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CRISPR-Cas9 Directed Genome Modification for Abiotic Stress Tolerance Rice; a Review

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Abstract

CRISPR Clustered regularly interspaced short palindromic repeats. CRISPR-associated protein (Cas) technology for site-specific genome editing has been used to precisely induce mutagenesis in a variety of plant species, including rice. Because Cas9 causes blunt-ended double-strand breaks, which are then repaired without substantial end-processing, a high fraction of mutations created by CRISPR/Cas9 is extremely short insertions and deletions. CRISPR/Cas9 is a powerful tool for targeted mutagenesis in rice, as well as some essential crops. One of the most demanding environmental restrictions affecting agricultural output around the world is salinity and drought. Because plant adaptation to abiotic stress is polygenic, much more rice genes have that play a critical role in abiotic stress response.

Introduction

The CRISPR-Cas system, the most recent genome editing approach, evolved from the adaptive immune system of bacteria and archaea, which allows organisms to adaptive immunity against the bacteriophage virus. CRISPR- clustered regularly interspaced short palindromic repeat was discovered- 2002. Though all CRISPR-Cas systems have DNA repeats, spacers, and Cas genes in common, the system has a great deal of variability due to fast evolution and horizontal gene transfer in nature. The CRISPR-Cas systems were classified using a multi-criteria approach based on characteristic Cas genes (Cas1, Cas2) and the sequence is very much similar to Cas proteins, Cas1, and also the structural arrangement of the system in the loci. In the genome-editing technique, accuracy in base editing has always been a challenge. By combining catalytically inactive Cas9 variants, dCas9 (dead Cas9), and Cas9 nickase to target deaminase domains and edit specific loci, efforts are being undertaken to improve the accuracy of gene editing schemes. Other than the CRISPR/Cas9 system, recent findings have revealed a variety of possible tools, including type V systems having the DNA-targeting Cas12 (Cpf1 or C2c1) effectors and RNA-targeting type VI systems containing the Cas13 (C2c2).

Rice genome editing with Cas9 system

All available internet tools were used to design and synthesize sgRNA, starting with the selection of the target gene. The sgRNA has been cloned into a plant binary vector along with the necessary Cas9 or Cas12 variants for transformation into target plant species using an acceptable approach such as Agrobacterium-mediated transformation. The presence of Cas9 or Cas12 and sgRNA would be tested after transformation on the putatively transformed plants. The plants are then screened for the desired targeted mutations using PCR-RE genotyping and DNA sequencing techniques, followed by the creation of transgenic seeds.

The Applications of CRISPR/Cas9 technology in plant science

In rice Gene research is a field of study that looks into how genes work. There are Abiotic stress (salt, drought, cold, heat, etc.) and biotic stress are (bacteria, viruses, fungi, etc.) conditions that are posing hazards to agriculture around the world. Crop breeders are always pursuing yield gains, quality improvement, and stress tolerance/ resistance as a result of the global population increase,

shortages of food, and degradation of the environment. Genome editing through CRISPR/Cas9-technology can be used for more than only functional genomics areas; it can also be used to improve crop types. CRISPR/Cas9 has the potential to be extremely useful in many aspects of plant breeding, both today and in the future.

The major challenges for CRISPR/Cas9 in Crop

CRISPR/Cas9 gene-editing system has a wide range of possible uses in plant science. The narrow pool of genes influencing essential agronomic features, which are a requirement for employing this technology, is a big barrier. In this regard, deciphering genomic sequencing data and targeting the genetic data for crop development are urgently needed. Other obstacles include inefficient transformation systems and difficulty with plant tissue cultures and regeneration, both of which necessitate an intricate, tedious, and time-taking process. The possibility for off-target consequences, as well as the safety concerns associated with CRISPR regenerated bioproducts, is currently being debated. Off-target effects are, fortunately, more acceptable in plants than in animals, particularly humans. Plant mutants having off-target effects can be found and deleted via segregation during successive crosses as detection technologies improve. Designing appropriate sgRNAs with a strong affinity for targeted reason sequences and selecting a Cas9 nuclease with high processivity, in combination with the proper experimental procedure, may help to overcome off-target effects in the future. The lack of market access for genome-edited crops is another important issue.

Conclusion

CRISPR/Cas9 technologies have opened up new possibilities in the domain of plant genetic manipulation. CRISPR has several desirable qualities for a proper gene editing system for site-specific mutagenesis, high specificity, high processivity, cheap cost, high efficiency, and very simple perform in the lab, which is not possible with old mutation techniques. The CRISPR system is also highly developed to the ZFN and TALEN gene-editing system because the Cas9 nuclease is guided by RNA rather than proteins. Because the CRISPR/Cas9 system is reasonably well known, its construct methods have been developed now to this day, and efforts to limit off-target effects have been made. Special toolkits for the easy creation of CRISPR/Cas9 systems are also being developed, the development approaches for CRISPR/Cas9 created cut screening it can also use a single transformation event to modify a single gene or a greater number of genes. Furthermore. All of these benefits have prompted the researcher's use of CRISPR/Cas9 in crop improvement and plant molecular research, particularly for traits related to high yield quality, biotic stress, and abiotic stress resistance. CRISPR/Cas9 is also appealing because it has the ability to produce gene-edited crops. As a result, CRISPR technology appears to be the best tool for plant molecular research area.

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