

## Phenotyping and genetic analysis of the Caucasian grape resistance to *Erysiphe necator*

Possamai T.<sup>(1)\*</sup>, Wiedemann-Merdinoglu S.<sup>(2)</sup>, Lacombe M. C.<sup>(2)</sup>, Dorne M. A.<sup>(2)</sup>, Merdinoglu D.<sup>(2)</sup>, De Nardi B.<sup>(1)</sup>, Migliaro D.<sup>(1)</sup>, Velasco R.<sup>(1)</sup>, De Mori G.<sup>(3)</sup>, Cipriani G.<sup>(3)</sup> and Testolin R.<sup>(3)</sup>

<sup>1</sup>CREA - Research Centre for Viticulture and Enology, viale XXVIII Aprile 26, 31015, Conegliano (TV), Italy

<sup>2</sup>INRAE, Université de Strasbourg, UMR 1131, 28 rue de Herrlisheim, 68000, Colmar, France

<sup>3</sup>Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, via delle Scienze 206, 33100, Udine (UD), Italy

### 1 Introduction

*Vitis vinifera* L., the Eurasian grapevine, because of its desirable fruit characteristics, is the most cultivated grapevine species worldwide. *Erysiphe necator* (syn. *Uncinula necator*) (Schw.) Burr., the causal agent of grape powdery mildew (PM), is native of North America and is one of the most devastating diseases affecting the viticulture. The pathogen has accidentally spread around the world and the lack of coevolution with *V. vinifera* has hindered the development of effective resistances in cultivated varieties. We investigated the resistance to *E. necator* in one Caucasian *V. vinifera* accession and the trait segregation in a breeding population.

### 2 Materials and methods

In 2018, the Caucasian variety ‘Shavtsitska’, reported as resistant to *E. necator* (Failla *et al.* 2016), was crossed with the susceptible variety ‘Glera’ at the CREA - Research Centre for Viticulture and Enology grape germplasm collection (Italy, 45°51'07.6"N, 12°15'28.6"E).

In 2019, seeds of the cross ‘Shavtsitska x Glera’ (population code 50042) were sown at INRAE-Centre Grand Est Colmar UMR 1131 (France). The origin and identity of seedlings were verified by SSR markers. The true-to-type progenies were grown in 2-liter pots in a mixture of sand-perlite-lapilli in greenhouse at 28°C (max temperature) with 16 h light and 8 h dark photoperiod. Shoots were periodically pruned to limit the vegetation and provided young apical leaves for phenotyping. Pests and diseases were managed by spraying every two weeks. Replicates of the parental plants were produced by wood cuttings. Several ‘control’ genotypes (characterized by different degree of resistance to *E. necator* and carrying specific *Run/Ren* loci), among which ‘RV1-22-8-78’ (‘RV1’ - carrying *Run1*), ‘Kishmish vatkana’ (‘K. vatkana’ - carrying *Ren1*), ‘Johanniter’ (carrying *Ren3* and *Ren9*) and ‘Cabernet sauvignon’ (‘Cabernet s.’ - carrying no resistance loci) were produced by softwood cuttings and added to the experiments.

The resistance to *E. necator* was studied by leaf discs bioassays in 2019 and 2020 prepared as described in Possamai *et al.* (2021). An *E. necator* single spore isolate obtained from susceptible infected plants in the greenhouse in 2018 was maintained and multiplied every ten days on young and disinfected leaves of ‘Cabernet s.’ in Petri dishes. For the phenotyping bioassays, sample discs of 1 or 2 cm of diameter, according to the type of the experiment, were excised with a cork borer from young and shiny leaves collected from a shoot apex of the studied plants. Leaf discs were placed in

Petri dishes on a wet filter paper disc on agar 10 g/l and inoculated with 600–800 conidia/cm<sup>2</sup> through a settling tower. Petri dishes were incubated in climatic chamber at 23°C with a photoperiod of 16 h light and 8 h dark.

