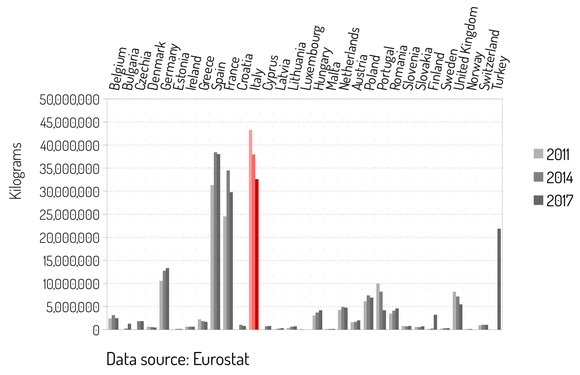
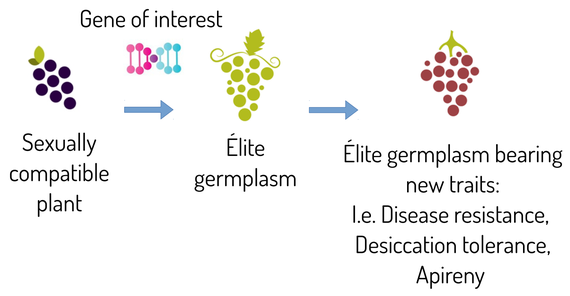
Genetic improvement of **grapevine** for **downy mildew resistance** through a **cisgenic** approach

Fungicides and Bactericides consumption in EU

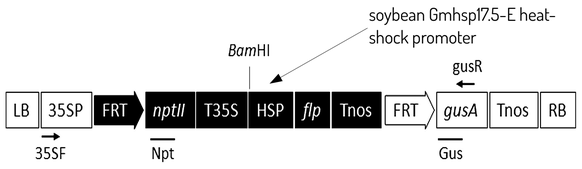


Cisgenesis

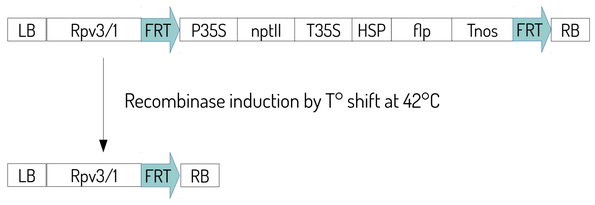


Rpv3-1 resistance

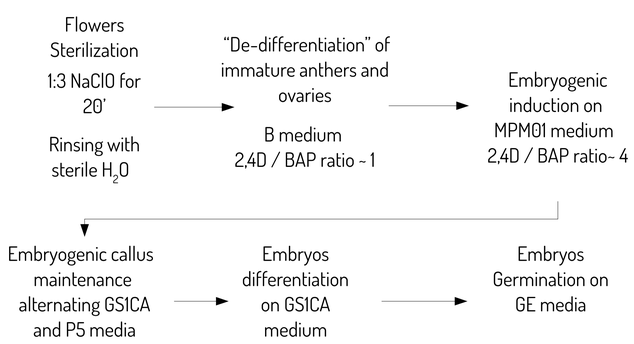
Heat shock induced *S. cerevisiae* Flp-FRT recombination system



Dalla Costa et al. 2016, Herzog et al. 2012



Somatic embryogenesis from flower tissues



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Modern biotechnological approaches toward sustainable viticulture

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Introduction

- Conventional breeding does not allow the introgression of single traits without compromising the genetic background that characterize an èlite cultivar.
- Cisgenesis make possible to introduce single genes from sexually compatible species, preserving all the already selected traits, avoiding the presence of transgenes usually perceived as unsafe by consumers.
- Downy mildew is one of the most severe disease in grapevine and it's caused by the oomycete *Plasmopara viticola*. To date, 27 QTLs have been associated with downy mildew disease resistance (*RPV1-RPV27*).
- Just in case of *RPV1* and *RPV3*, however, the underlying genes have been identified and characterized. Both belong to nucleotide-binding leucine rich repeat (NB-LRR) receptor family, acting as cytoplasmic pathogen sensors, triggering cell-death mediated defense at the infection sites.

Aims

- Introducing Rpv1 and Rpv3-1 resistance
- Reduction of the agrochemicals needs
- Evaluation of Rpv1 and Rpv3-1 resistance into the selected varieties

Preliminary results

- Flower tissues of Glera, Sangiovese, Pinot Nero, Chardonnay and Thompson seedless collected from field grown plants and from fruiting cuttings, will be used as explants for the induction of somatic embryos.

Collection dates	Variety	Explanted		Retained		
		Anthers	Ovaries	Anthers	Ovaries	Percent %
6.5 – 14.5 – 21.5 – 25.5 – 28.5 – 6.6 (T) – 8.7(T)	Glera	8775	1755	3125	625	35.6
6.5 – 18.5	Chardonnay	1350	270	375	75	27.8
6.5 – 13.5	Sangiovese	950	190	650	130	68.4
18.5 – 23.5 – 6.7 (T) –	Pinot nero	2625	525	1075	215	41.0
25.5	Thompson seedless	1425	285	450	90	31.6

Perspectives

- PCR products of candidate genes, including native promoter and terminator, will be cloned into a binary vector engineered with an inducible excision system.
- Agroinfiltration and Agrodrenching will be used to evaluate the efficacy of the candidate genes into the different genetic backgrounds of the selected cultivars.
- The gene construct will be used for the stable transformation of grapevine embryogenic calli through *A. tumefaciens* infection.
- Regenerated plantlets will hence be selected and checked for the presence and expression of candidate genes.
- For the removal of exogenous sequences, chemical or thermal induction of the excision system will be used.
- Resistance and susceptibility to downy mildew will be tested by leaf disc bioassay or whole leaves inoculation of *in-vitro* and acclimatized plantlets.