., 2020), improving the nutritional content will increase the ability to provide a more balanced diet for the population. The improvements being brought about in rice grains quality-based attributes via the use of targeted genomes editing is a quick, sustainable, as well as the cost-effective approach (Jena and Kim, 2020). However, many studies have demonstrated the successful use of genome editing to improve rice. Below are some examples of the improvement of rice traits through genome editing (Figure 2, Table 2).

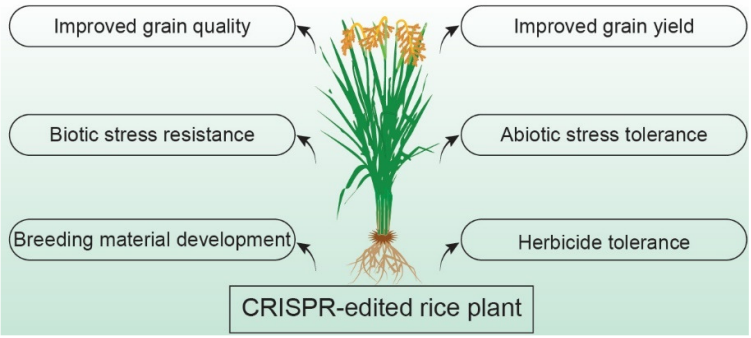

Figure 2. CRISPR-Cas mediated genome editing in rice for the improvement of different traits

Table 2. The genes and functions manipulated using the Modern Genetic Engineering tools in rice

Sr#	Gene manipulated	Technique employed	Mechanism	Transformation method	Result	References
1	*Gn1a*	CRISPR/Cas 9	NHEJ	Agrobacterium	Grain Number Increase	(Huang *et al*., 2018)
2	*DEP1*	CRISPR/Cas 9	NHEJ	Agrobacterium	Dense and erect panicle	(Huang *et al*., 2018)
3	*GS3*	CRISPR/Cas 9	NHEJ	Agrobacterium	Grain size increase	(Chan *et al*., 2020)
4	*OsBADH2*	TALENs	NHEJ	Agrobacterium	Increase in fragrance	(Shan *et al*., 2013)
5	*OsDEP1*	TALENs	NHEJ	Agrobacterium	Dense and erect panicle	(Shan *et al*., 2013)
6	*OsCKX2*	TALENs	NHEJ	Agrobacterium	Cytokines Dehydrogenase	(Shan *et al*., 2013)
7	*OsCKX2*	ZFN	NHEJ	Agrobacterium	Increase in the number of reproductive organs	(Li *et al*., 2013)
8	*TMS5*	CRISPR/Cas 9	NHEJ	Agrobacterium	Increase yield and sterility	(Zhou *et al*., 2016)
9	*SWEET14, SWEET11*	CRISPR/Cas 9	NHEJ	Agrobacterium	Bacterial Blight resistance	(Jiang *et al*., 2013)

10	*GS3, IPA1*	CRISPR/Cas 9	NHEJ	Agrobacterium	Improved grain size and yield	(Li *et al*., 2016)
11	*IPK1*	ZFN	NHEJ	Agrobacterium	Kernel quality increased	(Abdelrahman and Zhao, 2020)
12	*OsSPL14*	Base editing	NHEJ	Agrobacterium	Panicle branching and higher grain yield	(Miura *et al*., 2010)
13	*OsSPL16*	Base editing	NHEJ	Agrobacterium	Increase in Grain size, shape, and quality	(Wang *et al*., 2012)
14	*GRF4*	Base editing	NHEJ	Agrobacterium	Growth regulation and yield enhancement	(Hua *et al*., 2019)
15	*NRT1.1B*	Base editing	NHEJ	Agrobacterium	Nitrogen- use efficiency enhancement	(Lu and Zhu, 2017)
16	*OsNRT1*	Base editing	NHEJ	Agrobacterium	Nitrate transporter Gene	(Wang *et al*., 2018)
17	*OsSPL14*	Base editing	NHEJ	Agrobacterium	Panicle Development and number increase	(Jiao *et al*., 2010)
18	*OsKO2*	Prime editing	NHEJ	Agrobacterium	Gibberellin biosynthesis in rice	(Tang *et al*., 2020)
19	*OsPDS*	CRISPR/Cas9 /Cas12a	NHEJ	Agrobacterium	Carotein Yield enhanacement	(Banakar *et al*., 2020)
20	*D17/HTD1*	CRISPR/Cas9	NHEJ	Agrobacterium	Carotenoid Yield enhancement	(Lacchini *et al*., 2020)
21	*BBM1*	CRISPR/Cas9	NHEJ	Agrobacterium	Enables embryo formation from fertilized egg	(Khanday *et al*., 2019)
22	*REC8,OSD1,PAIR and MTL*	CRISPR/Cas9	NHEJ	Agrobacterium	For heterozygosity fixation and haploid induction	(Wang *et al*., 2019)
23	*SF3B1*	CRISPR/Cas9 directed evolution	NHEJ	Agrobacterium	Confers resistance to splicing inhibitors	(Butt *et al*., 2019)

Improving Rice Yield

Grain yield is a complex yet crucial trait for rice improvement. Grain yield is controlled by many genes known as quantitative trait loci (QTLs). Various attempts have been made to improve grain yield using CRISPR/Cas9 (Sedeek *et al*., 2019). Four yield related genes were successfully mutated in rice using CRISPR/Cas9, including *Gn1a* (gene for grain number), *DEP1* (DENSE AND ERECT PANICLE1; controls panicle architecture), *GS3* (regulates grain size), and *IPA1* (gene for plant architecture). Phenotypic

variation was successfully generated due to the presence of edited genes, highlighting the efficiency of CRISPR/Cas9 for the targeted editing of genes regulating complex traits such as grain yield (Li *et al*., 2016). In a study, two important QTLs, GRAIN SIZE3 (*GS3*) and Grain number 1a (*Gn1a*) were disrupted using CRISPR/Cas9 in five widely cultivated rice varieties. The same QTL was shown to have various (even contrasting) effects on grain yield in different genetic backgrounds (Shen *et al*., 2018). Similarly, three yield related QTL genes (*OsGS3*, *OsGW2*, and *OsGn1a*) were edited simultaneously using multiplex genome editing. The resulting triple mutants showed a 30–68% increase in yield per panicle. This type of approach can be used for the rapid breeding of QTLs in elite crop varieties (Zhou *et al*., 2019; Mao *et al*., 2021).

In another study, superior alleles of yield-related genes were developed using CRISPR/Cas9. The authors were interested in determining whether alleles created by gene editing could have stronger effects than natural alleles. Two yield-regulating genes, *Gn1a* and *DEP1*, were edited to develop superior alleles in rice. The mutant alleles of *Gn1a* and *DEP1* conferred higher yields than natural high-yield alleles, indicating that CRISPR/Cas9-based genome editing can be used to create superior alleles to improve current rice varieties (Huang *et al*., 2018).

Improving rice grains nutritional qualities

More than 3 billion people use rice as a staple food, especially in Asian countries where more than 70% of the world population lives. Deficiency of iron and vitamin B is the most frequent malnutrition found among them. Around 24,000 people die daily globally because of malnutrition (Khush, 2005). This is because people are eating the foods, which are deficient in terms of the required proteins, the energy levels, iron and zinc contents, vitamin A, and even iodine (Birla *et al*., 2017). Although much work was spent on improving key agronomic characteristics such as yield, genome editing was also used to improve rice's quality and nutritional value. This includes altering the compositions of oil and starch, controlling the fragrance, reducing accumulation of heavy metal and attempts to biofortify. This is where improvement in rice grains' nutritional qualities using the CRISPR/Cas9 model can help in overcoming the malnutrition issue.

