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Titolo della tesi

Mapping of resistance QTL to powdery mildew (*Erysiphe necator* Sch.) in the Caucasian grape varieties "Shavtsitska" and "Tskhvedianis tetra" (*Vitis vinifera* L.)

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Table of contents

List of acronyms

AFLP: amplified fragment length polymorphism

AUDPC: area under disease pressure curve

Avr: avirulence

BC: backcross

bp: bays pair

chr: chromosome

CIM: composite interval mapping

cM: centi-Morgans

dpi: days post-infection

ETI: effector triggered immunity

ETS: effector triggered susceptibility

GBS: genotyping by sequencing

GQ: genotype quality

HR: hypersensitive response

IM: interval mapping

Kb: kilobase

LG: linkage group

LOD: logarithm of odds

LRR: leucine rich repeat

MAB: marker assisted breeding

MAMP: microbial associated molecular patterns

MAS: marker assisted selection

Mb: megabase

MLO: mildew resistance locus O

MQM: multiple QTL mapping

MST: minimum spanning tree

NB: nucleotide binding

NGS: next generation sequencing

NHR: non-host resistance

OIV: International Organisation of Vine and Wine

PAMP: pathogen associated molecular patterns

PCD: programmed cell death

PCR: polymerase chain reaction

PRR: pattern recognition receptors

PTI: pattern triggered immunity

QTL: quantitative trait loci

R (-genes): resistance

RAPD: random amplification of polymorphic DNA

rAUDPC: relative AUDPC

RFLP: restriction fragment length polymorphism

ROS: reactive oxygen species

Ren: resistance to *Erysiphe necator*

Rpv: resistance to *Plasmopara viticola*

Run: resistance to *Uncinula necator*

SA: salicylic acid

SEM: scanning electron microscope

SNP: single nucleotide polymorphism

SSR: single sequence repeat

TSP: travelling salesperson problem

.vcf: variant call format

VMC*: Vitis* Microsatellite Consortium

Abstract

Vitis vinifera L. is the most cultivated grapevine species worldwide. *Erysiphe necator*, the causal agent of grape powdery mildew, is one of the main pathogens affecting the viticulture. *V. vinifera* has usually little genetic resistance against *E. necator* and grape production is highly dependent from agrochemicals. The main purpose of this work was the study and the mapping of the resistance to *E. necator* in the Caucasian *V. vinifera* germplasm. The biparental mapping approach was chosen to investigate the genetic basis of the trait and two F1 populations were developed by crossing Shavtsitska and Tskhvedianis Tetra with two susceptible *V. vinifera* varieties, Chardonnay and Glera. The phenotypic resistance of parental plants and offsprings was studied by using leaf discs bioassays and evaluating the infection features during the pathogen life cycle. Caucasian cross parents showed a resistance to *E. necator* and the trait segregated in their populations: the resistant genotypes delayed and limited the development of pathogen mycelium, sporulation and conidia but they did not halt completely the infections. A total of 184 seedlings of Shavtsitska cross population were genotyped through the Genotyping by Sequencing (GBS) technology allowing the development of two high-density linkage maps for the cross parents. QTL analysis revealed a major resistance locus on Shavtsitska linkage group 13. Such a QTL was associated with a reduced pathogen development as well as an enhanced plant necrotic response. The QTL explained up to 80.15% of the observed variability and was restricted in an interval of approx. 2.2 cM. The comparison with grape reference genome PN40024 located the QTL at about 18 Mb from the top of the DNA sequence on chromosome 13 and recombinants analysis restricted the locus in a region of 1.4 Mb. Some SSR located in the genomic region were used for genotyping the cross populations of the study and 103 further Caucasian varieties. Resistance associated SSR alleles were shared among Shavtsitska, Tskhvedianis tetra, resistant seedlings and 22 Caucasian varieties suggesting a widespread presence of the resistance trait in the Caucasian germplasm. The QTL isolated in Shavtsitska located in the region where the Ren1 resistance gene carried by several Central Asia *V. vinifera* was previously mapped. Further molecular analysis is needed to confirm whether different genes in such region, that in the reference genome PN40024 is rich of resistance motifs, control the resistance to *E. necator* in grape accessions of different geographic origin. Meanwhile, our findings

would provide new *V. vinifera* genetic sources for grape breeding programs aiming to obtain resistant elite cultivars.

Key words: Resistance genes, Linkage maps, Grape breeding, Mildew phenotyping, MAS

Other activities carried out during the doctoral program and related to grape genetic improvement for resistances to biotic stresses

I was involved for three years in the CREA-VE grape breeding program. The main activities to which I participated had been:

a) Identification of parental plants and crosses programming to produce new grapevine varieties resistant to *P. viticola* and *E. necator*;
b) Grapevine seedlings growing in greenhouse, in field plantation and evaluations of resistance to pathogens, agronomic and grape-related traits;
c) Phenotyping of seedlings for the resistance to *E. necator* in the greenhouse and for the resistance to *P. viticola* by *in vitro* detached leaf discs bioassay;
d) Markers assisted selection (MAS) of grape seedlings with SSR markers and optimization of a new rapid and low-cost protocol for the molecular screening of grape progeny.

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1 Introduction

1.1 The grapevine

Vitis vinifera L. has been the most cultivated grapevine species since ancient times. Since the beginning of its domestication, humans have selected the most promising genotypes and have applied cultural practices to manage its growth and production. The improvement and the success of *V. vinifera* cultivation has brought the species worldwide in all continents.

Nowadays, the grapevine is ones of the most widely grown fruit crop and occupies 7.4 million hectares. Some 78 million tonnes of fresh fruits are annually harvested, of which slightly more than half are reserved to the production of wine (57%) while the destination of the rest is for table grape (36%) and dried grape (7%) production (OIV, 2019).

In addition to its economic value, *V. vinifera* is a model organism for the study of perennial fruit crops and non-climacteric fruit ripening (Cantu & Walker, 2019).

1.1.1 Grapevines taxonomy

Grapevines of agronomic importance are classified in the order of *Vitales*, in the family of *Vitaceae*, that comprises hundreds of species, and within the genus *Vitis* L. The *Vitis* genus encompasses more than 70 species which are divided into the two subgenera *Muscadinia* and *Vitis* on the basis of some morphological and genetic diversities (Planchon, 1887; Moore & Wen, 2016; Lu et al., 2018).

The subgenus *Muscadinia* contains the two species *V. popenoei* and *V. rotundifolia*, which grow in the South-East of the North America and in Central America (Hickey et al., 2019). They mainly differ from other *Vitis* spp. for having continuous internodes, simple tendrils, prominent lenticels and smooth bark (Hickey et al., 2019). *Muscadinia* species differ from *Vitis* relatives for having 40 chromosomes (2n) instead of 38, for this reason the intergeneric crosses within *V. rotundifolia* results in chromosomal unbalanced offsprings (Patel & Olmo, 1955). *V. rotundifolia* is of particularly interest because being cultivated since five centuries (Olien, 1990) and used, more recently, in grape breeding programs due to its high resistance to many biotic stresses (Bouquet, 1981; Olmo, 1986).

Vitis spp., on contrary, are characterized by nodes with a diaphragm that separates the internodes, bifid or more branched tendrils and a thick and rough scaly bark (Chen et

al., 2007; Wen, 2007; Moore & Wen, 2016). The subgenus *Vitis* spp. is widespread throughout the Northern Hemisphere (Figure 1) and the centres of grape genetic diversity are located in the South-East of the North America and in East Asia (Chen et al., 2007; Wen, 2007; Moore & Wen, 2016). *Vitis* spp. can be divided in three groups based on their area of diffusion and genetic distances (Wen et al., 2018a):

1) American grapevines: about thirty species belong to this group and are spread between the tropical area of Central America and the cold regions of North America (Moore & Wen, 2016). Some species of temperate and cold climates (e.g. *V. aestivalis*, *V. berlandieri, V. champini*, *V. cinerea*, *V. labrusca*, *V. riparia*, *V. rupestris* and *V. solonis*) have found relevant uses as cultivated varieties, cross parent in breeding program and rootstocks;
2) Asian grapevines: this group includes at least 30 species (Chen et al., 2007). The most known are *V. amurensis*, *V. piasezkii*, *V. pseudoreticolata* and *V. romanetii* because have been recently studied or involved in grape breeding programs;
3) Euroasiatic or European grapevine: only *V. vinifera* belongs to this group, which exists both in wild (ssp. *sylvestris*) and cultivated (ssp. *vinifera*) form.

Figure 1. Native geographic distribution of the *Vitis* spp. (grey shading). Dashed lines indicate southern borders of the polar ice cap during the most recent ice age. Dash-dot lines indicate ice age refugia of the forest flora (Wan et al. 2013).

Many American and Asiatic species have overlapping distributions, so natural hybridization may occur where there are no ecological and phenological barriers. The systematics of *Vitis* spp. is based primarily on morphology, while molecular methods have only recently been introduced. However, the species classification is confused, possibly due to natural hybridizations and extreme morphological variations within the single species (Wan et al., 2013).

The family *Vitaceae* includes other fifteen genera, some examples are *Ampelocissus* (that is the closest to the genus *Vitis*; Wen et al., 2018b), *Pterisanthes*, *Tetrastigma*, *Landukia*, *Parthenocissus*, *Ampelopsis* and *Cissus*, which contain only species of ornamental importance.

