PhD School on Agriculture, Environment and Bioenergy

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

(XXXVI cycle, 2020-23)

Project draft

1. Field of interest

General arboriculture and tree crops (AGR/03)

Applied life Sciences and Non-Medical Biotechnology (LS9)

2. Project title

Selection of grapevine cultivars resistant to biotic stresses

3. Tutor (membro del Collegio dei Docenti)

De Lorenzis Gabriella

- Eventually: co-tutor/s

Lucio Brancadoro

4. Relevance of the topic and state of the art:

Downy mildew (DM) and powdery mildew (PM) are the two most destructive grapevine (*Vitis vinifera*) disease caused by *Plasmopara viticola* (Berk. and Curt.) Berl. and de Toni. and *Erysiphe necator*, respectively. 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 and PM agents 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 and PM in an environmental friendly way.

In this project, the validation of candidate genes (WP1) will be performed as well as the GWAS analysis on populations harboring DM and PM resistant traits (WP2).

5. Layout of the project (draft)

5.1. Materials & Methods:

WP1 - Validation of candidate genes: In Toffolatti et al. (2018, 2020) 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) and once their involvement in the plant-pathogen interaction will be ascertained, these target genes will be used to obtain resistance genotypes through genome editing protocols.

WP2 - GWAS analysis on populations harboring DM and PM resistant traits: In Bitzade et al. (2015) and Toffolatti et al. (2016), some grapevine cultivars resistant to DM and PM were identified. A number of progenies harboring DM and PM resistant traits have been developed so far. A couple of these progenies will be genotyped by the Vitis18kSNP genotyping (Laucou et al. 2018) array and phenotyped for their resistance to DM (Toffolatti et al. 2016) and PM (Barba et al. 2013), in order to identify the loci associated to the resistant traits.

5.2. Schedule and major steps (3 years):

Activities	First year	Second year	Third year
WP1	dsRNA assay	Genome editing and phenotyping of edited genotypes	
WP2	DM and PM phenotypic characterization		*Phenotypic characterization if necessary*
	Genotypic characterization		
			GWAS data analysis

6. Available funds (source and amount)

Financial support by iGrape project: 10,000.00 € / year.

7. Literature:

Barba P, et al. Grapevine powdery mildew resistance and susceptibility loci identified on a high-resolution SNP map. Theor. Appl. Genet. 2014, 127:73-84.

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.

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