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CRISPR-CAS MEDIATED CROP IMPROVEMENT IN RICE: A REVIEW

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ABSTRACT

Rice is the staple food crop that is grown across the globe due to its resilience to climatic conditions. Cereals are the predominant food grains consumed by the majority of the population across the globe, with rice alone feeding 50% of the global populace. In order to feed the geometrically rising population, there is a need for a dramatic upsurge in rice production levels. But climate change is one of the causes resulting in the rising of various biotic and abiotic stresses. The advancement of science had resulted in the development of Genome editing technologies like clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein 9 (Cas9), transcription activator-like effectors nucleases (TALENs), and zinc-finger nucleases (ZFNs). Among these techniques, due to ease of application, and high precision crisper-cas technology had emerged as a solution to overcome various obstacles in the journey of enhancing production levels of rice. In this, we will review various improvements made in the rice crop using crisper-cas which ultimately led to enhanced production.

Keywords **:** Adaptability, Genome editing, Biotic stress, and Abiotic stress.

Introduction

The limited population and the large area under cultivation during the early 90s had met then food demands. The pace of the rising population picked up during the mid-90s which led to a severe food crisis. The green revolution led agriculture dealt with the problem of achieving self-sufficiency in food grains. This achievement was the result of the high fertilizer responsiveness and high-yielding nature of the varieties developed. Among the several crops, rice is the one that provides the required number of calories and nutrition to more than half of the population. The availability of the complete sequence of the rice genome had changed the direction of the research from conventional to molecular-based breeding approaches. In support of the conventional approaches, the use of molecular approaches like Marker-assisted selection, QTL mapping, association mapping and genomic selection had begun. The molecular tools supplemented conventional breeding approaches had unveiled many undiscovered avenues in the field of rice research. As these are limited to the exploration of existing genetic variation, the crop improvement achieved through them has almost reached the plateau stage. In order to create the novel genetic variation in the gene pool earlier mutation breeding has been used which made it the ultimate source of variation. The lack of precision in general mutation breeding enhanced the need for a novel breeding approach that can modify or create variation with high precision. During this dark hour, Genome editing had been identified as the approach through which the genes can be modified in the native organism itself. This can be carried out by using various techniques like TALENS, Zinc finger nuclease (ZFNS), Clustered regularly interspaced palindromic sequences (CRISPR) and advancements like base and prime editing. Among the three approaches, the factors like ease of application and high precision of CRISPR enhanced its practical utility and application. Based on the various milestones achieved using this technology, it had been the ray of light, that can direct the future progress of agriculture on the path to success.

Genome Editing

It is the novel approach by which the existing genes are subjected to the targeted mutagenesis by which the desirable traits can be expressed in the genotypes of interest. The non-deployment of genes from foreign organisms is the major aspect of this approach that made it unique from transgenic. The process of gene editing entirely relied upon the functioning of the Site-directed nucleases (SDN'S) which create addition, deletion or substitution of nucleotides resulting in the targeted mutations resulting in the development of desirable traits. Based on the function of the SDN they had been classified into three types SDN1, SDN2 and SDN3. In the case of SDN1, once the double-stranded breaks are introduced in the DNA, the non-homologous end joining repair pathway will be initiated. In this particular pathway, as there is no role of the template DNA, the mutations resulting are random. But in the case of SDN2, the repair pathway is a homology-directed repair pathway (HDR) which will result in target-specific mutations. The activity of the SDN3 is entirely different from the above two in which the foreign genetic material is used as a template during the repair resulting in the introduction of foreign genetic material (EFSA GMO Panel, 2012a, Podevin *et al*., 2013). Based on

the above information, SDN1 and SDN2-based gene-edited plants do not contain foreign genetic material, but the SDN3 plants contain foreign genes. The SDN2-based functioning of the Crispr-cas application is one of the reasons for its high precision.

Functional Aspects of Crispr-cas

The term CRISPR was first coined in the year 2002; (Jansen *et al*., 2002) refers to tandem repeats flanked by non-repetitive DNA stretches that were first observed downstream of *Escherichia coliiap* (alkaline phosphatase isozyme) genes (Ishino *et al*., 1987). The importance of this technology in genome editing has been first demonstrated in the year 2012 in mammalian cells (Jinek *et al*., 2012). It is the natural defence existing in the bacteria and archaea, which prevents them from invasion of foreign organisms. When the host organism is invaded by foreign organisms, its DNA is made into fragments by Cas proteins and the fragments will be integrated into the crisper as new spacers. Once the same foreign organism attacks again, it will be recognized quickly with the help of crRNA, and paring will take place between them. This paring will guide the Cas protein to cleave the target sequences of foreign in advance which will result in the early protection of the host (Takahashi *et al.,* 1955).

