# Potential of New Plant Breeding Techniques for grapevine sustainability

**Loredana Moffa[1,2*], Gaetano Giudice[1,3], Giorgio Gambino[4], Irene Perrone[4], Chiara Pagliarani[4], Riccardo Velasco[1], Walter Chitarra[1,4], Luca Nerva[1,4]**

[1]Research Centre for Viticulture and Enology, Council for Agricultural Research and Economics (CREA-VE), Via XXVIII Aprile 26, 31015 Conegliano (Italy);
[2]University of Udine, Department of Agricultural, Food, Environmental and Animal Sciences (Di4A), Via delle Scienze 206, 33100, Udine (Italy);
[3]University of Milano, Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy (DiSAA), Via Celoria 2, 20133, Milano (Italy);
[4]Institute for Sustainable Plant Protection, National Research Council (IPSP-CNR), Strada delle Cacce 73, 10135 Torino (Italy).

## Introduction

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

We decided to apply CRISPR/Cas9 focusing on two susceptibility genes for each gene family:

- ✂ *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 hydraulic proprieties of xylem vessels (Allario *et al*., 2018).

In parallel, we are also applying cisgenesis to move the resistance locus **RPV3-1** (Resistance to *Plasmopara viticola*). This locus contains two different genes, ***TNL2a*** and ***TNL2b,*** that, when inserted in susceptible genotypes, conferred resilience to downy mildew (Foria *et al*., 2020). One of the drawbacks linked to classical *Agrobacterium tumefaciens* mediated transformation is the insertion of unrelated transgene such as selection marker genes that could cause toxicity or allergenicity to humans and animals, in addition to their potential hazards for the environment. In European Union plants obtained through CRISPR are considered as GMOs (Directive 2001/18/EC). 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 grapevine genetic transformation is the production of embryogenic calli. We collected inflorescence as described in Gribaudo *et al*., 2004 and we obtained embryogenic calli from different genotypes (Fig. 1).

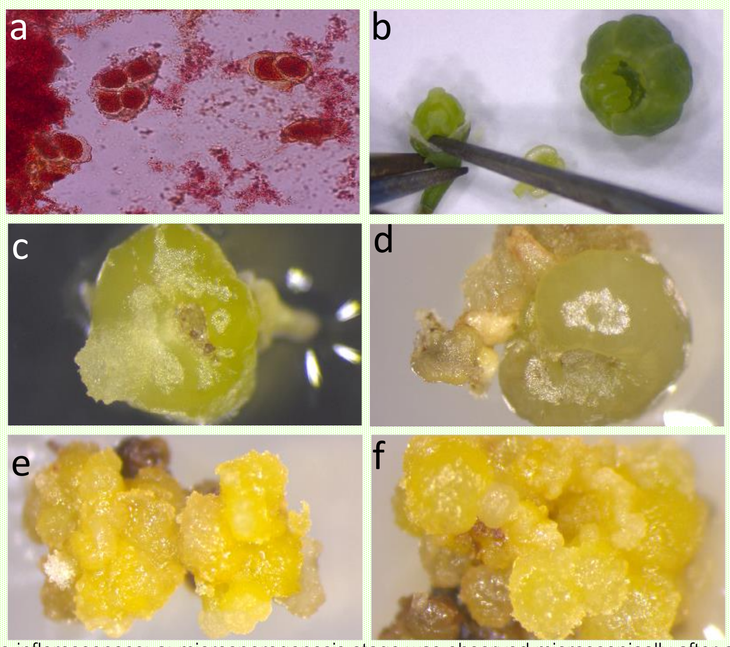


Fig. 1: Grapevine inflorescences: a: microsporogenesis stage was observed microscopically after anther squashing in Safranine-O; b: ovaries and anthers collecting phase; c-d: pre-embryogenic calli formation after 14-30 days post collected; e-f: embryogenic calli formation after 60-90 days

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.

