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CRISPR/CAS9 EDITING FOR IMPROVING THE NUTRITIONAL QUALITY OF TOMATO FRUITS

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Nowadays, the growing interest for well-being is influencing the choices of consumers addressing them to eat functional foods with high antioxidant potential. Cultivated tomato (*Solanum lycopersicum* L.) is an excellent source of antioxidants, such as ascorbic acid (AsA or Vitamin C), carotenoids, and phenolic compounds. Epidemiological results confirm that these antioxidant molecules are associated with a reduced risk of cancer, inflammation and cardiovascular disease. Over the years, 76 *Solanum pennellii* introgression lines (ILs) have been produced to reintroduce the untapped genetic variability from wild species into cultivated varieties. In the laboratories of the Department of Agricultural Sciences (University of Naples "Federico II") several sublines have been produced, characterized by the presence in homozygosity of a small portion of the genome of the wild species introduced into that of the cultivated variety M82. Some of these sublines showed good performances in terms of yield and fruit qualitative traits, in particular those for tomato processing. A subline coded R182 has been exploited to identify favorable alleles that can improve fruit quality traits in commercial varieties, including antioxidants content. This R182 has a small region (448 Kbp) of wild genome introgressed in the cultivated genetic background (M82). In this study, quantitative and qualitative analyses were performed on R182 and the parental line M82, which evidenced that the subline R182 has better performance in terms of yield and fruit qualitative characteristics. In particular, a higher content of ascorbic acid in R182 fruit compared with the parental line was detected. Among all the genes mapping in the R182 introgression region (Aliberti et al., 2020), we focused our attention on two genes, coding for a Nucleobase Ascorbate Transporter (NAT) and for a Major Facilitator Superfamily Protein (MFSP), which could play an indirect role in the biosynthesis and accumulation of AsA and in controlling other key qualitative traits. Using genome modification techniques (CRISPR/Cas9 technology) the function and mechanisms of action of these genes are being confirmed. The mutated lines were obtained thanks to the collaboration between the Department of Agricultural Sciences of the University of Naples "Federico II" and Metapontum Agrobios Research Center of ALSIA. The lines carrying CRISPR/Cas9 derived knock-out mutations of the NAT and MFSP genes were obtained by genetic transformation of the S. *lycopersicum* M82 and R182 lines. Mutants obtained are being characterized by molecular analysis and DNA sequencing. A *posteriori* analyses of the genomic editing experiments will be performed by characterizing and quantifying the insertion, deletion and homologous recombination events.