

LESS IS MORE: CRISPR/CAS9-BASED MUTATIONS IN DND1 GENE ENHANCE TOMATO RESISTANCE TO POWDERY MILDEW WITH LOW FITNESS COSTS

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powdery mildew, CRISPR/Cas9, DND1 gene, tomato resistance, fitness costs

Powdery mildew, caused by *Oidium neolycopersici*, is a devastating disease and a severe concern for plant productivity in tomato (*Solanum lycopersicum* L.). The presence of susceptibility (S) genes in plants facilitates pathogen proliferation and their disabling may help to provide a broad-spectrum and durable type of resistance. Previous research has shown that the DND1 susceptibility gene's impairment enhances plant resistance to a wide range of pathogens, causing a detrimental impact on plant fitness as side effect. To check the possibility of reducing the negative consequences of dnd1 mutation while bolstering plant response to diseases, a CRISPR-Cas9 vector, containing four single guide RNAs targeting three SLDND1 exons, was designed and introduced via *Agrobacterium-tumefaciens*-mediated transformation into the market cultivar "Moneymaker" (MM). Three T₁ lines (named E1, E3, and E4) were crossed with MM and then selfed to produce T_{F2} families. All the T_{F2} plants in homozygous state (dnd1/dnd1) showed reduced susceptibility to powdery mildew compared to the heterozygous (DND1/dnd1) and control (DND1/DND1) ones. Two events (E1 and E4) appeared as fully knock-out (K0) mutants, with trunked copies of DND1, exhibiting clear dwarf and auto-necrosis phenotypes. In contrast, one event (E3) showed a full-length protein with a 3 amino acids deletion, exhibiting a nearly normal height phenotype with fewer auto-necrosis spots. We thus compared the 3D structures of the reference and mutant proteins, observing a clear conformational change in the E3-derived mutant protein, likely affecting protein functionality. One dnd1/dnd1 T_{F2} line (E3) was whole-genome

sequenced through Illumina for deeper analysis; no off-target effects were underlined in the selected genomic regions, nor traces of Cas9 gene, which was eliminated by segregation. Our results confirmed, for the first time, reduced susceptibility to *O. neolycopersici* in tomato KO mutants of the *DND1* gene. More importantly, the E3 mutant event demonstrates that it is feasible to generate *dnd1* mutants with less pleiotropic effects for breeding crops resistant to a range of pathogens including powdery mildew.