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CRISPR/Cas Genome Editing to Improve Rice Production and Productivity

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Abstract

At present condition we are indeed to produce more quantity of rice to face the demand of rice requirement, but cultivation of rice crop and increase the rice production faces several hindrances *i.e.*, pest, diseases, salinity, water deficit, temperature *etc*. that significantly lower its production and productivity also another drawback of rice cultivation is required of more amount of water. Because of the genomic and bioinformatics advancements several effective new crop improvement techniques are developed, among those, an important one is genome editing technology especially CRISPR/Cas9 technique. By using these, rice plant with desirable traits such as panicle architecture, shoot architecture, grain characters, leaf structure traits *etc*. can be modified in the positive way to increase the rice grain yield either by editing of direct yield governing parameters or targeting to increase the tolerance mechanisms against different stresses which affect the rice productivity.

Keywords: Abiotic stress, Gene knocked in/out, Genome editing, Improved rice variety

Introduction

To maintain the food security for dense population, rice production and productivity should be significantly increased from the present production status. Due to climate change, land, air and water pollution, available good quality of water for irrigation and the crop cultivable area is drastically reduced year by year. Hence rice production should be increased without increasing the cultivable area; this only possible by escalating the rice yield per capita land area and cultivating crops which is using water more efficiently. For instance, producing of a one kg of cooking rice demands around three thousand litres of water and in rice because of drought up to 50% yield reduction is happen; while low temperature stress poses further threats to both the production and quality of rice. Additionally, economic value and consumer demand of rice varieties are increased by the creation of stress-tolerant cultivars and improvements to grain quality characteristics. In the past, traditional breeding techniques (mutagenesis and hybridization) or transgenic technology have enhanced rice grain quality, climatic resilience, disease resistance and production. Nevertheless, these methods are laborious, time-consuming, need big screens and are vulnerable to human prejudice. For example, the main disadvantages of mutagenesis in rice are that it can cause unintended, harmful mutations, leading to a loss of fitness or other undesirable traits. The main inconvenience of hybridization in rice are the high cost of seeds, inconsistent performance due to environmental factors and the fact that farmers cannot reuse the seeds from their harvest. In case of transgenic rice major problems faced are environmental risks like ecological imbalance and health concerns *i.e.*, potential allergens or antibiotic resistance and socio-economic issues like higher seed costs and the potential for corporate seed monopolies (Tabassum *et al*., 2021). To overcome the aforementioned rice improvement techniques and satisfy the world's expanding population's demand for rice, more potent, accurate, quick and reliable crop improvement techniques like genome editing will be needed.

Article History

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Genome Editing Technique

All the existing difficulties faced in crop improvement through conventional breeding can be overcome by powerful genome editing technology. By this technology it is possible to alter or modify a specific desired trait or selected multiple traits in any rice varieties within a short period of time hence, this new technology has a strong impact for fastening the crop improvement programs to increase the rice production. Genome editing technology (GET) modifies a gene, need to be improved by cleaving DNA *via* site-specific endonucleases (SSE). The Clustered Regularly Interspaced Short Palindromic Repeats with CRISPR associated enonucleases (CRISPR/Cas9) approach is garnering significant attention due to its cost-effectiveness, user-friendliness and high efficacy in multiple gene editing. Comprehensive information regarding gene sequence, function and QTL pertinent to desirable characters are essential to use genome editing technology (GET). CRISPR/Cas9 induces cleavage in both the DNA strands (double-stranded breaks) at specific sites of designated gene, which can be rectified by the host's repair mechanisms through either homology-directed repair (HDR) or non-homologous end joining (NHEJ). In HDR repairing method specific genes can be knocked in and modified in positive way, in case of NHEJ method gene knockouts can be done through sequence insertion and deletion (In/Del). Through this method more number of genes at multiple genomic locations can be modified, this multiplex genome editing is necessary to change more number of desirable characters (Zafar *et al*., 2020; Sivaji *et al*., 2023).

CRISPR/Cas9 System and How It Works?

CRISPR/Cas9 system is naturally existing immune system in bacteria that act against the invading foreign viral genetic material and protect the host. DNA endonuclease activity is exhibited by the Cas9 protein, which is responsible for making cleavage in the DNA double strands and binds to the guide RNA, which offers the sequence information; together they make a binary complex. Then the guide RNA in the binary complex will search the target host genome and bind where sequences of guide RNA detect its complementary sequences is called as the target DNA strand (the other strand has the nucleotide sequences similar to the guide RNA is called non target strand) and a precise cut has been made by Cas proteins. Cas9 protein cut the both target DNA and non target DNA from upstream of the protospacer adjacent motif (PAM) site (three nucleotides; most often it is NGG) present in the non-target DNA strand. This is known as the PAM sequence, situated in the non-target DNA strand present to the right downstream of the target DNA sequence. The PAM sequence is most important for Cas protein to make double stranded DNA brake (DSB) at precise position. Two nuclease domains present in Cas9 proteins, HNH and RuvC, make internal cuts at specified sites in the target DNA strand that are complementary to the guide RNA sequence and the non-target strand, respectively. So cas9 proteins produce DSB in the gene which is complementary to guide RNA and this is repaired using either NHEJ repair mechanism or HDR system. This NHAJ repair system deletions and insertions (InDels) are introduced randomly in cleavage. The HDR system is high specific develop precise genome modification at the DSB site using a homologous repair DNA strand (Le *et al*., 2022) (Figure 1).

