

GREEN FLESH AND TANGERINE CRISPR/CAS9 EDITED TOMATO LINES OBTAINED IN THE SAN MARZANO ITALIAN LANDRACE

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The Stay Green Protein (SGR) and Carotenoid Isomerase (CRTISO) genes were targeted using computationally designed gRNAs with the stable transformed CRISPR/Cas9 system, to reproduce the effects of loss of function mutations into the Italian tomato landrace San Marzano (SM). Tomato fruits of the green flesh (gf) mutant ripen to a brownish colour for the simultaneous presence of lycopene and chlorophyll, which is not degraded during fruit maturation, whereas tangerine (t) abolishes all-trans-lycopene in fruits, which appear orange, mainly due to the presence of pro-lycopene. These phenotypes represent a valid alternative to the red tomato because of their different nutraceutical and organoleptic qualities. Both mutations had already been introgressed into SM with four backcrosses, but there were still evident phenotypic differences, mainly due to the genetic distance between SM and the t donor parent. Genome editing is supposed/manages to overcome such drawback, having the capacity to maintain the whole SM background. Here, genotypic, phenotypic, and biochemical characterization was performed in gf and t introgressed and edited lines, in comparison with SM. The average gene editing efficiency in T0 lines was 67.8% and 71.4% for SGR and CRTISO respectively. Analysis of edited alleles (in T1) plants showed that gf did not confirm the mutations of the T0 generation, while t

mutations were stable and inherited in a Mendelian fashion. The phenotyping of the T1 and T3 edited lines showed maintenance of the SM phenotype in the edited lines, overcoming the limits of the backcross scheme. Furthermore, essential vegetative, flower and fruit traits of the gf and t mutation were properly recapitulated by the edited mutations. Interestingly, edited lines also showed added benefits in some cases, such as higher soluble solids content and longer shelf-life. To evaluate putative perturbations at the metabolome level, the fruit volatile metabolite fraction was studied, using HS-SPME/GC-MS. The analytical strategy allowed the unequivocal relative quantification of 69 volatile compounds (VOCs) by comparison of both mass spectra and retention index with those of authentic standards. Thirty-two of them showed statistically significant differences in at least one of the T1 lines studied. The gf volatile profile did not differ from SM. For t, the previously observed dissimilarity of the introgressed line attributed to benzenoids and terpenoids was not observed in the edited lines. Lastly, the diverse content of lycopene in t had no effect in volatile apocarotenoid formation, whose levels positively correlate with tomato flavour acceptability. These genotypes offer new opportunities to improve and diversify SM fruit quality by modifying its flavour, and therefore its nutraceutical properties, finally paving the way for new breeding plans aimed at re-evaluating local varieties by the use of new innovative strategies, such as CRISPR-Cas9.