# Accelerating the breeding of carrots (*Daucus carota* L.) by editing the centromeric histone H3 (CENH3) using CRISPR/Cas9

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Today`s world is faced with a number of hardships. The climate is changing at an accelerated speed. Droughts, floods and other weather extremes call for a fast-adapting agricultural landscape, while the world's population grows exponentially at the same time. Therefore, we focus on accelerating F1 hybrid breeding to create a wider variety of highly adapted cultivars to use the available natural resources as productive as possible. In our work we focus on carrot (*Daucus carota* L.), a subculture with a high content of secondary metabolites, that supports a colorful and wholesome diet with an additional long history as a model organism.

The production of genetically homogeneous parental lines through several subsequent steps of inbreeding takes up an excessive amount of time and resources. Other *in vitro* (e.g. anther or ovule culture) or *in situ* (e.g. wide hybridization or irritated pollen) techniques to produce haploid or double haploid progeny are inefficient in Apiaceae. We therefore propose to induce targeted mutations in the coding region for the centromeric specific histone H3 (CENH3) using the RNA guided endonucleases (RGEN) technique CRISPR/Cas9.

The equal distribution of chromosomes during cell division depends highly on CENH3, therefore its editing and putative loss of function could provoke uniparental genome elimination of the mutated parent during early embryogenesis when crossed with a plant that has functional CENH3.

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.

We found plants with a chimeric CENH3 genotype and variations of CENH3 accumulation after introducing an expression cassette for CRISPR/Cas9 via the agrobacterium *Rhizobium rhizogenes.* While testing for viable putative haploid inducer lines by self-crossings and crossings with wild type plants, we are establishing transformation systems using *Agrobacterium tumefaciens* to fasten the transformation process and to therefore be able to test additional target regions creating a higher number of putative haploid inducer lines in a shorter time.

We are also working on a DNA-free protocol to induce targeted mutations transiently. Here protoplasts are incubated with preassembled sgRNA and Cas9 proteins, allowing for mutated but non-transgenic lines with compromised CENH3.