CRISPR/Cas-based genome engineering

Friedrich Fauser[1], Simon Schiml[1], Jeannette Steinert[1], Gabriele Ilg[1] and Holger Puchta[1]
[1] Karlsruhe Institute of Technology (KIT), Botany II
Email of corresponding author: friedrich.fauser@kit.edu

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 *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 *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 *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 *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 *A. thaliana*.

Taken together, we provide the plant community with a highly efficient CRISPR/Cas system. Most notably, the *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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