Production of high oleic/low linoleic rice

Oleic acid is a valuable component of rice bran oil. Increasing the oleic acid content in rice bran oil would improve health and help prevent diseases. Oleic acid is converted to linoleic acid by the enzyme fatty acid desaturase 2 (FAD2) in plants. Rice contains three functional FAD2 genes, with OsFAD2-1 exhibiting the highest expression in seeds. By disrupting this gene, high oleic/low linoleic rice bran oil could be produced. Targeted mutagenesis of OsFAD2-1 via CRISPR/Cas9 led to the creation of OsFAD2-1 knockout rice plants with a 2-fold increase in oleic acid contents and no detectable linoleic acid, thereby improving the fatty acid composition of rice bran oil (Abe *et al*., 2018).

Fragrant rice

Fragrant rice is favored worldwide due to its high quality and excellent market value. Betaine aldehyde dehydrogenase (Badh2) is primarily responsible for fragrance in rice. The suppression of Badh2 induces the biosynthesis of 2-acetyl-1-pyrroline (2AP). TALEN-based genome editing was used to disrupt Badh2, resulting in an increase in 2AP levels from 0.35 to 0.75 mg/kg. This amount of 2AP is almost equal to that found in the aromatic rice variety that was used as a positive control (Shan *et al*., 2015; Hui *et al*., 2021).

CRISPR/Cas9 based genome editing of Badh2 was also used to develop fragrant rice in the Zhonghua 11 (indica rice variety) background. The first exon of Badh2 was edited to include an additional base (T), which led to an increase in 2AP contents. Such studies could help fast-track the breeding process of fragrant rice (Shao *et al*., 2017).

Rice with increased β-carotene accumulation

The biofortification of β-carotene is an important target for rice improvement. The *Osor* gene, an ortholog of the Orange (Or) gene in cauliflower, was targeted in rice using CRISPR/Cas9. Or is responsible for β-carotene accumulation in cauliflower curd. The directed modification of *Osor* via CRISPR/Cas9-based genome editing resulted in enhanced β-carotene accumulation in rice callus (Endo *et al*., 2019).

Rice with increased amylose content

Cereals with high amylose contents represent a good source of resistant starch (Regina *et al*., 2006). Foods with high resistant starch contents are nutritious and may reduce the risk for many diseases (Jiang *et al*., 2010). CRISPR/Cas9 technology was used for directed mutagenesis of SBEI and SBEIIb in rice; these genes control amylose content (Sun *et al*., 2017). Although there was no clear difference between sbeI edited plants and wild type, the sbeII edited rice plants showed considerably increased amylose and resistant starch contents, with increases up to 25 and 9.8%, respectively. This indicates the ability of CRISPR/Cas9 system to create high-amylose rice by editing SBEIIb gene.

Low-cadmium rice

The presence of heavy metals in food can be harmful to human health. Eliminating heavy metals from soil would lessen their accumulation in rice grains. In addition, genome editing could be exploited to reduce the levels of toxic metals in food crops. Cadmium (Cd) is extremely toxic and dangerous for human health (Clemens *et al*., 2013). Rice is a major source of calorie intake, and its grains often contain excessive amounts of Cd. Therefore, Cd accumulation should be controlled in rice. Since it is challenging to develop low-Cd rice via conventional breeding, there is a need to develop new strategies for generating low-Cd rice lines. CRISPR/Cas9 has been used to develop low-Cd rice lines by knocking out the metal transporter gene OsNramp5. The OsNramp5 mutants showed dramatically reduced Cd contents compared to the wild type. In field trials, the mutants contained <0.05 mg/kg Cd compared to 0.33 to 2.90 mg/kg in the control plants. The reduced Cd level was achieved without affecting yields (Tang *et al*., 2017).

In another study, CRISPR/Cas9-based editing was used to destroy OsNRAMP5 in two japonica rice varieties. Three independent OsNRAMP5 mutants were generated, and the effects of the mutations on Cd accumulation and plant growth were examined. Less Cd and Mn accumulated in the roots and shoots of the mutants compared to wild-type plants. However, in a paddy field experiment, many traits were affected in the mutants, including plant height, leaving to lower grain yields.

Therefore, the editing of OsNRAMP5 should be approached with caution. Factors such as pH, soil conditions, and water must be considered, as they might influence the Mn level in the soil, ultimately affecting grain yield (Yang *et al*., 2019). These findings pave the way for investigating how genome editing could be used to minimize heavy metal contamination in crops.

Red rice

Red rice contains high levels of the health-promoting nutrients proanthocyanidins and anthocyanins. Two genes, Rc and Rd, are involved in determining the red coloration of rice grains. The RcRd genotype of wild rice species *Oryza* rufipogon produces red pericarp tissue, whereas many varieties of cultivated rice produce white grains due to a frame-shift deletion of 14-bp in the 7th exon of Rc. CRISPR-Cas9 was recently used to bring back the recessive Rc allele by reversing the frame-shift deletion into an in-frame mutation, thus converting white pericarp rice varieties into red ones. The red grains obtained from the mutants contained high levels of proanthocyanidins and anthocyanidins with no notable difference in agronomic traits between the wild type and mutants, indicating that reversing the functioning of Rc has no negative effect on major agronomic traits in rice. This approach could therefore be used for crop improvement (Zhu *et al*., 2019).

Improving storability

Seed longevity and quality are compromised during storage due to the deterioration of grains, causing severe economic losses for farmers. Lipoxygenases (LOXs) negatively regulate seed storability by catalyzing the dioxygenation of polyunsaturated fatty acids to form hydroperoxide. TALEN-based genome editing was successfully used to mutate LOX3 in rice, resulting in LOX3 deficiency and improved seed storability (Ma *et al*., 2015).

Other Traits

Male-sterile lines for hybrid rice production

Disease-free male sterile lines have great potential for use in hybrid rice breeding. Using CRISPR/Cas9, mutations were introduced into male sterility-related genes *TMS5*, *Xa13*, and *Pi21*. The transgene-free mutants acquired in the T1 generation showed characteristics of thermosensitive genic male sterility and improved resistance to rice blast and bacterial blight. This approach provides a way to convert breeding materials into thermosensitive genic male sterile lines though gene editing, ultimately leading to accelerated breeding using male sterile lines (Li *et al*., 2019b).

Reducing seed dormancy

Genome editing was also used to reduce seed dormancy in rice. The viviparous-1 (OsVP1) gene, encoding a transcription factor involved in regulating of seed development and ABA signaling, was knocked out in rice using CRISPR-Cas9. The edited lines showed increased expression of ABA signaling genes, suggesting that genome editing could be effective for reducing seed dormancy in rice (Jung *et al*., 2019).

Genome editing to develop asexual reproduction in rice

Breeders often develop elite plant varieties with better yields, but these phenotypes are usually lost during genetic segregation. Various attempts have been made to preserve these phenotypes. Plants can sometimes form an embryo from a fertilized egg due the expression of "Baby Boom" (BBM1) in sperm cells. The expression of this gene in fertilized cells is due to a contribution from the male. Genome editing was used to remove the plant's ability to go through meiosis. Due to this disruption, the plant produced egg cells via mitosis, indicating that the full set of chromosomes was derived from the mother (Khanday *et al*., 2019). Subsequently, when the cells expressed BBM1, these plants developed egg cells able to generate embryos, which will then grow into clonal seeds. These plants could be reproduced clonally through seed propagation (Khanday *et al*., 2019). The strategy was also employed for clonal reproduction of F1 rice hybrids. Four genes, *REC8*, *PAIR*, *OSD1*, and *MTL* were edited using CRISPR/Cas9 for heterozygosity fixation and haploid induction. The plants produced in this way were able to proliferate clonally through seeds. This strategy can be exploited for the self-propagation of elite rice varieties developed by breeding (Wang *et al*., 2019).