1.1.2 Grapevine domestication and modern viticulture

Archaeological remains traced back the appearance of the genus *Vitis* to over sixty-five million years ago (Brown, 1962; Nie et al., 2010; Wan et al., 2013). Part of the speciation within the genus *Vitis* was probably determined by subsequent glacial and interglacial periods over millions of years: grapevines populations were segregated or gathered in different areas and forced to new ecological adaptations (Hewitt, 1996; Wan et al., 2013). Between Europe and West Asia only the species *V. vinifera* survived (Figure 1). Genetic studies evidenced the Anatolian Peninsula and Caucasus regions as diversity centres for *V. vinifera* subsp. *sylvestris* and the Near East as the possible centre of origin of the species (Arroyo-García et al., 2006; Grassi et al., 2006; Zecca et al., 2012). Caucasus grape germplasm in fact showed particular genetic patterns such as few relations with foreign *V. vinifera* accessions and small genetic distances between wild and cultivated *V. vinifera* (Imazio et al., 2013; De Lorenzis et al., 2015; Riaz et al., 2018; Sargolzaei et al., 2021).

The most ancients traces of *V. vinifera* cultivation and wine production were found in Caucasus and Near East and were dated to 4000-6000 years b.C (McGovern, 2003; Myles et al., 2011; McGovern et al., 2017). This geographical area would probably be the main centre of grape domestication. However, intraspecific hybridizations with European *V. vinifera* populations (Myles et al., 2011; Zecca et al., 2012; Riaz et al., 2018) and secondaries domestication in the Mediterranean basin (Arroyo-García et al., 2006; Myles et al., 2011) may also contributed to the origin and spread of viticulture.

V. vinifera was the only *Vitis* spp. having been truly domesticated from the wild relative plants. Human intervention occurred during thousands of years and throughout several generations (Zhou et al., 2017; Liang et al., 2019). Domestication syndrome for *V. vinifera* included a higher sugar content in the grape, an increased size of berry and bunch, changes in seed morphology and a shift from dioecious to hermaphroditic mating system (This et al., 2006). The domesticated plants with favourable agronomical traits differentiated *V. vinifera* ssp. *vinifera* from ssp. *sylvestris*.

V. vinifera genetic improvement continued through the selection of plants generated by intraspecific crosses or somatic mutations (This et al., 2006). Elite cultivars were historically selected and perpetrated, even over centuries, by vegetative propagation and then adopted by modern viticulture (e.g. Ramos-Madrigal et al., 2019). In the last five hundred years grapevine cultivation became widespread all around the world, the elite *V. vinifera* cultivars were mainly disseminated and nowadays they occupy considerable percentages of the vine-growing area (http://www.oiv.int/). However, thousands of *V. vinifera* grape varieties with different genetic profiles and ampelographic characteristics still exist (Alleweldt & Possingham, 1988; Robinson et al., 2012). It is assumed that the exact number constantly changes due to the disappearance of ancient accessions and the creation of new varieties from grape breeding.

Although *V. vinifera* is the most cultivated grape species because of desirable fruit characteristics, a wide range of pests and diseases impacts on its cultivation. *Daktulosphaira vitifoliae* Fitch (or grape phylloxera), *Plasmopara viticola* Berl. & De Toni (the causal agent of downy mildew) and *Erysiphe necator* Sch. (the causal agent of powdery mildew) are the most devasting plagues affecting *V. vinifera*. These diseases are native of North America and have been accidentally introduced in Europe, and in other countries around the world, starting from the mid of the 19th century. Unfortunately, the lack coevolution between *V. vinifera* and the pathogens resulted in the absence of effective resistances to alien biotic stresses in the European grapevine. *V. vinifera* cultivars in modern viticulture are grafted on rootstock resistant to *D. vitifoliae* and agrochemicals are used to minimize the impact of *P. viticola* and *E. necator*. The control of fungal diseases requires a careful agronomic management and up to twenty or more phytosanitary interventions. Agrochemicals represents worldwide a cost for viticulturist (e.g. Sambucci et al., 2019) and potential negative

impacts on human health and the environment (e.g. Komárek et al., 2010). For these reasons, winegrowers are increasingly looking for new solutions that limit the use of phytosanitary products (Pertot et al., 2017) and increase the sustainability of grape cultivation.

1.2 *Erysiphe necator:* the causal agent of grape powdery mildew

Powdery mildews are common diseases caused by filamentous fungi that affected more than 9000 dicot and over 650 monocot plant species (Inuma et al., 2007). *Erysiphe necator* Sch. (synonym *Uncinula necator* Burr.), telomorphic form, or *Oidium tuckeri,* anamorphic form, is an Ascomycete, obligate biotrophs, belonging to the order of *Erysiphales* and to the family of *Erysiphaceae*.

1.2.1 Reproductive biology and epidemiology

E. necator damages grape tissues throughout the entire vegetative season (Figure 2). Primary infections are caused either by ascospores originating from cleistothecia (main overwintering structure, Figure 3a) or, particularly in areas with mild winters, by mycelium survived in dormant buds (Pearson & Gartel, 1985). Ascospores and hyphae produce lobed appressoria which actively penetrates the plant tissues both by a mechanical pressure and an enzymatic activity on the epidermis cuticle and cell walls. The pathogen develops globular haustoria responsible for the nutrition of the pathogen in invagination of plant cell membranes (Manners, 1989). The fungus produces multiple hyphae and appressoria on the tissues of the plant developing a wide and complex mycelium. Under optimal conditions, multiseptated conidiophores emerge from the hyphae. Conidia are mainly wind dispersed and initiate secondary infections (asexual reproduction). *E. necator* differentiates cleistothecia when hyphae of compatible mating types get in contact (sexual reproduction). Cleistothecia contain from 4 to 6 asci and from 4 to 8 ascospores per asci. *E. necator* life cycle is shown in Figure 2 (Pearson & Goheen, 1988) and is extensively described in Gadoury et al. (2012).

Ascospore infections begin in spring when rainfalls exceed 2–3 mm and temperatures are above 10°C (Gadoury, 1990). The lower and upper temperature limits for the pathogen growth are 6° and 32°C, respectively (Carroll & Wilcox, 2003). *E. necator* develops rapidly from 23 to 30°C, with an optimum at 26°C, and with an average

atmospheric humidity of 85 %. *E. necator* is particularly vulnerable to ultraviolet radiation and consequently to direct sunlight (Willocquet et al., 1996).

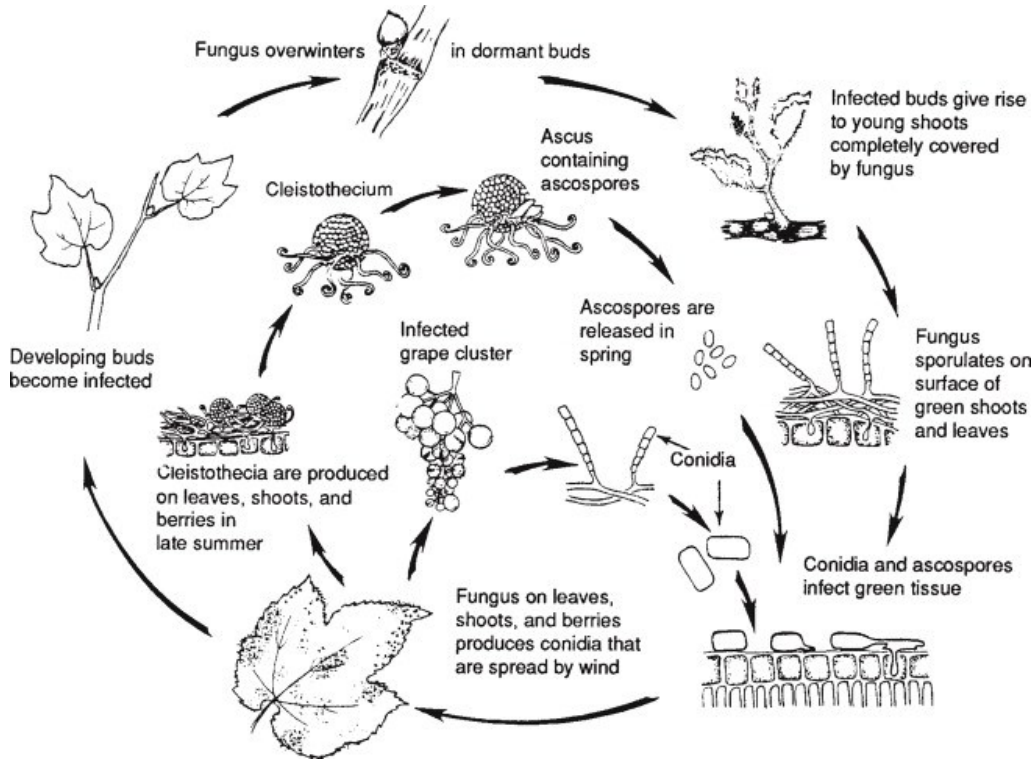

Figure 2 Cycle of *E. necator* (Pearson & Goheen, 1988).

