Abstract

Genome editing in barley - Fragment deletions induced by the CRISPR/Cas9 system in the ENGase gene

Designer nucleases (e.g. Zinc-Finger nucleases, TALENs, CRISPR/Cas9 System) are popular tools to induce mutations through double strand breaks or for the introduction of DNA sequences via homologous recombination. The CRISPR/Cas9 System from *Streptococcus pyogenes* is an increasingly popular tool for genome editing due to its ease of application. Here we demonstrate disruption of the barley (*Hordeum vulgare* cv. "Golden Promise") endo-N-acetyl-β-D-glucosaminidase (ENGase) gene using RNA directed Cas9 nuclease. ENGase plays a role in the de-glycolysation of N-glycoproteins in plants. Five single guide RNAs (sgRNAs) were designed for different target sites in the upstream part of the ENGase coding region. Targeted fragment deletions were induced by co-bombarding selected combinations of sgRNA with wild-type cas9 using separate plasmids, or by co-infection with separate *Agrobacterium tumefaciens* cultures. Genotype screening was carried out in the primary transformants (T0) and their T1 progeny to confirm the presence of site-specific small deletions/insertions (indels) and genomic fragment deletions between pairs of targets. Cas9-triggered site-specific mutations were induced with high efficiency (78%). The detected indels and fragment deletions were successfully transmitted to the T1 generation, and transgene free (sgRNA:cas9 negative) genome-edited homozygous ENGase knock outs were identified among the T1 progeny. The high mutation frequency confirms the exceptional efficiency of the system and its suitability for generating loss-of-function mutant lines. Immature pollen culture facilitated the generation of doubled haploids from genome edited plants and allowed the recovery of instantly homozygous, true breeding lines. Such plants may be used in functional genetics approaches in order to study e.g. the glycosylation pathway processes of recombinant proteins. For this, lines expressing the heavy and light chain of a recombinant antibody were crossed with ENGase knock-out lines. The hybrid progeny contains the two genes of interest in a hemi/heterozygous state. In order to produce instantly homozygous plants, the doubled haploid technique was applied. Among the regenerants plants were selected, which express the heterologous protein, but lack functional ENGase. Such progeny will be analysed for changes in the N-glycosylation pattern of the antibody.