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"Knowledge-based, Smart Agriculture and Contract Farming"

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Speech Topic: Precise Gene Editing Using Ribonucleoprotein-complexes

Abstract:

Targeted mutagenesis employing sequence-specific endonucleases (SSE) such as CRISPR/Cas technology has been demonstrated in various plant species. This technique's frontiers still lack predictability of the outcome since SSE-introduced double-strand breaks are resulting in insertions and deletions (InDels), which are itself not predictable. Using the cell's homology-directed repair (HDR) mechanism, a predicted allele exchange can be introduced into the loci by providing a synthetic repair template including the desired gene modification. One way to achieve this precise allele exchange is the use of ribonucleoprotein complexes (RNP). A synthetic sequence-specific gRNA and a Cas protein are assembled *in vitro* and transferred together with the allele exchange-specific repair template into the cell. To facilitate easy detection of homology-directed genomic modifications, we follow two approaches. Employing *Gfp*-transgenic barley plants, we use *Gfp*-specific guides for Cas9/Cas12a-mediated double-strand break induction and application of a custom *Yfp* repair template. After successful HDR, Yfp fluorescence can be used as a readout. In a second attempt, endogenous *LOX1* gene was targeted, and the *Eco*RI restrictions site was integrated for easier detection of HDR products.
Presented are data comparing SpCas9 and AsCas12a endonuclease in barley epidermal leaf cells considering their different features. To interfere with the ratio between preferred non-homologous end joining (NHEJ) and HDR, RNAi was used to repress key genes involved in NHEJ. RNAi constructs targeting *Ku70*, *Ku80* and *Ligase IV* genes were generated and used for transient and stable barley integration.
