Accelerating the breeding of carrots (*Daucus carota* L.) via CRISPR/Cas9

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Traditional plant breeding aims to develop plant cultivars that produce a high quality product with a constant yield, but the fast changing climate with more extreme conditions calls for a fast adaptation of crop varieties. While hybrid breeding results in a uniform progeny profiting from the heterosis effect, it is a time consuming challenge to produce homozygous parental lines which are needed for hybrid breeding in cross-pollinated crops like carrot (*Daucus carota* L.). To find a universal tool and to prevent the necessity of homozygous parental lines by back crossings, we aim to accelerate traditional F1 hybrid breeding by using the new plant breeding technique (NBPTs) CRISPR/Cas9.

To achieve (doubled) haploid and therefore completely homozygous parental lines in one generation, we target a gene that is involved in the first cell divisions of the early embryo. During cell division, the microtubules of the spindle apparatus attach to the kinetochore region of the centromere to distribute chromatids equally. Therefore, a compromised function of the centromere specific histone H3 protein (CENH3), as part of the kinetochore, can result in an uneven distribution of chromosomes. In our approach, one parental gamete carries chromosomes with compromised CENH3 due to mutations induced by CRISPR/Cas9 while the other gamete still carries the wild type CENH3. A compromised centromere function of the mutant allows for a uniparental genome elimination of the mutated parent genome when crossed with a wild type plant. The resulting (doubled) haploid progeny can directly be used in hybrid breeding.

We used *Rhizobium rhizogenes* to introduce a CRISPR/Cas9 expression cassette that induces mutations inside the CENH3 coding region. Immunostaining against CENH3 with specific antibodies showed a change in the phenotype, with mutated lines showing a weaker fluorescence compared to the wild type controls.

In order to facilitate this approach, we established a fast, transient and DNA-free method for genome editing in carrot by introducing preassembled sgRNA and Cas9 protein into carrot protoplasts via PEG mediated transformation. Since this complex degrades after around 48 h, the regenerated plant lines carries no foreign genes. A putative lethal effect of induced mutations was visible in a lower regeneration capacity of the protoplasts with only wild type regenerates. To screen for regeneration capacity and viability of mutated carrot lines, we are comparing different target regions inside the CENH3 gene.