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Review Article

CRISPR/Cas9 genome editing tool for rice crop improvement

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Food crop yield, quality, and tolerance mechanisms to biotic and abiotic factors are important aspects that contribute to food security. To feed about 10 billion people by 2050, high yielding climate-resilient rice cultivars with good grain quality must be created more quickly. Yield and quality, along with stress tolerance traits of the rice crop, have been improved by adopting various methods. Among these, in recent years, the yield of the crop has been improved marginally by utilizing conventional breeding methods. Mutation breeding is an important pathway that has created many novel variations and contributed towards isolating new high yielding genotypes in the rice crop. Forward and reverse genetic protocols have been engaged for the identification of genomic variants in conventional mutation breeding to characterize the novel variants to convert as functional markers for the development of new improved varieties. Generation of desired mutations in the desirable region of the genome of the crops is highly tedious through conventional breeding methods such as random mutagenesis since the gene manipulations happen randomly while the mutagenesis is done using physical and chemical mutagens. Also, it requires large mutant plant populations to isolate the desired mutants and mutations. The advancement of CRISPR/Cas9 genome editing technology rapidly replaces conventional random mutagenesis technologies, has the ability to multiplex genome editing to create novel variations for crop improvement programs, and reduces the time duration required for trait based crop improvement programs. In this review, significant gene manipulations employed through CRISPR/Cas9 for rice crop improvement in terms of yield and biotic and abiotic stress tolerance are discussed.

Key words: *CRISPR/Cas9, crop improvement, mutagenesis, mutations, genome editing, rice*

Introduction

Currently, the rice crop feeds and ensures food security for nearly half of the world's population. According to the FAO (Romero & Gatica-Arias, 2019), the world's projected population of ten billion will necessitate a 74% increase in food demand by 2050 due to the alarming population incremental phenomenon. More specifically, a 40% increase in rice consumption is anticipated (Milovanovic & Smutka, 2017). Even though there are many high-yielding varieties and hybrids of rice available right now, there is still a need to develop high-yielding rice varieties that can adapt to changing environments (Clarke and Zhang, 2013) in order to feed the world's growing population. Over the past few years, numerous conventional methods have been employed to increase rice yield (Khush, 2003; Brennan & Malabayabas, 2013; Peng et al., 2008; Denardin et al., 2019) in the face of the strain (Blum, 2009; Seo et al., 2020; Tripathi et al., 2012; Mackill et al., 2010). Genetic engineering methodologies are also used to improve the grain quality of rice crops (Ryoo et al., 2007; Zhang et al., 2013) along with salt stress (Yue et al., 2020) and drought (Li et al., 2021) tolerance. Conventional crop improvement methods such as in random mutagenesis, different types of physical and chemical mutagens are utilized to create new variation in the genome of crop plants. The identification of desired genotypes contributes to crop improvement after the creation of the mutant population. The maintenance

screening, and development of a random mutagenized population are extremely time-consuming and laborious. While attempting to improve related traits in crops through specific genome mutations, sometimes fail to achieve the desired genotypes.

The development of technology for manipulating genes, the combination of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and the CRISPR-associated protein Cas9 is an excellent tool which is capable for generating novel genomic variations in targeted regions (Zafar et al., 2020) and also it is comparatively superior than TALENs (Zafar et al., 2020). CRISPR/Cas9 technology rapidly replaces random mutagenesis crop improvement programs and is extremely useful for multiplex genome editing to create novel variations. CRISPR/Cas9 technology facilitates trait-based crop development by causing double strand breaks in the DNA sequence in the targeted location. In terms of crop improvement, CRISPR/Cas9 is the unavoidable tool. In particular, the application of this technology to rice (*Oryza sativa*) will result in the creation of novel genotypes that will improve yield and biotic and abiotic stress tolerance. Since rice serves as a model plant for functional genomic analysis, the utilization of CRISPR/Cas9 paves the way for the enhancement of related traits in other cereal crops. Enhanced specificity of CRISPR/Cas9 on the genome editing in the DNA sequence is being utilized for the identification of the function of the genes and molecular breeding strategies. This technology requires very short time to create the new variations to develop new cultivars and improve the existing cultivars with improved performance in terms of yield and other related traits for the efficient crop production.

