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IMPROVING THE NUTRITIONAL QUALITY OF TOMATOES THROUGH CRISPR/CAS9-MEDIATED MUTAGENESIS

FRANCESCA S.*, SEVI F.***, AUFERIO G.*, OLIVIERI F.*, FIORE A.**, BARONE A.*, CHIAIESE P.*, D'AGOSTINO N.*, RIGANO M. M.*

*) Department of Agricultural Sciences, University of Naples Federico II, Portici, Naples, Italy
**) Italian National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA), Casaccia Research Centre, Rome, Italy

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Ascorbic acid (AsA) is an essential antioxidant for humans who need to get it from food. Tomato (Solanum lycopersicum L.) fruits are rich in antioxidants and other bioactive compounds, including AsA. The increase in AsA content can be achieved by amplifying its biosynthesis or by inhibiting its degradation. In plants, AsA can be synthesized via four pathways: the D-mannose/L-galactose pathway, the D-glucose pathway, the myo-inositol pathway, and the alternative D-galacturonate pathway. Most of the genes and enzymes in those pathways have been identified in tomato. About 25 years ago S. lycopersicum cv. M82 X S. pennellii introgression lines (ILs) were produced.

In our laboratory, various sub-lines have been generated and characterized which accumulate a greater quantity of AsA in fruits. Recently, we have identified an ascorbate oxidase gene (AO) in the wild region of the IL7-3 that regulates the accumulation of AsA in tomato fruits. Further investigations on ILs allowed to demonstrate that pectin methylesterases (PMEs), play a key role in the biosynthesis of AsA using pectin-derived D-galacturonic acid as metabolic precursor. Indeed, it is likely that the gene knock-out of pectin methylesterase inhibitors (PMEIs) can lead to an increase in AsA content.

We constructed CRISPR/Cas9 vectors for the two target genes and genetic manipulation was mediated by Agrobacterium tumefaciens strain EHA105 on

cotyledon explants (cv Red Setter and Moneymaker). As for the PMEI target gene, a total of 39 antibiotic resistant and Cas9-positive T0 lines were selected, but only four heteroallelic mutants (two with -1/-1 bp deletion and two -2/-5 bp deletion) were identified by Sanger sequencing analysis. At present, the edited plants have been transferred in vivo to obtain T1 seeds. As for the AO gene, we obtained regenerated explants on selection media and the evaluation of the transformation efficiency is ongoing. Soon the fruits of the edited plants will be subjected to morpho-physiological and biochemical analysis to evaluate the AsA levels and the nutritional characteristics of the berries.

In parallel, the mutagenesis efficiency of vectors carrying single guide RNAs for the AO target gene was evaluated by hairy root production. For this purpose, the tomato explants were co-cultured with transgenic A. rhizogenes strain ATCC 15834 for two days and then subcultured in a selective medium. Hairy roots were observed after three weeks of co-cultivation and when they reached 1 cm in length, they were singularly picked and cultivated first in a solid growth medium and then in a liquid medium. The use of hairy roots as a biotechnological tool is useful not only for the validation of plant transformation vectors, but also for early phenotyping currently in progress.