Transgene-Free Edited Rice and Safety Concerns

For genome editing, the Cas9 and the sgRNA that provides target specificity are generally expressed from transgenes that are integrated into the host genome. However, to maintain the stable phenotypes of edited plants, the CRISPR/Cas9 construct that is inserted in the genome must be removed, as the presence of CRISPR/Cas9 creates problems when trying to differentiate previously generated mutations from newly generated mutations. The chances of off-target effects also increase if CRISPR/Cas9 is present in the genome. Furthermore, genome-edited crops should be transgene free if regulatory approval is needed for commercial applications (Ahmad *et al*., 2021). Therefore, after modifying the genome, various methods are used to remove

CRISPR/Cas9 components. This step is also required for the social acceptance of genome editing in agriculture. Here, we review some of the methods that have been developed to avoid off-target effects and make edited crops transgene free.

Conventional approaches for removing the transgene

Transgene removal has been performed using molecular methods such as the FLP/FRT, Cre/loxP, and piggy Bac transposon systems. Other conventional approaches have also been used, including segregation. The content of the parental line can be maintained by backcrossing or selfing. Many studies have reported the development of Cas9-free lines after genome editing through segregation (Xu *et al.*, 2015; Gao *et al.*, 2016; Pyott *et al.*, 2016; Nekrasov *et al.*, 2017; Zaidi *et al.*, 2018).

Editing in GERM LINE CELLS

Germ line cells with the desired mutations can be edited in order to develop transgene-free plants. The CRISPR construct can be delivered to germ line cells using viral vectors, thereby creating the desired mutations in these cells. The seeds from these germline-edited plants transfer the targeted mutations to the next generation. This system can be used to develop germ line-edited plants, but it is less efficient for crop plants (Ali *et al.*, 2015; Zaidi and Mansoor, 2017).

Transgene-free genome editing by transiently expressing CRISPR-Cas9

Another genome-editing method for use in plants that are regenerated from callus is transient expression of CRISPR-Cas9, which can be introduced as RNA or DNA. This system, which is based on transient expression, is highly effective and precise for generating transgene-free plants. This method has been used in many crop plants (Zhang *et al.*, 2016; Hamada *et al.*, 2018) and can be used for transgene-free genome editing in rice (Figure 3).

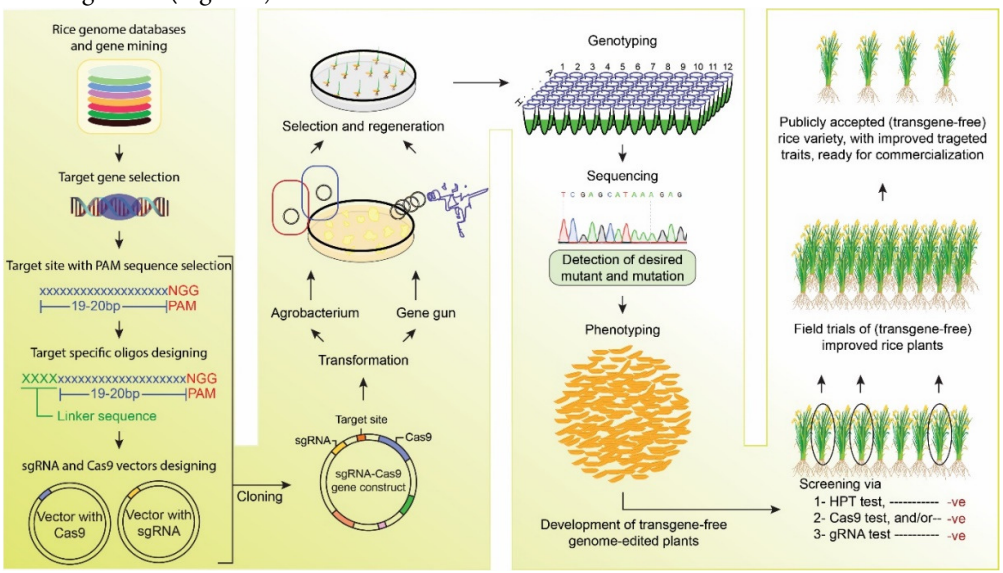

Figure 3. Development of transgene-free genetically edited rice plants for commercialization through genetic segregation

The gRNA will be designed for targeted modification and during construct development appropriate transgene-free method will be chosen (e.g., transient expression of Cas9, RNP or suicide transgene method etc.). Ligation of gRNA with Target gene site. The developed construct along with gRNA Ligated with target genes is completed and can be delivered to plant by Agrobacterium, biolistic or transfection. Once the construct is delivered into the plant. It will perform its function by modifying the target site in the rice genome. After modification the transgene will be removed from the plants through mutant screening. The regenerated plants will be screened for targeted modification without

transgene in field trial. The plants developed in this way will be with desired traits without any transgene integration in the genome, so can be labeled as Transgene Free genetically edited plants by regulatory authorities.

Suicide transgene method

Although several methods are effective for generating plants, they are degraded soon, leaving little time for performing targeted mutation. Therefore, another system was developed that vigorously and automatically eradicates CRISPR/Cas9-containing plants but provides sufficient time for the CRISPR system to perform the desired genome editing. A pair of suicide transgenes was employed to efficiently kill pollen and T0 plants containing a CRISPR/Cas9 construct. This strategy was successfully used in rice to generate targeted mutations and isolate transgene-free plants. This technique greatly reduces the time and labor needed to detect transgene-free plants containing mutations produced by CRISPR/Cas9 (He *et al*., 2018).

Conclusions

Rice is an excellent model-based system for the functional and adaption-based genomics studies. This is because rice has a smaller genome size along with high availability of different genetic resources as well as higher transformational efficiencies, and even greater genomic similarities with the other cereals humans consume. Hence, rice has become an increasingly popular subject in the testing of the efficiencies of different types of the applicable genome's editing technologies. This has allowed researchers to study the various functions associated with the various genes as well as the demonstration of the potential (as well as success) in rice quality improvement. Premium quality rice grain is the demand of a growing population with better living standards. It is the application of various genome editing tools such as ZFN, TALENs, and especially CRISPR/Cas9 which has broadened rice researches and brought in newer opportunities to develop the novel varieties which have shown not only improved productivities but also qualities. Being efficient, and easy to handle, the CRISPR/Cas9 system has all genome editing capabilities, e.g., knock-in, knockout, knockdown, and expression activation. This system has tremendous untapped potential, has formed an ever-expanding genetic toolbox for plant biologists to investigate functional genomics, and is a helping hand for breeders to integrate important genes into the genomes of important crops. The successful application of CRISPR/Cas9 for tissue engineering and human stem cell modification has led to further developments in the field of precise genome editing. The ability to target multiple genes via multiplexed genome editing strategies can facilitate pathway-level research to engineer complex multi genic rice grain quality attributes. Previously, few studies have been conducted that are related to targeted mutagenesis for rice grain quality improvement. The pathways of rice grain quality are not well understood, and they can be investigated for the genetic mechanisms controlling quality attributes. The development of novel regulatory components from naturally existing peripherals (genes, promoters, *cis*-regulatory elements, small RNAs, and epigenetic modifications) can facilitate the engineering of regulatory pathways for different elements of rice grain quality. The rapid shift of research toward the utilization of CRISPR/Cas9 systems for targeted mutagenesis could be a promising approach for overcome barriers to breeding improved quality rice. In addition, true successful crop improvement via genome editing requires the cultivation of edited crops in the field. However, most of the genome-edited crops reported to date have not yet reached the field due to biosafety and regulatory issues (Ahmad *et al*., 2021). Whether genome-edited plants should be considered as transgene free is still a matter of debate. However, there is support for the notion that these plants could be labeled as Transgene Free or non-GMO genetically edited plants (Figure 3), because technically there is no difference between plants produced through genome editing vs. conventional breeding transgene-free protocols have been established.

