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TARGETED MUTAGENESIS BY CRISPR/CAS9 ON SLDET1 GENE AS A TOOL TO IMPROVE THE NUTRITIONAL VALUE OF TOMATO FRUIT

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The tomato *SlDET1* gene is the orthologue of the *Arabidopsis* nuclear protein DE-ETIOLATED 1 (DET1) gene and it has been proposed encoding a negative regulator of the phytochrome signal transduction. Mutations in *SlDET1* gene, such as a C-to-T mutation in exon 11, or an alternative splicing causing a 9 bp-deletion in exon 11, have been found in tomato *high pigment* (*hp2*) mutants. The phenotypes of *hp2* mutants are characterized by light hypersensitivity, displaying elevated levels of anthocyanins in the seedlings, shorter hypocotyls, and more deeply pigmented fruits compared with wild-type plants. Mutations in the 5'-terminal part of the gene generate instead very severe phenotypes in terms of plant growth and survival. In *hp2* mutants, throughout fruit ripening, genes related to chloroplast biogenesis and structural genes involved in phytonutrients (e.g., carotenoids and flavonoids) biosynthesis are up-regulated. Such up-regulation put the plastid biogenesis as an important determinant of phytonutrient overproduction in the *hp2* mutant fruits.

In this study, we developed a strategy to introduce mutations by CRISPR/Cas9 on *SlDET1* that may allow the accumulation of bioactive compounds of nutritional interest in fruits, but resulting in less severe phenotypes than those known so far. For this purpose, sgRNA guides were designed on two different sites of exon 11, and were assembled coupled in our construct. To estimate the rate of targeting efficiency of our sgRNA guides, we used the hairy roots transient assay in tomato, which is a fast and reliable tool to study the possible editing mediated by CRISPR/Cas9. We obtained a range of mutations on *SlDET1* with the both guides, confirming their efficiency, and we applied our approach to tomato stable transformation. Molecular analyses are ongoing to identify targeted mutagenesis on *SlDET1* gene in tomato stable transformants.

These results can contribute to the generation of tomato mutants that could be different from the previously described *hp2* ones, with the high-pigmented fruits during development and ripening. The CRISPR/Cas9-mediated genome editing represents a novel biotechnological strategy to generate new different tomato lines with high levels of important phytonutrients for human health, such as carotenoids and flavonoids, thus improving the nutritional value of this worldwide important crop.