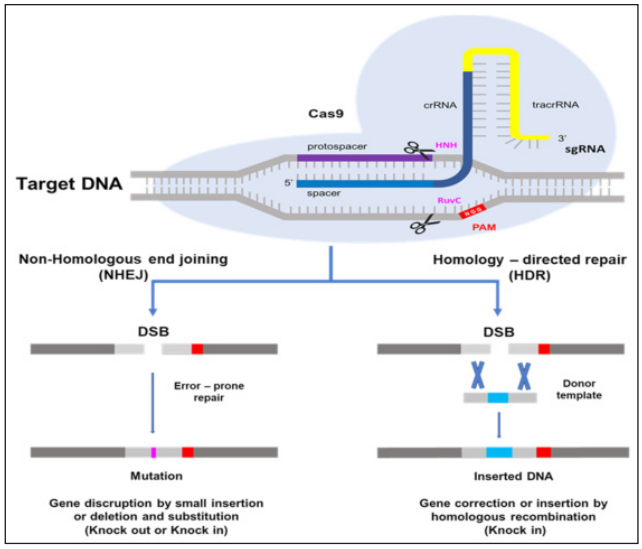

Figure 1: NHEJ and HDR method of gene modification and desirable traits can be modified Crisper/Cas genome editing in rice

Applications of CRISPR/Cas9 System for Rice Improvement

It is used to improve various useful traits which are either involved directly yield improving traits such as shoot, panicle, root architecture *etc*. or indirectly *i.e*., yield affecting traits such as resistant to adverse climate conditions, to obtain more yields in rice crop (Figure 2). By genome editing method genes of rice, which governed plant architecture, panicle architecture, important signal and metabolic pathways *etc*. can be modified in order to increase grain yield, high quality and nutrition fortification, herbicide tolerance, rice with early maturation and long day flowering, salinity resistance, water stress tolerance, flood resistance, heat tolerance, submergence resistance and biotic stress tolerance (disease, insect and nematode). Because abiotic stress, tolerance are governed by various parameters and several genes, developing of abiotic stress-resistant/tolerant crops is really difficult. In comparison to wild type rice plants, CRISPR/Cas9-mediated modification of several genes of rice plants increased over all grain yield, enhanced

