

UNIQUE RESISTANCE TRAITS AGAINST DOWNY MILDEW FROM THE DOMESTICATION CENTER OF GRAPEVINE

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***Plasmopara viticola*: an enemy of grapevine**

Downy mildew is one of the most severe diseases of *Vitis vinifera*, the grapevine species cultivated worldwide for wine fresh fruit and raisin production. It is caused by the oomycete *Plasmopara viticola*, a polycyclic pathogen able to grow only on grapevine causing damages to all green tissues of the host: leaves, shoots, inflorescences and bunches (Gessler *et al.*, 2011). Due to the high susceptibility of *V. vinifera*, fungicide application is unavoidable to obtain an adequate yield, in terms of quality and quantity. Fungicide application is however associated with risks for human health and the environment. To achieve a sustainable grapevine production, there is the need to develop alternative and complementary disease control measures. The cultivation of grapevine varieties resistant to *P. viticola* could help reducing the fungicide use.

Resistance traits from the grapevine domestication center

Traditionally, resistance is found in American and/or Asian grapevine species that co-evolved with the pathogen or with a close pathogen species. The hybrids obtained by crossing *V. vinifera* with those grapevine species, however, do not meet the standard criteria for the production of high quality wines, which can be obtained only with pure *V. vinifera* cultivars. With the aim of finding sources of resistance in *V. vinifera*, our research group screened a large collection of Georgian grapevine accessions, that are characterized by a high genetic variability (Sargolzaei *et al.*, 2021), and found a black cultivar, named Mgaloblishvili, that showed mild (or absent) symptoms in field and reduced disease severity and sporulation in laboratory trials (Toffolatti *et al.*, 2016). Aim of our research was, then, the characterization of the resistance mechanism of Mgaloblishvili, before exploiting it for breeding.

Putative resistance mechanism of Mgaloblishvili against *P. viticola* infection

Transcriptome analysis of grapevine genes allowed us to draw the putative resistance mechanism of Mgaloblishvili (Toffolatti *et al.*, 2018), that is based on: i) recognition of the pathogen through PAMP (Pathogen Associated Microbial Pattern) receptors and effector receptor (rust resistance kinase LR10); ii) recognition of the damages caused to the host by the pathogen during infection through DAMP (Damage Associated Microbial Pattern) receptors; and iii) synthesis of antimicrobial compounds, mainly terpenes (Ricciardi *et al.*, 2021), and structural barriers through cell wall reinforcement.

Apart from the detection of the first resistance genes of *V. vinifera*, for the first time the existence of a susceptibility gene in the grapevine species was discovered (Toffolatti *et al.*, 2020). Susceptibility genes facilitate the compatibility between plant and pathogen and are essential for their interaction and the disease development. Therefore, their silencing results in pathogen resistance (Zaidi *et al.*, 2018; Thatcher *et al.*, 2012).

GWA (Genome-wide association) analysis allowed us to identify three resistance *loci* in Georgian cultivars located on chromosomes 14 (*locus* Rpv29), chromosome 3 (*locus* Rpv30) and chromosome

16 (*locus* Rpv31). These *loci* include genes that are involved in defenses from biotic stress and in particular in pathogen recognition (rust resistance kinase LR10), cell wall reinforcement and signal transduction (Sargolzaei *et al.*, 2020).

What is happening from the pathogen point of view?

Based on the analysis of *P. viticola* transcriptome, we moreover identified the pathogenicity genes involved in the modulation of the plant immune system (Toffolatti *et al.*, 2020). In particular, genes encoding for four cytoplasmic effectors, twenty apoplastic effectors and forty proteins with signal peptide for secretion were overexpressed by *P. viticola* in the interaction with the *V. vinifera* cultivars Mgaloblishvili and Pinot noir.

New prospective for the modern viticulture

In conclusion, putative grapevine resistance and susceptibility genes were identified. These genes could be exploited for achieving *V. vinifera* varieties that are resistant to *P. viticola* and that can be, at the same time, cultivated also for producing Protected Designation of Origin (PDO) wines. Genetic improvement for resistance could be performed through traditional or, better, innovative breeding techniques, such as the transgene-free system CRISPR, that could allow us to transform already existing cultivars from susceptible to resistant. The *loci* associated with resistance will be used to screen collections of *V. vinifera* to identify other cultivated or wild *V. vinifera* accessions possessing it and will be used for Marker Assisted Selection (MAS) breeding. Finally, to avoid the selection of *P. viticola* strains that are able to overcome resistance, we aim at developing new and safe target-specific fungicides, such as peptide aptamers, acting against *P. viticola* genes with no effect on beneficial off-target organisms. The aptamers, that are short chains of amino acids, will contribute to control the pathogen, that possesses a great evolutionary potential due to its polycyclic behavior and the occurrence of sexual reproduction, and reduce the selection pressure exerted by the resistant plants on the pathogen.

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