Scientists optimize prime editing for rice and wheat

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Many genetic and breeding studies have shown that point mutations and indels (insertions and deletions) can alter elite traits in crop plants. Although nuclease-initiated homology-directed repair (HDR) can generate such changes, it is limited by its low efficiency. Base editors are robust tools for creating base transitions, but not transversions, insertions

or deletions. Thus, there is a pressing need for new genome engineering approaches in plants.

David R. Liu and his colleagues at Harvard University developed a new genome editing approach, prime editing, which uses engineered Cas9 nickase (H840A)-reverse transcriptase (RT) fusion proteins paired with a prime editing guide RNA (pegRNA) that encodes the desired edit in human cells.

Recently, a research team led by Prof. Gao Caixia of the Institute of Genetics and Developmental Biology of the Chinese Academy of Sciences reported the optimization of a prime editing system (PPE system) for creating desired point mutations, insertions and deletions in two major cereal crops, namely, rice and wheat. The main components of a PPE system are a Cas9 nickase-RT fusion protein and a pegRNA.

Using the PPE system, these researchers produced all 12 kinds of single base substitutions, as well as multiple point mutations and small DNA insertions and deletions at 9 rice and seven wheat sites in protoplasts, with efficiencies up to 19.2%. The efficiency of PPE was strongly affected by the length of the primer binding site (PBS) and RT template.

Although byproducts (off-target effects) were generated by the PPE system, they can be reduced by optimizing RT template length. Moreover, using a PPE system optimized for plants, they found that the original RT could be replaced by CaMV-RT (from the cauliflower mosaic virus) and retron-derived RT (from E. coli BL21). Prime editing efficiency was also improved at some targets by using their PPE-Ribozyme (PPE-R) and by incubating at 37 C.

Furthermore, Gao and her collaborators were able to create stable mutant rice plants carrying G-to-T point mutations, multinucleotide substitutions, and a number of desired 6-nt deletions, with a mutant

production efficiency approaching 22%. It is noteworthy that these three types of mutation are very difficult to produce with current editing tools.

"Although the efficiency of the PPE system is lower than that of base editors, it is still an appealing new tool for creating all 12 types of single-point mutation, mixtures of different substitutions, and insertions and deletions. The system thus has great potential for plant breeding and functional genomics research," said Dr. Gao.

The scientific paper, entitled "Prime genome editing in rice and wheat," was published in *Nature Biotechnology* on March 16, 2020.

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