Efficient induction of inversions in plant genomes using the CRISPR/Cas system

Carla Schmidt, Michael Pacher and Holger Puchta
Karlsruhe Institute of Technology, Botanical Institute, Karlsruhe
E-mail of corresponding author: carla.schmidt@kit.edu

CRISPR/Cas mediated genome engineering in plants has mainly concentrated on the knockout of genes and the induction of deletions. Sequence inversions occur often during plant genome evolution - also in related cultivars - making the inverted regions inaccessible for plant breeders as a result of fixed linkages. Thus, it is important to set up technologies that allow us to induce inversions within chromosomes in a directed and efficient way in plants.

In the current research, we induced two DSBs in a distance of about 3 - 18 kb using the Cas9 protein from *Staphylococcus aureus* (*Sau*Cas9) for efficient DSB induction on four different loci in the model plant *Arabidopsis thaliana*. The expression of the Cas9 nuclease was controlled by the constitutive PcUbiquitin4-2 promoter from *Petroselinum crispum* (PcUbi4-2) and by the egg-cell specific promotor (EC-P) of *A. thaliana*. The approach, driven by the PcUbi4-2 promotor, was analysed in T1 using digital droplet PCR (ddPCR) to quantify the amount of deletions and inversions in wildtype and *ku70-1* mutants. It was possible to generate deletions with frequencies up to 7 % and inversions up to 2 %. Additionally, we defined via deep sequencing the patterns of junction formation in wildtype and *ku70-1* mutants. Like for deletions, in the majority of cases for inversions re-joining of the cut junctions occurs without further mutations. Surprisingly, in plants deficient in KU70, which is essential for classical non-homologous end joining (NHEJ), inversion induction is enhanced. However, most junctions - that often contain micro homologies - are imperfect with insertions and mainly deletions. Using the egg cell specific expression we were able to induce heritable inversion formation at different loci and at distances between 3 and 18 kb in the percent range. By screening individual lines heritable inversion events up to the 10 % range can be regularly achieved. Most of the events contained the inversion with scarless junctions and without any sequence change within the inverted region making the technology attractive for applications in crop plants.