1.2.2 Symptomatology

E. necator grows on all grapevine green tissues (Figure 3). Powdery mildew is displayed as visible whitish powdery colonies on plant organs. During the infection, the colonies gets thicker, larger and more intensely coloured due to the pathogen mycelium growth and sporulation. The leaves are usually colonized in the adaxial surface and if infected early can grow irregularly and twistedly (Figure 3b-c). Leaves seem characterized by an ontogenic resistance: young leaves with a thin cuticle have a higher susceptibility, while older leaves are less vulnerable to *E. necator* infections (Doster, 1985; Merry et al., 2013). More or less numerous plant necrosis can be observed under the mycelium (Figure 3d). *E. necator* overwintering in dormant buds gives rise to "flag shoots" the following spring (Figure 3e). The inflorescences, when hit, undergo floral abortion and can shrivelled completely. Infected young berries can break off during growth (Figure 3f) and become more prone to rot (e.g. *Botrytis* infections). Grape berries can be infected as long as they do not reach 8% of sugar content, while established colonies can sporulate until sugar content reaches 15%

(Gadoury et al., 2003). Grape yield is qualitatively damaged when plant canopy is reduced and when grape acquires negative organoleptic properties. Damages became quantitative with the definitive loss of inflorescences and clusters.

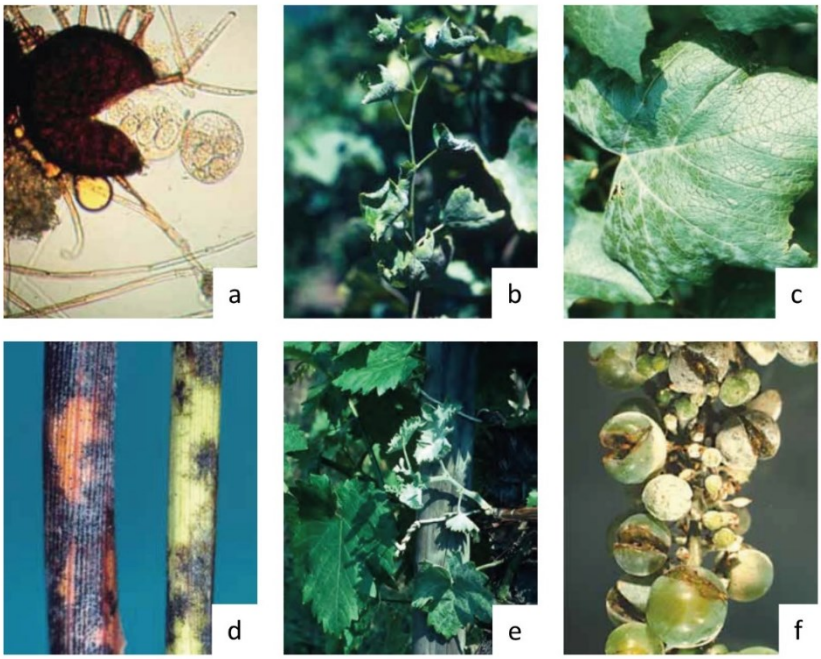

Figure 3 A) Microscopic view of cleistothecia releasing ascospores; B) Distortion of infected young leaves; C) Whitish powdery coating on leaf; D) Dark lesions on green and woody shoots; E) Flag shoots; F) Shrivelled and cracked fruit (Wilcox 2003).

The assessment of powdery mildew symptoms and grapevines susceptibility/resistance to *E. necator* are carried out in the field (e.g. Cadle-Davidson et al., 2011; Barba et al., 2015; Pap et al., 2016), in greenhouse (e.g. Pap et al., 2016; Agurto et al., 2017), and *in vivo* on either detached leaves or leaf discs (e.g. Staudt, 1997; Blanc et al., 2012; Pap et al., 2016). The disease severity may be assessed through visual scoring of the frequency and extensions of pathogen infections obtaining both qualitive and quantitative data (e.g. OIV 2009). More specific details on the plant-pathogen interaction are usually collected through observation at the stereomicroscope, microscope with different staining protocols of plant tissues (e.g. Aniline-Blue and Trypan-Blue in Feechan et al., 2011 and Agurto et al. 2017), and scanning electron microscope (SEM) (e.g. Leinhos et al., 1997; Blanc et al., 2012). Microscopes allow the observation of *E. necator* conidia, appressoria, hyphae and conidiophores, thus the evaluation of pathogen life cycle (e.g. Leinhos et al., 1997); while the staining may also elucidate the plant reactions to the pathogen, for instance the components of the plant hypersensitive response (HR) (e.g. Feechan et al., 2011; Agurto et al. 2017).

1.2.3 Pathogenic specialization and population biology

E. necator isolates show a differential capability to infect *Vitis* spp. and they are usually better adapted to the species from which they are obtained (Gadoury & Pearson, 1991; Gadoury et al., 2012). For example, Frenkel et al. (2010) described that among 38 isolates collected from *Vitis* spp., only the 10 isolates collected from *V. rotundifolia* showed the ability to form new colonies on *V. rotundifolia*. Moreover, *E. necator* isolates can differ in the rate of colony expansion on different accessions of the same *Vitis* sp. Pathogenic specialization could depend on the action of single plant resistance genes (Lebeda et al., 2016), or on the pathogen's ability to overcome the host's quantitative resistance (Gadoury & Pearson, 1991), or perhaps both.

In Europe and Australia, two genetically distinct *E. necator* groups have been identified, called A and B (or I and III), due to their differentiation based on genetic markers and biological patterns (e.g. Délye et al., 1997; Brewer & Milgroom, 2010; Csikós et al., 2020). Group A is often associated, but not exclusively, with overwintering mycelium and "flag shoots" symptoms. It is usually found at the beginning of the epidemic and characterized by little genetic diversity. Group B appears to generate mainly from ascospore infections and to be frequent late in the epidemic. The two groups may differ in their aggressiveness on *V. vinifera*, but group A seems more aggressive for some infection components while group B for others components (Miazzi et al., 2008; Montarry et al., 2008). *E. necator* populations in Italy and France are often composed of only one group but the two groups may coexist in the same vineyard with variable frequencies (e.g. Miazzi et al., 2008; Montarry et al., 2008). The genetic differentiation maintained between the groups reflects a lack of recombination between them under natural conditions.

E. necator populations in the eastern North America are more diversified (on the west coast only the group B is present) and show many different genetic haplotypes, within them there are isolates genetically identical to group A and other closer to group B (Brewer & Milgroom, 2010). These evidences indicate that European and Australian *E. necator* ancestors likely came from at least two different native populations of the eastern North America (Brewer & Milgroom, 2010). Recently, Gur et al. (2021) identified an *E. necator* strain genetically differentiated from any known group in Europe and North America; they proposed that this *E. necator* population would be founded from a non-American source and possibly an Asian one. However, a more in-

depth study is necessary to confirm such a hypothesis that is completely new in comparison to common notions on *E. necator* origin and centres of differentiation described above.

Finally, a review on *E. necator* genetic, comprising the information on the molecular marker genetic variability, barcoding, and genome sequencing, are described in the paper of Pirrello et al. (2019).

1.3 Plants immunity and responses to the pathogens

In nature, plants are constantly exposed to attacks from many potential pathogens which exhibit different infection strategies and lifecycles. Plants have deployed several defence mechanisms that make them immune to most possible pathogens and susceptible only to a relatively small number of adapted microbes.

A disease resistance joint to all individuals of a plant species deployed against all genetic variants of a given pathogen is the most common form of plant immunity and it is called non-host resistance (NHR) (Nürnberger & Lipka, 2005). NHR occurs when the pathogen is not specifically recognized by the plant or when the nutritional needs of the pathogen are not present. In this case, the plant has several successive layers of effective protective mechanisms, comprising both constitutive barriers (physical and chemical) and inducible reactions (Lipka et al., 2008).

Pathogens need to evade or suppress plant defences to establish an interaction for their own advantage. Jones & Dangl (2006) proposed a model to describe the plant-pathogen interaction that suggests a sequential intervention of plant defences based onto two main recognition mechanisms (the known four phased zigzag model; Figure 4).

The first and most general plant response (non-specific), and for example involved in the inducible components of NHR (Lipka et al., 2008), is based on the recognition of compounds called elicitors, or Pathogen (or Microbial) Associated Molecular Patterns (PAMP or MAMP), which are common to many pathogens and infection processes. The main PAMPS are generally chitin or ergosterol for fungi, flagellin for bacteria and plant cell walls polysaccharide residues hydrolysed by exogenous enzymes (Jones & Takemoto, 2004). Plants perceive elicitors through transmembrane-extracellular protein receptors, or Pattern Recognition Receptors (PRR), which trigger the Pattern Triggered Immunity (PTI). Downstream cell-autonomous responses of PTI include, for instance, production of Reactive Oxygen Species (ROS), antimicrobial substances and

ethylene, ion fluxes, transcriptional induction of pathogenesis related genes, protein phosphorylation, strengthening of cell walls and callose depositions (Bittel & Robatzek, 2007; Kwon 2010).