A histochemical study was carried out and three leaf discs of 1 cm of diameter per individual were evaluated at 1, 2, and 3 days post infection (dpi) for several bioassays. Trypan-Blue staining was carried out as described in Possamai *et al.* (2021). After Trypan-Blue staining, 1 cm discs were evaluated by bright-field microscopy (x100) and one-hundred germinated conidia per disc were categorized in 4 classes: 0 = conidia + appressoria; 1 = conidia + primary hypha; 2 = conidia + primary and secondary hypha; and 3 = conidia + three hyphae and/or branched hyphae. Classified data were utilized to fit linear models (LM) by software R (R Core Team, 2017) and to compare the pathogen development on cross parents and control plants. A preliminary study on the production of callose depositions in response to *E. necator* was carried out by Aniline-Blue staining as described in Agurto *et al.* (2017) with little modifications: leaf discs were cleared with 3 dips of 30 min in an ethanol 96% - acetic acid 100% solution (3:1 by volume) and rinsed twice for 30 min in a 0.15 M K<sub>2</sub>HPO<sub>4</sub> solution; then, discs were stained in 0.15 M K<sub>2</sub>HPO<sub>4</sub> and 0.01% Aniline-Blue solution for 1-2 h; finally, discs were washed once in water and stored in lactoglycerol overnight. Callose depositions were observed by epifluorescence microscopy using a DAPI filter (x200). Observations under a scanning electron microscope (SEM) were also carried out.

Phenotyping of the population 50042 was finalized on 264 seedlings and in three replicated tests. In each replicate one disc of 2 cm per genotype and up to four discs per parental plant were prepared. PM infection was evaluated at 3-5-7-10 dpi. At each dpi, four area per leaf disc were scored by stereomicroscope (x64) for: pathogen mycelium growth, sporulation intensity, mean number of conidia per conidiophore and presence or absence of plant necrosis. Pathogen mycelium and sporulation were scored according to the OIV 452-1 descriptor (2009) with some modifications: 9 = absence of pathogen structures in the area; 7 = presence of few-short hyphae/few conidiophores; 5 = mycelium/conidiophores sparse with low density or spread in colonies; 3 = dense mycelium/conidiophores on most of the leaf disc area; and 1 = dense mycelium/conidiophores covered all the observed area.

At 10 dpi leaf discs were suspended in 300 ul of Tween-20 water solution (0,05 % volume/volume) and conidia in suspension counted through the Malassez Counting Chamber. Conidia counts were square root transformed. The relative

\*Corresponding author: [tyrone.possamai@crea.gov.it](mailto:tyrone.possamai@crea.gov.it)

Area Under Disease Pressure Curve (rAUDPC) (Jeger and Viljanen-Rollinson, 2001) was calculated for *E. necator* mycelium growth and sporulation intensity starting from the discs averaged score per dpi. Standard broad-sense heritability ( $H^2$ ) was calculated by 'inti' R package (Lozano-Isla 2021).

Genetic analysis on 'Shavtsitska' and its cross population was carried out as described in Possamai *et al.* 2021. Briefly, DNA was extracted with the DNeasy 96-well DNA extraction kits (Qiagen, Hilden, Germany). The GBS data were generated following an upgraded Elshire *et al.* (2011) method. The reads were aligned to the 12X.2 *V. vinifera* reference genome 'PN40024' (Canaguier *et al.*, 2017) and the SNP calling by Stacks software (Catchen *et al.*, 2013). The pseudo-testcross markers (Grattapaglia & Sederoff, 1994) selection and the linkage analysis were performed using a custom pipeline in R mainly based on the 'ASMap' package (Taylor *et al.* 2017). Genotypic and phenotypic data were utilized together to carried out the QTL analysis using 'qtl' R package (Broman *et al.*, 2003). Individual and averaged experimental data were all investigated. Interval Mapping (IM) was carried out by scanone function (model = normal and method=em). Makeqtl and fitqtl functions (model = normal, formula=y~Q1) were used to fit the final QTL models and calculate the trait narrow-sense heritability ( $h^2$ ).

### 3 Results and discussion

Several Caucasian *V. vinifera* were described as resistant to *E. necator* (Failla *et al.*, 2016). Preliminary checks did not identify for such accessions relationship to known resistance sources (data not shown). Therefore, to investigate and characterize the Caucasian resistance determinants, the cultivar 'Shavtsitska' was chosen, crossed to generate a breeding population and the progeny studied in several leaf discs bioassays. The Caucasian grape showed a partial resistance to *E. necator* that was inherited in the progeny and associated to a mayor QTL in chromosome 13.

In Trypan-Blue staining about 1-3% of the conidia did not germinated. 'Shavtsitska' and 'RV1' early delayed the pathogen growth and at 1 dpi they showed a higher amount of conidia in classes 0 and 1 in comparison to 'Glera' and 'Cabernet s.' (Fig.1a). At 2 dpi 'Shavtsitska', 'K. vatkana' and 'RV1' showed most conidia in classes 0, 1 and 2 while 'Glera' and 'Cabernet s.' had conidia with longer and ramified hyphae and in class 3 (Fig. 2b). At 3 dpi, all plants except 'RV1', which halted the pathogen growth according to other genotypes carrying the *Run1* locus (Feechan *et al.*, 2011; Pap *et al.* 2016; Agurto *et al.*, 2017), showed most conidia in class 3, but on resistant plants hyphae were shorter and had fewer ramifications. Significant phenotypic differences were calculated between susceptible and resistant plants at 3 dpi but they were less important compared to the observations at 2 dpi (Fig. 3c).