Yield improvement

Grain yield is a complex trait yet an essential and inevitable strives of rice crop improvement programmes. Yielding ability of the rice is controlled by polygenes and majorly contributed by grain numbers/panicle, panicle number/plant and 1000 grain weight (Xing & Zhang, 2010). These days, rice assortments utilizing genome-altering innovations contain new genotypes of yield-related qualities. Shan et al., 2014 reported the potential of CRISPR/Cas9 framework to increase the grain yield of the rice crop. CRISPR/Cas9 is used to modify the genes viz., *Gn1a*, *DEP1*, *GS3*, and *IPA1* which are regulatory genes for the traits, the architecture of the plant and panicle, number of grain per panicle and size of the grains. Various research undertakings have been made to additionally foster grain yield using CRISPR-Cas9 (Sedeek et al., 2019). Grain size is one of the most important parameters influencing rice grain quality and yield potential. It has sparked considerable interest among molecular biologists and breeders for yield improvement (Wang et al., 2015). Wang et al. (2022) targeted miR396 site present in the CS2 gene which regulates a few essential traits such as seed shattering, grain size, grain quality and nitrogen use efficiency and abiotic stress response and resulted in a gain of function in the $GS2^{E}$ mutant. The mutant showed multiple beneficial trait expressions on grain size and yield; also, the thousand grain weight was increased by 23.5%, and consequently, the yield was increased by 10.4%.

Biotic and abiotic stress

Targeting regulators and genes which are responsible for the biotic and abiotic resistance or tolerances in the crop plants for the modification or to generate the allelic forms could be a best way for the development of the biotic and abiotic resistance or tolerances plants. Since rice genomes are available with excellent sequence quality increases the possibility of selection and modification of the specific genes.

Table 1. Recent applications of CRISPR/Cas9 technology on biotic stress tolerance enhancement in rice

Diseases	Targeted gene	Citation
Blast	*OsDjA2* and *OsERF104*	Távora et al., 2022
Blast	*Bsr-d1*, *Pi21* and *ERF922*	Zhou et al., 2022
Bacterial blight	*Os8N3*	Kim et al., 2019
Bacterial blight	*EBEs* (Promotor regions of *OsSWEET14*)	Zafar et al., 2020
Bacterial blight	*OsSWEET14*	Zeng et al., 2020

Currently, CRISPR/Cas9 genome editing technology hastens crop development programmes regarding biotic and abiotic stress resistance or tolerances genotypes (Table 1). The main challenges for crop plants during both vegetative and reproductive stages are biotic and abiotic stress to express maximum productivity. Blast is the most adverse

disease in rice crop caused by *Pyricularia oryzae* (syn. *Magnaporthe oryzae*) one and reducing crop yield worldwide (Jain et al. 2017). Cultivation of rice varieties with multiple key resistant genes for blast disease is the most commonly used method to manage this disease.

Cultivation of rice resistant cultivars with single or multiple key resistance (R) genes is the most commonly used and most environmentally friendly approach to control *P. oryzae* infection. Távora et al., 2022 knocked out *OsDjA2* and *OsERF104* genes and explained about the level of disease resistance in the mutant Nipponbare rice plants which showed reduced number of blast lesions and reduced percentage of diseased leaf area compared to control plants. A *indica* TGMS line (LK638S) used for the generation of mutations in the genes *Bsr-d1*, *Pi21* and *ERF922* and created single as well as triple mutants through CRISPR/Cas9 (Zhou et al., 2022). The results of this experiments revealed that *ERF922* mutants possessed strongest resistance nature against blast disease. The gene *Os8N3* is otherwise known as *OsSWEET14*, which is functioning as sugar transporter. Kim et al. (2019) targeted EBEs of *Os8N3* for knocked out this gene and conferred the bacterial leaf blight disease resistance in the Kitaake (*Oryza sativa* L. ssp. *Japonica*) cultivar. In Basmati varieties, bacterial blight caused by *Xanthomonas oryzae* pv. *Oryzae* led to considerable yield losses. Zafar et al., 2020 targeted the four EBSs which are residing in the promotor region of *OsSWEET14* genes for the CRISPR Cas9 editing and created TALEs (*AvrXa7*, *PthXo3*, and *TalF*). The deletions in the *EBE* of *AvrXa7* revealed the resistance against locally prevailing *Xoo* strains. Zeng et al. (2020) utilized CRISPR/Cas9 and created different mutant allelic natures of *OsSWEET14* genes in the rice cv. Zhonghua 11 (*CR-S14*) and conferred broad spectrum disease resistance against Asian and African *Xoo* strains. At the same time, the yielding potential of the genotypes are not disturbed due to the disruption in the *OsSWEET14*, but the plant height was increased.

