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TUNING OF A GOLDEN BRAID-BASED TOOL FOR CRISPR-CAS9 GENE KNOCK-OUT IN TOMATO AND EGGPLANT

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A number of genome editing technologies have been developed in recent years. Among them the CRISPR (clustered regularly interspaced palindromic repeats)/Cas9 has emerged, as it represents a system that is markedly easier to design, specific, efficient and well-suited for high-throughput and multiplexed gene editing for a variety of organisms.

We report on the application of the CRISPR/Cas9 system integrated into the GoldenBraid (GB) cloning standard tool to target the *greenflesh/sgr1* in tomato (*Solanum lycopersicum* L.), as well as polyphenol oxidase (PPOs) genes in eggplant (*Solanum melongena* L.).

Tomato was used as a model species for assessing the efficiency of the GB tool, since targeting the disruption of *sgr1* with Cas9 results in a distinctive, immediately recognizable greenflesh phenotype characterized by the lack of chlorophyll degradation in mature fruits and senescent leaves. In T_0 plants, knocked-out edited alleles (monoallelic as well as biallelic mutants), were obtained in 63% of transformed plants.

Eggplant berries are rich in antioxidant phenolics which, after cutting, become available to polyphenol oxidase enzymes (PPOs) catalyzing their oxidation and giving rise to browning of the fruit flesh to detriment of berry quality for both fresh consumption and industrial transformation. Disruption of PPOs genes with Cas9 might thus result in genotypes with high content in phenolics, but reduced browning of the fruit flesh. Ten PPO genes (named PPO1-10) were isolated and characterized, also thanks to the recent availability of a high quality and annotated eggplant genome sequence. Their qPCR expression profiles were assessed in the fruit flesh and peel of three eggplant commercial varieties, immediately and 30 min after cutting. Increases of transcripts at 30 min after cutting were spotted in PPO1, PPO3, PPO4 and PPO5 genes.

A GoldenBraid CRISPR Cas9 tool carrying 2 guide RNAs: one targeting PPO1 and 3, the other PPO 4,5 as well as PPO6 (due to the high homology between these gene family members) was developed. Three eggplant varieties were selected for *Agrobacterium*-mediated transformation on the basis of high polyphenol content and *in vitro* regenerated shoots from callus were obtained.