Necrosis and callose depositions were early, frequently and intensively produced at all penetration sites in 'RV1' as attended for *Run1* (Feechan *et al.*, 2011; Pap *et al.* 2016; Agurto *et al.*, 2017). 'Shavtsitska' showed only frequent and intense necrosis from 2 dpi beneath the appressoria of both conidia and hyphae (Fig 2a). In 'K. vatkana', carrying *Ren1*, the necrosis reaction was less frequent and less intense (Qiu *et al.* 2015), and probably activated in post-penetration

(Hoffmann *et al.* 2008), accompanied by callose depositions at 2-3 dpi and in particular beneath the primary appressoria (Fig. 2b). Finally, 'Johanniter', carrying *Ren3* and *Ren9*, and the susceptible plants showed only a weak necrotic response (more frequent closed to the conidia appressoria in 'Johanniter' discs).

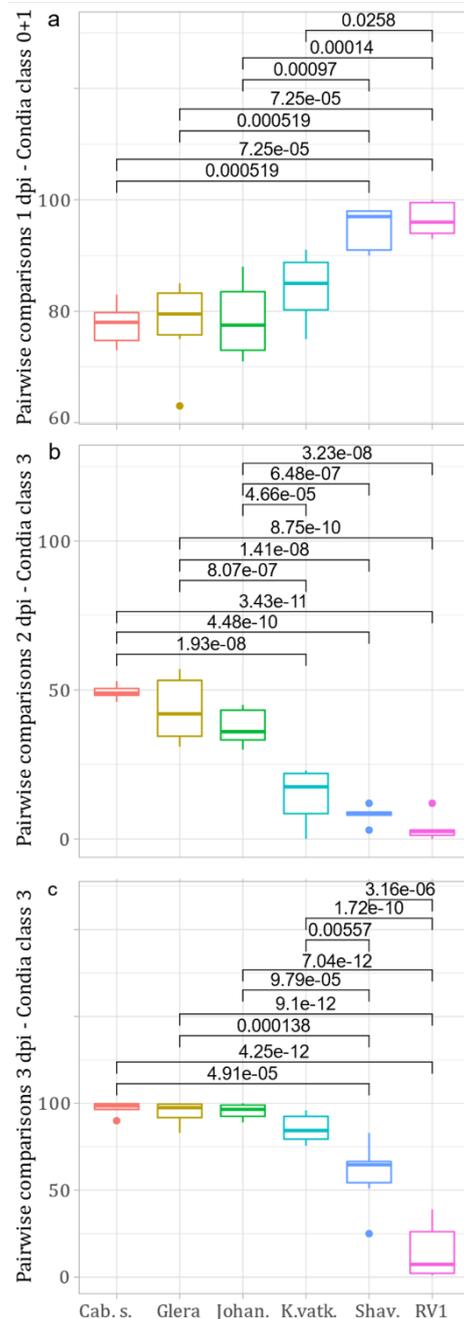


Figure 1: Box-plots for 1 (a), 2 (b) and 3 (c) dpi Trypan-Blue experiments data with significant differences from pairwise comparisons between the studied varieties ( $p$ -value < 0.05 for Tuckey HSD tests).

Observations made at SEM showed that on resistant plants 'RV1', 'Shavtsitska' and 'K. vatkana' had multilobed and

multiple appressoria were early and frequently present (Schnee *et al.* 2008) confirming the reaction at the infection sites. On ‘Shavtsitska’ discs, we observed that *E. necator* conidia were able to establish a successful interaction on straight hairs but not on prostrate hairs suggesting a possible role of trichomes as physical barrier (Niks & Rubiales, 2002). ‘Shavtsitska’ displayed from 3 to 10 dpi a partial resistance to *E. necator* with limitation of pathogen development (Fig. 3) and a frequent and increasing necrotic response: mycelium growth was delayed through all the experiments (rated up to

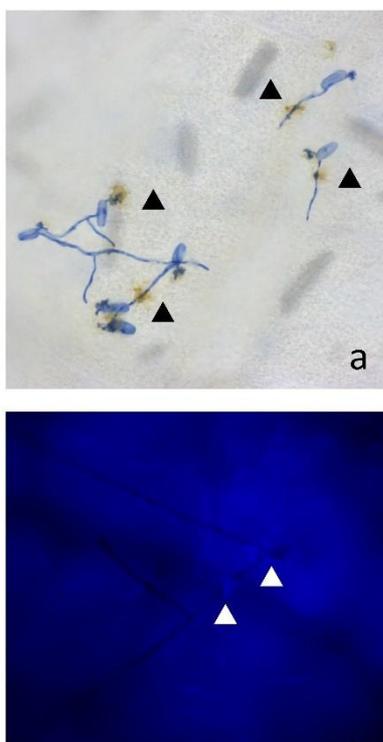


Figure 2: Necrosis production on ‘Shavtsitska’ (a) and callose depositions on ‘K. vatkana’ (b).

