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CRISPR/CAS9 MEDIATED MUTAGENESIS OF POLYPHENOL OXIDASE GENES IN EGGPLANT FOR THE IMPROVEMENT OF THE BERRY QUALITY

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Eggplant (*Solanum melongena*) berries are rich in phenolic acids, mainly chlorogenic acid. The polyphenol oxidase enzymes (PPOs) catalyse the oxidization of phenolic acids after cutting and cause the browning of the fruit flesh, with a negative impact on their quality for both fresh consumption and industrial transformation.

Among the PPO genes present in eggplant genome (named *ppo*1-10), four of them (*ppo1-3-4* and *5*) highlighted a strong increase in transcript levels in the flesh of the berries after cutting.

A CRISPR/Cas9 toolkit was developed within the GoldenBraid cloning standard, allowing simple and practical assembly of constructs for gene editing. An eggplant breeding line of the variety 'Black Beauty' and a double haploid of the variety 'Ecavi' were selected for *Agrobacterium*-mediated transformation. Seed-derived cotyledons were transformed with a CRISPR/Cas9 construct targeting a conserved region of *ppo4-5* and, due to the high homology of the gene family members, *ppo6* as well.

Genotyping of transformed plants through Sanger and Illumina deep sequencing of amplicons, revealed that the plants were successfully edited in PPO 4,5 and 6 *loci,* with the insertion of a single nucleotide as the most frequent mutation. Illumina analyses highlighted that our gRNAs induced no additional mutations in the host genome due to off-target activity.

The mutations were stably inherited in the progeny in which some individuals, due to segregation, no longer carried out the transgene construct. Fruits analyses of T_1 plants in respect to browning/PPO activity and metabolic profile of phenols are in progress. Furthermore an assessment of potential secondary effects of the induced mutation will be assessed.

Our results demonstrate that the CRISPR/Cas9 system is an efficient tool for generating stable and heritable modifications in eggplant.