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Research Journal of Agricultural Sciences
An International Journal

P- ISSN: 0976-1675
E- ISSN: 2249-4538

Volume: 12
Issue: 05

Res Jr of Agril Sci (2021) 12: 1543–1546

CARAS

Res. Jr. of Agril. Sci. (Sep-Oct 2021) 12(5): 1543–1546
www.rjas.org

Case Study

Abiotic Stress Tolerance Enhancement in Rice through CRISPR/Cas9 Approach

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Received: 08 Jun 2021 | Revised accepted: 06 Aug 2021 | Published online: 09 Sep 2021

A B S T R A C T

Rice is an important staple food crop and it is cultivated in many countries. To reach sufficient productivity many biotechnological strategies are being applied to improve the productivity by increasing the quality and quantity of the crop. There are some abiotic factors which are extremely affecting the growth of the crop and its productivity. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas system is considered as one of the specific and effective types of genomic editing in plant biotechnology. This helps in studying the gene function and creating new rice varieties which shows tolerance against different abiotic factors i.e., drought, salinity, high and low temperatures, etc. The review aims to provide recent information about abiotic stress tolerant varieties of rice produced by genome editing using CRISPR-Cas9 system.

***Key words*:** CRISPR/Cas9, Abiotic stress, Rice, Genome editing, Stress tolerance

Rice is a basic food source in more than 100 countries around the world. Rice is considered as a strategic crop plant by the Food and Agriculture Organization (FAO). The scientific name for rice is *Oryza sativa*. *Oryza sativa* is the most common species and is subdivided into the long-grain *indica*, and short-grain *japonica*. In 2017, world production of paddy rice was 769.7 million metric tons by China and India with a combined 49% of this total. Other major producers were Indonesia, Bangladesh and Vietnam. Rice provides 21% of energy and 15% of protein for humans, so its quantity and quality require major attention [7]. These two strategies could be improved by biotechnological approaches. One of the pros for cultivating rice is that it can be grown in a wide range of environments, even in areas not suitable for other crops [2]. But there is a great effect on the crop yield due to biotic and abiotic factors. The plants in reproductive stages are more sensitive to these stresses and thus affect the yield of many important plant species [11]. To get rid of these factors, and to obtain good productivity many modifications were done genetically. Abiotic stress is one dreadful factor that affects the growth and development and leads to less yield. Genome editing allows the introduction of deletions, insertions or base substitutions by causing damage, double-strand breaks (DSBs), in targeted deoxyribonucleic acid (DNA). Generally, plant cells follow two strategies to repair the damage, the preferred repair mechanism in higher plants is non-homologous end-joining (NHEJ), which mainly causes insertions or deletions and can result in frameshift mutations. A second repair mechanism, homologous recombination, occurs when a template with homologous sequence surrounding the DSB is available and is used for DSB repair, resulting in gene replacement [5]. The most preferred choice to edit plant genome for improving the tolerance against the abiotic stress is clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated nuclease 9 (Cas9) system. It leads genome editing by precise manure with minimal or no effect on growth and development of plants.

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CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) CAS system

Many breeding techniques had developed to increase the crops productivity which is time consuming processes [6]. The important crop improvement strategies are required which is easy, fast, efficient, and accurate in obtaining new genome edited crops, one of such genome editing techniques is CRISPR-Cas9. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a family of DNA sequences found in the genome of prokaryotic organisms such as bacteria and archaea. They are used to detect and destroy DNA from similar bacteriophages during subsequent infections. Hence these sequences play an important role in the antiviral defense mechanism of prokaryotes and provide a form of acquired immunity [2]. Cas9 /CRISPR-associated

