Designer nuclease mediated gene knockout in barley – a TALEN approach

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Gene targeting is a breakthrough technology that will greatly facilitate the functional validation of genes and offers versatile novel possibilities for crop improvement. Recently, transcription activator-like effector nucleases (TALENs) have rapidly developed into a powerful genome targeting tool. TALENs are customizable fusion proteins of DNA binding domains from transcription activator-like effectors and *Fok*I endonuclease, that cause double strand breaks (DSBs) at a user-defined genomic position. DSBs are then processed by the cell's DNA repair machinery, which is error-prone to some extent and thus leaves mutated target sites behind. To establish TALEN-mediated gene targeting in a cereal crop, a *gfp* specific TALEN pair was designed *in silico* and respective expression cassettes were generated. These were then transferred by means of *Agrobacterium* to embryogenic pollen cultures obtained from barley carrying a homozygous single-copy of the *gfp* reporter gene. In this setup, a deleterious mutation of just one *gfp* allele can be phenotypically detected thanks to the haploid nature of the pollen-derived target cells. Screening for loss of fluorescence as well as sequencing of the *gfp* gene of TALEN-transgenics did indeed result in the identification of *gfp* knockout mutants. More than 10 percent of the *gfp* plants that proved to be retransformed with TALEN-coding sequences showed independent mutations in the *gfp* gene. In particular, deletions of 4 to 36 nucleotides and in one case an additional 8-nucleotide duplication were found. In almost all mutants, at least one of the TALEN binding motifs were affected by the genetic alterations so that the TALENs were no longer able to bind and digest the modified target site. Mutants that were further analysed showed faithful sexual transmission of the altered *gfp* allele with no segregation of the knockout phenotype seen in pollen and progeny. This indicates that modification of the *gfp* gene has taken place prior to genome duplication, which rendered the mutant allele homozygous in these plants. In this context it is particularly remarkable that TALENs act as mutagen *in planta* rather than giving rise to recombinant DNA *sensu strictu*. In addition, our results may also pave the way for the establishment of even more sophisticated procedures using TALENs to precisely edit cereal and other crop plant genomes based upon DSB repair *via* homologous recombination.