The various requirements of CRISPR technology to carry out the cleavage process are (i) a gRNA which is a synthetic oligonucleotide sequence of 20 nucleotides that bind to the target DNA and (ii) a Cas9 nuclease enzyme that cleaves 3–4 bases after the protospacer adjacent motif (PAM; generally 5′ NGG; Jinek *et al*., 2012). The Cas9 nuclease is composed of two domains, (a) RuvC-like domains and (b) an HNH domain, with each domain cutting one DNA strand. Based on the number of protein subunits along with crRNA in the ribonucleoprotein (RNP) complex the CRISPR-Cas system is classified into two classes i.e., class I and class II, which are further classified into three types each. Type I, III, and IV in class I and type II, V, and VI in class II (Makarova *et al*., 2015). The class I system contains multiple protein subunits along with crRNA, while the class II system contains only one protein and crRNA to target the invading viral RNAs (Shmakov *et al*., 2015). The Cas9 protein in the type II CRISPR-Cas system processes the pre-crRNA with the help of tracer RNA and RNase III, while the Cas12 and Cas13 proteins of type V and VI systems, respectively, process the pre-crRNA themselves (Deltcheva *et al*., 2011; Dong *et al*., 2016; Dong *et al*., 2017).

Role of Crispr-cas in Crop Improvement

Once the potential of Crispr-cas had been revealed, it is used for the improvement of various traits for creating novel variations for non-available traits which are desirable. The overall achievements of the CRISPR-cas approach in crop improvement of rice had been classified under different sections like an improvement in yield, improvement in biotic and abiotic stress tolerance and improvement in quality and nutritional traits. Apart from these, there are some other achievements.

Improvement for Yield

The yield is the major objective of any breeding program which will ultimately benefit the farmer. There is a need for an enormous rise in yield levels of rice to meet the future needs of the growing population. The yield-related genes like *Gn1a* (gene for grain number), *DEP1* (DENSE AND ERECT PANICLE1; controls panicle architecture), *GS3* (regulates grain size), and *IPA1* (gene for plant architecture) are targeted for mutation using CRISPR-CAS and mutants developed were featured with enhanced grain number, dense erect panicles, and larger grain size, respectively leading to higher yields. The mutant alleles of Gn1a and DEP1 were developed using CRISPR-Cas9-based genome editing and those resulted in higher yields in comparison to natural high-yield alleles (Huang *et al*., 2018). The CRISPR/Cas9 system was used for targeting the miR396 recognition site of the rice *GS2* gene, which synthesizes the growth-regulating factor 4 *(OsGRF4)* and regulates multiple agronomic traits including grain size, grain quality, nitrogen use efficiency, abiotic stress response, and seed shattering. mutant named GS2E within the miR396-targeted sequence was identified which showed increased expression of *GS2*(Wang *et al*., 2022)

Improvement for Biotic stress Resistance

The yield level of rice damaged by biotic factors is around 52% of global production. Around 31% of this damage is caused due to losses resulting from the infection of diseases like bacterial blight (caused by *Xanthomonas oryzae*), blast (caused by *Magnaporthe grisea*), sheath blight (caused by *Rhizoctonia solani*), and tungro disease (tungro bacilliform virus and tungro spherical virus) (Park *et al*., 2008). Apart from this, the unpredictable climatic changes are leading to the development of new virulent races.

BLAST

Among several diseases caused by rice, the first and the foremost is the Rice blast. It is caused by the fungal pathogen *Magnoporthe grisea*is a destructive disease, causing 10-30 yield losses annually which was around 157 million tonnes across the globe. In order to develop resistance to blast, CRISPR- CAS was used to install mutations in the genes *Bsr-d1*, *Pi21* and *ERF922.* The developed single or triple mutants showed enhanced resistance to blast (Zhou *et al*., 2022). Similarly, the two exons of the OsSEC3A gene were targeted using CRISPR-cas with two sgRNAs and the edited rice plants resulted are with enhanced immunological responses to blast disease.