Authors' Contributions

A.Z. and Z.S conceived the idea. S.A., Z.S., and P.H., provided technical expertise to strengthen the basic idea. A.Z., S.A., and J.T collected the literature for this review. A.Z and S.A. wrote the manuscript. S.A. reproduced and prepared the illustrations. A.Z. and S.A streamlined the idea. S.A., J.T., Z.S., and P.H proofread and provided intellectual guidance. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Abdelrahman M, Zhao K (2020). Genome editing and rice grain quality. The Future of Rice Demand: Quality Beyond Productivity. Springer, pp 395-422.

Abe K, Araki E, Suzuki Y, Toki S, Saika H (2018). Production of higholeic/low linoleic rice by genome editing. Plant Physiology and Biochemistry 131:58-62. *https://doi.org/10.1016/j.plaphy.2018.04.033*

Ahmad S, Tang L, Shahzad R, Mawia AM, Rao GS, Jamil S, ... Tang S (2021). CRISPR-based crop improvements: A way forward to achieve zero hunger. Journal of Agricultural and Food Chemistry 69(30):8307-8323. *https://doi.org/10.1021/acs.jafc.1c02653*

Ahmad S, Shahzad R, Jamil S, Tabassum J, Chaudhary MAM, Atif RM, ... Tang S (2021). Regulatory aspects, risk assessment, and toxicity associated with RNAi and CRISPR methods. In: CRISPR and RNAi Systems. pp 687-721, Elsevier. *https://doi.org/10.1016/B978-0-12-821910-2.00013-8*

Ahmar S, Gill RA, Jung KH, Faheem Am Qasim MU, Mubeen M, Zhou W (2020). Conventional and molecular techniques from simple breeding to speed breeding in crop plants: Recent advances and future outlook. International Journal of Molecular Sciences 21(7):2590. *https://doi.org/10.3390/ijms21072590*

Ain QU, Chung JY, Kim YH (2015). Current and future delivery systems for engineered nucleases: ZFN, TALEN and RGEN. Journal of Controlled Release 205:120-127. *https://doi.org/10.1016/j.jconrel.2014.12.036*

Anzalone AV, Randolph PB, Davis JR, Sousa AA, Koblan LW, Levy JM, Chen P, Wilson C, Newby GA, Raguram A (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. Nature 576:149-157. *https://doi.org/10.1038/s41586-019-1711-4*

Ansari A, Wang C, Wang J, Wang F, Liu P, Gao Y (2017). Engineered dwarf male-sterile rice: a promising genetic tool for facilitatingrecurrent selection in rice. Frontiers in Plant Science 8:2132. *https://doi.org/10.3389/fpls.2017.02132*

Ali Z, Abul-Faraj A, Li L, Ghosh N, Piatek M, Mahjoub A, ... Mahfouz MM (2015). Efficient virus-mediated genome editing in plants using the CRISPR/Cas9 system. Molecular Plant 8:1288-1291. *https://doi.org/10.1016/j.molp.2015.02.011*

Azizi P, Osman M, Hanafi M, Sahebi M, Rafii M, Taheri S, . . . Yusuf M (2019). Molecular insights into the regulation of rice kernel elongation. Critical Reviews in Biotechnology 39(7):904-923. *https://doi.org/10.1080/07388551.2019.1632257*

Banakar R, Schubert M, Collingwood M, Vakulskas C, Eggenberger AL, Wang K (2020). Comparison of CRISPR-Cas9/Cas12a ribonucleoprotein complexes for genome editing efficiency in the rice phytoene desaturase (OsPDS) gene. Rice 13(1):1-7. *https://doi.org/10.1186/s12284-019-0365-z*

Birla DS, Malik K, Sainger M, Chaudhary D, Jaiwal R, Jaiwal PK (2017). Progress and challenges in improving the nutritional quality of rice (*Oryza sativa* L.). Critical Reviews in Food Science and Nutrition 57(11):2455-2481. *https://doi.org/10.1080/10408398.2015.1084992*

Boch J, Scholze H, Schornack S, Landgraf A, Hahn S, Kay S, Lahaye T, Nickstadt A, Bonas U (2009). Breaking the code of DNA binding specificity of TAL-type III effectors. Science 326:1509-1512. *https://doi.org.10.1126/science.1178811*

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

Butt H, Eid A, Ali Z, Atia MA, Mokhtar MM, Hassan N, ... Mahfouz MM (2017). Efficient CRISPR/Cas9-mediated genome editing using a chimericsingle-guide RNA molecule. Frontiers in Plant Science 8:1441. *https://doi.org/10.3389/fpls.2017.01441*

Butt H, Zaidi SSA, Hassan N, Mahfouz M (2020). CRISPR-based directed evolution for crop improvement. Trends in Biotechnology 38:236-240. *https://doi.org/10.1016/j.tibtech.2019.08.001*

Brooks C, Nekrasov V, Lippman ZB, Van Eck J (2014). Efficient gene editing in tomato in the *f*irst generation using the clustered regularly interspaced short palindromic repeats/CRISPR associated9 system. Plant Physiology 166:1292-1297. *https://doi.org/10.1104/pp.114.247577*

Chen X, Lu X, Shu N, Wang S, Wang J, Wang D, Guo L, Ye W (2017). Targeted mutagenesis in cotton *Gossypium hirsutum* L. using the CRISPR/Cas9 system. Scientific Reports 7:44304. *https://doi.org/10.1038/srep44304*

Calingacion M, Laborte A, Nelson A, Resurreccion A, Concepcion JC, Daygon VD, . . . Bassinello PZ (2014). Diversity of global rice markets and the science required for consumer-targeted rice breeding. PloS One 9(1):e85106. *https://doi.org/10.1371/journal.pone.0085106*

Cassandri M, Smirnov A, Novelli F, Pitolli C, Agostini M, Malewicz M, . . . Raschellà G (2017). Zinc-finger proteins in health and disease. Cell Death Discovery 3(1):17071. *https://doi.org/10.1038/cddiscovery.2017.71*

Clemens S, Aarts MG, Thomine S, Verbruggen N (2013). Plant science: the key to preventing slow cadmium poisoning. Trends in Plant Science 18:92-99. *https://doi.org/10.1016/j.tplants.2012.08.003*

Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Zhang F (2013). Multiplex genome engineering using CRISPR/Cas systems. Science 339:819-823. *https://doi.org/10.1126/science.1231143*

Cui Y, Hu X, Liang G, Feng A, Wang F, Ruan S, . . . Chen D (2020). Production of novel beneficial alleles of a rice yield-related QTL by CRISPR/Cas9. Plant Biotechnology Journal 18(10):1987. *https://doi.org/10.1111/pbi.13370*

Chen Yu, Zhu A, Xue P, Wen X, Cao Y, Wang B, Shah L, Chang S, Zhang Y (2020). Effects of GS3 and GL3.1 for grain size editing by CRISPR/Cas 9 in rice. Rice Science 27(5):405-413. *https://doi.org/10.1016/j.rsci.2019.12.010*

