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NPBTs FOR SUSTAINABLE VITICULTURE MANAGEMENT TO BIOTIC AND ABIOTIC STRESS

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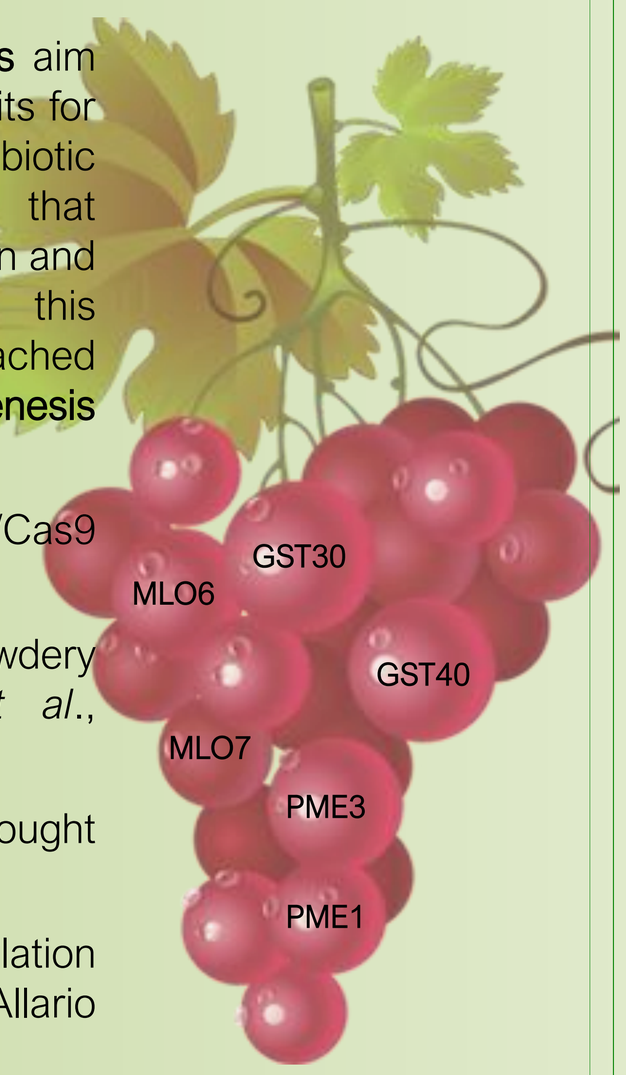
Introduction

The **New Plant Breeding Techniques** aim to overcome traditional breeding limits for plant improvement to biotic or abiotic stress, satisfy European policies that encourage a pesticides use reduction and more sustainable agriculture. In this framework great benefit could be reached through **CRISPR/Cas9** and **cisgenesis** technologies.



We decided to apply CRISPR/Cas9 focusing on susceptibility genes:

- *MLO6* and *MLO7* involved in powdery mildew interaction (Pessina *et al*., 2016);
- *GST30* and *GST40* involved in drought resilience (Chen *et al*., 2012);
- *PME1* and *PME3* involved in regulation of woody hydraulic proprieties (Allario *et al*., 2018).

In parallel to genome editing, we also applied cisgenesis to move the resistance locus **RPV3-1** (Resistance to *Plasmopara viticola*). This locus contains two different genes ***TNL2a*** and ***TNL2b*** that were inserted in susceptible genotypes (Foria *et al*., 2020).
One of the drawbacks linked to classical *Agrobacterium tumefaciens* mediated transformation is the insertion of unrelated transgene. To overcome these limits, we exploit an inducible excision system based on a **Cre-lox** recombinase technology controlled by a heat-shock inducible promoter (**HSP**) that will be activated once the transformation event(s) will be confirmed.

Materials and Methods

The first step in genetic transformation is embryogenic calli production. We evaluated inflorescence development stage as described in Gribaudo *et al*., 2004, through microscopy assay and embryogenic calli formation Fig. 1.

