Epigenetic footprints of CRISPR/Cas9-mediated genome editing in plants

U.S. DEPARTMENT OF ENERGY | Office of Science

Background

CRISPR/Cas9 has been widely applied to various plant species accelerating the pace of plant genome editing and precision breeding in crops. Unintended effects beyond off-target nucleotide mutations are still somewhat unexplored. Epigenetic changes such as DNA methylation plays important roles in various biological processes. There is no published study exploring epigenetic changes in genome-edited organisms.

Approach

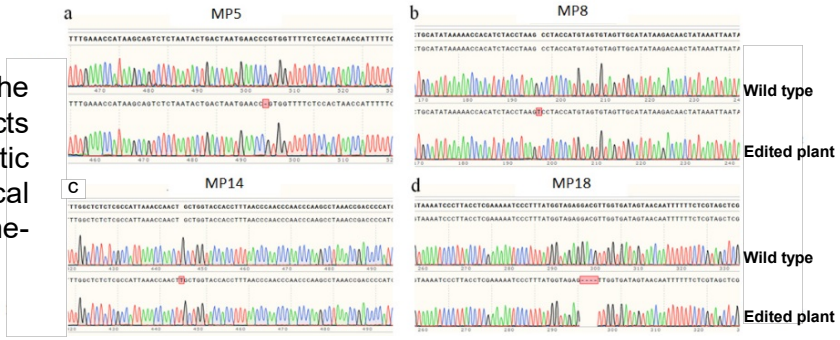
- Directed mutagenesis was performed via CRISPR/Cas9 in four individual *Arabidopsis* gene promoters: MP5, MP8, MP14, & MP18.
- DNA methylation of the edited plants in the locus-specific edited genes was compared to that of the wild-type control plants by bisulfite genomic sequencing.
- Epigenetic profiling was conducted by analyzing DNA methylation patterns of promoter regions flanking target sequences.

Outcomes

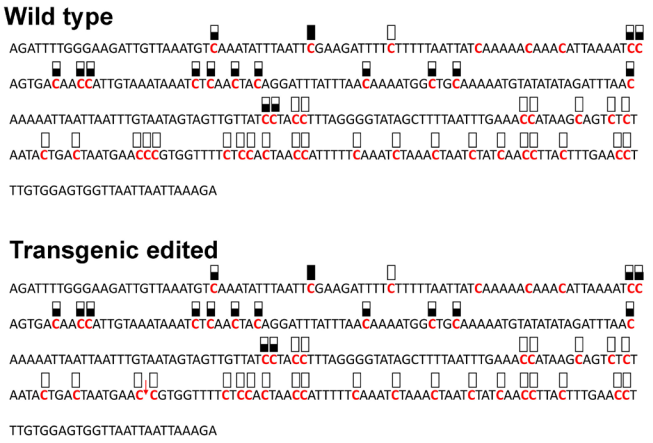
- Phenotypes of wild-type and transgenic edited plants were similar.
- Methylation patterns of promoter regions flanking target sequences were identical among wild-type and transgenic edited plants.
- There was no effect of mutation type on epigenetic status.
- No off-target mutations were detected in candidate sites.
- CRISPR/Cas9 did not leave an epigenetic footprint on either the immediate gene-edited DNA and flanking DNA or introduce off-target mutations.

Significance

The present study answers an important question for basic science as well as risk assessment in plant genome editing. It provides experimental evidence important to the technology development of the CRISPR/Cas system by showing no apparent epigenetic changes. Given the increased usage of CRISPR/Cas9, determining unintended effects beyond off-target mutations such as methylation status presented here provides further insights into the application of this precise genome editing platform.



Chromatograms from edited plants. The red boxes in the figures indicate mutation locus and type. **a**) Contains a single deletion, **b**) & **c**) each has an added nucleotide of thymine, and **d**) contains a four-nucleotide deletion.



Representative methylation pattern of the MP5 locus in wild type and transgenic edited plants. Solid boxes indicate that the cytosine at this position were methylated (>90%), open boxes indicate that cytosine methylation was not detected (<10%), and half-shaded boxes indicate that the cytosine was methylated at a range of 40-60%. Arrow in transgenic edited indicates the one nucleotide "C" deletion.

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