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HIGH PIGMENT-2 CRISPR/CAS9 EDITED TOMATO LINES OBTAINED IN DIFFERENT GENETIC BACKGROUNDS

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high intensity light, high pigment tomato mutant, San Marzano landrace, traditional variety

Several photomorphogenic mutants have been described in tomato (Solanum lycopersicum) and among them, mutants carrying the monogenic recessive high pigment mutations (hp-1, hp-1w, hp-2, hp-2j, hp-2dg). Such variants are characterized by their exaggerated light responsiveness, higher anthocyanin levels, shorter hypocotyls, and more deeply pigmented fruits when compared with wild-type plants. This work is focused on the hp-2 mutant of S. lycopersicum, which is known to stimulate a higher content of flavonoids, carotenoids, and anthocyanin in leaves and fruits. Indeed, hp mutants are characterized by an increased number and size of chloroplasts, which is at the basis of an enhancement of the photosynthesis required to increase pigment accumulation. Gene editing was used to modify the De-Etiolated 1 (DET) target gene, which is involved in the transmission of light signals in a larger complex of interacting proteins. The stable transformed CRISPR/Cas9 system had the aim to reproduce the effects of the partial loss of function hp-2 mutation firstly in Microtom (MT), in Moneymaker (MM) and then in the Italian tomato landrace San Marzano (SM). This phenotype represents a valid alternative to the red tomato because of its increased

nutraceutical and organoleptic qualities. The hp-2 mutation had already been introgressed into SM and MT through backcrossing, but the genome editing is supposed to overcome pleiotropic drawbacks, having the capacity to maintain the whole chosen background.

Two sgRNA guides were designed on the exon 11 and were assembled coupled in a Golden Gate Level 1 acceptor construct (pICH47751 and pICH47761). A final binary vector containing Cas9-expression cassette and kanamycin-resistance-expression (NptII) cassette, was used to generate the CRISPR–Cas9 construct. The sgRNAs efficiency was then tested by a hairy-roots transient expression assay, with 7% of samples showing a precise 56 pb deletion on the target site.

For the stable transformation, we used an in-house protocol and the LBA4404 Agrobacterium tumefaciens strain. We obtained a single T0 MT rooted plant, which corresponds to the 4% of shoots obtained. Regarding SM, the 7% of shoots rooted, for a total of 6 plants, but only three survived and resulted Cas-9 positive and edited. One of them showed anthocyanin spots on the superior leaf trichomes, which is a phenotype reported in hp-2 mutations. This work offers new opportunities to improve and diversify the tomato fruit quality by modifying its pigmentation, and therefore its nutraceutical properties, finally paving the way for new breeding plants aimed at re-evaluating local varieties using new innovative strategies, such as CRISPR-Cas9.