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CRISPR/CAS9-MEDIATED MUTAGENESIS TO IMPROVE THE NUTRITIONAL QUALITY OF TOMATO FRUITS.

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Tomato (*Solanum lycopersicum* L.) berries are a rich source of antioxidants and other bioactive compounds, including Ascorbic Acid (AsA), which plays a key role in human health. Plants produce AsA through several biochemical pathways and many genes and enzymes in those pathways have been identified in tomato. About 25 years ago introgression lines (ILs) or sub-lines were produced, in which genomic regions of *S. lycopersicum* cv. M82 were replaced with the corresponding segments of the wild *S. pennellii* genome. In our laboratory, several sub-lines have been produced and characterized that accumulate high content of AsA in the fruits. Recently, we identified a gene in the wild region of the IL7-3 that encodes an ascorbate oxidase (AO) and that regulates the accumulation of AsA in tomato fruits. Further investigations on ILs have allowed demonstrating that pectin methylesterases (PMEs) and pectin methylesterases inhibitors (PMEIs) play a fundamental role in the biosynthesis of AsA using the D-galacturonic acid derived from pectins as metabolic precursor.
Within this motivating context, we would like to exploit the great potential of the genome editing techniques to accelerate breeding programs with the aim of improving the organoleptic and nutritional (increase in AsA content) quality of tomato fruits. To achieve our goals, we selected three target genes, potentially involved in the AsA accumulation and encoding an ascorbate oxidase, a laccase (LAC) and a PMEI, respectively. We designed single-guide RNA molecules for all candidate genes and constructed CRISPR/Cas9 plasmids for knock-out experiments. We used different strategies to design sgRNA expression plasmids: LAC and AO were targeted with four individual sgRNAs, while PMEI with a single guide. Genetic transformation of tomato cotyledons (Red Setter and MoneyMaker) was mediated by *A. tumefaciens* strain LBA4404. At present, we obtained two Cas9+ plants for the LAC gene and seven for the AO gene. The Cas9+ plants were micropropagated and *in vivo* transferred to obtain T1 seeds. Sanger sequencing was performed to confirm gene knock-out. Other genetic transformations are ongoing for these two target genes in order to increase the number of successfully edited plants. As for PMEI, we obtained 34 regenerated explants on selection media and the validation of the Cas9+ events is ongoing. In the near future, morpho-physiological and biochemical analysis (i.e., plant phenotyping; evaluation of the AsA content and total antioxidant activity) on the fruits harvested from the mutated plants will be performed to verify the improvement in the nutritional quality of tomato fruits.