Establishment of genome engineering in tobacco aiming to develop novel haploid technology

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Haploid technology is widely used in basic and applied research to produce genetically fixed plants in one step. However, current methods have diverse limitations, such as species and genotype dependency, which is rather unlikely to be overcome through the improvement of existing protocols. The aim of this project is to establish novel haploid technology in tobacco, which relies on uni-parental genome elimination as consequence of modified functionality of the centromere-specific histone CENH3. This principle was previously found and experimentally demonstrated in *Arabidopsis thaliana* by Ravi and Chan (2010) who showed that upon crossing of the wild-type with a parent that expresses a modified derivative instead of the native *CENH3*, the genome of this so called inducer line is eliminated during subsequent zygotic embryogenesis. The duplication of the remaining haploid wild-type genome results in fertile and entirely true-breeding plants (doubled haploids). Since CENH3 is highly conserved in eukaryotes, the method could be adopted in many crop species. The concept of the present study includes the functional knock-out of the native *CENH3* and its partial complementation by functionally attenuated *cenh3*-derivatives. *Nicotiana benthamiana*, which serves as a well-manageable experimental model for dicot species, is used to test those candidate *cenh3*-derivatives. The *CENH3* gene of *N. benthamiana* had been cloned, two chimeric constructs assembled and transgenic plants carrying one of the modified *CENH3s* were produced. In addition, transcription activator-like effector nucleases (TALENs) targeting the native *NbCENH3* were designed to knock-out the native CENH3 via site-directed mutagenesis. However, in the plants carrying one of the *cenh3*-derivatives and both TALEN-units, mutations could not be detected in the TALEN target region until now. For this reason, we alternatively use RNA-guided endonucleases (RGENs) to target the native *NbCENH3*. To establish the RGEN technology in tobacco, a *gfp*-specific RGEN construct was expressed in plants carrying a single copy of the *GFP* reporter gene and sequencing of *GFP* indeed revealed various indels in its target site.

Literature: Ravi M and SWL Chan (2010): Haploid plants produced by centromere-mediated genome elimination. Nature 464, 615-619