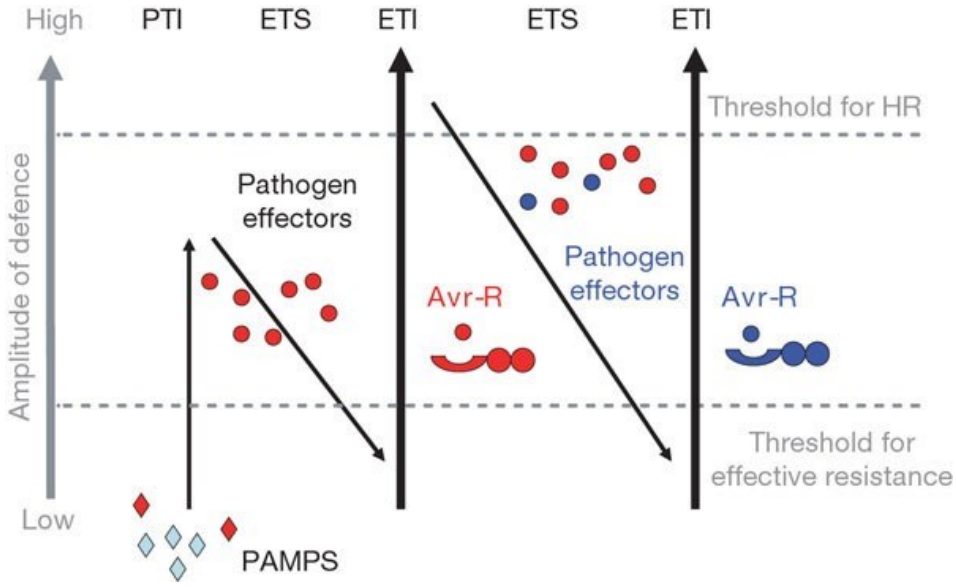

Figure 4 *Zigzag model for plant-pathogen interaction (Jones & Dangl*

The second plant response is the host induce resistance that is usually specific and based on an adaptive interaction. Pathogens release into the plant cells apoplast and cytoplasm disease effector molecules, also called avirulence (Avr) proteins (originating from the pathogen Avr-genes), to disturb the elicitors recognition and the PTI induction by the plant: Avr-proteins enhance the microbial fitness and can cause the Effector Triggered Susceptibility (ETS). Hosts can perceive pathogen effector by R-protein, produced by R-genes (Dangl & Jones, 2001), and trigger a defence reaction called Effector triggered immunity (ETI). Effectors are recognized by plants in different ways: by a direct interaction (Keen, 1990), by the perception of the interaction between an effector and a target protein (Dangl & Jones, 2001), by the detection of a protein alteration that trap the effector (van der Hoorn & Kamoun, 2008) or other more complex interactions (Petit-Houdenot & Fudal, 2017). ETI is often associated with the hypersensitive response (HR) - programmed cell death (PCD) (Greenberg & Yao, 2004), which is effective against obligate biotrophs pathogens, but not against necrotrophs (Glazebrook, 2005). In ETI, local and systemic synthesis of salicylic acid (SA) is well known to induce R-genes expression and to enhance the resistances against microbial pathogens (Metraux et al., 1990; Maleck et al., 2000).

While the PAMP and PRR are relatively stable and heritable, the components of ETS and ETI are object of diversification and selection due to the continuous co-evolution of plants and pathogens (Bent & Mackey, 2007). Natural selection drives pathogens to avoid ETI either by shedding or diversifying the effectors, or by acquiring additional effectors that suppress the response. Natural selection for plants results in developing new R-genes and consequent proteins to trigger ETI responses again. Genes tandem duplications and ectopic duplications followed by local rearrangements and genes conversion are important processes that influence the evolution of R-genes families (Leister, 2004; Mcdowell & Simon, 2006; Marone et al., 2013; Panchy et al., 2016). To date, at least five different classes of R-genes are known (Van Ooijen et al., 2007) and the largest class is represented by the gene family that encodes Nucleotide Binding Leucine Rich Repeat (NB-LRR) proteins (Hammond-Kosack & Jones, 1997; Dangl & Jones, 2001).

Plant resistances can be classified in two categories based on the number and effect of the genes that control the trait. Resistances are quantitative when multiple genes contribute together to the expression of the trait (polygenic resistance). Plants with quantitative resistances display different degrees of susceptibility to the pathogen which depend on the number and strength of the genes possessed by the individual. Polygenic resistances are often durable due to the nature of associated barriers, the contribution of multiple genes (usually between three and five) and non-specificity against single pathogen strains (Poland et al., 2009). Resistances controlled by one gene are defined as qualitative because each gene is decisive for the ability of the plant to defend itself (monogenic resistance). R-genes typically provide performing qualitative resistances but they are often limited to a specific pathogen isolate and characterized by a lack of durability due to the continuous evolution of the pathogen (Parlevliet, 2002). Resistances to biotic stresses can be improved in term of duration and efficacy by combining, or 'stack', two or more resistance sources. This process is named genes pyramiding (Mundt, 2018).

1.3.1 Grapevine sources of resistance to *E. necator*

Species belonging to the genus *Vitis* display susceptibility, partial resistance or complete resistance to *E. necator* encoded by various defence barriers and mechanisms.

Grapevines resistant to *E. necator* have been phenotypically identified since 19th century in American *Vitis* spp. co-evolved with the pathogen. For instance, *V. candicans* and *V. rotundifolia* were usually described as totally resistant species (Staudt 1997; Feechan et al. 2011) while *V. riparia* and *V. rupestris* as partially resistant (Cadle-Davidson et al., 2011; Feechan et al., 2011). Variable levels of resistance have been recorded within the species *V. aestivalis*, *V. cinerea* and *V. labrusca* (Cadle-Davidson et al. 2011).

Grapevines resistant to *E. necator* have been also described within East Asia *Vitis* species (Wan et al., 2007; Gao et al., 2016). For example, *V. amurensis, V. piazezkii* and *V. romanetii,* have been characterized to be highly resistant (Wan et al., 2007; Gao et al., 2016); on the contrary, *V. palmata* and *V. yenshanensis* have often been described as susceptible species (Staudt, 1997; Riaz et al., 2013). As within American species, some Asian grapevines showed wide intraspecific variability for the resistance trait (Wan et al., 2007).

V. vinifera varieties have been considered for a long time such as susceptible to *E. necator* (Gaforio et al., 2011). However, a partial resistant to *E. necator* have been recently described for some *V. vinifera* accessions from Central Asia (Hoffmann et al., 2008; Coleman et al., 2009; Riaz et al., 2013). These include both wild and cultivated genotypes.

Several defence mechanisms are involved in the control of *E. necator*. Resistance to *E. necator* penetration have been described for many grape accessions and it was associate to grapevine PTI (Feechan et al., 2011; Qiu et al., 2015). Resistance to *E. necator* penetration appeared to deploy multiple responses such as production of callose papillae (Heintz & Blaich, 1990; Agurto et al., 2017; Hu et al., 2019), ROS species (Agurto et al., 2017) and phytoalexins (Fung et al., 2008; Schnee et al., 2008). PCD, on the other hand, seems to be expressed in post-penetration and it has been the most frequent reaction described in the interaction between grapevines and *E. necator* (Feechan et al., 2011; Qiu et al., 2015; Agurto et al., 2017; Zendler et al., 2017; Hu et al., 2019). Enhanced constitutive expression of genes encoding pathogenesis related proteins (e.g. b-1,3-glucanase and chitinase) and the regulation of SA-mediated systemic response have both been described as potential factors of an increased resistance to *E. necator* (Fung et al., 2008; Hu et al., 2019). Defence mechanisms against *E. necator* have appeared to be shared within resistant grapevines but to differentiate

in time and intensity of expression (Feechan et al., 2011; Agurto et al., 2017). Finally, it has also been suggested that epicuticular waxes may influence pathogen development on tolerant genotypes (Özer et al., 2017).

1.4 Genetic mapping and mining of quantitative trait loci

Over the past century, several milestones have been achieved in the field of genetic (e.g. discovery of DNA sequencing and polymerase chain reaction (PCR); Sanger & Coulson, 1975; Mullis et al., 1986) and many approaches have been developed to accelerate crops genetic study. Nowadays, next-generation-sequencing (NGS; Schuster, 2008) increased the scale of research by offering the possibility of collecting extensive structural and functional genomic information, as well as enabling the whole genome sequencing of many plant species.

1.4.1 DNA markers

Advances in genetic have provided the discovery of DNA marker. DNA markers represent the variation in nucleotide sequence between individuals, populations, species or higher taxonomic level (Winter & Kahl, 1995). DNA markers occupy a specific genomic position called locus (plural loci) and have variants in the DNA sequence called alleles. They arise from different classes of DNA mutations, such as substitutions (point mutations), rearrangements (insertions or deletions) or errors in replication of tandemly repeated DNA (Collard et al., 2005), and may be detected with different strategies.

DNA markers have numerous applications and are involved, for example, in identifications of individuals, analysis of population diversity, systematics and molecular phylogeny, marker assisted selection (MAS), development of linkage maps and investigation of quantitatively inherited traits (Weising, 1995; Winter & Kahl, 1995; Collard et al., 2005). Many DNA marker have been utilized in the last few decades (e.g. RFLP (Botstein et al., 1980), RAPD (Williams et al., 1990) and AFLP (Vos et al., 1995)) but in genetic analysis, two classes of markers, that are Simple-Sequence-Repeats (SSR; Bell & Ecker, 1994) and Single-Nucleotide-Polymorphisms (SNP; Rafalski, 2002), became the predominant ones.