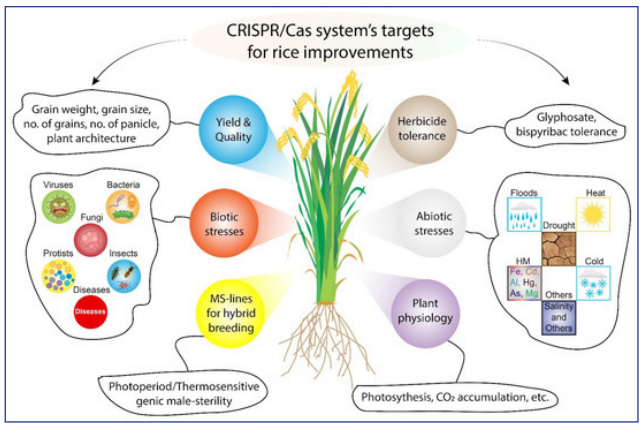

Figure 2: CRISPR/Cas genome editing to improve traits of rice for higher yield

Table 1: CRISPR/Cas gene editing in rice for yield improvement

Sl. No.	Type of genome editing	Traits/ genetic factors targeted in wild type	Gene/ QTL edited	Characters improved in mutants	Results
1	sgRNA/Cas9-mediated multiplexed editing	Three panicle, Grain and plant architecture regions	Dense and Erect Panicle (DEP1), Grain Size 3 (GS3), Grain number 1a (Gn1a) and a plant architecture QTL, Ideal Plant Architecture1 (IPA1)	Erect panicle, improved grain size and number of grains, more/less number of tillers and reduced plant height	Improved grain yield
2	sgRNA/Cas9-mediated multiplexed editing	Three panicle architecture regions	Panicle architecture genes, PIN family of auxin efflux carrier-like gene 5b (OsPIN5b) genes	Increased the panicle length	Increasing the rice grain yield
3	sgRNA/Cas9-mediated multiplexed editing	Grain width/ weight	GW2, GW5, GW6 and GW8 and GS3	Enhanced grain width, weight, size	More grain yield
4	sgRNA/Cas9-mediated multiplexed editing	ABA receptor gene	OsPYL1, OsPYL4, OsPYL6 and OsPYL9	Increased the number of grains by 31%	Higher yield
5	CRISPR/Cas9-mediated loss-of-function mutation using	-	Waxy gene controlling Rice gel consistency (GC) and gelatinization temperature (GT)	-	Decrease amylose content and convert rice to glutinous types without altering other desired agronomic characteristics
6	CRISPR/ Cas9-based multiplexed editing	Leaf architecture	SEMI-ROLLED LEAF 1 and 2 (OsSRL1 and OsSRL2)	To curled leaves, reduced number of stomata, stomatal conductance, transpiration rate and malondialdehyde (MDA) content	Conferred drought tolerance
7	CRISPR/Cas9-mediated knockout	Transcription factors	R2R3-type MYB transcription factor, OsMYB	-	Increased the grain yield and cold tolerance
8	CRISPR/Cas9-mediated knockout	Transcription factors	Zinc finger TF, drought and salt tolerance (DST) and a miRNA, OsmiR535	-	Enhanced drought, salinity and osmotic stress tolerance
9	sgRNA/Cas9 system based knockout	Transcription factors	Amino acid B-type response regulator TF and OsRR22	By improving the shoot architecture; thus, highlighting the utility of TF targeting for improving abiotic stress tolerance in rice	Salinity tolerance
10	sgRNA/Cas9 system based knockout	Coding sequences or exons	CDS or exons of Ethylene Response Factor, OsERF922 polyketide synthase-encoding genes, OsRSY1 and OsALB1 and PYRICULARIA ORYZAE RESISTANCE 21 (Pi21)	-	Enhanced resistance against rice blast, *M. oryzae*.

Sl. No.	Type of genome editing	Traits/ genetic factors targeted in wild type	Gene/ QTL edited	Characters improved in mutants	Results
11	CRISPR/Cas9-mediated targeting	Coding sequences or exons	CDS or exons of Sugar will Eventually be Exported Transporters (SWEET) gene family members, OsSWEET11 (also called Os8N3) and OsSWEET14	-	Increased the resistance against bacterial leaf blight (BLB) caused by *X oryzae pv. Oryzae* (XOO)
12	CRISPR/Cas9-mediated targeting	Eukaryotic Translation Initiation Factor	eIF4G, a host S gene	Improved the resistance against or rice tungro disease (RTD) caused by rice tungro spherical virus (RTSV)	The disease resistance was observed in terms of less disease symptoms, improved yield and agronomic performance
13	CRISPR/Cas9-mediated knockout	-	Acetolactate Synthase (OsALS) gene conferred resistance against imazapic (IMP) and imazethapyr (IMT)	-	Conferred resistance against imazapic (IMP) and imazethapyr (IMT) herbicides
14	CRISPR/Cas9-mediated knockout and multiplexed editing	-	Knockout of the OsALS gene and multiplexed editing of OsALS and FTIP1e genes	-	Increased resistance against bispyribac sodium and imazamox herbicides, respectively
15	CRISPR/Cas9 mediated knock-in/ replacement	-	5-enolpyruvylshikimate-3-phosphate synthase (OsEPSPS) gene	-	Increased the glyphosate resistance
16	CRISPR/Cas9 mediated knock down	-	Rice annexin gene *OsAnn3*	-	Cold tolerance

resistance to drought, cold, flood, high heat, salinity stresses and several herbicides such as bispyribac, sodium imazamox and glyphosate (Table 1).

Two new rice types, *viz*., DRR Rice 100 (Kamla) and Pusa DST Rice 1, were developed in India utilizing CRISPR-Cas genome-editing technology for the first time in the history. Based on Samba Mahsuri (BPT 5204), ICAR-IIRR, Hyderabad, created the DRR Rice 100 (Kamala) variety. It matures twenty days before than normal wild type variety and aims to enhance the number of grains each panicle. An another variety, Pusa DST Rice 1, based on MTU 1010, was created by ICAR-IARI, New Delhi and is resistant to saline and alkaline soils, increasing yield by up to 20% (Tabassum *et al.*, 2021; Tripathy *et al.*, 2021).

Conclusion

CRISPR/Cas system, possess the ability to increase crop yield by improving its desirable characters with precise manner; hence it is efficient and superior to traditional breeding methods. Due to its versatile genome editing capabilities, including InDels, gene knock out, gene knock in and direct substitutions at specific site, CRISPR/Cas technique has surpassed other GETs in case of developing optimal plants and domestication of crops, such as the creation of climate smart rice and other crops. Furthermore, this new and promising delivery techniques employed in recent gene editing methods will result in transgene-free products, thereby addressing ethical, regulatory and commercialization challenges. The application of GETs in functional genomics, when combined with other methods, will give answers to the many unsolved question regarding to increase the crop production and productivity and contribute to achieving the hunger free society.

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