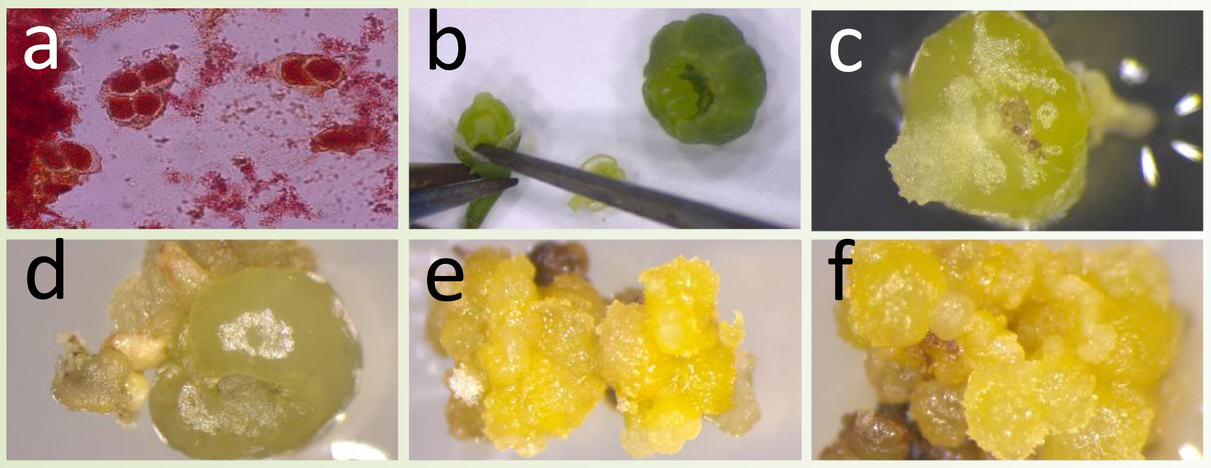


Fig. 1: grapevine inflorescences: a: Microsporogenesis stage was observed microscopically after anther squashing in Safranine-O; b: ovaries and anthers collecting phase; c-d: pre-embryogenic calli formation after 14-30 days post collected; e-f: embryogenic calli formation after 60-90 days.

To promote T-DNA removal we introduced an inducible excision system based on a **Cre-lox recombinase** technology controlled by a HSP. This system was used both for cisgenesis and genome-editing constructs (Fig. 2 and 3). We introduced two gRNAs for each gene in genome editing constructs and *TNL2a* and *TNL2b* in the pNS13 plasmid for cisgenesis.

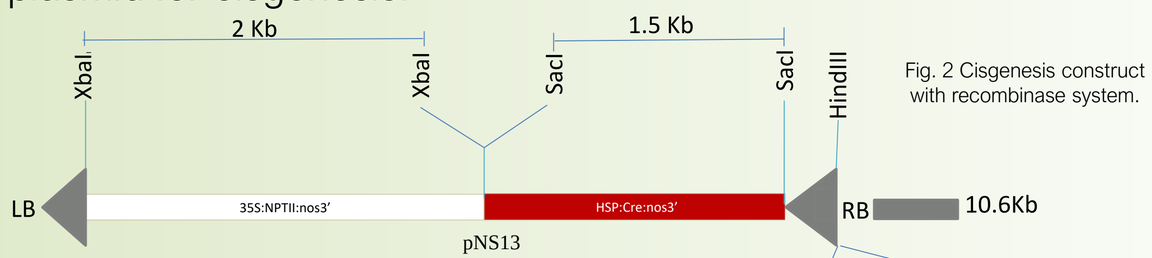


Fig. 2 Cisgenesis construct with recombinase system.

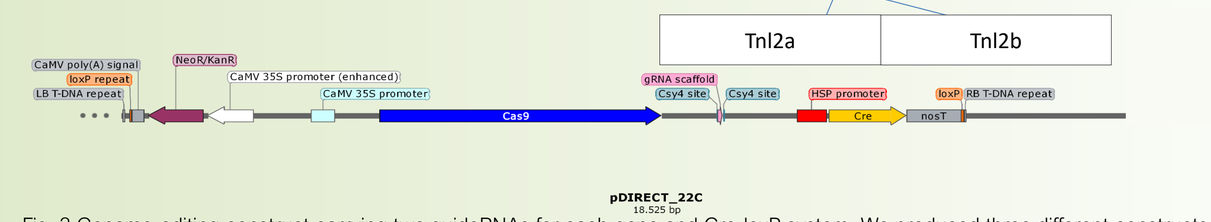


Fig. 3 Genome-editing construct carrying two guideRNAs for each gene and Cre-loxP system. We produced three different constructs one for each gene family.

Results

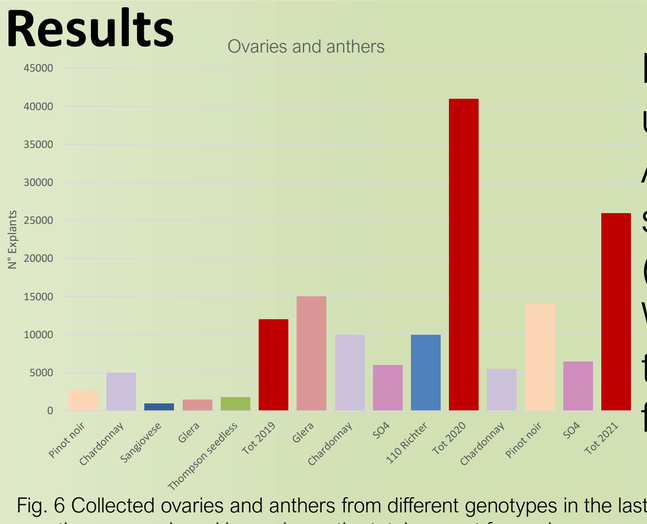


Fig. 6 Collected ovaries and anthers from different genotypes in the last three years. In red have shown the total amount for each year.

