

THE APPLICATION OF NEW PLANT BREEDING TECHNIQUES (NPBTs) IN GRAPEVINE CULTIVATION

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In the current global context of environmental change, New Plant Breeding Techniques (NPBTs) have emerged with the aim of overcoming the limitations of traditional breeding methods for improving plants resistance to biotic and abiotic stresses. These techniques align with European Policies that advocate for reduced chemical inputs and a more sustainable approach to agriculture. In our study, we applied genome editing using the CRISPR/Cas9 system in grapevine, focusing on genes associated with susceptibility to specific diseases. To counteract powdery mildew, we targeted two genes, *VvML07* and *VvML06*, which belong to the *Mildew Locus 0* (ML0) family. Additionally, we directed our attention to the *Non-Expressed Protein 3* (NPR3), which plays a pivotal role in defense responses against pathogenic biotrophic fungi, negatively affecting the accumulation of salicylic acid. In parallel to genome editing, we employed cisgenesis to introduce the resistance locus *RPV3-1* (Resistance to *Plasmopara viticola*) into economically significant grape cultivars (i.e., Chardonnay). This locus consists of two distinct genes, *TNL2A* and *TNL2B*, which were inserted together using their native promoters and terminators. To address one of the limitations associated with conventional *Agrobacterium tumefaciens*-mediated transformation, namely the insertion of unrelated transgenes, we utilized an inducible excision system based on Cre-Lox recombinase

technology. This system is regulated by a heat-shock inducible promoter that will be activated once the transformation and/or editing event(s) are confirmed, facilitating the removal of CRISPR/Cas components and selection markers in both the genome editing and cisgenic approaches. To date, the plants regenerated are under scrutiny to evaluate the editing efficiencies and the copy number inserted in the genome. The plants identified as edited are now under acclimatization and will be studied under laboratory condition while waiting for the possibility to evaluate them in field.