SSR (or microsatellites) are PCR-based markers consisting of short (1-6 bp) motifs tandemly repeated present in the DNA sequence. SSR alleles depend on variations in the number of repeats of the core motif. They originate from sequence spilt-strands,

miss pairing during DNA replication, repair, and recombination (Tautz & Renz, 1984; Schlötterer & Tautz, 1992). Microsatellite markers are abundant, highly polymorphic and have multiple codominant alleles. However, the development of correctly operative markers can be expensive and time-consuming. Furthermore, SSR analysis and data acquisition are difficulty automatable.

SNP are sequence-based markers and consist of single nucleotide variation between individuals. There are three categories of SNP: transitions (C/T or G/A), transversions (C/G, A/T, C/A, or T/G) or small insertions/deletions (indels). Thus, SNP can be bi-, tri- or tetra-allelic but most of the times they are biallelic. SNP are the most abundant form of genetic polymorphism in genomes, provide excellent density and are suitable for high-automation. SNP have been fully exploited with NGS which have let the development of many methods to genotype large numbers of markers and samples (Miller et al., 2007; Davey et al., 2011; Elshire et al., 2011).

1.4.2 Linkage maps

Linkage, or genetic, maps represent the chromosomes of a given species, where the distances between genes and markers instead of being physical are based on the frequency of recombination. Genetic maps are often constructed by analysing the segregation of molecular markers in a progeny generated by a controlled cross: low recombination frequencies between markers are indicative of markers located close each other on a chromosome; markers with larger recombination frequencies, approximately 50%, are considered to be located far away on a chromosome or in different chromosomes. The relative order of markers and their distances are inferred by the contemporary analysis of recombination frequency of arrays of close markers.

The size of the cross population depends on the definition of the genetic map required for the study case and it can range from one hundred to thousands of individuals. Larger populations show more chromosomal cross-over events and allow to generate more precise linkage maps (i.e. improved marker positions and order); however, the larger the population, the higher the cost and the complexity of the analysis. There are different crossing strategies to produce mapping populations (McCough & Doerge, 1995). Backcross (BC) and F2 populations generated by crossing inbred lines are the simplest and fastest mapping populations to develop for self-pollinating species, alternatively, recombinant inbred lines, doubled haploid or multiparent populations

can be produced, but they require longer time and complex procedure to be generated (Collard et al., 2005; Sehgal et al., 2016). In cross-pollinating species, where the production of inbred lines is prevented by self-incompatibility or by inbreeding depression like in grape and in many perennial crops (Einsen & Pratt, 1975), mapping populations are produced by crossing two heterozygous parents. Such a cross is called pseudo test-cross and allows to produce a genetic map for each parent.

In the past, different types of markers such as RFLP, AFLP and SSR have been used for the construction of linkage maps. Nowadays, SNP becoming the most exploited markers for developing linkage maps due to advance strategies for their discovery and genotyping and bioinformatics facilities for their analysis (e.g. Li, 2011; Pfender et al., 2011; Poland & Rife, 2012; Liu et al., 2014). NGS and SNP are well adapted to any cross population.

In linkage analysis, markers and their segregation in the offsprings are verified according to parental patterns and generally they segregate in a Mendelian way. Linkage between markers is usually calculated using odds ratios (i.e. the ratio of linkage versus no linkage) and are expressed as logarithm of odds (LOD) value (Risch, 1992). Linked markers are grouped together into linkage group (LG), which represent entire or partial chromosomes. Many efficient algorithms are available to order the marker on LG (e.g. Wu et al., 2008; Monroe et al., 2017) while genetic distances are usually calculated with Kosambi (Kosambi, 1943) or Haldane (Haldane, 1919) mapping function. Map units are expressed in centi-Morgans (cM). The relation between genetic and physical distances is not linear because cross-overs occur with different frequencies along the chromosome.

1.4.3 QTL mapping in biparental population

Most agronomic traits are quantitative and are controlled by the collective effects of numerous genes that may have different positions on chromosomes. These genes are called quantitative trait loci (QTL). The most important use of linkage maps is to locate QTL in the genomes.

QTL analysis consists in detecting the association between individuals' phenotypes and markers position and allele. QTL detection requires the segregation of polymorphic markers and the expression of different phenotypes in the offsprings. There are several analysis methods for detecting QTL which have different advantages and

disadvantages (Tanksley, 1993; Liu, 1998; Broman & Sen, 2009). Single-marker analysis is the simplest method, it compares the phenotypic means of allele-based groups of individuals marker by marker. Interval mapping (IM), similarly but with more statistical power, tests single QTL models but by using information from both markers flanking an interval (Lander & Botstein, 1989; Broman & Sen, 2009). Composite interval mapping (CIM) includes in the IM analysis additional genetic markers and allows the scanning for multiple QTL (e.g. Jansen, 1993). Multiple QTL analysis methods (e.g. model selection approach and Bayesian QTL mapping; e.g. Hoeschele et al., 1997 and Broman & Speed, 2002) have the advantages of reducing models residual variation, better detecting loci with low effects, separating linked QTL and identifying interactions between QTL (Broman & Sen, 2009).

Results for QTL analysis are typically presented using LOD scores. Higher LOD correspond to greater evidences for the presence of a QTL in the region, the highest peak in the LOD profile identifies the most likely location for the QTL at the stake (Collard et al., 2005). QTL are generally accepted when they exceed a threshold of 3.0 or more. Alternatively, the significance threshold may be calculated using permutation tests (Churchill & Doerge, 1994; Broman & Sen, 2009). QTL regions are defined inside confidence intervals that can be determined, for example, with LOD support interval criteria (Lander & Botstein, 1989), Bootstrapping (Liu, 1998) and Bayes credible interval (Sen & Churchill, 2001). QTL are described as major or minor based on the proportion of the phenotypic variation explained (thresholds usually range from 10 to 20 %). Major QTL are stable across experiments while minor QTL are not, as they appear erratically in different years, environments and replicated tests.

Factors influencing QTL detection are the genetic properties of the loci, the size of the segregating population, external effects and experimental errors both in genotyping and phenotyping of the cross population (Tanksley, 1993). Close genes affecting the same trait could be difficult to resolve and are usually detected as a single QTL (pleiotropic QTL). QTL in different positions can interact positively for a trait (additive effect), as well as an allele at a locus can modify the effect of another QTL by deviating the attended phenotype (epistatic effect). An increase in population size provides gains in linkage maps quality thus in definition and resolution of major and minor QTL (i.e. QTL position, effect and confidence interval) (Tanksley, 1993). On contrary, genotyping errors and missing data negatively affect the genetic mapping and together with

erroneous phenotypic data can deviate QTL analysis. Phenotypic evaluations in different experimental conditions and independent analysis of closely-related segregating populations can verify, confirm and improve the robustness of a QTL mapping study (Lander & Kruglyak, 1995; Collard et al., 2005).

1.5 Grape genetics and breeding for resistances to diseases

Since the 19th century grapevine interspecific crossing became a widespread practice to produce new grape varieties and introgress into *V. vinifera* the resistant traits of *Vitis* wild species. However, grape breeders encountered many difficulties, due for instance to the complex genetic base of the agronomical traits of interest, linkage drag phenomenon, self-fertilization and long-time between generations (juvenility of the progeny). Many resistant grape varieties were produced, but the limited knowledge of the genetic of elite varieties and resistance traits and the empirical selection of cross parents, provide only a small number of promising varieties.

Genetic research on grapevine presents more challenges compared to herbaceous and self-pollinating species (e.g. high heterozygosity level and inbreeding depression). However, grapevine has become one of the most studied plants (Cantu & Walker, 2019). *V. vinifera* have been used in many pioneering genetic studies and often adopted as model species for perennial crops. For instance, *V. vinifera* is the second sequenced woody plant and the sixth ever (Jaillon et al., 2007).

DNA markers were one of the first genetic information acquired in grapevine molecular studies. In particular, RFLP, RAPD and AFLP were the first markers discovery and efficiently used in grape genetic mapping and QTL analysis (Lodhi et al., 1995; Doligez et al., 2002; Fischer et al., 2004).

SSR have been the most utilized DNA markers in grape research. Numerous grapevine SSR, also transferable among *Vitis* spp. (e.g. Sefc et al., 1999), was developed in independent works starting from 1990s (e.g. Thomas & Scott, 1993; Bowers et al., 1996; 1999; Sefc et al., 1999;). One of the most relevant contributes to SSR discovery derived from the international cooperation of the *Vitis* Microsatellite Consortium (VMC) started in 1998 that discovered about one thousand SSR markers. After that, the sequencing of the *V. vinifera* PN40024, a highly inbred line (Jaillon et al., 2007), allowed to access to thousands of SSR markers sparse on grape genome. Up to several hundred of SSR were utilized to develop grape linkage maps (e.g. Doligez et al., 2002; Fischer et

al., 2004; Pap et al. 2016; Zendler et al., 2017). Compared to the first implemented markers, SSR permitted the unification of grape linkage groups, anchoring the genetic and physical maps and developing a set of markers suitable for comparative studies (Cipriani et al., 2011).

SNP are the most recent markers introduced in grapevine genetic research (e.g. Troggio et al. 2007). Sequencing of the heterozygous Pinot variety showed that SNP are dense on the grape genome: their frequency ranging from one SNP every 60 bp to one SNP every 250 bp for a total of two million potential SNP (Velasco et al., 2007). Until the introduction of NGS tools, the discovery, validation and genotyping of SNP in grapevine were more challenging and demanding in genetic activities. NGS has enabled also in grapevine studies to generate huge amounts of sequence data, to apply high-throughput systems for SNP analysis and develop high-density linkage maps (e.g. Teh et al., 2017; Delame et al., 2019; Sapkota et al., 2019). SNP chips, or microarrays, have been the first technology used for acquiring genome-wide genotype data of thousands of SNP (e.g. Myles et al., 2010). Subsequently, SNP genotyping methods based on genotyping by sequencing (GBS) have been also proposed in grape research (e.g. Hyma et al., 2015).

