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Prime Editor: A New Genome Editing Tool for Agriculture

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Owing to the adverse climatic conditions and different biotic and abiotic stresses, there is an urgent need to breed the crops for the foreseen challenges. The crop breeding approaches like cross breeding and mutation breeding take longer time and demand handling of large populations making it labor intensive. Transgenic breeding, though takes lesser time to develop promising variants, is withheld by regulatory issues from utilization in crop improvement. Genome editing technologies: have recently been established as powerful tools for creating targeted mutations in crop plants. Among them, Prime editing is very recent and an advanced version of CRISPR/Cas system.

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/ Cas system

The CRISPR/Cas system involves the specific introduction of targeted sequence variation by making DSBs in the genome of the plant. However, the system lacks the ability to make predictable changes making it unsuitable for curing the gene sequences (Chen *et al*., 2019).

Base editors

Base editing technology enables conversion of a specific base into another with utmost efficiency. Cytosine base editors (CBEs) and adenine base editors (ABES) mediates conversion of CG into TA base pair, and AT into GC base pair, respectively. Base editing can bring about only transition mutations and cannot insert or delete a stretch of sequence in the genome.

Prime editors

Prime editing was developed by Anzalone *et al*. (2019) which can introduces InDels and all 12 base-to-base conversions (both transitions and transversions) without inducing double stranded breaks (DSBs). It can be used efficiently for plant genome editing.

Types of prime editors

1. PEI-wild type M-MLV RT fused to the C terminus of Cas9 (11840A) nickase
2. PE2-Cas9 (H840A) with pentamutant M-MLV RT (D200N/L603 W/T330P/ T306K/ W313F)
3. PE3-PE2 with additional simple gRNA to simultaneously nick the non-edited strand

Components and mechanism of prime editors

Prime editing uses Cas9 nickase (Cas9n) fused to reverse transcriptase and engineered pegRNA (a prime editing guide RNA), which consists of a primer binding site (PBS), the desired edited sequence, and a sequence that recognizes the target DNA. The Cas9n is recruited to the target DNA sequence by pegRNA, and then nicks the PAM-containing DNA strand. The 3' end of the nicked DNA strand hybridizes to the PBS of pegRNA, priming reverse transcription of the desired edited-sequence on the pegRNA by reverse transcriptase fused to Cas9n. Hybridization between the target DNA and the reverse transcription product produces a 3 flap with edited-sequence or 5' flap with unedited sequence. The 5' flap is cleaved preferentially by the endonuclease, and the 3' flap is ligated to the DNA strand. The heteroduplex DNA is repaired by the endogenous DNA repair process, resulting in stable incorporation of the edited sequence into the genome.

Case Studies

Tian *et al*. (2018) developed a non-transgenic herbicide tolerant watermelon variety by editing. a single base C to T in Acetolactate synthase (*ALS*) gene, using CRISPR/Cas9-mediated base editing technology with an efficiency of 23%.

Butt *et al*. (2020) demonstrated the efficient utilization of prime editors (PE2 and PE3) to generate herbicide resistant rice by making targeted single and double base pair changes in *ALS* gene. Almost equal numbers of shoots were recovered from PE2 and PE3 suggesting that PE3 did not increase editing efficiency in plants.

Li *et al*. (2020) established an efficient prime editing system for rice with desired edits in both exogenous (hpt*II* gene) and endogenous (OsEPSPS gene) genes. They observed the precision of editing efficiency of 9.38% and 2.22% respectively. No off-target effects were found at the potential off-target site.

Conclusion

Prime editing technology has potential for application in gene correction and knockouts, protein engineering and directed molecular evolution by virtue of which it can do wonders in the field of plant biology. It would be a promising technology for plant genome engineering, especially because prime editing can achieve efficient knock-in of DNA fragments in plant cells. Despite some technical limitations and challenges, it is evident that prime editing will play a leading role among the many genome editing technologies for crop improvement in near future.

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