Haploid induction after targeted mutagenesis of *CENTROMERIC HISTONE 3* in barley

Stefan Hiekel, Maia Gurushidze, Sindy Schedel, Takayoshi Ishii, Andreas Houben and Jochen Kumlehn

Ag Pflanzliche Reproduktionsbiologie,
Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK)
hiekel@ipk-gatersleben.de

Abstract: Genetically stable lines can be produced either by time-consuming and laborious selfing over numerous generations or in just one step by employing haploid technology. Due to various constraints of current haploid technology (e.g. genotype-dependency, challenging cell culture procedures, recombination bias in DH-populations) there is a strong demand for alternative or even universally useful methods applicable in many crop species. Therefore, we aim to establish a novel method in the model cereal crop barley based upon uni-parental genome elimination as a result of a functional modification in the centromere-specific histone 3 (CENH3). In *Arabidopsis*, the replacement of native CENH3 by an altered derivative (GFP-*tailswap*-CENH3) was demonstrated to result in plants having a certain capacity of producing haploid progeny. Crossed with any CENH3 wild type plant of the same species, these lines trigger the elimination of their own chromosomes during early embryo development (Ravi and Chan, 2010). To produce such inducer-lines for barley, we stably expressed a *GFP-tailswap-HvCENH3α* transgene and confirmed the localization of its product to all barley centromeres. In addition, we created functional knock out (KO) alleles of *HvCENH3α* via targeted mutagenesis using RNA-guided endonucleases (RGENs). Crossing of *cenh3α* KO mutants with wild type barley results in the elimination of the *cenh3α* KO allele-carrying genome, which, via embryo rescue, can entail the formation of haploid plants.

Literature:

Ravi, M. and Chan, S.W. 2010.
Haploid plants produced by centromere-mediated genome elimination. Nature 464:615-618.