Targeted modifications of centromeric histone H3 (CENH3) by using CRISPR/Cas9 in carrots (*Daucus carota* L.)

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Plant breeding needs to evolve constantly to address the growing demands concerning not only the yield but also the biotic and abiotic behavior of crops. At the same time it is facing climate change and a decrease in arable land area. While a lot of studies about improving breeding strategies focus on major crops like cereal or maize we bring attention to accelerate the breeding of carrot (*Daucus carota* L.), a subculture with a high content of secondary metabolites which makes it a great addition to a colorful and wholesome diet.

The main breeding method of carrot is F_1 hybrid breeding. However, the production of genetically homogeneous parental lines through several subsequent steps of inbreeding takes up a lot of time and resources. The production of haploid parental lines by tissue culture techniques has been proven to be inefficient in plants of the family of *Apiaceae.* Interspecific hybridization is yet unknown to achieve haploids in *Daucus* species. We therefore propose to apply the RNA guided endonucleases (RGEN) technique CRISPR/Cas9 to modify the kinetochore specific centromeric histone H3 (CENH3) which is crucial for the proper segregation of chromosomes during cell division.

In eudicots CENH3 consists of a highly conserved C-terminal histone fold domain (HFD) and a N-terminal tail that varies between species in its length and sequence. Modifications and possible loss of function of CENH3 to provoke uniparental genome elimination during early embryogenesis has been proposed as a new plant breeding technique (NPBT) for haploid induction.

We target different regions of the CENH3 sequence and compare mutated lines in their expression and accumulation of CENH3 as well as in their function as putative haploid inducer lines.

The introduction of an expression cassette for CRISPR/Cas9 by agrobacterium-mediated plant transformation via *Rhizobium rhizogenes* resulted in a high number of transgenic hairy root lines. We therefore screened hairy root lines for mutations induced by the non homologous end joining (NHEJ) pathway in the target region prior to somatic embryogenesis to identify highly mutated lines. Changes in the accumulation of CENH3 in mutated lines were visualized by staining with a specific antibody in cytogenetic studies.

We found changes in the geno- and phenotype of CENH3 in transgenic hairy root lines and produced transgenic plants carrying a variety of mutations in the targeted region inside of the CENH3 sequence.