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GENE EDITING OF PMR4 PROMOTES RESISTANCE IN TOMATO VARIETIES TOWARDS LATE BLIGHT (LB) ATTACKS

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Late blight (LB) is a devastating plant disease affecting tomato (*Solanum lycopersicum* L.) and striking many species in the Solanaceae family. It is caused by the etiological agent *Phytophthora infestans* and the control of this pathogen extensively relies on fungicide application, leading to economic losses and environment impact.

Since this kind of pathogen can take advantage of plants' susceptibility genes (S-genes) to enter the cell and to assist its proliferation, the disabling of S-genes may provide a broad-spectrum and durable type of resistance. To this scope, the CRISPR/Cas9 technology was our best choice to obtain resistant plants.

Powdery Mildew Resistant 4 (PMR4) gene has been selected, since previous studies in potato showed the ability of PMR4 knock-down mutants to be resistant to LB. In order to verify whether the effect of disabling of the *PMR4* gene confers resistance to LB even in tomato, this gene been disabled in two widely cultivated varieties: 'San Marzano' (SM) and 'Oxheart' (OX). CRISPR mutants were generated by using 4 sgRNAs targeting the FKS1dom1 and Glucan-synthase domain of the PMR4 protein in order to maximize gene editing efficiency.

Overall, 87 tomato pmr4 transformants were generated (T0 generation) of which 70 in SM and 17 in OX. They were tested and 26 SM and 9 OX mutants resulted positive for the Cas9 gene genomic integration. All the genotypes were then evaluated using a Detached-Leaf Assay (DLA) using *P. infestans*, with 88% of SM and 44% of OX mutants showing reduced disease symptoms, compared with their wild types. The Sanger/TIDE analysis was performed on the edited target regions to assess the editing efficiency on each sgRNA, focusing only the mutants with the higher level of tolerance. Sequencing analysis revealed an efficient editing at the target loci, 3 edited plants with large deletions (~2kb) and a prevalence of indels (mainly deletion of 7 nucleotides) as predominant mutations introduced.

The six more promising edited plants were subjected to Illumina whole genome sequencing (WGS), in order to precisely assess: i) the editing efficiency, ii) the emergency of any off-target events and iii) the number/position of Cas9 gene integration.