PhD School on Agriculture, Environment and Bioenergy

(http://sites.unimi.it/dottorato_aab/)

(XXXIX cycle, 2023-26)

Project draft

1. Field of interest

AGR03

2. Project title

Identification of genes related to grapevine response to biotic stresses

3. Tutor (membro del Collegio dei Docenti)

Gabriella De Lorenzis

- Eventually: co-tutor/s

Silvia Laura Toffolatti

4. Relevance of the topic and state of the art

Downy mildew (DM) is one of the most destructive grapevine (*Vitis vinifera*) disease caused by *Plasmopara viticola* (Berk. and Curt.) Berl. and de Toni. Cultural practices are scarcely effective in reducing disease incidence, whereas regular fungicide treatments more efficiently protect grapevine to DM and PM. Nevertheless, the intensive use of chemicals becomes more and more restrictive (EU Directive 2009/128) due to their high costs, their risk on human health and their negative environmental impact due to the chemical residues detected in grapes, soil and aquifers. Recently, resistant phenotypes to DM agent were reported in Caucasian germplasm (Bitzade et al. 2015, Toffolatti et al. 2016) and some candidate key genes related to the plant pathogen interaction have been identified (Toffolatti et al. 2018, Toffolatti et al. 2020). Exploitation of these traits in grapevine breeding could be one of the possible ways to find a valid alternative to chemicals for disease management and to achieve an effective protection to DM in an environmental friendly way. In this project, the validation of candidate genes will be performed following two approaches: i) dsRNA; ii) genome editing.

5. Layout of the project (draft)

5.1. Materials & Methods:

WP1 - Validation of candidate genes *via* dsRNA: In Toffolatti et al. (2018, 2020) and Marcianò et al. (2023) some resistance and susceptibility genes were identified in Mgaloblishvili and Pinot noir cultivars. Their validation will be carried out through dsRNA assay (Dubrovina et al. 2019; Marcianò et al. 2021).

WP2 - Validation of candidate genes *via* genome editing: target genes validated in WP1 will be used to obtain resistance genotypes through genome editing protocols. To speed up the procedure, calli will be edited and the phenotyping will be performed on edited calli. Once the validation is over, genes will be used to edit embryos.

5.2. Schedule and major steps (3 years):

Activities	First year	Second year	Third year
WP1	dsRNA assay and phenotyping of plants treated with dsRNAs		
WP2	Genome editing on calli and phenotyping of edited genotypes		Genome editing on embryos

6. Available funds

Financial support by INVINIVERITAS project and Transition grant: 10,000.00 €/year.

7. Literature

Bitsadze N, et al. Screening of Georgian grapevine germplasm for susceptibility to downy mildew (*Plasmopara viticola*). Vitis 2015, 54:193–196.

Dubrovina A, et al. Induction of transgene suppression in plants via external application of synthetic dsRNA. Int. J. Mol. Sci. 2019, 20:1585.

Laucou V, et al. Extended diversity analysis of cultivated grapevine *Vitis vinifera* with 10K genome-wide SNPs. PLoS ONE 2018, 13:1–27.

Marcianò D, et al. RNAi of a Putative Grapevine Susceptibility Gene as a Possible Downy Mildew Control Strategy. Front. Plant Sci. 2021, 12:667319.

Marcianò D, et al. Influence of Nitrogen on Grapevine Susceptibility to Downy Mildew. Plants 2023, 12:263.

Toffolatti SL, et al. Evidence of resistance to the downy mildew agent *Plasmopara viticola* in the Georgian *Vitis vinifera* germplasm. Vitis 2016, 55:121–128.

Toffolatti SL, et al. Unique resistance traits against downy mildew from the center of origin of grapevine (*Vitis vinifera*). Sci. Rep. 2018, 8:12523.

Toffolatti SL, et al. Novel aspects on the interaction between grapevine and *Plasmopara viticola*: dual-RNA-Seq analysis highlights gene expression dynamics in the pathogen and the plant during the battle for infection. Genes 2020, 11:261