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TRANSGENE-FREE CRISPR-CAS AND CISGENESIS APPROACHES FOR RESISTANCE TO POWDERY AND DOWNY MILDEW IN GRAPEVINE

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The development of disease-resistant tree crops is essential to sustainable agriculture, particularly under increasing biotic stresses driven by climate change. This project explores a dual strategy to confer resistance to powdery mildew (PM) and downy mildew (DM) in grapevine employing (i) a transgene-free CRISPR-Cas genome editing platform based on ribonucleoprotein (RNP) delivery, and (ii) a cisgenic transformation approach utilizing native resistance (R) genes and their regulatory elements. Both approaches rely on efficient production of embryogenic callus and regeneration of modified grapevine plants.

Our CRISPR-Cas RNP system enables precise gene editing without the integration of foreign DNA, targeting key susceptibility (S) genes known to be involved in PM and DM pathogenesis–specifically, *MLO* and *DMR6* genes, respectively.

In parallel, we are implementing a cisgenic strategy to introduce naturally occurring R genes from sexually compatible and disease-resistant donor genotypes. This strategy uses a binary vector system carrying a heat-inducible and site-specific recombinase system to remove exogenous DNA sequences such as the selectable marker gene. Following antibiotic selection, regenerated plants containing the cisgenic construct are subjected to controlled heat shock treatments to activate the recombinase. The resulting plants retain only the native R genes, while exogenous DNA is removed by excision.

We present initial results demonstrating successful simultaneous editing of one *MLO* and two *DMR6* genes. In cisgenic lines, we report stable expression of the *Resistance to Plasmopara viticola1*

(RPV1) gene. Both approaches are designed to align with emerging regulatory frameworks and preserve the heterozygous genetic backrounds of elite grapevine cultivars such as Chardonnay and Merlot, which would be otherwise disrupted through conventional breeding.

All resulting lines from both cisgenic and gene editing strategies will undergo sequencing to assess and ensure the absence of genetic, genomic and chromosomal side-effects that may arise from nuclease activity, T-DNA integration, and tissue culture.