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REGULATION OF ASCORBATE BIOSYNTHESIS IN TOMATO USING GENOME EDITING APPROACHES

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Ascorbic acid (AsA), also known as L-ascorbic acid or Vitamin C, is one of the most important antioxidant compounds in plants. Tomato (*Solanum lycopersicum*), which contributes strongly to human nutrition, is one of the most important AsA sources in the human diet. The regulation of AsA biosynthesis in tomato fruit is not well understood and genome editing approaches could be helpful to clarify the role of specific genes in controlling the AsA content in red ripe fruit. The aim of the present work is the functional validation of three genes potentially involved in biosynthetic pathways leading to AsA accumulation: one UDP-glucoronic-acid-4-epimerase (UglcAE) and two GDP-L-galactosephosphorylases (GGP1 and GGP2). The first gene was chosen because it maps to one of the *S. pennellii* introgression sub-lines, which carries a QTL for high AsA content in fruit (Rigano et al., 2018). The other two genes, involved in the L-Galactose pathway for ascorbate biosynthesis, were chosen since it has been demonstrated that ascorbate concentrations in Arabidopsis and tomato are determined via posttranscriptional repression of GGP (Laing et a., 2015).

The functional validation of the UGlcAE was carried out through its over-expression in M82 tomato. In S. lycopersicum M82 the UGlcAE is represented by gene fragment of 192 bp, but in S. pennellii two identical genes encoding proteins with the UGlcAE family amino acid sequence motifs and an unusual intron in the genomic sequence. The expression analysis of this gene in the red ripe fruits of transformed plants, showed that the S. *pennellii* UglcAE was expressed only in M82 over-expressed fruits. Fruits of these transgenic lines will be assayed for ascorbate content.

The potential role of the two GGP genes in the regulation of ascorbate biosynthesis in tomato was investigated by CRISP/Cas9 mutants affecting the regulatory ORF upstream GGP genes. Most GGP plants showed a phenotype similar to Money Maker, although three of them were phenotypically different compared to the parental genotype, not only for the leaves (thicker than Money Maker), but also for the unusual flower structure; in addition these plants showed difficulties in producing normal fruits, as well as parthenocarpic fruits. As for GGP T_0 genotyping, several mutations were found in GGP1 and GGP2 plants. Finally, a preliminary ascorbate evaluation on red ripe fruits evidenced that one mutant with a missense mutation in the 5' ORF showed the statistically high values in terms of total and oxidised AsA compared to Money Maker. GGP T_1 genotyping is in progress and ascorbate evaluation will be carried out on GGP leaves and red ripe fruits compared to Money Maker controls. Further analysis will be carried out to identify the metabolic profiles of GGP fruits.