5); latent period ended at 7 dpi and sporulation was poor and rated between 7 and 5; the mean rAUDPC was 0.74 +/- 0.14 for mycelium growth and 0.95 +/- 0.08 for sporulation intensity; 1-2 conidia per conidiophore and between 0 and 2.1 x 10<sup>4</sup> conidia/cm<sup>2</sup> were counted at 10 dpi. ‘Glera’ was not able to inhibit the *E. necator* development (Fig. 3): mycelium grew fast and spread over the whole discs (score 1); sporulation was produced at 5 dpi with final scored of 3 and 1; the averaged rAUDPC resulted 0.33 +/- 0.13 and 0.67 +/- 0.12 for mycelium growth and sporulation, respectively; up to 6 conidia per conidiophore and between 4.1 x 10<sup>3</sup> and 6.7 x 10<sup>4</sup> conidia/cm<sup>2</sup> were counted at 10 dpi.

‘Shavtsitska’ showed a degree of resistance to *E. necator* between ‘RV1’ carrying *Run1* (totally resistant with no pathogen sporulation; Pauquet *et al.* 2001; Feechan *et al.* 2011; Pap *et al.* 2016) and ‘K. vatkana’ carrying *Ren1* (partially resistant with complete pathogen life cycle; Hoffman *et al.* 2008) (Fig. 3).

Some 264 seedlings of population 50042 were phenotyped, 158 three times and 106 twice. The offspring usually displayed the same resistance phenotype among replicates

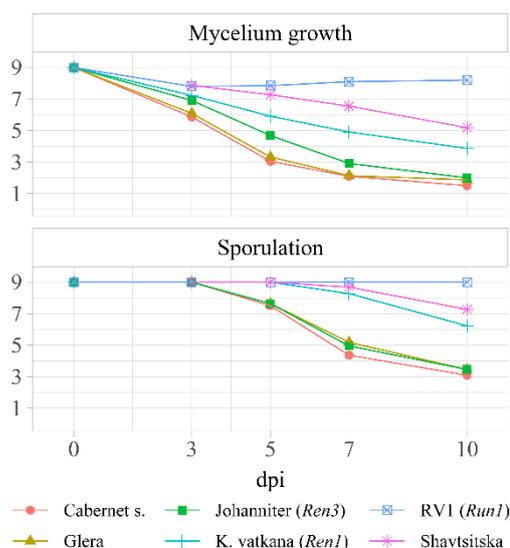


Figure 3: Behavior of *E. necator* infection on the studied varieties.

(maximum Spearman coefficients of correlation between data of 0.59-0.69) and the trait segregated in a Mendelian way with a ratio of 1:1. About 45-50% of the individuals showed a susceptible-like phenotype while the remaining had variable level of partial resistance with some of them very close to ‘Shavtsitska’ (Fig. 4; Tab. 1). The phenotypic data distribution was usually continuous but clearly bimodal for several series of data, as for qualitative segregation of *Run1* and *Ren6* loci in Pauquet *et al.* (2001) and Pap *et al.* (2016) (Fig. 4). Broad-sense heritability data confirmed that the Caucasian resistance strongly affected the pathogen development, in particular its sporulation, and evidenced the quality and reproducibility of the trait phenotyping strategy (Tab.1).

Resistance variable	mean	std	min	max	H <sup>2</sup>
Mycelium g. 5 dpi	4.82	1.41	1.89	8.17	0.67
Sporulation 7 dpi	6.01	1.84	2.91	9.00	0.83
Necrosis 5 dpi	0.31	0.30	0.00	1.00	0.59
√(conidia/cm <sup>2</sup> )	232.61	91.17	47.61	479.39	0.59
rAUDPC myc. g.	0.46	0.14	0.22	0.85	0.63
rAUDPC spo.	0.73	0.13	0.50	1.00	0.83

Table 1: Statistics and heritability recorded for the resistance variables observed on cross population.

