Toward next generation of genome editing in plants

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Abstract

Recent advances in genome editing technologies have substantially improved our ability to make precise changes in eukaryotic genomes. Sequence-specific nucleases (SSNs), particularly the Clustered Regularly Interspaced Short Palindromic Repeats / CRISPR associated proteins (CRISPR/Cas) system, are revolutionizing our ability to interrogate the function of the genome and can potentially be used to improve agronomically important traits in plants. In most eukaryotic cells, the non-homologous end joining (NHEJ) pathway generates insertions and deletions during DNA double-stranded breaks (DSBs) repair. In the presence of a DNA template with homology to the sequences flanking the DSB location, homology-directed repair (HDR) can seal the DSBs in an error-free manner. However, HDR pathway is generally much less efficient than the NHEJ pathway in higher plants even if DNA template exists. I have been trying to improve both NHEJ and HDR mediated genome editing in higher plants.

Improvement of CRISPR/Cas system for plant genome editing

To streamline and facilitate rapid and universal use of CRISPR/Cas-based genome editing in plants, we prepared CRISPR/Cas vector series, in which different promoters and terminators are used for sgRNA and Cas expression and different selection marker expression cassettes are combined. Our vectors are used in over than 100 laboratories in Japan and foreign country. In addition, we revealed that not only target sequences and expression constructs of Cas9 and sgRNA, but also the length of culture period of transgenic calli affected mutation efficiency in rice[1)]. Extension of the culture period was effective for obtaining mutated regenerated plants and this knowledge is useful for other plant species in which calli are used for transformation.

Wild type *Streptococcus pyogenes* Cas9 (*Sp*Cas9) induces mutation near "GG" sequence. We revealed that *Sp*Cas9 harboring seven amino acid substitutions could induce mutations near "G" [2)].

Improvement of HDR mediated genome editing in plants

We established HDR mediated genome editing system in which CRISPR/Cas9 mediated DSBs induction, suppression of NHEJ repair pathway by DNA ligase 4 (Lig4) knockout and supply of donor DNA were combined in rice and confirmed that stacking of these factors was effective for the efficient HDR mediated genome editing[3)]. Transient suppression of NHEJ pathway seems better for keeping plant genome stability, so the improvement is still on going. I hope to establish universal and efficient HDR mediated genome editing system in plants near future.

1) Mikami M, Toki S, Endo M (2015) Plant Cell Reports 34 (10): 1807-1815.
2) Endo M et al. (2018) Nature Plants *in press*
3) Endo M, Mikami M, Toki S (2016) Plant Physiology 170 (2): 667-677.