Bacterial Leaf Blight

Next to the blast, this is a prominent disease that is caused by the pathogen *Xanthomonas oryzae* pv. *Oryzae* is causing a 10-20% reduction in the annual yield losses (Zhang *et al*., 2013). The site-specific mutations were generated in the UPT box using CRISPR/Cas12a technology which hampered the protein binding of transcription-activator-like effectors (TAL) and gene activation. The genome-edited rice developed with improved bacterial blight resistance (Yu *et al*., 2021). The regulatory role of the OsPFT1 gene in rice sheath blight disease development is validated by the CRISPR-Cas9 gene editing tool by developing the OsPFT1 gene knockout. The mutants developed are characterized by resistance to sheath blight (Shah *et al*., 2019)

Improvement for abiotic stress resistance

Drought stress

Among the several abiotic stressors, it is one of the serious limiting factors impacting rice production. It is observed that more than one-third of the global cultivated

area is affected by drought stress (Rijsberman, 2006). Plants show several responses when exposed to drought situation among which leaf rolling is one of the immediate responses observed which reduces the impact of drought by decreasing water loss through transpiration. Crispr-cas was used to produce rolled leaf mutants by targeting *Semi-rolled leaf1, 2* (*SRL1* and *SRL2*) genes which showed drought tolerance (Liao, 2019).

Heat stress

In addition to the drought, the Heat stress resulting from the uncontrolled climatic changes is questioning global food security by having a large impact on rice production levels. The knockouts of the gene *OsNAC006* were developed using the CRISPR-Cas9 system resulting in heat sensitivity in rice. It elucidated the prominent role of *OsNAC006* in heat stress responses which can be exploited in further crop improvement (Wang *et al*., 2020).

Salt stress

Next to the drought and heat stress, Salt stress is one of the major global problems, which had an impact on 20% of the global cultivable land which is about to increase with climate vagaries (Munns, 2005). Earlier it has been reported that the salt tolerance increased dramatically when the OsRR22 gene, encoded 696–amino acid B-type response regulator transcription factor is knocked out (Takagi *et al*., 2015). This identified that is a potential target for crisper in order to enhance salt tolerance (Zhang *et al*., 2019). Similarly, in the process of identifying the role of OsbHLH024 in salinity tolerance of rice, the Crispr-cas approach was employed for generating the base deletion of adenine (A), nucleotide generating osbhlh024 mutant (A91).The exposure of this particular mutant to salt conditions showed a salinity tolerant phenotype (Alam *et al*., 2022). These improvements in rice in terms of salinity tolerance will bring the uncultivable land into cultivation which will add a shoulder to the wheel of enhancing global rice production.

Cold stress:

It is the major obstacle to rice cultivation in tropical and subtropical zones (Van Nguyen, 2006). The impact of the cold stress was very high in the early developmental stages of plant growth. Crispr-cas was used to target the gene *OsPRP1* and the knockout mutants generated were sensitive to cold tolerance. This confirmed the role of the *OsPRP1*gene in enhancing cold tolerance which is a future area of thrust while breeding for cold tolerance (Nawaz *et al*., 2019).

Crop improvement for herbicide tolerance

The scarcity of human resources and high cost of manual labour had limited the option of manual weeding, which enhanced the usage of herbicides. The minute errors in usage of non-selective herbicides are resulting in the damage of the crop. This recognised the requirement of the selective herbicides. The CRISPR/Cas9 based manipulation of OsPUT1/2/3 greatly conferred paraquat resistance in rice without obvious yield penalty (Lyu *et al*., 2022). The Crispr-cas installed mutation in the ALS gene disabled the interaction between the ALS protein and IMI herbicides which developed tolerance to IMI herbicides (McCourt, 2006, Lonhienne, 2018). The CRISPR/Cas9 was employed to modify 5-enolpyruvylshikimate-3- phosphate synthase (EPSPS) which resulted in two amino acid substitutions (T102I and P106S). This mutation conferred resistance to the herbicide glyphosate (Li *et al*., 2016).

