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


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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 accidently 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-lapillli 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 Ren/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/cm² 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 K2HPO4 solution; then, discs were stained in 0.15 M K2HPO4 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
Area Under Disease Pressure Curve (AUDPC) (Ieger 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 hereditability (*H²*) 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 12X2 *V. vinifera* reference genome ‘PN40024’ (Canaguier et al., 2017) and the SNP calling by Stacks 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 fit the final QTL models using a custom pipeline in R.

### 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 major 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. vatkanca’ 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. vatkanca’, 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).

![Box-plots](image_url)

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. vatkanca’ 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 thorough all the experiments (rated up to 158; partially resistant with 201‘x 10 conidia per conidiophore; 0.12 growth was delayed thorough all 1 to 6 conidia per conidiophore and between 0 and 2.1 x 10 conidia/cm² 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 10³ and 6.7 x 10³ conidia/cm² were counted at 10 dpi.

‘Shavtsitska’ showed a degree of resistance to *E. necator* between ‘RV1’ carrying Ren1 (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 (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 Ren1 and Ren5 loci in Pauquet et al. (2001) and Pap et al. (2016) (Fig. 4). Broad-sense hereditability 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).

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 10⁴ conidia/cm² 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 10³ and 6.7 x 10³ conidia/cm² were counted at 10 dpi.

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<table>
<thead>
<tr>
<th>Resistance variable</th>
<th>mean</th>
<th>std</th>
<th>min</th>
<th>max</th>
<th>H²</th>
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<tbody>
<tr>
<td>Mycelium g. 5 dpi</td>
<td>4.82</td>
<td>1.41</td>
<td>1.89</td>
<td>8.17</td>
<td>0.67</td>
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<tr>
<td>Sporulation 7 dpi</td>
<td>6.01</td>
<td>1.84</td>
<td>2.91</td>
<td>9.00</td>
<td>0.83</td>
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<tr>
<td>Necrosis 5 dpi</td>
<td>0.31</td>
<td>0.30</td>
<td>0.00</td>
<td>1.00</td>
<td>0.59</td>
</tr>
<tr>
<td>√(conidia/cm²)</td>
<td>232.61</td>
<td>91.17</td>
<td>47.61</td>
<td>479.39</td>
<td>0.59</td>
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<td>rAUDPC myc. g.</td>
<td>0.46</td>
<td>0.14</td>
<td>0.22</td>
<td>0.85</td>
<td>0.63</td>
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<tr>
<td>rAUDPC spo.</td>
<td>0.73</td>
<td>0.13</td>
<td>0.50</td>
<td>1.00</td>
<td>0.83</td>
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</table>

Table 1: Statistics and hereditability 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 hereditability; $h^2$) 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%).

<table>
<thead>
<tr>
<th>Observed variables</th>
<th>Chr</th>
<th>LOD</th>
<th>Pos.</th>
<th>$h^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycelium g. 5 dpi</td>
<td>13</td>
<td>40.17</td>
<td>47.0</td>
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<td>Sporulation 7 dpi</td>
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<td>61.45</td>
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<td>Necrosis 5 dpi</td>
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<td>$\sqrt{c_1+c_2}$</td>
<td>13</td>
<td>28.68</td>
<td>46.7</td>
<td>0.51</td>
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<td>rAUDPC - mycelium g.</td>
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<td>46.7</td>
<td>0.61</td>
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<tr>
<td>rAUDPC - sporulation i.</td>
<td>13</td>
<td>64.88</td>
<td>47.0</td>
<td>0.80</td>
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</tbody>
</table>

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.
References


13. Lozano-Isla F. 2021. inti: Tools and Statistical https://doi.org/10.1038/s1015634617334