protein 9 is an enzyme that uses CRISPR sequences as a guide to recognize and break the specific strands of DNA that are complementary to the CRISPR sequence. Cas9 enzymes together with CRISPR sequences form the basis of a technology known as CRISPR-Cas9 which can be used to edit genes within organisms [26]. Degradation of exogenous DNA is first observed in Escherichia coli [9]. Later it was reported in other bacteria like Shigella dysenteriae, Salmonella enteric and Mycobacterium tuberculosis [8], [16]. CRISPR/Cas9 is an RNA- mediated adaptive immune system that provides resistance against genetic attacks and stores the history of infection in the form of spacer sequences. These spacers function along with Cas9 endonuclease proteins to recognize and destroy the exogenous DNA. The significant contribution of CRISPR/Cas9 technology to genome editing is by establishing a modified version of CRISPR/Cas9. This modified version of CRISPR/Cas9 is made up of the customizable single strand RNA (sgRNA), which is the fusion product of crRNA and tracrRNA. This combination will result in a Cas9/sgRNA complex that targets and initiates DSB at specific DNA sequences [10].

Abiotic stress tolerance

The intensive increase in biotic and abiotic stresses (high and low temperature, salinity, drought, and heavy metal stresses) is due to global warming that leads to decrease in crop production [24]. Many biotechnological approaches are adopted to increase the quality and quantity of rice as well as its resistance to pests, diseases and environmental stresses. Drought, salinity, heat, excess of salts or toxic metals, such as aluminium, arsenate, and cadmium, in the soil are major environmental factors that significantly influence the growth of plants and lead to a decline in plant productivity [17], [20]. Current efforts to improve the abiotic stress tolerance of plants resulted in significant achievements. Abiotic stresses disturb normal morpho-biochemical and physiological processes; hence this directly affects yield. The response to these stresses varies according to genotype [23].

