Screening for adaptation to resistant grapevine accessions in *Plasmopara viticola* population of north-eastern Italy

Marone Fassolo[1,2], E., Maddalena[1,2], G., and S.L. Toffolatti[1*]

[1]Università degli Studi di Milano, Dipartimento di Scienze Agrarie e Ambientali, via Celoria 2, 20133, Milano, Italy
[2] Università degli Studi di Milano, Dipartimento di Bioscienze, via Celoria 26, 20133, Milano, Italy

1 Introduction

Downy mildew is one of the most severe diseases of *Vitis vinifera*, the grapevine species cultivated worldwide for wine and fruit production (Gessler *et al.,* 2011). It is caused by the oomycete *Plasmopara viticola*, a polycyclic pathogen and an obligate parasite of grapevine. The infections of bunches, shoots and leaves cause quantitative and qualitative yield reductions (Fig. 1). The premature plant defoliation also affects the survival of the plant during winter.

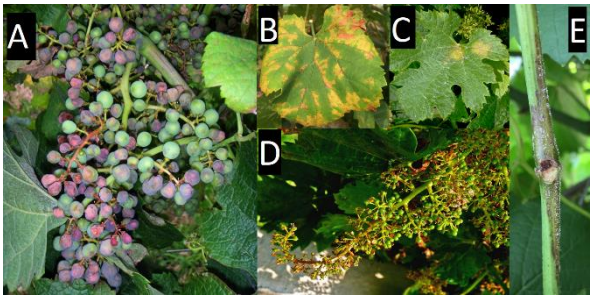

Figure 1: Symptoms of downy mildew on bunches (A, D), leaves (B, C) and shoots (E).

P. viticola originated in North America and was introduced into Europe, and from there in the rest of the world, at the end of XIX Century (Fontaine *et al*., 2021). Due to the high susceptibility of the commonly cultivated *V. vinifera* varieties, the management of downy mildew is mainly based on the application of suitable agronomic practices and fungicides, that are associated with risks for human health and the environment. The cultivation of resistant varieties can help increasing the sustainability of viticulture by reducing the number of fungicide sprays necessary to control the disease. Breeding for resistance is traditionally achieved by crossing the susceptible *V. vinifera* species with the resistant species that are found among the American or Asian germplasm. The updated list of resistant grapevine varieties and the resistance *loci* is available at https://www.vivc.de/index.php?r=qtls-vivc%2Findex.

Bianca, an interspecific hybrid possessing 78% *V. vinifera* genes and 12% genes from American grapevine species (Di Gaspero and Cipriani, 2002), is among the most well-known resistant cultivars. It harbours the *Rpv3* QTL. However, the resulting hybrids cannot be cultivated for the production of Designation of Origin wines in Italy. Moreover, taking into consideration the pathogen adaptation to host resistance genes, we're always in a need for new resistance genes. Durability of resistance is a major problem in dealing with the cultivation of tree plants resistant to pathogens. Indeed, in recent years, *P. viticola* strains able to overcome resistance imposed by one QTL have been already found for Bianca and Regent (*Rpv3* QTL) (Delmotte *et al*., 2014; Peressotti *et al*., 2010; Toffolatti *et al*., 2011).

The screening of a large collection of Georgian grapevine accessions, that are characterized by a high genetic variability, allowed to discover a black cultivar, named Mgaloblishvili, that showed reduced disease severity in field and laboratory trials (Toffolatti *et al*., 2016). Microscopy and comparative transcriptome analyses highlighted that the putative resistance mechanism of Mgaloblishvili is different from that present in the resistant control variety, Bianca (Toffolatti *et al*., 2018). Indeed, the pathogen infection was blocked at 3 days post inoculation (dpi) and did not induce the hypersensitive response commonly observed in Bianca. Moreover, different genes were modulated not only by Mgaloblishvili in response to *P. viticola* inoculation, but also by *P. viticola* during the interaction with the Caucasian accession (Toffolatti *et al*., 2018; 2020).
Aim of our research was, then, the characterization of the durability of Mgaloblishvili resistance, before exploiting it for breeding. Mgaloblishvili harbours the *Rpv29* QTL (Sargolzaei *et al*., 2020) and in this study, its resistance levels to *P. viticola* isolates of north-eastern Italy was assessed and compared to that of Pinot noir (susceptible control) and Bianca (resistant control).

2 Materials and methods

Potted plants of Cabernet sauvignon, Pinot noir, Mgaloblishvili and Bianca were grown in greenhouse. Seventy-two *P. viticola* isolates were obtained by serial dilution of sporangia collected from infected leaves of 14 vineyards located in north-eastern Italy (Fig. 2). The isolates were propagated on healthy leaves of cv Cabernet sauvignon. The third to the fifth leaves starting from the apex of the shoots of Mgaloblishvili, Pinot noir and Bianca were sampled for the experimental inoculation. Three leaves were sampled per plant and three leaf discs were cut from each leaf and placed in Petri dishes. 10 µL droplets of sporangia suspension ($5x10^4$ sporangia/mL) were inoculated in the centre of each leaf disc. The infection level was estimated as percentage of sporulating area (PSA) by image analysis of the leaf area covered by sporulation 5-13 days after incubation in growth chamber at 22°C with 8/16 h photoperiod (Hernandez *et al*., 2021). *P. viticola* isolates were grouped according to four phenotype classes (Table 1), depending on presence/absence and the visual density of sporulation at 7 dpi (Fig. 3; Table 1).