Durai S, Mani M, Kandavelou K, Wu J, Porteus MH, Chandrasegaran S (2005). Zinc finger nucleases: Custom-designed molecular scissors for genome engineering of plant and mammalian cells. Nucleic Acids Research 33:5978-5990. *https://doi.org/10.1093/nar/gki912*

Endo A, Saika H, Takemura M, Misawa N, Toki S (2019). Anovel approach to carotenoid accumulation in rice callus by mimickingthe cauliflower orange mutation via genome editing. Rice 12:1-5. *https://doi.org/10.1186/s12284-019-0345-3*

Feng Z, Mao Y, Xu N, Zhang B, Wei P, Yang DL, ... Zhu JK (2014). Multigeneration analysis reveals the inheritance, specificity, and patterns of CRISPR/Cas induced gene modifications in *Arabidopsis*. Proceedings of the National Academy of Sciences 111(12):4632-4637. *https://doi.org/10.1073/pnas.1400822111*

Gaj T, Gersbach CA, Barbas III CF (2013). ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends in Biotechnology 31(7):397-405.*%20https:/doi.org/10.1016/j.tibtech.2013.04.004*

Gao X, Chen J, Dai X, Zhang D, Zhao Y (2016). An effective strategyfor reliably isolating heritable and Cas9-free arabidopsis mutants generatedby CRISPR/Cas9-mediated genome editing. Plant Physiology 171:1794-1800. *https://doi.org/10.1104/pp.16.00663*

Gao F, Shen XZ, Jiang F, Wu Y, Han C (2016). DNA-guided genome editing using the *Natronobacterium gregoryi* Argonaute. Nature Biotechnology 34:768-773. *https:/doi.org/10.1038/nbt.3547.*

Gasiunas G, Barrangou R, Horvath P, Siksnys V (2012). Cas9–crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. Proceedings of the National Academy of Sciences 109(39):E2579-E2586. *https://doi.org/10.1073/pnas.1208507109*

Hua K, Tao X, Zhu JK (2019). Expanding the base editing scope in rice by using Cas9 variants. Plant Biotechnology Journal 17(2):499-504. *https://doi.org/10.1111/pbi.12993*

Hamada H, Liu Y, Nagira Y, Miki R, Taoka N, Imai R (2018). Biolisticdelivery-based transient CRISPR/Cas9 expression enables in planta genomeediting in wheat. Scientific Reports 8:14422. *https://doi.org/10.1038/s41598-018-32714-6*

Huang L, Zhang R, Huang G, Li Y, Melaku G, Zhang S, . . . Zhang Y (2018). Developing superior alleles of yield genes in rice by artificial mutagenesis using the CRISPR/Cas9 system. The Crop Journal 6(5):475-481. *https://doi.org/10.1016/j.cj.2018.05.005*

Hui S, Li H, Mawia AM, Zhou L, Cai J, Ahmad S ... Hu P (2021). Production of aromatic three-line hybrid rice using novel alleles of BADH2. Plant Biotechnology Journal 20(1):59-74. *https://doi.org/10.1111/pbi.13695*

Iaffaldano B, Zhang Y, Cornish K (2016). CRISPR/Cas9 genome editing of rubber producing dandelion *Taraxacum kok-saghyz* using *Agrobacterium rhizogenes* without selection. Industrial Crops and Products 89:356-362. *https://doi.org/10.1016/j.indcrop.2016.05.029*

Jena KK, Kim S-R (2020). Genomics, biotechnology and plant breeding for the improvement of rice production. In: Accelerated Plant Breeding. Springer, Volume 1, pp 217-232.

Jia H, Zhang Y, Orbovic V, Xu J, White F, Jones J, Wang N (2017). Genome editing of the disease susceptibility gene CsLOB1 in citrus confers resistance to citrus canker. Plant Biotechnology Journal 15:817-823. *https://doi.org/10.1111/pbi.12677*

Jia H, Wang N (2014). Targeted genome editing of sweet orange using Cas9/sgRNA. PloS One 9:e93806. *https://doi.org/10.1371/journal.pone.0093806*

Jiang W, Zhou H, Bi H, Fromm M, Yang B, Weeks DP (2013). Demonstration of CRISPR/Cas9/sgRNA-mediated targeted gene modification in Arabidopsis, tobacco, sorghum and rice. Nucleic Acids Research 41(20):e188-e188. *https://doi.org/10.1093/nar/gkt780*

Jiang H, Lio J, Blanco M, Campbell M, Jane J-l (2010). Resistant-starch formation in high-amylose maize starch during kernel development. Journal of Agricultural and Food Chemistry 58:8043-8047. *https:/doi.org/10.1021/jf101056y*

Jiao Y, Wang Y, Xue D, Wang J, Yan M, Liu G, . . . Li J (2010). Regulation of OsSPL14 by OsmiR156 defines ideal plant architecture in rice. Nature Genetics 42:541-544. *https://doi.org/10.1038/ng.591*

Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E (2012). A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. Science 337(6096):816-821. *https://doi.org/10.1126/science.1225829*

Jung YJ, Lee HJ, Bae S, Kim JH, Kim DH, Kim HK, ... Kang KK (2019). Acquisition of seed dormancy breaking in rice (*Oryza sativa* L.) via CRISPR/Cas9-targeted mutagenesis of OsVP1 gene. Plant Biotechnology Reports 13:511-520. *https://doi.org/10.1007/s11816-019-00580-x*

Kamburova VS, Nikitina EV, Shermatov SE, Buriev ZT, Kumpatla SP, Emani C, Abdurakhmonov IY (2017). Genome editing in plants: an overview of tools and applications. International Journal of Agronomy 7315351. *https://doi.org/10.1155/2017/7315351*

Kim H, Kim JS (2014). A guide to genome engineering with programmable nucleases. Nature Reviews Genetics 15:321-334. *https://doi.org/10.1038/nrg3686*

Kim YB, Komor AC, Levy JM, Packer MS, Zhao KT, Liu DR (2017). Increasing the genome-targeting scope and precision of base editing with engineered Cas9-cytidine deaminase fusions. Nature Biotechnology 35:371-376. https://doi.org.10.1038/nbt.3803.

Khanday I, Skinner D, Yang B, Mercier R, Sundaresan V (2019). A male expressed rice embryogenic trigger redirected for asexual propagation through seeds. Nature 565:91-95. *https://doi.org.10.1038/s41586-018-0785-8*

Khan Z, Khan SH, Mubarik MS, Sadia B, Ahmad A (2017). Use of TALEs and TALEN technology for genetic improvement of plants. Plant Molecular Biology Reporter 35(1):1-19. *https://doi.org/10.1007/s11105-016-0997-8*

Khush GS (2005). What it will take to feed 5.0 billion rice consumers in 2030. Plant Molecular Biology 59(1):1-6. *https://doi.org/10.1007/s11103-005-2159-5*

Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR (2016). Programmable editing of target base in genomic DNA without double-stranded DNA cleavage. Nature 533:420-424. *https://doi.org/10.1038/nature17946*

Kohli A, Miro B, Balié J, d'A Hughes J (2020). Photosynthesis research: a model to bridge fundamental science, translational products, and socio-economic considerations in agriculture. Journal of Experimental Botany 71(7):2281-2298. *https://doi.org/10.1093/jxb/eraa087*

Kopischke S, Schüßler E, Althoff F, Zachgo S (2017). TALEN-mediated genome-editing approaches in the liverwort *Marchantia polymorpha* yield high efficiencies for targeted mutagenesis. Plant Methods 13:20. *https://doi.org/10.1186/s13007-017-0167-5*

