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Poster Communication Abstract – 7.13

GENOME EDITING IN ALFALFA BY INTRON TARGETING USING CRISPR-CAS9

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Genome editing in autotetraploid plants such as alfalfa (2n=4x=32) has the potential to modify all four homologous copies of a gene of interest at the same time. In this work, we aim to accomplish precise gene editing by homologous recombination (HR) mediated by CRISPR-Cas9 in alfalfa. As a proof of concept, we target the glutamate-1-semialdehyde aminotransferase (GSA) gene by introducing a point mutation that confers gabaculine resistance. The use of two selection systems will increase the chances of success: the conventional selectable marker system *npt*II–kanamycin allows to select transformation events, and subsequently the GSA–gabaculine selection system allows selection for the HR events. We set out to introduce two point mutations in the GSA sequence, one in the third exon (conferring gabaculine resistance) and one in the first intron (providing a diagnostic restriction site). Therefore, we choose a target sequence in the second intron, so that any sequence modification in the target site should not affect gene function. Considering the genomic GSA sequence (start-stop), the chosen target site leaves about 460 bp as the left (5') homologous recombination region (LHR) and about 1000 bp as the right (3') HR region (RHR). Agrobacterium-mediated transformation of the *M. sativa* genotype RSY1 yielded 9 kanamycin-resistant events. A second regeneration cycle was performed with gabaculine selection in an attempt to enrich for homologous recombination events. No clear-cut gabaculine resistant event was observed, indicating that HR did not occur or that any HR-derived

allele is not capable to confer resistance; in fact, gabaculine resistance was previously obtained by constitutive overexpression of the mutant gene, whereas in HR events the mutant gene would be expressed by its edogenous promoter. Restriction enzyme-based screening also did not show the presence of HR events. However, TIDE (Tracking of Indels by Decomposition) analysis clearly indicates that we have obtained 4 editing (indel mutation) events (44.4%). Sequencing of cloned alleles is underway to confirm this result, to characterize the mutation types and zygosities, and to reveal any HR-edited allele.

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