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## REDUCED SUSCEPTIBILITY TO DOWNY MILDEW OF DMR6 GENE-EDITED GRAPEVINE PLANTS AND DEVELOPMENT OF DNA-FREE EDITED MUTANTS

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The application of New Breeding Techniques (NBTs) in *Vitis vinifera* holds the promise of improving agronomic traits while preserving the genotype of elite cultivars. One of the most important traits when considering the sustainability of viticulture is the production of pathogen-resistant clones; in particular, to the oomycete *Plasmopara viticola,* the causal agent of downy mildew (DM), a disease that leads to considerable production losses every year.

To this final goal, taking advantage of previous knowledge in other species, we edited members of the *Downy mildew resistance 6* gene family in two table grape cultivars, Crimson seedless and Sugraone, to dissect the role they play in DM resistance. Our editing results indicated that reduced susceptibility to the pathogen (assessed by in planta inoculation assays) is obtained by knocking-out VviDMR6-1, but to a larger extent both VviDMR6-1 and VviDMR6-2 genes in the same plant. Instead, impairing mutations in the single VviDMR6-2 gene was ineffective. Considering the role of DMR genes in the catabolism of salicylic acid (SA), we measured the levels of free SA and found an increased constitutive level in the double *dmr6-1_dmr6-2* mutant, which might explain the partially tolerant phenotype.

Although the dmr6 edited mutant of Crimson seedless and Sugraone were obtained by delivering the CRISPR-Cas9 editing machinery via conventional Agrobacterium-mediated delivery and thus are transgenic plants, public acceptance, regulatory burdens and chimerism issues are pushing towards the

development of editing technologies with no presence of exogenous DNA. To overcome these issues, we recently developed a single-cell-based technology to obtain non-transgenic and edited grapevine plants by coupling the DNA-free transfection of the CRISPR/Cas9 editing machinery with an efficient protocol of plant regeneration from protoplasts in the same table grape cultivars. The regenerated, non-chimeric plants were edited on the downy- and powdery-mildew susceptibility genes, VviDMR6-2 and VviMLO6 respectively, either as single or double mutants. The challenge ahead is transferring this protocol to multiple grapevine varieties by tuning the already established procedure, and gathering knowledge on the gene targets regulating other relevant traits.