Kuang Y, Li S, Ren B, Yan F, Spetz C, Li X, . . . Zhou H (2020). Base-editing-mediated artificial evolution of OsALS1 in planta to develop novel herbicide-tolerant rice germplasms. Molecular Plants 13. *https://doi.org/10.1016/j.molp.2020.01.010*

Lacchini E, Kiegle E, Castellani M, Adam H, Jouannic S, Gregis V, Kater MM (2020). CRISPR-mediated accelerated domestication of African rice landraces. PloS One 15(3):e0229782. *https://doi.org/10.1371/journal.pone.0229782*

Li ZK, Chen B, Li XX, Wang JP, Zhang Y, Wang XF, ... Ma ZY (2019). A newly identified cluster of glutathione S-transferase genes provides *Verticillium* wilt resistance in cotton. The Plant Journal 98(2):213-227. *https://doi.org/10.1111/tpj.14206*

Li C, Zhang R, Meng X, Chen S, Zong Y, Lu C, ... Gao C (2020). Targeted,random mutagenesis of plant genes with dual cytosine and adenine base editors. Nature Biotechnology 38:875-882. *https://doi.org/10.1038/s41587-019-0393-7*

Li H, Li J, Chen J, Yan L, Xia L (2020). Precise modifications of both exogenous and endogenous genes in rice by prime editing. Molecular Plant 13(5):671-674. *https://doi.org/10.1016/j.molp.2020.03.011*

Li J, Meng X, Zong Y, Chen K, Zhang H, Liu J, . . . Gao C (2016). Gene replacements and insertions in rice by intron targeting using CRISPR-Cas9. Nature Plants 2:16139. *https://doi.org/10.1038/nplants.2016.139*

Li Z, Liu ZB, Xing A, Moon BP, Koellhoffer JP, Huang L, Ward RT, Clifton E, Falco SC, Cigan AM (2015). Cas9-guide RNA directed genome editing in soybean. Plant Physiology 169:960-970. *https://doi.org/10.1104/pp.15.00783*

Li S, Zhao B, Yuan D, Duan M, Qian Q, Tang L, . . . Li C (2013). Rice zinc finger protein DST enhances grain production through controlling Gn1a/OsCKX2 expression. Proceedings of the National Academy of Sciences 110(8):3167-3172. *https://doi.org/10.1073/pnas.1300359110*

Li JF, Norville JE, Aach J, Mccormack M, Zhang D, Bush J, Church GM, Sheen J (2013). Multiplex and homologous recombination-mediated genome editing in *Arabidopsis* and *Nicotiana benthamiana* using guide RNA and Cas9. Nature Biotechnology 31:688. *https://doi.org/10.1038/nbt.2654*

Li Y, Fan C, Xing Y, Yun P, Luo L, Yan B, . . . He Y (2014). Chalk5 encodes a vacuolar H+-translocating pyrophosphatase influencing grain chalkiness in rice. Nature Genetics 46:398-404. *https://doi.org/10.1038/ng.2923*

Li T, Liu B, Spalding MH, Weeks DP, Yang B (2012). High-efficiency TALEN-based gene editing produces disease-resistant rice. Nature Biotechnology 30:390. *https://doi.org/10.1038/nbt.2199*

Lin Q, Zong Y, Xue C, Wang S, Jin S, Zhu Z, ... Gao C (2020). Prime genome editing in rice and wheat. Nature Biotechnology 38:582-585. *https://doi.org/10.1038/s41587-020-0455-x*

Liu J, Nannas NJ, Fu F-f, Shi J, Aspinwall B, Parrott WA, Dawe RK (2019). Genome-scale sequence disruption following biolistic transformation in rice and maize. The Plant Cell 31(2):368-383. *https://doi.org/10.1105/tpc.18.00613*

Lopez-Obando M, Hoffmann B, Gery C, Guyon-Debast A, Teoule E, Rameau C, ... Nogue F (2016). Simple and e*ffi*cient targeting of multiple genes through CRISPR-Cas9 in *Physcomitrella patens*. G3: Genes, Genomes, Genetics 6:3647-3653. *https://doi.org/10.1534/g3.116.033266*

Lu Y, Zhu J-K (2017). Precise editing of a target base in the rice genome using a modified CRISPR/Cas9 system. Molecular Plant 10(3):523-525. *https://doi.org/10.1016/j.molp.2016.11.013*

Lv C, Huang Y, Sun W, Yu L, Zhu J (2020). Response of rice yield and yield components to elevated [CO_2]: a synthesis of updated data from FACE experiments. European Journal of Agronomy 112:125961. *https://doi.org/10.1016/j.eja.2019.125961*

Lawrenson T, Shorinola O, Stacey N, Li C, Ostergaard L, Patron N, Uauy C, Harwood W (2015). Induction of targeted, heritable mutations in barley and Brassica oleracea using RNA-guided Cas9 nuclease. Genome Biology 16:258. *https://doi.org/10.1186/s13059-015-0826-7*

Ma L, Zhu F, Li Z, Zhang J, Li X, Dong J, Wang T (2015). TALENbased mutagenesis of lipoxygenase LOX3 enhances the storagetolerance of rice (*Oryza sativa*) seeds. PLoS One 10:e0143877. *https://doi.org/10.1371/journal.pone.0143877*

Ma X, Zhang Q, Zhu Q, Liu W, Chen Y, Qiu R, ... Liu YG (2015). A robust CRISPR/Cas9 system for convenient, high-efficiency multiplex genome editing in monocot and dicot plants. Molecular Plant 8:1274-1284. *https://doi.org/10.1016/j.molp.2015.04.007*

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

Mao T, Zhu M, Sheng Z, Shao G, Jiao G, Mawia AM, ... Hu P (2021). Effects of grain shape genes editing on appearance quality of erect-panicle geng/japonica rice. Rice 14(1):1-7. *https://doi.org/10.1186/s12284-021-00517-5*

Miao J, Guo D, Zhang J, Huang Q, Qin G, Zhang X, . . . Qu L-J (2013). Targeted mutagenesis in rice using CRISPR-Cas system. Cell Research 23(10):1233-1236. *https://doi.org/10.1038/cr.2013.123*

Mishra R, Joshi RK, Zhao K (2018). Genome editing in rice: recent advances, challenges, and future implications. Frontiers in Plant Science 9(1361). *https://doi.org/10.3389/fpls.2018.01361*

Mishra R, Zhao K (2018). Genome editing technologies and their applications in crop improvement. Plant Biotechnology Reports 12:57-68. *https://doi.org/10.1007/s11816-018-0472-0*

Mishra R, Joshi RK, Zhao K (2020). Base editing in crops: current advances, limitations and future implications. Plant Biotechnology Journal 18(1):20-31. *https://doi.org/10.1111/pbi.13225*

Miura K, Ikeda M, Matsubara A, Song X-J, Ito M, Asano K, . . . Ashikari M (2010). OsSPL14 promotes panicle branching and higher grain productivity in rice. Nature Genetics 42(6):545-549. *https://doi.org/10.1038/ng.592*

Monsur MB, Shao G, Lv Y, Ahmad S, Wei X, Hu P, Tang S (2020). Base editing: the ever expanding clustered regularly interspaced short palindromic repeats (CRISPR) tool kit for precise genome editing in plants. Genes 11(4):466. *https://doi.org/10.3390/genes11040466*

Nekrasov V, Staskawicz B, Weigel D, Jones JD, Kamoun S (2013). Targeted mutagenesis in the model plant *Nicotiana benthamiana* using Cas9 RNA-guided endonuclease. Nature Biotechnology 31:691-693. *https://doi.org/10.1038/nbt.2655*

Nishitani C, Hirai N, Komori S, Wada M, Okada K, Osakabe K, ... Osakabe Y (2016). Efficient genome editing in apple using a CRISPR/Cas9 system. Scientific Reports 6(1):1-8. *https://doi.org/10.1038/srep31481*

Nordin Y (2008). Golden rice and other biofortified food crops for developing countries: challenges and potential. FAO, Rome, Italy.

