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Poster Communication Abstract – 1.14

USE OF THE CRISPR/CAS9 TECHNIQUE TO INCREASE THE ASCORBIC ACID CONTENT IN LETTUCE LEAVES

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Among the Genome Editing techniques CRISPR/Cas9 is reported as more efficient in targeting specific regions of the gene sequence. It is divided in 2 components, Cas9 nucleases and a sgRNA that form a complex in which 20 bp of the gRNA guides the Cas9 to a specific target DNA using Watson-Crick base pairing. The double stranded break (DSB) created by Cas9 is repaired in the cell either by error prone Non-Homologous End-Joining (NHEJ), resulting in small random insertion/deletions at the cleavage site, or by high fidelity Homology-Directed Repair (HDR), resulting in precise modification at the site of the double stranded breaks using a homologous repair template.

With the aim to increase ascorbate in Lactuca sativa the CRISPR/Ca9 technique was used to knock out three genes encoding negative regulators of the Ascorbic Acid (AsA) biosynthesis pathway. The three genes are: CSN5B, CSN8 (encoding two subunits of the protein complex named Photomorphogenic COP9 Signalosome, (CSN) and Monodehydroascorbate reductase (MDHAR) gene. CSN5B and CSN8 proteins are involved in the ubiquitination of GDP-D-Mannose pyrophosphorylase (GMP) enzyme in the dark and in its degradation through the 26S proteasome, leading to a decreased AsA content in Arabidopsis leaves. The MDHAR gene is involved in the recycling pathway of AsA and one of its isoforms has been shown to act as a negative regulator of ascorbate production in tomato. In lettuce genome, four different isoforms of MDHAR gene have been identified and we decided to knockout all the isoforms for a total of six target genes. Four gRNAs were selected for each target gene and assembled into a plasmid using the Golden Gate Cloning technique. For this experiment, every L2 plasmid created contains the gRNAs cassette (four

gRNAs with the tracrRNA), Cas9 gene, kanamycin resistance gene and dsRED gene to check the in vivo expression of the plasmid.

Each plasmid was delivered into the plant via the Agrobacterium tumefaciens strain LBA4404, infecting 4-days-old lettuce cotyledons. 139 plants were obtained through the regeneration process, of which 122 were transformed. The sequencing of target genes showed that fourteen plants showed mutations: two mutants from MDHAR1 plants, seven from MDHAR2 plants, one from MDHAR3 plants, three from MDHAR4 plants and one from CSN8 plants. No editing has been highlighted for the CSN5B gene. Most of the mutations led to frameshift alteration resulting from indels (base insertion and deletion), generating a stop codon in the coding sequence that could lead to truncated or no longer functional proteins, thus knocking out the gene sequence.

In addition, the transformed T1 plants have flowered, and a significant number of seeds have already been collected. The AsA content in T2 plants homozygous for the mutations will be evaluated using biochemical assays.