*Corresponding author: silvia.toffolatti@unimi.it

Significant differences among the rank-transformed PSA values recorded at 13 dpi by the members of the different classes on the three cultivars were assessed by Kruskal-Wallis test and pairwise comparison by SPSS v. 26 (IBM Analytics, Milano).

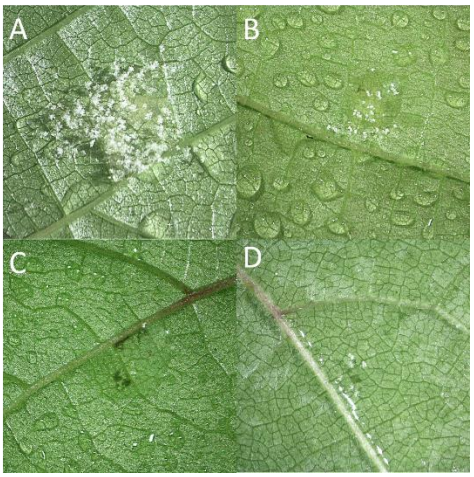

Figure 3: Pictures of leaf tissues of Pinot noir (A), Mgaloblishvili (B) and Bianca (C, D) seven days after *P. viticola* inoculation with a suspension of sporangia in the centre of the image. Diffused sporulation (high sporulation, HS) is visible on Pinot noir (A), while limited sporulation (low sporulation, LS) is present on Mgaloblishvili (B). On Bianca, necrotic spots with little (D) or without (no sporulation, N) (C) sporulation were observed.

3 Results

Based on the presence of sporulation, *P. viticola* strains could be grouped into four phenotypic classes: in the first class were placed 19 isolates that were able to affect only the susceptible reference; in the second class were grouped 9 isolates able to infect both Pinot noir and Bianca; in the third class, were placed 16 isolates causing disease on Pinot noir and Mgaloblishvili; and in the fourth one were grouped 28 isolates that were able to infect all the cultivars, with different disease intensity levels. Based on the detection of sporulation, the latency period was 5-7 days long on Pinot noir and 7-13 days long on Mgaloblishvili and Bianca.

Table 1**.** Phenotypic classes assigned to the isolates based on the presence and intensity of sporulation* on Pinot noir (P), Bianca (B) and Mgaloblishvili (M) and number of strains included in each class

Class	**P**	**B**	**M**	**Strains n.**
1	HS	N	N	19
2	HS	LS	N	9
3	HS	N	LS	16
4	HS	LS	LS	28

*HS=high intensity of sporulation; LS=low intensity of sporulation; N=no sporulation;

Variable levels of disease severity in the three cultivars could be observed at 13 dpi. In all phenotypic classes, the PSA values were significantly higher on Pinot noir (16.7%<PSA<39,9%), than on Mgaloblishvili (3.8%<PSA<22.9%) and Bianca (0.2%<PSA<1%) (Table 2). The strains included in group 3 and 4 showed significantly higher PSA values on Mgaloblishvili than on Bianca.

Table 2. Average PSA (%) values (± standard error, SE) recorded at 13 dpi by the strains grouped in each class (strains n.) and results of statistical analysis*

Class	Strains n.	Cultivar	PSA (%) ± SE
1	19	Bianca	0.2 ± 0.2 a
		Mgaloblishvili	3.8 ± 3.8 a
		Pinot noir	16.7 ± 6.6 b
2	9	Bianca	1.0 ± 0.9 a
		Mgaloblishvili	4.1 ± 3.6 a
		Pinot noir	22.5 ± 8.6 b
3	16	Bianca	0.4 ± 0.2 a
		Mgaloblishvili	22.9 ± 7.9 b
		Pinot noir	39.9 ± 10.2 c
4	28	Bianca	1.0 ± 0.6 a
		Mgaloblishvili	19.9 ± 5.8 b
		Pinot noir	33.6 ± 7.1 c

*Different letters indicate significant differences among PSA values (P<0.04)

4 Conclusions and future perspectives

In general, reduced disease severity occurred in both resistant accessions. Even if the strains included in groups 3 and 4 showed significantly higher PSA values on Mgaloblishvili than on Bianca, the values ranged between 20-23%. These values are in line with those previously reported on the Mgaloblishvili and confirm that the cultivar is able to significantly reduces, but not completely inhibiting, the pathogen infection (Toffolatti *et al*., 2016). This suggests that Mgaloblishvili and Bianca maintained their resistant behaviour towards the tested strains.

However, this study also highlighted that *P. viticola* isolates are potentially able to overcome resistance imposed by two different QTLs, since sporulation was often visible at 13 dpi also on resistant cultivars. The adapted strains showed a longer latency period and a reduced disease severity, indicating a fitness penalty or a partial ability to overcome resistance. Since the achievement of resistant cultivars is a hard task and their cultivation is the major solution for reducing the impact of the management of downy mildew, it is of fundamental importance not only to combine different resistance genes in a single cultivar, but also to follow an integrated disease management strategy to control the adapted pathogen individuals. In the future, it would be interesting to identify the pathogenicity mechanism of the adapted and non-adapted strains to develop innovative

fungicides, such as short peptides and small RNAs, able to specifically target the pathogen genes/proteins (Rosa *et al*., 2021; Marcianò *et al*., 2021).

Figure 2: Location of the sampled vineyards and number of isolates obtained (map from google maps).

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