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CRISPR/CAS9-MEDIATED KNOCK-OUT OF TOMATO DMR6 GENE TO CONFER TOLERANCE AGAINST PATHOGENS

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A promising strategy to confer broad-spectrum and long-lasting resistance against pathogens in plants is based on the disabling of susceptibility genes (S-genes). These genes, required by the pathogen to infect a plant at some step of the infection process, support plant/pathogen compatibility. The S-gene Downy Mildew Resistance 6 (SlDMR6), which is related to salicylic acid catabolism, negatively regulates defence genes' expression. In Arabidopsis and potato, DMR6 confers tolerance against Phytophthora capsici and Phytophthora infestans, thus we assessed its role of DMR6 in affecting in tomato the susceptibility against late blight (LB) in tomato caused by P. infestans.

Two homologues (dmr6-1 and dmr6-2) were identified in tomato and, based on their transcription activation in response to P. infestans, dmr6-1 was selected as the most promising target. In order to induce knock-out mutations, a multiplexing CRISPR/Cas9 approach based on three gRNAs designed on dmr6-1 was applied.

The experimental work led to the production of five in vitro plantlets. Illumina deep sequencing of the target sites revealed the successful editing of dmr6 in all of them , with the insertion of 1bp as the most frequent observed mutation. After selfing, mutated alleles were stably

inherited in ten T1 progeny. One plant was found carrying mutations in homozygous state in all the target regions and, due to the segregation of Cas9, the absence of transgene. A whole genome re-sequencing (Illumina) of this mutant was also performed to verify the absence of off-target genomic effects.

T1 mutants were then evaluated through a Detached-Leaf Assay (DLA) using P. infestans and all of them showed reduced disease symptoms if compared with their wild types. The tolerance of mutant lines against O. neolycopersici, B. cinerea, A. alternata and C. fulvum will be tested in future experiments.