Osakabe K, Osakabe Y, Toki S (2010). Site-directed mutagenesis in Arabidopsis using custom-designed zinc finger nucleases. Proceedings of the National Academy of Sciences 107:12034-12039. *https://doi.org/10.1073/pnas.1000234107*

Potrykus I (2008, September). Golden Rice—from idea to reality. Bertebos Prize lecture. In: Bertebos Conf., 7–9 September.

Petolino JF, Worden A, Curlee K, Connell J, Moynahan TLS, Larsen C, Russell S (2010). Zinc finger nuclease-mediated transgene deletion. Plant Molecular Biology 73:617-628. *https://doi.org/10.1111/pbi.12941*

Puchta H (2004). The repair of double-strand breaks in plants: mechanisms and consequences for genome evolution. Journal of Experimental Botany 56:1-14. *https://doi.org/10.1093/jxb/eri025*

Puchta H (2017). Applying CRISPR/Cas for genome engineering in plants: The best is yet to come. Current Opinion in Plant Biology 36:1-8. *https://doi.org/10.1016/j.pbi.2016.11.011*

Pyott DE, Sheehan E, Molnar A (2016). Engineering of CRISPR/Cas9-mediated potyvirus resistance in transgene-free *Arabidopsis* plants. Molecular Plant Pathology 17:1276-1288. *https://doi.org.10.1111/mpp.12417*

Rai KM, Ghose K, Rai A, Singh H, Srivastava R, Mendu V (2018). Genome engineering tools in plant synthetic biology. In: Current Developments in Biotechnology and Bioengineering. Elsevier, pp 47-73.

Rees HA, Liu DR (2018). Base editing: precision chemistry on the genome and transcriptome of living cells. Nature Reviews Genetics 19(12):770-788. *https://doi.org/10.1038/s41576-018-0059-1*

Regina A, Bird A, Topping D, Bowden S, Freeman J, Barsby T, ... Morell M (2006). High-amylose wheat generated by RNA interference improves indicesof large-bowel health in rats. Proceedings of the National Academy of Sciences 103:3546-3551. *https://doi.org/10.1073/pnas.0510737103*

Ren C, Liu X, Zhang Z, Wang Y, Duan W, Li S, Liang Z (2016). CRISPR/Cas9-mediated *effi*cient targeted mutagenesis in Chardonnay *Vitis vinifera* L. Scientific Reports 6:32289. *https://doi.org/10.1038/srep32289*

Riaz A, Kanwal F, Ahmad I, Ahmad S, Farooq A, Madsen CK, ... Alqudah AM (2022). New hope for genome editing in cultivated grasses: CRISPR variants and application. Frontiers in Genetics 13:866121. *https://doi.org/10.3389/fgene.2022.866121*

Romero FM, Gatica-Arias A (2019). CRISPR/Cas9: development and application in rice breeding. Rice Science 26(5):265-281. *https://doi.org/10.1016/j.rsci.2019.08.001*

Sauer NJ, Narvaez-Vasquez J, Mozoruk J, Miller RB, Warburg ZJ, Woodward MJ, ... Sanders SL (2016). Oligonucleotide mediated genome editing provides precision and function to engineered nucleases and antibiotics in plants. Plant Physiology 170:1917-1928. *https://doi.org/10.1104/pp.15.01696*

Sedeek KEM, Mahas A, Mahfouz M (2019). Plant genomeengineering for targeted improvement of crop traits. Frontiers in Plant Science 10:114. *https://doi.org/10.3389/fpls.2019.00114*

Shan Q, Zhang Y, Chen K, Zhang K, Gao C (2015). Creation of fragrant rice by targeted knockout of the Os BADH 2 gene using TALEN technology. Plant Biotechnology Journal 13:791-800. *https://doi.org/10.1111/pbi.12312*

Shan Q, Wang Y, Li J, Gao C (2014). Genome editing in rice and wheat using the CRISPR/Cas system. Nature Protocols 9(10):2395-2410. *https://doi.org/10.1038/nprot.2014.157*

Shan Q, Wang Y, Li J, Zhang Y, Chen K, Liang Z, ... Qiu JL (2013a). Targeted genome modi*fi*cation of crop plants using a CRISPR-Cas system. Nature Biotechnology 31:686-688. *https://doi.org/10.1038/nbt.2650*

Shan Q, Wang Y, Chen K, Liang Z, Li J, Zhang Y, . . . Zheng X (2013). Rapid and efficient gene modification in rice and *Brachypodium* using TALENs. Molecular Plant 6(4):1365-1368. *https://doi.org/10.1093/mp/sss162*

Shao G, Xie L, Jiao G, Wei X, Sheng Z, Tang S, ... Hu P (2017). CAS9-mediated editing of the fragrant gene Badh2 in rice. Chinese Journal of Rice Science 31:216-222. *https://doi.org/10.16819/j.1001-7216.2017.6098*

Shen L, Li J, Fu Y, Wang J, Hua Y, Jiao X, Yan C, Wang K (2017). Orientation improvement of grain lengthand grain number in rice by using CRISPR/Cas9 system. Chinese Journal of Rice Science 31:223-231.

Shukla VK, Doyon Y, Miller JC, Dekelver RC, Moehle EA, Worden SE, ... Urnov FD (2009). Precise genome modification in the crop species Zea mays using zinc-finger nucleases. Nature 459:437-441. *https://doi.org/10.1038/nature07992*

Shimatani Z, Kashojiya S, Takayama M, Terada R, Arazoe T, Ishii H, ... Kondo A (2017). Targeted base editing in rice and tomato using a CRISPR-Cas9 cytidinedeaminase fusion. Nature Biotechnology 35:441. *https://doi.org/10.1038/nbt.3833*

Sun Y, Jiao G, Liu Z, Zhang X, Li J, Guo X, ... Xia L (2017). Generation of highamylose rice through CRISPR/Cas9-mediated targeted mutagenesis of starch branching enzymes. Frontiers in Plant Science 8:298. *https://doi.org/10.3389/fpls.2017.00298*

Sugano SS, Shirakawa M, Takagi J, Matsuda Y, Shimada T, Hara-Nishimura I, Kohchi T (2014). CRISPR/ Cas9-mediated targeted mutagenesis in the liverwort *Marchantia polymorpha* L. Plant and Cell Physiology 55:475-481. *https://doi.org/10.1093/pcp/pcu014*

Svitashev S, Young JK, Schwartz C, Gao H, Falco SC, Cigan AM (2015). Targeted mutagenesis, precise gene editing and site-speci*fi*c gene insertion in maize using Cas9 and guide RNA. Plant Physiology 169:931-945. *https://doi.org.10.1104/pp.15.00793*

Stadler LJ (1928). Genetic effects of X-rays in maize. Proceedings of the National Academy of Sciences 14(1):69-75. *https://doi.org/10.1073/pnas.14.1.69*

Tabassum J, Ahmad S, Hussain B, Mawia AM, Zeb A, Ju L (2021). Applications and potential of genome-editing systems in rice improvement: current and future perspectives. Agronomy 11(7):1359. *https://doi.org/10.3390/agronomy11071359*