Together with ‘Shavtsitska’ and ‘Glera’, a total of 184 offsprings were genotyped. Maternal and paternal maps were built separately by using the data of 183 individuals and 2,291 and 2,627 markers, respectively. ‘Shavtsitska’ genetic map covered 1,205 cM while ‘Glera’ genetic map 1,315 cM, both split into 19 LG. SNP kept a good coverage on most of the LG, with only few gaps, in particular for ‘Shavtsitska’, remained. Marker with distorted segregation were observed on ‘Glera’ LG 13. Finally, the maps showed a close

correlation between the SNP genetic order and their physical position on grape reference genome and were therefore considered reliable for a QTL analysis.

The QTL analysis identified a strong resistance to *E. necator* in the Caucasian variety, and none in ‘Glera’. The interval mapping (IM) located the major QTL in ‘Shavtsitska’ chr 13 at about 47 cM from the top (Tab. 2). LOD peak varied from 5.87 to 64.88 according to variable observed, experiment replicate and the time course (dpi) considered. The averaged data for 5-7 dpi observations (as suggested by Blanc *et al.*, 2012) and the rAUDPC indexes provided the best LOD values. QTL models explained between 50.68 and 80.15% of the observed phenotypic variance (narrow-sense heritability;  $h^2$ ) and included the locus in 2.2 cM interval in ‘Shavtsitska’ map, that corresponds to 1.4 Mb on the grape reference genome (contained within the SNP13\_16797000 and the SNP13\_18213673). Ratio between narrow and broad sense heritability ( $h^2/H^2$ ) showed that the resistance QTL explained almost all of the genetic variation component resulting from the phenotyping (up to the 97%).

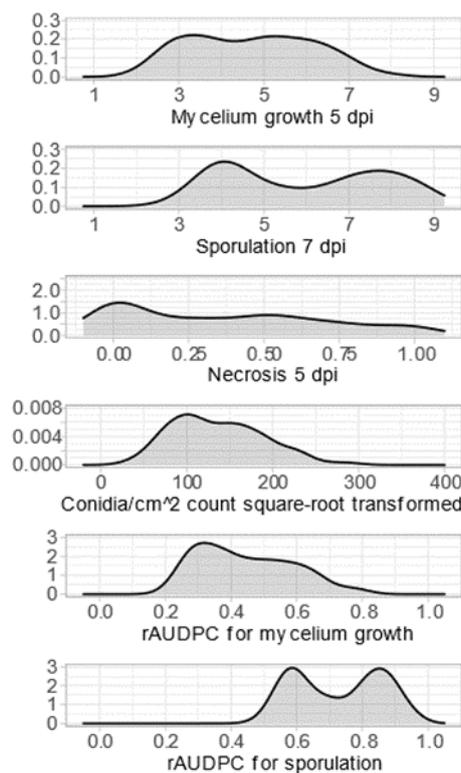


Figure 4: Phenotypic data distributions for the main resistance variables recorded for cross population 50042 (y-axis density values).

Observed variables	Chr	LOD	Pos.	$h^2$
Mycelium g. 5 dpi	13	40.17	47.0	0.63
Sporulation 7 dpi	13	61.45	47.0	0.78
Necrosis 5 dpi	13	31.65	46.7	0.55
$\sqrt{(\text{conidia}/\text{cm}^2)}$	13	28.68	46.7	0.51
rAUDPC - mycelium g.	13	37.72	46.7	0.61
rAUDPC - sporulation i.	13	64.88	47.0	0.80

Table 2: Results of QTL analysis for ‘Shavtsitska’.

#### 4 Conclusions

The resistance to *E. necator* of ‘Shavtsitska’, a Caucasian grapevine variety, significantly lowers the severity of foliar PM infection in controlled conditions. Riaz *et al.* (2020) characterized a similar phenotypic resistance in several wild Caucasian *V. vinifera* and located the genetic basis of the trait in the chromosome 13. Evidences showed the genetic inheritance of resistance in both wild and cultivated *V. vinifera*. However, we considered the inheritance of the trait in the domestication process as not intentional because no one reported PM disease in Europe and Asia before the 19th century. Instead, natural or intentional selection may have taken place after *E. necator* introduction from North America favouring in such a way the maintenance of the trait in the cultivated varieties.

Caucasian grapes may be of interest in grape breeding (Sargolzaei *et al.*, 2020) because they have a ‘vinifera’ genetic background and pleasant agronomic characteristics. Therefore, the Caucasian resistance to *E. necator* could be introgress in breeding programs in one or limited cross generations, in the perspective of producing new elite cultivars with pyramided resistance genes.

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