For grapevine, as for other perennial plants, construction of linkage maps is based on pseudo-testcross mapping strategy (Grattapaglia & Sederoff, 1994). It has been the first (Lodhi et al., 1995) and, with some exception, the most utilized approach to develop grape genetic maps. In the pseudo-testcross strategy two heterozygous parents are crossed, thus in the F1s the heterozygous segregating markers for the parents are separately analysed as in backcross populations. Segregation of dominant markers, such as RAPD and RFLP, may be used to construct a map for each parent but does not allow to build the parental homologous linkage groups. Instead, co-dominant markers, for example SSR, usually permit to build the parental maps, the homologous linkage groups and an integrated linkage map.

More than 160 linkage maps from grape populations have been published. Their length usually varied between 1000 and 1500 cM, which could be considered a reference range for the length of *Vitis* map, although several factors of genetic or environmental origin can affect it (Vezzulli et al., 2019a). Genetic maps have only been developed within the *Vitis* genus and one of the main uses have been the QTL detection (for this purpose more than 50 grape populations have been analysed; Vezzulli et al. 2019a).

Considered the impact of pest and diseases in viticulture, research has long focused on the discovery of resistance QTL to biotic stresses. Resistances were mainly identified from American and Asiatic *Vitis* spp. (e.g. Table 1) but several loci were also discovered in *V. vinifera* (Hoffmann et al., 2008; Sargolzaei et al., 2020). To date thirty-one loci of resistance to *Plasmopara viticola* (Rpv), twelve loci of resistance to *E. necator* (named resistance to *Erysiphe necator* - Ren or resistance to *Uncinula necator* – Run; Table 1) were identified (http://www.vivc.de). Finally, also Mildew resistance loci O (MLO), acting as susceptibility genes, demonstrated that they can control the resistance to *E. necator* in grapevines (Pessina et al., 2016).

Resistance loci were mapped in many genomic positions and confer different features to the resistance trait. Some loci co-localized in grape genome but showed different resistance haplotypes, resistance levels (e.g. Foria et al., 2018; Possamai et al. 2020) and/or specificities toward pathogen strains (e.g. Riaz et al., 2011; Feechan et al., 2015) as well. Regarding the resistance to *E. necator,* Run1 (Pauquet et al., 2001), Ren4 (Ramming et al., 2011), Ren5 (Blanc et al., 2012) and Ren6 (Pap et al., 2016) are associated to a total resistance. Ren1 (Hoffman et al., 2008) and Ren7 (Pap et al., 2016) were characterized as mayor QTL capable but to confer a partial resistance to the pathogen. Finally, Ren3 (Welter et al., 2007), Ren9 (Zendler et al., 2017; Zendler et al., 2020) and Ren10 (Teh et al., 2017) were identified as minor loci. In *V. vinifera,* up to 459 R-genes were characterized (Di Gaspero & Cipriani, 2002; Velasco et al., 2007; Goyal et al., 2020). Major resistance QTL were often located in region of the PN40024 genome enriched in R-genes, in particular encoding NBS-LRR proteins (e.g. Coleman et al., 2009; Venuti et al., 2013; Foria et al., 2020), and other genes involved in metabolic pathway associable to plants defence mechanisms (e.g. polyphenols synthesis; Vezzulli et al., 2019b). However, only one gene (*MRun1*) responsible for the resistance to *E. necator* was cloned and functionally characterized until now (Feechan et al. 2013).

QTL mapping of grapevine agronomic traits and grape qualities identified only a few major loci which concern flower sex (e.g. Battilana et al., 2013), berry colours (e.g. Costantini et al., 2015), seedlessness (e.g. Doligez et al., 2002), berry dimensions (e.g. Doligez et al., 2002; Fischer et al., 2004) and terpenols content (e.g. Duchêne et al., 2009). Many other traits resulted under the control of minor loci such as grape phenology (e.g. Fischer et al., 2004; Zyprian et al., 2016) and sugar and acids grape content (e.g. Chen et al., 2015). Consequently, most of the agronomic traits were poorly

reproducible between studies and resulted non-practical for breeding (Vezzulli, et al., 2019a).

Table 1 Resistance loci to *E. necator* identified in grapevine.

Locus	Chr	Position [Mb]	Original species	Reference
Run1.1	12	13.1-20.4	*V. rotundifolia*	Pauquet et al., 2001
Run1.2				Feechan et al., 2015
Run2.1	18	20.9-26.9	*V. rotundifolia*	Riaz et al., 2011
Run2.2				
Ren1	13	18.4	*V. vinifera*	Hoffmann et al., 2008
Ren2	14	26.9	American *Vitis* spp.	Dalbó et al., 2001
Ren3	15	4.9-10.9	American *Vitis* spp.	Welter et al., 2007
Ren4	18	26.9	*V. romanetii*	Ramming et al., 2011
Ren5	14	4.8	*V. rotundifolia*	Blanc et al., 2012
Ren6	9	8.6-9.1	*V. piasezkii*	Pap et al., 2016
Ren7	19	0.2-0.9	*V. piasezkii*	Pap et al., 2016
Ren8	18	26.8	American *Vitis* spp.	Zyprian et al., 2016
Ren9	15	1.4	American *Vitis* spp.	Zendler et al., 2017
Ren10	2	79.0	American *Vitis* spp.	Teh et al., 2017

Over the past twenty years, QTL studies have enabled significant progresses in grape breeding. One of the most important results have been to provide effective markers tightly linked to QTL and traits of interest to be applied in the marker assisted selection (MAS) and marker-assisted breeding (MAB). Grape seedlings MAS have been widely applied to follow and combine the resistance loci to biotic stresses, in particular for Rpv and Ren loci (e.g. Eibach et al., 2007; Merdinoglu et al., 2018). The genes of resistance to *E. necator* more commonly exploited are Run1, Ren1, Ren3 and Ren9 (e.g. Agurto et al., 2017; De Nardi et al., 2019; Zini et al., 2019). Genes pyramiding is important in grape breeding because it may: increase the ability of genotypes to limit the pathogen infections (Venuti et al., 2013; Agurto et al., 2017; Zini et al., 2019; Possamai et al., 2020); provide a broad resistance against many pathogen strains (Feechan et al., 2015); and secure the trait from the possible effects of pathogen adaptive evolution (e.g. Peressotti et al., 2010; Cadle-Davidson, 2019).

The new knowledge on grape resistant sources to biotic stresses, the novelties in the genetic field and the varieties selected over the course of a century, have recently allowed grape breeders, to develop many new promising varieties resistant to *P.*

viticola and *E. necator*. The adoption of such varieties has proved to be an effective and complete solution for sustainable viticulture. Resistant varieties may respond to the increasingly complex social and regulatory context of plant and environment protection by reducing the number of agrochemicals and phytosanitary interventions applied against pathogens (Pedneault & Provost, 2016). However, additional research works are essentials to mine new genetic variants and genotypes useful for breeding programs.

2 Aims of the PhD project

The main object of the project has been the study and the mapping of the resistance trait to *E. necator* in the Caucasian *Vitis vinifera* L. germplasm maintained at the CREA-VE grapevines collection in Susegana, Italy. The main purposes were:

(i) to evaluate the phenotypic resistance to *E. necator* of Shavtsitska and Tskhvedianis Tetra varieties by using leaf discs bioassays and evaluating the infection features during the pathogen life cycle;

(ii) to use the two Caucasian varieties to map the QTL controlling the resistance to *E. necator* through controlled crosses with susceptible cultivars and the phenotypic and molecular analysis of the offsprings;

3 Materials and methods

Several Caucasian grapevines exhibited a resistance to *E. necator* in previous studies (Failla et al. 2016). A test carried out with molecular markers on a collection of Caucasian varieties (*Vitis vinifera* L. subsp. *vinifera*) held at CREA-VE showed that neither Run1 nor Ren1 nor Ren3/Ren9 resistance genes were carried by this germplasm (data not shown). We decided therefore to study and to map the resistance to *E. necator* on two of those accessions as described below.

3.1 Plant material

In 2018, the Caucasian varieties Shavtsitska and Tskhvedianis tetra (T. tetra), reported as resistant to *E. necator,* were crossed with the two susceptible varieties Glera and Chardonnay (Table 2). The crosses were performed at the CREA-VE grape germplasm collection in Susegana, Italy (45°51'07.6"N 12°15'28.6"E) where five individuals of many Caucasian grapevine accessions are conserved (Table S1). Reciprocal cross combinations were tried by reversing the seed parent and the pollen donor to increase the possibilities of obtaining a suitable number of seeds.

Flowers of the seed parents were emasculated (calyptra and anthers were manually removed) and protected against contamination from alien pollen with paper bags; inflorescences of male parents were collected and used for the pollination of the castrated bunches of seed parents. After fruit-set, paper bags were replaced by tulle bags. The bunches from seed-parent were harvested at physiological ripeness. The seeds were extracted and treated overwinter as described in Conner (2008) by eliminating the floating seeds and stratifying the remainder in moist sand in a refrigerator at 4°C.