Tang X, Sretenovic S, Ren Q, Jia X, Li M, Fan T, . . . Liu L (2020). Plant prime editors enable precise gene editing in rice cells. Molecular Plant 13(5):667-670. *https://doi.org/10.1016/j.molp.2020.03.010*

Tang L, Mao B, Li Y, Lv Q, Zhang L, Chen C, ... Zhao B (2017). Knockout of OsNramp5 using the CRISPR/Cas9 system produces low Cd accumulating indica rice without compromising yield. Scientific Reports 7:14438. *https://doi.org/10.1038/s41598-017-14832-9*

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

Townson J (2017). Recent developments in genome editing for potential use in plants. Bioscience Horizons: The International Journal of Student Research 10. *https://doi.org/10.1093/biohorizons/hzx016*

Townsend JA, Wright DA, Winfrey RJ, Fu F, Maeder ML, Joung JK, Voytas DF (2009). High-frequency modification of plant genes using engineered zinc-finger nucleases. Nature 459:442-445. *https://doi.org/10.1038/nature07845*

Urnov FD, Rebar E J, Holmes MC, Zhang HS, Gregory PD (2010). Genome editing with engineered zinc finger nucleases. Nature Reviews Genetics 11(9):636-646. https://doi.org/10.1038/nrg2842

Wang S, Wu K, Yuan Q, Liu X, Liu Z, Lin X, . . . Qian Q (2012). Control of grain size, shape and quality by OsSPL16 in rice. Nature Genetics 44(8):950. *https://doi.org/10.1038/ng.2327*

Wang C, Liu Q, Shen Y, Hua Y, Wang J, Lin J, ... Wang K (2019). Clonal seeds from hybrid rice by simultaneous genome engineering of meiosis and fertilization genes. Nature Biotechnology 37:283-286. *https://doi.org/10.1038/s41587-018-0003-0*

Wang W, Hu B, Yuan D, Liu Y, Che R, Hu Y, . . . Chu C (2018). Expression of the nitrate transporter gene OsNRT1.1A/OsNPF6.3 confers high yield and early maturation in rice. The Plant Cell 30(3):638-651. *https://doi.org.10.1105/tpc.17.00809*

Wang M, Wang S, Liang Z, Shi W, Gao C, Xia G (2017b). From genetic stock to genome editing: gene exploitation in wheat. Trends in Biotechnology 36:160-172. *https://doi.org/10.1016/j.tibtech.2017.10.002*

Wang L, Wang L, Tan Q, Fan Q, Zhu H, Hong Z, Zhang Z, Duanmu D (2016b). Efficient inactivation of symbiotic nitrogen fixation related genes in *Lotus japonicus* using CRISPR-Cas9. Frontiers in Plant Science 7:1333. *https://doi.org/10.3389/fpls.2016.01333*

Waltz E (2016). CRISPR-edited crops free to enter market, skip regulation. Nature Biotechnology 34:582. *https://doi.org/10.1038/nbt0616-582*

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

Wang S, Zhang S, Wang W, Xiong X, Meng F, Cui X (2015b). Efficient targeted mutagenesis in potato by the CRISPR/Cas9 system. Plant Cell Reports 34:1473-1476. *https://doi.org/10.1007/s00299-015-1816-7*

Wenefrida I, Utomo HS, Blanche SB, Linscombe SD (2009). Enhancing essential amino acids and health benefit components in grain crops for improved nutritional values. Recent Patents on DNA & Gene Sequences 3:219-225. *https://doi.org/10.2174/187221509789318405*

Xu R, Li J, Liu X, Shan T, Qin R, Wei P (2020). Development of a plant prime editing system for precise editing in the rice genome. Plant Communications 1(3):100043. *https://doi.org/10.1016/j.xplc.2020.100043*

Xu R, Yang Y, Qin R, Li H, Qiu C, Li L, . . . Yang J (2016). Rapid improvement of grain weight via highly efficient CRISPR/Cas9-mediated multiplex genome editing in rice. Journal of Genetics and Genomics 43(8):529-532. *https://doi.org/10.1016/j.jgg.2016.07.003*

Xu R-F, Li H, Qin R-Y, Li J, Qiu C-H, Yang Y-C, ... Yang JB (2015). Generation of inheritable and "transgene clean" targeted genome-modifiedrice in later generations using the CRISPR/Cas9 system. Scientific Reports 5:11491. *https://doi.org.10.1038/srep11491*

Xu Y, Wang F, Chen Z, Wang J, Li W, Fan F, . . . Yang J (2020). CRISPR/Cas9-targeted mutagenesis of the OsROS1 gene induces pollen and embryo sac defects in rice. Plant Biotechnology Journal 18(10):1999. *https://doi.org/10.1111/pbi.13388*

Xu W, Zhang C, Yang Y, Zhao S, Kang G, He X, ... Yang J (2020). Versatile nucleotides substitution in plant using an improved prime editing system. Molecular Plant 13:675-678. *https://doi.org/10.1016/j.molp.2020.03.012*

Zafra MP, Schatoff EM, Katti A, Foronda M, Breinig M, Schweitzer AY, ... Dow LE (2018). Optimized base editors enable efficient editing in cells, organoids and mice. Nature Biotechnology 36:888-893. *https://doi.org/10.1038/nbt.4194*

Zaidi SS, Mukhtar MS, Mansoor S (2018). Genome editing: targeting susceptibility genes for plant disease resistance. Trends in Biotechnology 36:898-906. *https://doi.org/10.1016/j.tibtech.2018.04.005*

Zaidi SS, Mansoor S (2017). Viral vectors for plant genome engineering. Frontiers in Plant Science 8:539. *https://doi.org/10.3389/fpls.2017.00539*

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

Zong Y, Wang Y, Li C, Zhang R, Chen K, Ran Y, ... Gao C (2017). Precise base editing in rice, wheat and maize with a Cas9-cytidine deaminase fusion. Nature Biotechnology 35:438-440. *https://doi.org/10.1038/nbt.3811*

Zhang B, Yang X, Yang C, Li M, Guo Y (2016a). Exploiting the CRISPR/Cas9 system for targeted genome mutagenesis in *Petunia*. Scientific Reports 6:20315. *https://doi.org/10.1038/srep20315*

Zhang Y, Liang Z, Zong Y, Wang Y, Liu J, Chen K, ... Gao C (2016). Efficient and transgene-free genome editing in wheat through transient expression of CRISPR/Cas9 DNA or RNA. Nature Communications 7:12617. *https://doi.org/10.1038/ncomms12617*

Zhang H, Zhang J, Lang Z, Botella JR, Zhu JK (2017). Genome editing—principles and applications for functional genomics research and crop improvement. Critical Review in Plant Science 36:291-309. *https://doi.org/10.1080/07352689.2017.1402989*

Zhang F, Maeder ML, Unger-Wallaced E, Hoshaw JP, Reyon D, Christian M, ... (2010). High frequency targeted mutagenesis in *Arabidopsis thaliana* using zinc finger nucleases. roceedings of the National Academy of Sciences 107:12028-12033. *https://doi.org/10.1073/pnas.0914991107*

Zhou H, He M, Li J, Chen L, Huang Z, Zheng S, . . . Zhao B (2016). Development of commercial thermo-sensitive genic male sterile rice accelerates hybrid rice breeding using the CRISPR/Cas9-mediated TMS5 editing system. Scientific Reports 6(1):1-12. https://doi.org/10.1038/srep37395

Zhang Y, Pribil M, Palmgren M, Gao C (2020). A CRISPR way for accelerating improvement of food crops. Nature Food 1:200-205. *https://doi.org/10.1038/s43016-020-0051-8*