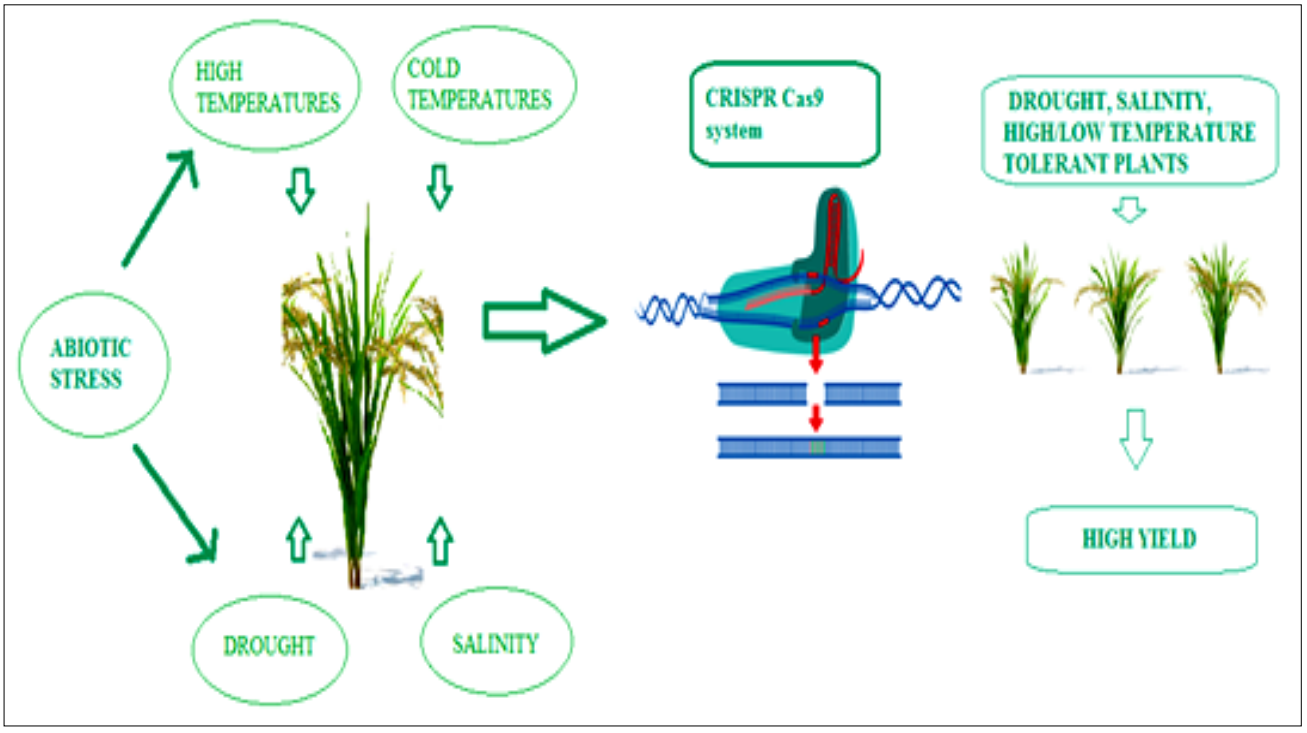

Fig 1 Pictorial representation of various stresses imparted on rice plant and the stress tolerance enhancement through CRISPR/Cas 9 mediated genome editing, subsequently resulted in the increased yield under stress conditions

Developing drought tolerance in rice

Many drought tolerant mutants were developed by incorporation of different genes by using CRISPR–Cas technology. Multiple mutant alleles of drought and salt tolerance (DST) genes are created by using CRISPR-Cas9 gene editing in indica rice cv. MTU 1010. Two different gRNAs were used to target regions of DST protein that might be involved in protein–protein interaction. This 366 bp deletion led to the deletion of amino acid residues from 184 to 305 in frame, and hence the mutant was named as dstD184–305. The dstD184–305 mutant produced leaves with broader width and reduced stomatal density, and thus enhanced leaf water retention under dehydration stress [22]. Similarly Abscisic acid (ABA) is involved in regulating drought tolerance in plants, *Arabidopsis thaliana* enhanced response to ABA1 (ERA1) encodes the β-subunit of farnesyl transferase and regulates ABA signalling and the dehydration response. *ERA1* is an important gene for enhancing drought tolerance in numerous crops, *osera1* mutant lines, in rice harboring CRISPR/Cas9-induced frameshift mutations, exhibit similar leaf growth as control plants but increased primary root growth. The *osera1* mutant lines also display increased sensitivity to ABA and an enhanced response to drought stress through stomatal regulation [18]. OsPYL9 was mutagenized through CRISPR/Cas9 in rice. Mutant lines appear to accumulate higher ABA, antioxidant activities, chlorophyll content, leaf cuticular wax, and survival rate, whereas a lower malondialdehyde level, stomatal conductance, transpiration rate, and vascular bundles occur under stress conditions. The OsPYL9 mutants showed an increase in grain yield under both drought and well-watered field conditions [21]. Rice (*Oryza sativa*) drought-responsive AP2/ERF transcription factor *OsERF71*, which is expressed predominantly in the

root meristem, pericycle, and endodermis. Over-expression of *OsERF71*, either throughout the entire plant or specifically in roots, resulted in a drought resistance phenotype at the vegetative growth stage, indicating that over-expression in roots was sufficient to confer drought resistance [4]. SNF 1-RELATED PROTEIN KINASE 2 (SnRK2) is a family of plant-specific protein kinases which is the key regulator of hyper-osmotic stress signalling and abscisic acid (ABA)-dependent development in various plants. Among the rice subclass-I and -II SnRK2s, osmotic stress/ABA–activated protein kinase 2 (SAPK2) may be the primary mediator of ABA signaling. SAPK2 increased drought tolerance in the following two ways: i.e., by reducing water loss via the accumulation compatible solutes, promoting stomatal closure, and upregulating the expression levels of stress-response genes such as OsRab16b, OsRab21, OsbZIP23, OsLEA3, OsOREB1 and slow anion channel (SLAC)-associated genes such as OsSLAC1 and OsSLAC7; and by inducing the expression of antioxidant enzyme genes to promote ROS-scavenging abilities that will ultimately decrease ROS damages [14].

