NEW PLANT BREEDING TECHNOLOGIES TOWARDS A MORE SUSTAINABLE VITICULTURE

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Background and Objectives

European grapevine cultivars are highly susceptible to many pathogens that are managed through a large pesticide use as reported in Fig. 1.

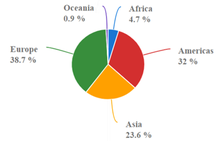
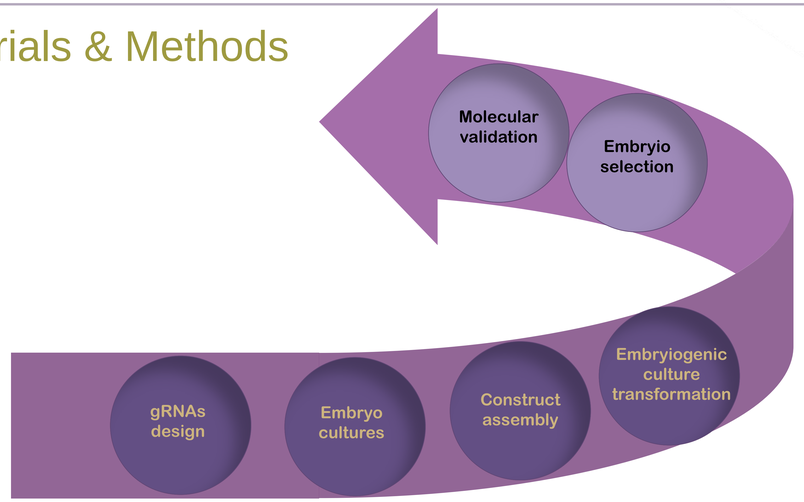


Fig.1: Fungicides and bactericides + (Total) by continent Average 2000 – 2018. Source: FAOSTAT

In this framework, the European policies encourage a pesticides use reduction which pass also through the genetic improvement of cultivated plants; Among others technique, grapevine genetic improvement can greatly benefit from New Plant Breeding Technologies. In specific, we focused our work to reduce grape fungal susceptibility, by producing knock-out plants from embryogenic calli using CRISPR/Cas9 technology.

In the present work the main goal is knocking-out two susceptibility genes belong to *Mildew Locus O* gene family: *VvMLO7* and *VvMLO6.* These genes seem to be involved in *Erysiphe necator* interaction (Pessina et al., 2016).

Materials & Methods



Preliminary results

1. Embryogenic calli obtainment for different genotypes: Chardonnay, Glera, Sangiovese, Pinot noir, SO4 and 110 Richter Fig. 2. Evaluation of inflorescence development stage as described in Gribaudo et al., 2004, through microscopy assay and embryogenic calli formation. Fig. 3.

Fig. 2: ovaries and anthers collected during 2019 and 2020.

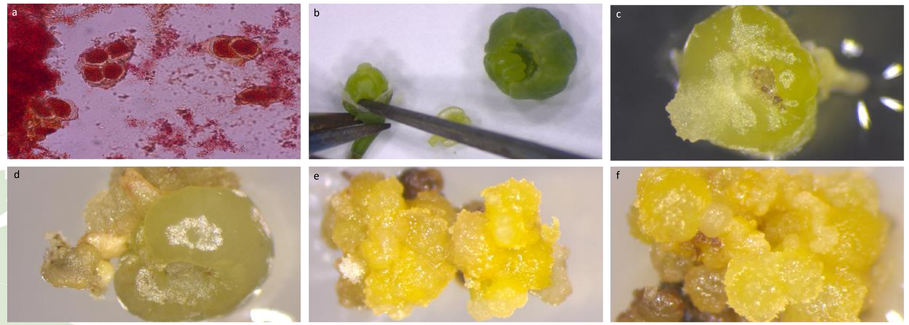


Fig. 3: grapevine inflorescences: a: Microsporogenesis stage was observed microscopically after anther squashing in Safranine-O; b: ovaries and anthers collecting phase; c-d: pre-embryogenic calli formation after 14-30 days post collected; e-f: embryogenic calli formation after 60-90 days.

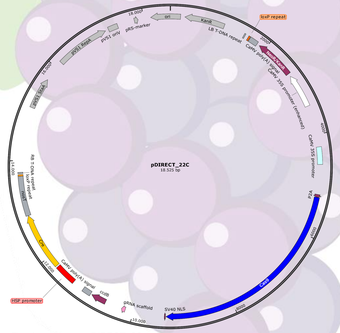


Fig. 4: construct pDIRECT_22c after recombinase system insertion.

2. Construct assembly: the entry vector pDIRECT_22c (Čermák et al., 2017) was modified to insert two specific gRNAs for each gene and a recombinase system in order to remove marker genes after the editing will be confirmed. This approach is based on a recombinase technology involving the Cre-loxP system from the P1 bacteriophage under a heat-shock inducible promoter. The final construct is shown in Fig. 4.

3. Embryogenic calli transformation mediated by *Agrobacterium tumefaciens:* the binary vector for the knock-out *of VvMLO7+6* genes were transferred into *Agrobacterium tumefaciens* GV3101 strain.

Conclusions

The NPBTs, and in specific CRISPR/Cas9 systems, display the potential to revolutionize the agricultural research field especially in crop such as grapevine. In this study we produced several embryogenic calli transformation in order to knock-out two susceptibility genes to *Erysiphe necator* the causal agent of powdery mildew. Further studies are ongoing to enhance grape abiotic stress resilience using a similar approach.

References

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