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## UNVEILING THE ROLE OF CRISPR/CAS9-GENERATED MDHARS MUTANTS IN LACTUCA SATIVA

ROGO U.*, VIVIANI A.**, PUGLIESI C.*, FAMBRINI M.*, MASCAGNI F.*, CAVALLINI A.*, NATALI L.*, MICHELMORE R. W.***, GIORDANI T.*

*) Department of Agriculture, Food and Environment, University of Pisa, Pisa, Italy
**) [] Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy
***) The Genome Center, University of California, Davis, USA

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Ascorbic acid plays a vital role as an antioxidant and nutrient in plants, contributing to the regulation of reactive oxygen species (ROS) levels and cellular redox balance. Total ascorbic acid (T-AsA) consists of reduced form (AsA) and oxidized forms, including monodehydroascorbate (MDHA) and dehydroascorbate (DHA). AsA synthesis in plants encompasses four biosynthesis pathways, with the Smirnoff-Wheeler pathway being the principal one. Additionally, a crucial recycling pathway contributes to maintaining a reduced and oxidized form ratio balanced. Several genes are involved in this pathway, including *monodehydroascorbate reductase* (*MDHAR*) isoforms responsible for reconverting MDHA in AsA. Using the CRISPR-Cas9 system, we selectively knocked out the *MDHAR1-4* genes in *Lactuca sativa* (cv. Cobham Green), enabling investigation into the functional implications of these genes. We extensively characterized the phenotypic and genotypic traits of the resulting mutant genotypes (M1, M2, M3, and M4).

In various plant species, both down-expression and over-expression of *MDHAR* isoforms have shown significant roles in responding to biotic and abiotic stresses. Thus, our study aims to explore the impact of each MDHAR isoform on AsA content, gene expression, and physiological responses under standard and stress conditions in lettuce. It was observed, under standard conditions, a statistical decrease in both T-AsA and AsA only for the mdhar3 mutant (M3 plants) at the 14-day leaf stage, while no significant

differences were observed at the 30-day leaf stage. qRT-PCR analysis demonstrated high expression levels of *LsMDHAR2* and *LsMDHAR4* gene isoforms in both 14-day and 30-day-old wild-type lettuce plants. In contrast, *LsMDHAR1* and *LsMDHAR3* isoforms exhibited minimal transcription levels. Moreover, mdhar mutants exhibited a significant decrease in the gene target transcriptions. Notably, deactivating one isoform often resulted in a reduction of expression in other isoforms.

Since salinity and light intensity are critical factors influencing the biosynthesis and recycling pathways of AsA, we are studying the response of these mutants under specific stress conditions.

The comprehensive investigation into *MDHAR* isoforms and their pivotal role in the ascorbic acid recycling pathway within lettuce not only unlocks tremendous potential for scientific advancements but also offers a compelling opportunity to revolutionize crop quality and face the pressing challenges posed by climate change.