In 2019, seeds of the crosses “Shavtsitska x Glera” (population code 50042) and “Chardonnay x Tskhvedianis tetra” (population code 50041) were sown at INRAE-Centre Grand Est Colmar UMR 1131 SVQV (Colmar, France). The plantlets were grown in rockwool substrate in greenhouse until their belonging to the population was genetically verified by means of molecular markers. After that, part of the true-to-type progeny of each cross was repotted in 2-liter pots in a mixture of sand – perlite – lapilli and kept in greenhouse. The plants were feeded with a nutritive solution and grown at 28°C with 16 h light and 8 h dark photoperiod. Shoots were periodically pruned to limit

the vegetation and guarantee the presence of young apical leaves for the phenotyping bioassays. Pests and diseases were managed by spraying every second week.

Replicates of the parental plants were produced in Colmar in 2019 from wood cuttings and grown in 2-liter pots together with their progeny. Several “control" genotypes (characterized by different degree of resistance to *E. necator* and/or carrying specific Run/Ren loci) among which RV1-22-8-78 (RV1), Kishmish vatkana (K. vatkana), Johanniter and Cabernet sauvignon (Cabernet s.) were added to the experiments to check the effectiveness of the powdery mildew infections (Table 2). Control plants were periodically produced from green cuttings and grown in greenhouse under the same conditions of the cross populations.

Table 2. Grape varieties studied in the phenotyping bioassays with their origin, degree of resistance to *E. necator* and known resistance gene. “P” identifies the cross parents and “C” the control varieties.

Grape variety	**Origin of the plant**	**Degree of resistance to *E. necator***	**Known resistance gene**
Shavtsitska (P)	*V. vinifera* Caucasian variety	Partially resistant	?
Tskhvedianis tetra (P)	*V. vinifera* Caucasian variety	Partially resistant	?
Glera (P)	*V. vinifera* Italian variety	Very susceptible	No one
Chardonnay (P)	*V. vinifera* French variety	Very susceptible	No one
RV1-22-8-78 (C)	2nd generation backcross *V. vinifera* x *M. rotundifolia*	Totally resistant	Run1
Kishmish vatkana (C)	*V. vinifera* Central Asia variety	Partially resistant	Ren1
Johanniter (C)	Variety with American *Vitis* species in the pedigree	Limited resistant	Ren3/Ren9
Cabernet sauvignon (C)	*V. vinifera* French variety	Very susceptible	No one

3.2 Phenotypic evaluation of the resistance to *E. necator*

3.2.1 *E. necator* strain maintenance and inoculation protocol

The phenotypic resistance of parental plants and offsprings was studied by using leaf discs bioassays prepared as described in Miclot et al. (2012) and Calonnec et al. (2013)

with some modifications. A single-spore isolate of *E. necator* was obtained in 2018 from *V. vinifera* plants cultivated in the greenhouse in Colmar (isolate Colmar 2-2018). The isolate was maintained and multiplied on leaves of Cabernet sauvignon for bioassay inoculations. For this purpose, every ten days some young and shiny leaves (3-8 cm of diameter) of the two varieties were:

1) Disinfected by incubation for 4 min in 50 g/l sodium hypochlorite water solution and rinsing in three consecutive baths of water for 4 min each;
2) Placed on a medium containing 10 g/l agar and 0.015 g/l Natamycin in Petri dishes with the adaxial surface up;
3) Inoculated by blowing *E. necator* conidia from ten-days-old infected leaves through a settling tower.

For phenotyping bioassays, young, shiny and expanded leaves of 2-4 cm of diameter from the shoot apex were collected. Leaves were disinfected as described above and sample discs of 1 or 2 cm of diameter (according to the type of the experiment) were excised with a cork borer. Leaf discs were accommodated in Petri dishes (a wet filter paper disc was added between the leaf material and the agar medium) and inoculated with 600–800 conidia/cm^2 of the *E. necator* isolate Colmar 2-2018 from ten-days-old infected leaves.

Inoculated Petri dishes were incubated in climatize chamber at 23°C with a photoperiod of 16 h light and 8 h dark.

3.2.2 Trypan-Blue staining and SEM observations of *E. necator* infection

A histochemical and a scanning-electron-microscope (SEM) study were carried out to investigate the plants response and the pathogen development in the first three days post-infection (dpi). In particular, the parents Shavtsitska and Glera, some of their progeny showing different level of resistance to *E. necator* and the controls genotypes (RV1-22-8-78, Kishmish vatkana, Johanniter and Cabernet sauvignon) were analysed in these experiments.

In the histochemical bioassays, three leaf discs of 1 cm of diameter per individual (seventeen offsprings, two parents and four controls) were analysed at each sampling date: two replicated data for 1, 2, and 3 dpi were obtained between June 2019 and July 2020. The fungal proliferation was studied by Trypan-Blue staining as described in Vogel & Somerville (2000) and Agurto et al. (2017) with little modifications. Leaf discs

were cleared with an ethanol-96% and acetic acid-100% solution (3:1 by volume) followed by three consecutive washes of 30 min. Decolorized discs were stored in lactoglycerol (glycerol 99,5 %, lactic acid 90% and water 1:1:1 v:v:v) for 12 h at room temperature. Discs were then stained with a Trypan-Blue water solution (0,01 % weight/volume) for 15 min and then stored again in lactoglycerol. Discs were mounted in slides for bright-field microscopy visualization by Zeiss Axio Imager M2 (Zeiss, Oberkochen, Germany) with x100 magnification. One-hundred germinated conidia per disc were categorised in 4 classes according to their development (Figure 5):

a) 0 = conidia showing only the appressoria;

b) 1= conidia showing the primary hypha;

c) 2 = conidia showing the primary and the secondary hypha;

d) 3 = conidia showing three hyphae and/or branched hyphae.

Classification results were verified and the most discriminant data were utilized to fit three linear model (LM), one for each dpi, and to compare the parental and control individuals. The statistical analysis was performed with software R (R Core Team, 2017).

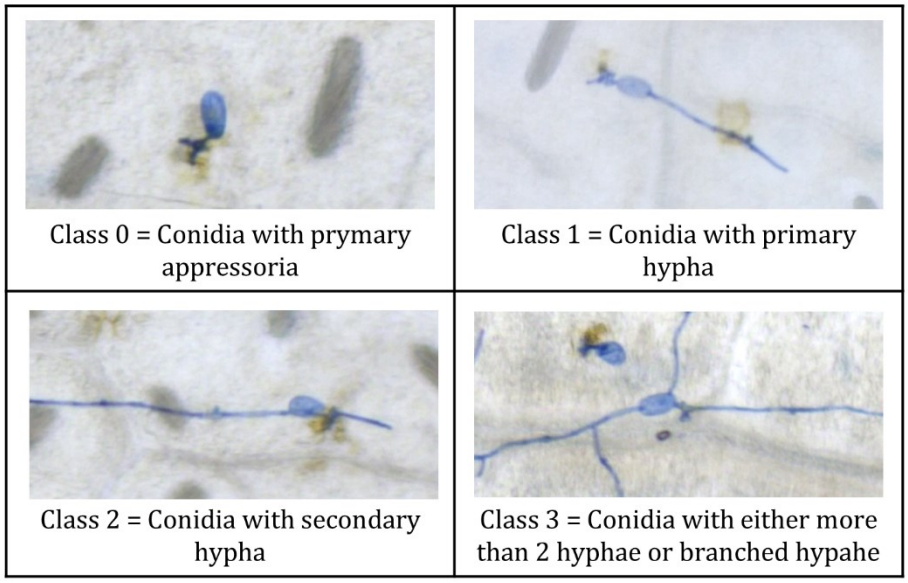

Figure 5 Representative conidia for Trypan-Blue staining experiments classification.

Observations with SEM (1-2-3 dpi) were carried out in July 2019. Leaf discs of 1 cm of diameter for Shavtsitska, Glera, ten of their offsprings and the controls were prepared and inoculated as described in section 3.2.1. Portions of the discs (1/2 to 1/4) were upload in Hitachi TM-1000 (Hitachi, Tokyo, Japan) scanning-electron-microscope and observed until a clear idea on the infection development was obtained.

3.2.3 Populations phenotyping for the resistance to *E. necator*

Phenotyping of the populations 50042 (Shavtsitska x Glera) and 50041 (Chardonnay x Tskhvedianis tetra) was carried out between May and July 2019. A total of 264 plants of the population 50042 and 67 plants of the population 50041 were studied in three and two replicated tests respectively. In each experiment the cross parents and the controls genotypes (RV1-22-8-78, Kishmish vatkana, Johanniter and Cabernet sauvignon) were also characterized.

The phenotyping was carried out on leaf discs of 2 cm of diameter prepared and inoculated as described in section 3.2.1. In each replicate one disc per progeny and up to four discs per parental and control plants were prepared. Powdery mildew infection was evaluated at 3-5-7-10 dpi for population 50042 and at 2-4-7-9-11 dpi for population 50041. At each dpi, four area on the leaf discs were scored for: pathogen mycelium growth, sporulation intensity, mean number of conidia per conidiophore and presence-absence of plant necrosis. Pathogen mycelium and sporulation were scored with two independent scales with five classes each, according to OIV (2009) with some modifications:

a) 9 = absence of pathogen structures in the area;
b) 7 = presence of few-short hyphae/few conidiophores;
c) 5 = mycelium/conidiophores sparse with low density or spread in colonies;
d) 3 = dense mycelium/conidiophores on most of the leaf disc area;
e) 1 = dense mycelium/conidiophores covered all the observed area.

