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CRISPR/CAS9 MEDIATED MUTAGENESIS OF POLYPHENOL OXIDASE GENES IN EGGPLANT

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

Following a survey of the eggplant genome, ten *ppo* genes (named *ppo*1-10) were isolated. Their qPCR transcript levels were assessed in the fruit flesh and peel of eggplant varieties, immediately and 30 min after cutting. The *ppo1-3-4* and *5* highlighted strong increases in gene transcription.

We used a CRISPR/Cas9 system, integrated into the GoldenBraid (GB) cloning tool, to knock-out the *ppo* genes. Cotyledons of the Black Beauty variety and of a double haploid line (DH) derived from the commercial hybrid 'Ecavi' were transformed with a CRISPR/Cas9 construct targeting a conserved region of *ppo4-5* as well as *ppo6* (due to the high homology between these gene family members).

Mutations at the target sites were assessed by sequencing genomic DNA extracted from DH Ecavi *calli* and Black Beauty *in vitro* regenerated shoots. Mutations found in edited plantlets were predominantly small deletions, with 1 bp being the most frequent. One Black Beauty edited plantlet showed the highest editing efficiency for all the three *loci*: i.e. 66% for *ppo4*, 75.3% for *ppo5* and 55% for *ppo6*.

We investigated potential off-target effects through deep sequencing of amplicons of the putative off-target sites identified *in silico*. Illumina analyses highlighted that no additional mutations are induced in the off-target-sites.

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