

## NEW INSIGHTS ON THE ROLE OF *SLDMR6-1* IN DROUGHT AVOIDANCE IN TOMATO

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*Solanum lycopersicum* L., DOWNY MILDEW RESISTANCE 6, susceptibility genes, drought stress, ecophysiological measurements

The DOWNY MILDEW RESISTANCE 6 (DMR6) protein is a 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase, involved in salicylic acid (SA) metabolism. In tomato its inactivation was found to increase SA levels and to confer disease-resistance against several pathogens. Due to the potential role of SA as an abiotic stress-tolerance enhancer, we conducted preliminary experiment to test the tolerance to drought stress in *SLDMR6-1* tomato mutants generated through the CRISPR/Cas9 technique.

A CRISPR/Cas9 multiplexing strategy, based on the use of three gRNAs, was applied to induce knock-out mutation of *dmr6-1* in plants of the tomato cultivar 'San Marzano'. Whole genome resequencing highlighted that one selected T1 plant did not carry any foreign DNA sequences but only indels at DMR6-1 target locus, making it indistinguishable from plants where natural mutations might occur. The average SNP number across not edited and edited lines was comparable, as well as the average mutation rate.

Mutant genotypes were analysed for their capability to respond to drought stress. Both wild-type (WT) plants and *SLDMR6-1* mutants were subjected to water deprivation for 7 days. While the WT plants exhibited severe wilting and chlorosis, *Sldmr6-1* mutants showed fairly turgid leaves and maintained

higher soil Relative Water Content (RWC). Ecophysiological measurements highlighted that *SlDMR6-1* mutants adopted a water saving behaviour by reducing assimilation rate (A), transpiration rate (E), and stomatal conductance (Gs) while increasing Water Use Efficiency (WUE). Compared to WT, the *SlDMR6-1* mutants under drought stress showed up-regulation of the anti-oxidant genes *SlPOD* and *SlGST* as well as down-regulation of *SlCYP707A2* gene involved in ABA catabolism.

Additionally, the *SlDMR6-1* mutants were tested with *Phytophthora infestans*, the causal agent of Late Blight. For the first time, our results showed that knocking-out the *DMR6* gene in tomato led to reduced susceptibility to *P. infestans*.