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CRISPR/CAS9-MEDIATE EDITING OF *PSY1* AND *CRTR-B2* IN TOMATO

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CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein) is becoming the most powerful genome editing system in crop breeding following its rapid development in human and animal genome research. This system can be used to inactivate or modulate the expression of genes in order to understand their function and regulation, as well as to generate new traits useful for improving resistance to pathogens, tolerance to abiotic stresses or increasing yield.

We have applied this method in tomato to generate new allelic variants for the *Psy1* and *CrtR-b2* genes that encode the phytoene synthase 1 and the beta carotene hydroxylase 2 enzymes involved in the biosynthesis of carotenoids in fruits and flowers. *CrtR-b2* and *Psy1* were chosen as targets because their knockout is easily detectable through the visual inspection of the plant as a change of petal or fruit colors. The overall editing rate across the two genes was 84% and the characterization of mutations allowed the identification of 34 new alleles across the four transformation experiments (D'Ambrosio *et al.* Transgenic Res (2018). https://doi.org/10.1007/s11248-018-0079-9). Characterization of the new allelic variants was performed on the T0 plants through the direct sequencing of the PCR amplified target DNA or by cloning and sequencing the allelic variants. The cloning of target region amplified fragments revealed that about 40% of all the transformed plants were indeed chimeric, that is they carried multiple alleles at low frequency. The presence of more than two allelic variants in the DNA of a plant could only be due to the presence of leaf sectors with different genotypes in the sample collected for DNA extraction. Characterization of T1 progenies from some chimeric plants was performed in order to verify if all the allelic variants detectable in a T0 mother plant have the same probability of being inherited by the progeny.