Targeted modification of gene function via homology-directed genome editing in barley

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Programmable endonucleases like transcription activator-like effector nucleases (TALENs) or RNA-guided nucleases (RGNs) open up new opportunities for targeted modifications in eukaryotic genomes. These endonucleases comprise a customizable DNA-binding module and a DNA-cleaving module which enables them to cut DNA at predefined target sites so that genetic modifications can be achieved through error-prone DNA-repair. In addition, more sophisticated applications of programmable endonucleases involve the use of a DNA repair template facilitating homology-directed repair (HDR) so as to create predefined rather than random DNA sequence modifications. Beside gene knock-out approaches, we have demonstrated that also a targeted allele conversion can be achieved in barley after TALEN- or RGN-mediated double strand break induction by concomitant provision of a repair template leading to an exchange of a single amino acid which itself entails an altered protein function. In addition, a test system based on transient expression in plant cells was developed to validate the activity of TALEN or RGN constructs which greatly facilitates the optimization of construct design as well as the preselection of effective endonuclease variants prior to their application using stable transformation.