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Editing the Genome for Salt Tolerance in Rice

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Abstract

Rice is considered as a major food crop in the World and provides 20% of the world's dietary energy. Development of abiotic stress tolerance rice genotypes including salt tolerance is very much necessary for sustainable rice production under climate change scenario. Ground salt is one of the most important barriers to rice production worldwide, especially in coastal areas. Rice has benefited from new breeding technologies, such as the CRISPR-led evolution, CRISPR-Cas, and basic editors, have recently been used in rice to achieve successful genome sequencing. In this way we can focus on the editing of genome for salt tolerance rice and find out the best source based on its conventional and advanced method to improve its resistant effect along with its productivity which can be widespread all over the sites.

Introduction

To achieve the future food security we have to produce 40% more rice for by 2050 (Ray *et al.*, 2013). To achieve this goal, breaking the yield barriers, improving climate resilience, biotic and abiotic stress tolerance cultivars we have to develop. Therefore, along with the molecular breeding tools, use of genome editing technologies is necessary to sustain rice production. Genome editing is a precision mutagenesis tool for functional genomics and crop improvement. New genome classification methods are being developed because rice is a good model for performance analysis model due to its short genetic makeup and close syntenic interactions with other grain plants. Currently, rice is being used to model crop system for many functional genomics studies by using genome editing technologies such as Transcriptional Activator-Like Effector nucleases (TALEN) and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated endonuclease Cas9 (CRISPR/Cas9) (Miao *et al.*, 2018). The risk of the rice crop being in excess of salt is the result of the combined action of a few stress-response genes that interact with other components of the stress signal transmission mechanism. Numerous salt tolerance quantitative trait loci were identified and few of them had been transferred into popular rice genotypes background *via* marker-assisted selection (MAS) or Marker-assisted backcross breeding (MABB). During the past two decades, many salt-related genes (*SKC1*, *DST*, *OsRR22*, *OsHAL3*, *P5CS*, *SNAC2*, and *OsNAP*) have been successfully cloned. Among them, the *OsRR22* gene encodes a 696-amino acid B-type response regulator transcription factor that is involved in both cytokinin signal transduction and metabolism; its loss of function has been reported to significantly increase salt tolerance (Takagi *et al.*, 2015). CRISPR-Cas technology helps to develop elite cultivars with desirable alleles by precision genome editing. With its low

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genome size and close links to other cereal plants, rice is a useful modelling program to test genomics performance. As a result, rice genome planning technologies such as CRISPR-Cas9, CRISPR-Cas12a, and basic planning are being developed. New research continues to develop unique rice genome transformation techniques, such as basic editors and master editing, in addition to the standard genetic modification that requires the establishment of a DSB and using the NHEJ or HDR method to be adapted.

Editing Mechanism Used for Salt Tolerance of Rice

CRISPR/Cpf1 has many advantages over CRISPR/Cas9, making it the most advanced and complete planning technology. CRISPR/Cpf1 receives T-rich sequence (5′-TTTN-3 or 5′-TTN-3′) PAM 5′ at the end of the target sequence, while CRISPR/Cas9 requires G-rich (5′-NGG-3′) PAM sequence at the end of 3′ of the target area, resulting in better cracking performance (Feng *et al*., 2013). Without the need for tracrRNA, the complexity of Cpf1–crRNA can effectively break down targeted DNA into the CRISPR/Cpf1 system. Compared to the 100 ntsgRNA used in the CRISPR/Cas9 system, the crNA 40-45 nucleotides in length including that of replication and space is good enough to allow genetic modification. Salt is one of the main problems preventing the cultivation of rice worldwide. The development of salt-tolerant rice varieties is a very natural way to control salt. To improve salt tolerance in rice, CRISPR-Cas9 was used to regulate *OsRR22*. Agronomic parameters and salt tolerance were tested in the dynamic lines. Compared with wild-type plants, flexible rows showed higher salt tolerance in the seedling category.

Base editing is also necessary for the improvement in saltol rice. While genetic mutation in plants with homology directed repair (HDR) may be a good strategy for genetic modification, the frequency and effectiveness of genetic DNA delivery and targeted genetics or genetic modification are both negative. The CRISPR/Cas9-based foundation planner strategy is one that allows for the direct and indirect conversion of one target base into another without the need for a DSB or donor template (Zhang *et al*., 2019). A 20-bp nucleotide sequence in the first exon of OsRR22 (GenBank Accession No BR000251.1) was selected as the target area to develop a specific targeted gene for OsRR22 in the gene. Cas9-OsRR22-gRNA binary plasmid was created using the CRISPR/Cas9 vector. Agrobacterium-mediated transformation was used to modify rice type WPB106 per vector. A total of nine WPB106 mutants were obtained from 14 T0 hygromycin-resistant transgenic WPB106 plants using a site-specific PCR and Sanger sequence (64.3 percent). At sowing and reproductive stages, salt stress has a profound effect on rice. Ionic imbalances, dehydration, osmotic stress, and oxidative damage are all caused by high levels of salt. As a result, it is important to determine which QTLs are most accurate and which candidate genes are involved. For tolerant genotypes, a panel of potential candidate genes, found in meta-QTL sites and expressed differently in salt stress conditions, are presented. There are twenty-three unknown genes, five of which have CBS or cupin backgrounds respectively. For example, a gene with a CBS domain was found in Chr2: M-QTL1 and was controlled at the top of the roots. It is thought to play a role in salt and oxidative tolerance by opening up chloride channels, according to a previous study. A few salt-responsive compounds (TFs) from different families, including *TIFY*, *MYB*, *HSF*, *HOX*, *WRKY*, *AP2*, and *GRAS*, have been found both in meta-QTL regions and between DEGs, and have been proven to play a important role in salt tolerance of rice. Ionic and osmotic homeostasis; Vehicles such as *HKT1* (Na^+/K^+ transporter), *NCX* (sodium/calcium exchanger), and *TIP2-1* were also among the potential genes (aquaporin). Many of the important genes involved in the release of toxins were found among DEGs found in meta-QTL sites, including hydrolase, oxidoreductase, and peroxidase.

Conclusion

A fundamental advantage of genome planning technology is that the contracts are designed to remove transgenes, resulting in no distinction between genetically modified plants and those that are genetically engineered. Scientists are constantly striving to improve the genome system by acquiring new proteins or by filtering existing ones. Cas9 has recently been circularly approved for the production of protease-activated ProCas9s. ProCas9 can detect and respond to protease cell activity, which is common during viral infection. This improved approach will make genome editing safer and more efficient. The CRISPR/Cas9-mediated mutations have the potential to generate genetically challenging genes, transform many areas, and produce massive removal. Further study of these attractive genes may reveal useful information that can be used to improve salt tolerance in certain genotypes by genetic engineering or by genetic engineering. In addition to agronomic activity, a complete examination of NILs in saline-affected soils will reveal natural benefits such as photosynthetic efficiency achieved by salt resistance.

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