Abiotic stress-tolerant rice cultivars must be developed if rice production is to be sustained in the face of rising salt-affected areas, diminishing freshwater supplies, and climate change. EMS mutants and transgenics have been used to validate the function of several rice genes. Frequently, a large number of these positive alleles are not accessible to rice which must be essentially developed. It will be used for introgression breeding to resolve the abiotic stress susceptibility of improved rice cultivars. Salinity is one of the most significant abiotic stresses affecting rice production worldwide. Cultivating salinity-tolerant cultivars is the most environmentally friendly and cost-effective method for managing salinity. For target-site genome editing, CRISPR/Cas9 systems have become increasingly popular in recent years to develop the salinity tolerance crop varieties. CRISPR/Cas9 employed targeted mutagenesis in the *OsRR22* was improved the salinity tolerance in the japonica rice cultivar WPB106 (Zhang et al., 2019). Kim et al. (2023) paved the way of generating targeted mutagenesis in the gene OsPUB7 through CRISPR/Cas9 to develop drought tolerance and abiotic stress rice in the future. Alam et al. (2022) reported that knocking out the *OsbHLH024* transcription factor conferred salinity tolerance. A deletion mutation in mutant A91 caused an increased level of expression in the genes *OsHKT1, OsHKT3, OsHAK7*, and *OsSOS1* under salt stress.

Using CRISPR-Cas9 gene editing in *indica* rice cv. MTU1010, generated mutant alleles of the drought and salt tolerance (*DST*) gene (Santosh Kumar et al., 2020). Two distinct gRNAs have been utilized to generate *DST* mutant alleles. The function of the DST protein might be affected the protein–protein interaction functions. Loss of function mutation in the *DST* mutants revealed decreased stomatal density which is caused by the downregulation of the stomatal development genes *SPCH1, MUTE*, and *ICE1*. The mutants exhibited moderate osmotic stress tolerance and elevated salt stress tolerance. Through improving the morphology and rolling trait in the leaf of the rice will help to get sustainable crop yield under the water deficit conditions. Mutation created in the *Semi-rolled leaf1,2* (*SRL1* and *SRL2*) genes generated the leaf rolling traits during the water deficit stress in the plants(Liao et al., 2019). The results revealed that the chlorophyll content, transpiration rate, stomatal conductance, vascular bundles (VB), stomatal number, and agronomic traits of homozygous mutants were all reduced, while the number of panicles and bulliform cells (BCs) were increased. The hybridization of mutant and its restorer showed a phenotype of semi-rolled leaves, more panicles, more grains per panicle, and more yield per plant.

Zeng et al. (2020) utilized the CRISPR/Cas9 targeted mutagenesis in the genes *OsPIN5b* (panicle length), *GS3* (grain size) and *OsMYB30* (cold tolerance). This experiment reported the improved yielding ability of the rice crop along with improved tolerance to cold stress. Park et al., 2022 adopted CRISPR/Cas9 to edit the *Oryza sativa Senescence-associated protein* (*OsSAP*) and it is otherwise called as drought induced genes and concluded that it is an efficient and novel tool to generate novel variation in the genes in the targeted region in rice crop. CRISPR/Cas9 mediated

afp1 mutants were analysed (Tianshun et al., 2021) and the results showed that Plant height and seed setting rate were lower in *afp1* mutants under normal conditions, but number of tillers/plant and length of the panicle were significantly increased. Interestingly, single plant yield of the mutant plant was showed significant variations. The *afp1* mutants had a lower ABA sensitivity and decreased water loss rate while compared with parent plant. The resistance to heat, drought, and osmotic stress were significantly increased.

Conclusion

Though many conventional crop breeding methodologies contribute to food security, increasing crop productivity is an important issue to address in the given scenario of increasing population and climatic fluctuations. A rapid method is needed to study the gene functions and generation of new variations in crop plants to develop new cultivars with improved yields as well as improved biotic and abiotic stress tolerances. The advent of genome editing tools, especially CRISPR/Cas, hastens the speed of the aforementioned objectives. It helps to edit the targeted genomic regions for the trait specific crop improvement program in a short time. From a scientific perspective, the label-free transgenic mutants and varieties produced by CRISPR/Cas are identical to those produced by natural mutation or conventional mutagenesis. Hence, the CRISPR/Cas technology could be exploited further for the development of new and improved varieties with the adaptation of unpredictable climatic changes to face the food security crisis in the future.

Author contributions

SG: Conceptualized and developed this manuscript. BRR, AS & DA: Edited this manuscript and fine-tuned it.

Competing interests

The authors have declared that no conflict of interest exists.

Ethics approval

Not applicable

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