CRISPR/Cas-based genome engineering

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The CRISPR/Cas system is becoming the major tool for targeted mutagenesis in eukaryotes to induce either double-strand breaks (DSBs) or single-strand breaks at preselected genomic sites. Thus, homologous recombination (HR) can be enhanced and targeted mutagenesis can be achieved by error-prone non-homologous end joining (NHEJ). Recently, we were able to demonstrate heritable targeted mutagenesis in \textit{Arabidopsis thaliana} as well as the first application of a Cas9 nickase in plants. Using a natural nuclease and marker genes, we also developed an \textit{in planta} gene targeting (GT) strategy in which both the GT vector and the target locus are activated simultaneously via DSB induction during plant development.

We demonstrate that the \textit{in planta} GT strategy can be used for natural genes by Cas9-mediated DSB induction. We were able to integrate a resistance cassette into the ADH1 locus of \textit{A. thaliana} via HR. Heritable events were identified using PCR-based genotyping, characterized by Southern blotting and confirmed on the sequence level.

Moreover, a major concern is the specificity of the CRISPR/Cas system. Off-target effects might be avoided using two adjacent sgRNA target sequences to guide the Cas9 nickase to each of the two DNA strands, resulting in the formation of a DSB. By amplicon deep sequencing, we demonstrate that this Cas9 paired nickase strategy has a mutagenic potential comparable to that of the nuclease. We also demonstrate the stable inheritance of such mutations in \textit{A. thaliana}.

Taken together, we provide the plant community with a highly efficient CRISPR/Cas system. Most notably, the \textit{in planta} GT strategy does not rely on efficient transformation and regeneration procedures, indicating the benefit for application in crop plants to improve elite cultivars.

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