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Transgene-free targeted mutation in rapeseed (Brassica napus L.) via transient CRISPR-Cas9 expression in protoplasts

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In Brassica napus, only a few studies are published on genome editing which all rely on stable transformation via A.tumefaciens. Transgenic approaches have the disadvantage that the constitutive expression of cas9 and sgRNA support the regeneration of chimeric plants carrying different mutations and might increase the chance of off-targets. Outcrossing of the CRISPR/ Cas expression cassette allows the selection of transgene-free progenies with site-specific mutations. However, this prolongs the whole process to select suitable mutants. Therefore, in several crop plants transgene-free approaches are already developed either via transient expression of the CRISPR/Cas components or via DNA-free transfer of the ribonucleoprotein complex.

In rapeseed, we demonstrate first time that through transient CRISPR/Cas expression in protoplasts mutated plants can be regenerated without transgene integration.

As a model gene we used the CLA1 gene for site-directed mutagenesis of the amphidiploid genome of Brassica. Cla1 encodes the 1-deoxy-d-xylulose-5-phosphate synthase which is required for chloroplast development. For transient expression of CRISPR/ Cas9 components we designed a multiplex vector which allow the targeting of all 4 alleles in the allotetraploid rapeseed genome. We improved the PEG-mediated transfection efficiency in rapeseed protoplasts using GFP as reporter gene. On average more than 25% of transfected protoplasts showed green fluorescence. Additionally, plant regeneration from transiently transfected protoplasts was optimized. Routinely, shoots can be induced from >50% of protoplast-derived calli.

We regenerated more than 1.000 plants from two transfection experiments. Around 865 plants were analyzed for mutations in the CLA-1 gene via PCR and capillary electrophoresis. Randomly, mutant lines were selected for DNA sequencing. Around 5% of the regenerated plants showed mutations in at least one allele. Deletions (1 to 104 bp) and insertions (1 to 156 bp) were detected. Most mutated plants (35%) carried deletions of around 100 bp corresponding to the distance of the genome specific guide RNA pair. According to statistical calculation, four albino plants were regenerated which show deletions in all 4 alleles. In the meantime, the efficiency of our rapeseed mutation platform could be further shown by three other targets.