

CRISPR-Cas9 gene editing unlikely to cause off-target mutations in plants, study shows

CRISPR-Cas9 enabled genome engineering has great potential for improving agriculture productivity, but the possibility of unintended off-target edits has evoked some concerns. Here we employ a three-step strategy to investigate Cas9 nuclease specificity in a complex plant genome. Our approach pairs computational prediction with genome-wide biochemical off-target detection followed by validation in maize plants.

Our results reveal high frequency (up to 90%) on-target editing with no evidence of off-target cleavage activity when guide RNAs were bioinformatically predicted to be specific. Predictable off-target edits were observed but only with a promiscuous guide RNA intentionally designed to validate our approach. Off-target editing can be minimized by designing guide RNAs that are different from other genomic locations by at least three mismatches in combination with at least one mismatch occurring in the PAM proximal region. With well-designed guides, genetic variation from Cas9 off-target cleavage in plants is negligible, and much less than inherent variation.

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The work described herein illustrates that CRISPR-Cas9 is remarkably specific and efficient at generating on-target genome edits While CRISPR-Cas9 has the potential to generate off-target cutting in genomic sites that are substantially similar to the target site, off-target edits are likely to be negligible in the background of existing natural variation and continuous unintended changes being generated during the plant breeding process.

Finally, regardless of the breeding method, standard practices of commercial crop development include advancement of candidate lines following extensive agronomic evaluations specific for a given crop. This has proven to be an effective tool to eliminate plants with undesirable characteristics resulting in crops with a history of safe use. Therefore, concerns related to specificity of CRISPR-Cas9 technology in crop improvement have little relevance.

Read full, original article: [CRISPR-Cas9 Editing in Maize: Systematic Evaluation of Off-target Activity and Its Relevance in Crop Improvement](#)