Embryogenic calli were used to performe Agrobacterium-mediated stable transformation (Fig. 7).
We obtained putative transformed embryos for each transformation.

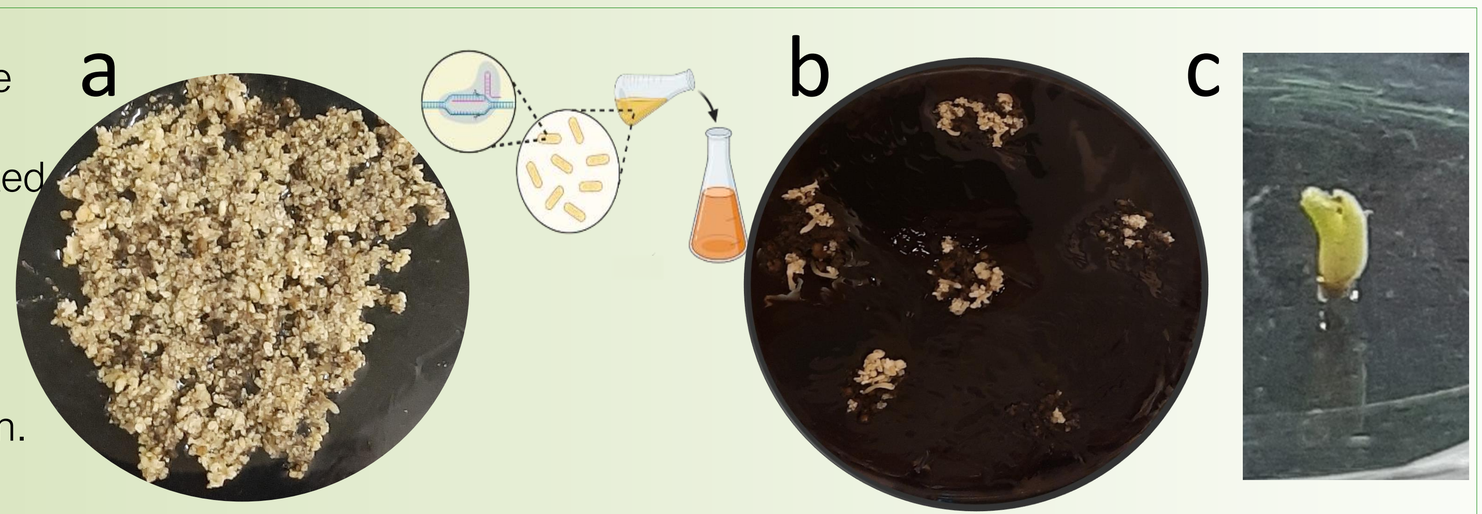


Fig. 7 Agrobacterium-mediated transformation: a: embryogenic calli formation; b: first embryos formation; c: regenerated embryo.

Conclusions

The **NPBTs** display the potential to revolutionize the agricultural research field especially in crops such as grapevine. Here we applied **genome editing** to knock-out three genes family in independent transformation: ***MLO***, ***GST*** and ***PME***. We also applied **cisgenesis** in order to insert resistance genes to *Plasmopara viticola:* ***TNL2a*** and ***TNL2b***.

References:

Allario T, Tixier A, Awad H, Lemaire C, Brunel N, Badel E, Barigah TS, Julien J-L, Peyret P, Mellerowicz EJ, *et al.* 2018. *PtxtPME1* and homogalacturonans influence xylem hydraulic properties in poplar. *Physiologia Plantarum* **163**: 502–515.

Chen J-H, Jiang H-W, Hsieh E-J, Chen H-Y, Chien C-T, Hsieh H-L, Lin T-P. 2012. Drought and Salt Stress Tolerance of an Arabidopsis Glutathione *S* -Transferase U17 Knockout Mutant Are Attributed to the Combined Effect of Glutathione and Abscisic Acid. *Plant Physiology* **158**: 340–351.

Foria S, Copetti D, Eisenmann B, Magris G, Vidotto M, Scalabrin S, Testolin R, Cipriani G, Wiedemann-Merdinoglu S, Bogs J, *et al.* 2020. Gene duplication and transposition of mobile elements drive evolution of the Rpv3 resistance locus in grapevine. *The Plant Journal* **101**: 529–542.

Gribaudo I, Gambino G, Vallania R. 2004. Somatic embryogenesis from grapevine anthers: The optimal developmental stage for collecting explants. *American Journal of Enology and Viticulture* **55**: 427–430.

Pessina S, Lenzi L, Perazzolli M, Campa M, Dalla Costa L, Urso S, Valè G, Salamini F, Velasco R, Malnoy M. 2016. Knockdown of MLO genes reduces susceptibility to powdery mildew in grapevine. *Horticulture Research* **3**: 16016.