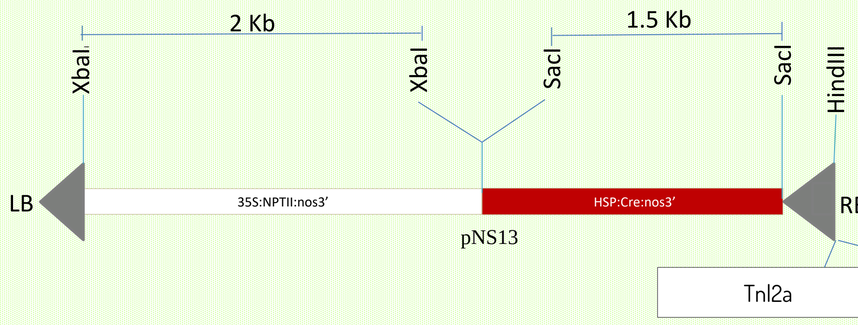


Fig. 2 Cisgenesis construct with recombinase system.

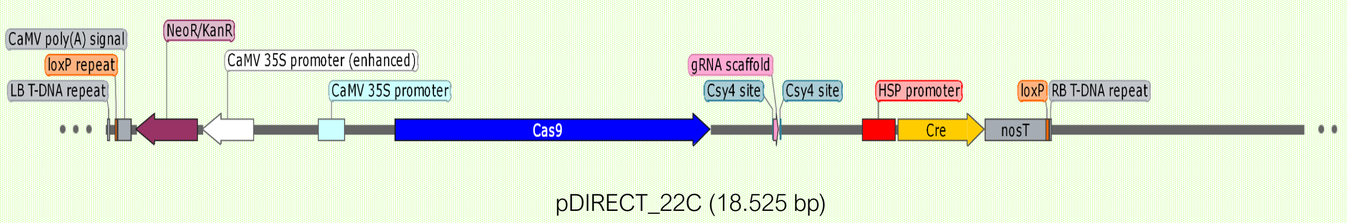


Fig. 3 Genome-editing construct carrying two guideRNAs for each gene and Cre-loxP system. We produced three different constructs one for each gene family.

## Results

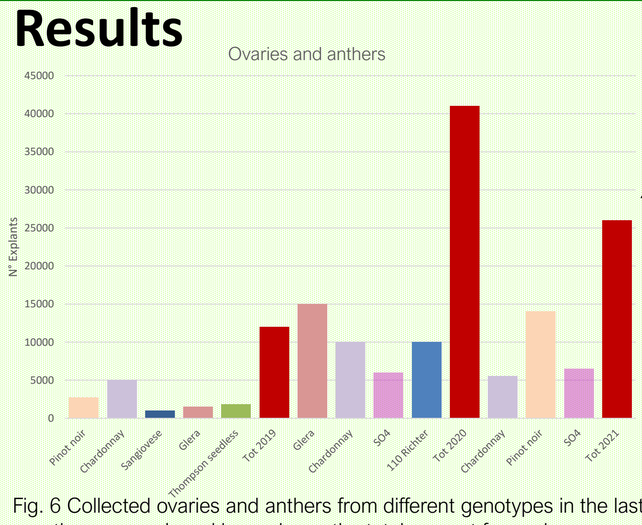


Fig. 6 Collected ovaries and anthers from different genotypes in the last three years. In red have shown the total amount for each year.

Embryogenic calli (Fig. 6) were used to performe *Agrobacterium*-mediated stable transformation (Fig. 7). We obtained different transformed plants for each construct.


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

## Ongoing activities

Fig.8 Regenerated plant.

Regenerated plants (Fig. 8) were evaluated for T-DNA presence (Fig. 9).

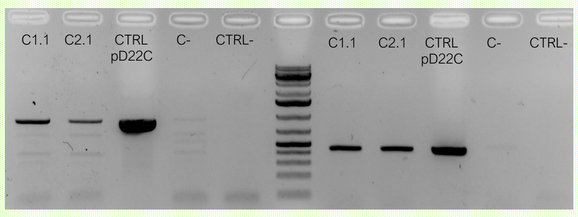


Fig.9 Screening PCR in Chardonnay edited lines.

The confirmed lines were micropropagated and tested under different temperature conditions for T-DNA excision (Fig. 10).

Fig.10 Micropropagated plant.

## Conclusions

The **NPBTs** display the potential to revolutionize the agricultural research field especially in woody 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:

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**Reference contacts:** loredana.moffa@crea.gov.it, luca.nerva@crea.gov.it