Developing salinity tolerance in rice

The dstD184–305 mutation induced by CRISPR-Cas9 method in DST gene in indica rice cv. MTU1010 phenocopied EMS-induced dst (N69D) mutation reported earlier in japonica cultivar. The Cas9-free dstD184–305 mutant exhibited moderate level tolerance to osmotic stress and high level of salt stress in seedling stage [22]. Similarly, the improvement of the salinity tolerance in rice by engineering a Cas9-OsRR22-gRNA expression vector, targeting the OsRR22 gene in rice. Nine mutant plants were identified from 14 T0 transgenic plants Sequencing showed that these plants had six mutation types at the target site, all of which were successfully transmitted to the next generations. Mutant plants without transferred DNA (T-DNA) were obtained via segregation in the T_1 generations. At the seedling stage, the salinity tolerance of T2 homozygous mutant lines was significantly enhanced compared to wild-type plants [1]. In *Oryza sativa*, *OsSAKP2* gene editing significantly increased the tolerance of rice plants to salt [14].

Developing cold tolerance in rice

A new Cold tolerant mutant of rice was developed by editing for excellent cold tolerance using CRISPR- Cas9 system. OsMYB30 is a gene which is edited by targeting two sites with high efficiency 63% of OsMYB30 site-1, 58% of OsMYB30 site-2, subsequently these mutants showed excellent cold tolerance [25].

Table 1 Data showing some of the edited genes to enhance stress tolerance in rice

Crop	Improved traits	Target gene	Results	References
Indica rice cv. MTU1010	Drought and salt tolerance	DST gene	Cas9-free dstD184–305 mutant exhibited moderate level tolerance to osmotic stress and high level of salt stress in seedling stage.	[22]
Oryza sativa Nipponbare	A panicle length, grain size gene, Cold tolerance	OsPIN5b, GS3, OsMYB30	Increased in the panicle length, grain size and excellent cold tolerance	[25]
Oryza sativa	Drought tolerance	ERA1	enhanced response to drought stress	[18]
Oryza sativa L.	Drought Tolerance and Grain Yield	OsPYL9	OsPYL9 mutants showed an increase in grain yield under both drought and well-watered field conditions	[21]
Oryza sativa	Salinity tolerance	OsRR22	Improvement in salinity tolerance	[1]
Oryza sativa L. japonica.	Drought tolerance and salinity	OsSAKP2	Increased drought tolerance and salinity tolerance	[14]

CONCLUSION

Abiotic stress had become a great disadvantage and affected the yield of the rice. Genome editing using CRISPR-Cas9 had shown an effective hope for producing new mutants in rice which are tolerant to abiotic stress [12-13] [19]. CRISPR-Cas9 technology is generating a genome-wide mutant library that can be used for identifying gene functions and for genetic improvement [15]. The CRISPR-guided mutagenesis of OsPYL9 has great potential for improving drought resistance and the yield of rice together. In Indica mega rice cv. MTU1010, the dstD184–305 mutant showed reduced stomatal density accompanied by an increase in leaf water retention under dehydration stress and during seedling stage stress tolerance assay, dstD184–305 mutant exhibited moderate level of tolerance to osmotic stress and high level of tolerance to NaCl stress [22]. In Oryza sativa Nipponbare, OsPIN5b, GS3, OsMYB30 which are edited for increasing the panicle length, grain size and excellent cold tolerance, while in *O. sativa* L. Japonica, editing of the OsSAKP2 gene resulted in increased drought tolerance and salinity tolerance [14]. For Oryza sativa genome editing at ERA1 gene causes enhanced response to drought stress [18], at OsPYL9 gene resulted in increase in grain yield under both drought and well-watered field conditions [21], at OsRR22 gene resulted in Improvement in salinity tolerance [1]. By applying this genome editing technique we can reach the demand of the crop by crossing all barriers and can increase the productivity and supply the sufficient quantity of food all over the nation.

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