Improvement for Quality and Nutritional

Micronutrients

Malnutrition is one of the major factors which is impeding not only the progress of building a healthy society but also the economic development. Mostly, the malnutrition i.e., lack of nutrient availability is observed in case of micronutrients, which reminded the need for enhancing them in the food grains. In this process, it was identified that *GRAIN WIDTH and WEIGHT2* (*GW2*) gene encodes a RING-type E3 ubiquitin ligase controls the grain weight in cereal crops. The endosperm of CRISPR/Cas9 based *GW2* knockout (KO) mutants in Indica (var. MTU1010) displayed a thick aleurone layer with enhanced grain protein content. The accumulation of essential dietary minerals (Fe, Zn, K, P, Ca) in the endosperm of rice grain is an additional benefit derived from the loss of function of *OsGW2* (Achary *et al*., 2021).

Resistant Starch

Amylose and Amylopectin are two components of the starch. The presence of high amounts of un branched amylopectin results in formation of resistant starch which is beneficial for human health. It releases the glucose in sequential manner inhibiting rapid postprandial glucose responses (Raben *et al*., 1994). The SbeII mutants developed by using Crispr-cas showed higher proportion of long chains producing debranched amylopectin. It resulted in noteworthy increase in amylose and resistant starch contents to as higher as 25.0 and 9.8%, respectively (Sun *et al*., 2017). By this modification the problem of diabetics can be managed to certain extent (Wilcox 2005; Wang *et al*., 2019).

Scented Rice

The quality of aroma is an additional superior quality of the rice grain which fetches superior price to the product. So, there is need to incorporate the trait of aroma in rice. The Betaine aldehyde dehydrogenase 2 (*OsBADH2*) is the gene regulating the biosynthesis of 2-acetyl-1-pyrroline (2-AP) involved in regulating the aroma in fragrant rice. Under the genetic background of the japonica Ningjing 1 (NJ1) and indica Huang Huazhan (HHZ) varieties, the new alleles of *BADH2*(*nj1-cr* ^{BADH2}*-1*, *nj1-cr* ^{BADH2}*-2*, *hhz-cr* ^{BADH2}*-1* and *hhz-cr* ^{BADH2}*-2*) were created by CRISPR/Cas9 gene editing technology. This enhanced the 2-AP content compared with wild-type, which led to the development of moderate fragrance (Hui *et al*., 2022).

Rice Bran

It is one of the by-products of the rice obtained after milling. It is rich in dietary fibre and had various applications in food industry and other value-added products. The balance of Oleic acid and linoleic acid is one of the factor which had impact on its utility. So, in order to improve its practical utility. The (*FAD2*) gene synthesized fatty acid desaturase enzyme, which regulates the balance of Oleic acid and linoleic acid in rice grains. Among the three functional *FAD2* genes, the Crispr-cas-led disruption of *OsFAD2-1* resulted ina double-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).

Low Cadmium Rice

The levels of arsenic and cadmium minerals in the food are to be monitored, as their excess concentration leads to toxicity. In order to prevent this CRISPR-Cas9 led knock out of the metal transporter gene OsNramp5 resulted in its mutants which possessed 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. This reduction in Cd had no effect on yields (Tang *et al*., 2017).

Red Rice

In the case of *Oryza rufipogon*, the red colour of pericarp tissue has resulted from the combined gene action of the genes Rc and Rd. But during the course of evolution there took place a frame-shift deletion of 14-bp in the 7th exon of Rc. This resulted in the development of white rice grains in the cultivated species. The deletion of the rc allele was reversed by using the Crispr-cas, which resulted in the development of red coloured pericarp in the cultivated ones (Zhu *et al*., 2019).

Conclusion

The enormously rising population which is to be fed and the enhanced stress factors due to the climatic irregularities which had drastic impacts on crop cultivation are to be addressed in future. There is need to enhance the production levels to feed the future populace. Among the several crops as rice major one providing nutrition to the more than half of the global populace. So, the improvement in rice has to be major concern. The crop improvement activities had been carried out using conventional approaches but the results obtained were not so supportive to the present conditions. Later, the results obtained by using molecular breeding approaches had been positive. Till now only existing genetic variation is being exploited, but in order to overcome above constraints there required a novel approach to creative new variation in the gene pool which can utilised for crop improvement. This led to utilisation of genome editing approaches which generate targeted mutations resulting in the desirable traits. Among this Crispr-cas has been identified as approach with most practical utility. Based on above mentioned improvements made in rice using crispr-cas, undoubtedly it is boon for scientific community.

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