A DNA-free editing approach to help viticulture sustainability: dual editing of *DMR6-1* and *DMR6-2* enhances resistance to downy mildew

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Abstract

The sustainability of viticulture hinges on maintaining quality and yield while reducing pesticide use. Promising strides in this direction involve the development of clones with enhanced disease tolerance, particularly through the knockout of plant susceptibility genes. Knocking out of *Downy Mildew Resistant 6* (*DMR6*) led to increased levels of endogenous salicylic acid (SA), a regulator of immunity, resulting in enhanced tolerance to Downy Mildew (DM) and other diseases in various crops.

Mutations in both *DMR6-1* and *DMR6-2* genes were introduced into two grapevine cultivars using CRISPR-Cas9 using two methods. In the first case, transgene delivery mediated by *A. tumefaciens* was employed, while in the second case, we developed a 'single-cell technology' for gene editing, creating non-transgenic grapevine mutants through the regeneration of protoplasts previously edited with the CRISPR/Cas9 ribonucleoprotein.

We tested the susceptibility of single and double mutants to DM through artificial inoculation assays on detached leaves and whole plants. Our findings indicate that a simultaneous mutation in both *DMR6-1* and *DMR6-2* is needed to significantly enhance resistance to DM, with the double mutant (*dmr6-1-dmr6-2*) outperforming either single mutant in both cultivars. Elevated levels of endogenous SA were only observed in the double mutant, while single mutation in *DMR6-1* or *DMR6-2* proved ineffective. Collectively, our data highlight the need for a double knockout to achieve appreciable results against DM-susceptibility.

Currenlty, we are adapting the 'single-cell technology' to generate edited vines from various agronomically relevant cultivars. In parallel, we are assessing the performance of plants edited in different susceptibility genes.

Keywords: DMR6; grapevine; DNA-free; gene editing; downy mildew; susceptibility gene.