Observations were made by stereomicroscope Zeiss Stemi 508 (Zeiss, Oberkochen, Germany) at x64 magnification. Representative photos at each dpi were taken with Zeiss Axio Zoom V.16 (Zeiss, Oberkochen, Germany).

After the last evaluation, the discs were stored in 1.5 ml tubes at -20°C. Subsequently conidia were suspended in 300 ul of Tween-20 water solution (0,05 % volume/volume) and counted with Malassez Counting Chamber. Conidia counts data were square root transformed (RQSP) before QTL analysis.

The Area Under Disease Pressure Curve (AUDPC) (Jeger & Viljanen-Rollinson, 2001) was calculated for *E. necator* mycelium growth and sporulation intensity starting from the discs averaged score per dpi. Data were finally expressed as relative AUDPC (rAUDPC).

3.3 DNA extraction and quantification

For each germinated seedling and cross parent, a single young expanded leaf (1-2 cm of diameter, about 50 mg of tissue) was collected in 96-well plates. Plates were maintained for 1 min in liquid nitrogen and the leaf tissues immediately grinded to a fine powder by Tissue-Lyser II instrument (Qiagen, Hilden, Germany) (30 Hz for 45 s for two times).

Total DNA was extracted with the DNeasy 96-well DNA extraction kits (Qiagen, Hilden, Germany). Modifications were made to the manufacturer's protocol to improve DNA yield and quality as follows: PVP-30 (1.5% weight/volume) was added to the lysis buffer (AP1) prior to heating and elution was performed with 80 ul of buffer (AE) heated at 65°C.

DNA concentration and quality (260/280 and 260/230 ratios) were measured with Nanodrop 1000 (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

DNA of the offsprings selected for the Genotyping by Sequencing (GBS) analysis (see below) was also quantified with Qubit 3.0 (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and samples with a concentration above 100 ng/ul were diluted to 75 ng/ul. DNA integrity was verified by gel electrophoresis at 1% agarose medium EEO with GelRed 1:10000 (Biotium, Fremont, California, USA). Finally, about 1500-3000 ng of DNA were dried at 65°C for 2 h and delivered for the GBS analysis.

3.4 SSR markers analysis

The SSR markers VVMD5 (Bowers et al., 1996), VVMD27, VVMD28 (Bowers et al., 1999), VrZag79 (Sefc et al., 1999) and VMCNG4b9 (*Vitis* Microsatellite Consortium) were used to screen the cross populations for contaminants. Forward primers were 5'-end labelled with different fluorescent dyes (6-FAM, HEX, NED and VIC). PCR reactions were carried out in a 8 µl volume containing 15 ng of DNA, 2.5 mM MgCl2, 150 µM of each dNTP, 0.125 to 0.50 µM of each primer, 1x Buffer Gold and 0,20 U of Taq DNA polymerase (AmpliTaq Gold, Thermo Fisher Scientific, Waltham, Massachusetts, USA) and with the following thermal profile: 94°C for 10 min, followed by 35 cycles at 92°C for 45s, 57°C for 60s, 72°C for 90s, and final elongation of 5 min at 72°C. PCRs were performed in a ProFlex PCR System (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and followed run by capillary electrophoresis on a 3500 Series

Genetic Analyzer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) to separate the amplicons.

The SSR SC8-0071-014 and Sc47_20 (Coleman et al., 2009) were screened in a subsample of individuals of the cross populations and in some Caucasian varieties, conserved at CREA-VE (Table S1), as described in De Nardi et al. (2019).

All PCR fragments were analysed with GeneMapper 4.0 software (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

3.5 Libraries preparation and SNP calling

High quality DNA of Shavtsitska, Glera and their cross progeny was delivered to "The Elshire Group" (Palmerston North, New Zealand) for the libraries preparation, the GBS analysis and the SNP calling. The GBS data were generated following the Elshire et al. (2011) method with the following modifications: 100 ng of genomic DNA and 3.6 ng of total adapters were used; the genomic DNAs were restricted with ApeKI enzyme and the libraries were amplified with 18 PCR cycles. The libraries were analysed by Illumina HiSeq X (Illumina, San Diego, California, USA) that generates 150 bp paired end reads. The demultiplexing based on combinatorial barcoding was performed with the Kevin Murray's axe-demux v.0.3.3 (Murray & Borevitz, 2018). The reads for both ends of the pair-end data were combined into individual per-sample files and aligned to the 12X.2 *V. vinifera* reference genome PN40024 (Canaguier et al., 2017) using Bowtie2 v.2.4.1 (Langmead & Salzberg, 2012). The alignments were subsequently analysed with Stacks v.2.5 (Catchen et al., 2013) to output the final SNP dataset in a .vcf file. To verify the results, the variants were put through the Kinship using GBS with Depth adjustment program (KGD) v.0.7.0 (Dodds et al., 2015).

3.6 Genetic mapping

The pseudo-testcross markers (Grattapaglia & Sederoff, 1994), that are markers heterozygous in one parent and homozygous in the other one, selection and the linkage analysis were performed using a custom pipeline mainly based on the software R (R Core Team, 2017).

Initially, the .vcf file was analysed by Perl (Wall et al., 2000) scripts described in Hyma et al. (2015). Genotyping errors were corrected as follow: genotypes homozygous for the major allele with genotype quality (GQ) < 20 or homozygous for the minor allele

with GQ > 20 were converted to missing data; genotypes homozygous for the minor allele with GQ < 20 were converted to heterozygous. Then, SNP having a genotyping rate above 80%, an error rate lower than 5% and two segregating alleles were retained. Shavtsitska and Glera genotypes were extracted with the vcfr R package v. 1.11.0 (Knaus & Grünwald, 2017) to complete the second filtering step. In brief, SNP that were, either homozygous, or heterozygous, or had missing data in both parents were eliminated; as well as SNP not matching in the parental replicated samples were filtered. Markers were grouped into chromosomes (chr) based on SNP physical positions on the grape reference genome (Canaguier et al., 2017) and their distribution was verified. SNP were divided into two datasets according to their segregation from Shavtsitska and Glera in order to build the two parental linkage maps (pseudo-testcross strategy; Grattapaglia & Sederoff, 1994).

Progeny genotypes were organized in backcross (BC) format to access to qtl R package v. 1.46.2 functions (Broman et al., 2003). The dataset was checked and duplicated samples were eliminated as well as markers with a genotyping rate below 90%, significant distorted segregations (p-values for chi-square tests <0.01) and co-located. Marker association and marker order were analysed with the Minimum Spanning Tree (MSTmap) algorithm (Wu et al., 2008) in mstmap function (ASmap package v. 1.0.4, Taylor & Butler, 2017) with the following parameter: bychr = TRUE, dist.fun = "kosambi" (Kosambi, 1943), p.value = 0.000001, objective_function = "ML", noMap.dist = 15, noMap.size = 5, mvest.bc = no and detectBadData = no. After that, genotyping errors were corrected to missing data based on genotypes logarithm of odds (LOD) scores (LOD>3) as described in Broman et al. (2003). Markers were manually verified and SNP in weak linkage to their neighbouring markers (with high recombination fraction and low independence LOD for the association) were eliminated from the dataset; as well as individuals with an excess of crossover/double crossover. Then, SNP order and distances were recalculated with MSTmap algorithm. Final marker distances were calculated with Lander-Green algorithm (i.e. the Hidden Markov Model) of est.map command and Kosambi mapping function. Final marker order was verified with tspOrder function based on the robust Travelling Salesperson Problem (TSP) algorithm (TSPmap package v. 1.0.0, Monroe et al., 2017). Parental genetic maps LG were renamed and inverted according to the reference genome sequences.

In parallel, parental maps were developed with JoinMap 4.0 (Van Ooijen, 2006) to verify the results of the custom pipeline analysis.

3.7 QTL analysis

Genotypic and phenotypic data of the population 50042 (Shavtsitska x Glera) were utilized together to carried out the QTL analysis using qtl R package v. 1.46.2 (Broman et al., 2003).

Data collected at 3-5-7-10 dpi for the pathogen mycelium growth, sporulation intensity and presence-absence of plant necrosis were firstly investigated. After that, conidia counts and the rAUDPC indexes were also explored. Individual and averaged experiment data were all analysed. QTL analysis was carried out for both cross parents as following described.

Interval Mapping (IM) was carried out calculating the multipoint genotype probabilities (calc.genoprob function with step=1 and error.prob=0.005) and testing single QTL presence at each position by scanone function (model = normal and method=em (Lander & Botstein, 1989)). The LOD significance threshold (p-value < 0.05) for each model were determined by permutation tests (n.perm = 1,000).

Scanone results were also verified through other more robust analysis method available in qtl package (i.e. method = sim and method = rhk). Non-parametric model (model=np), based on Kruskall-wallis test (Kruglyak & Lander, 1995), were tested when phenotypic data residuals were on the edge of normal distribution.

The search for more independent and/or interacting resistant loci was refined with addqtl, stepwise (QTL model selection approach; Broman & Speed, 2002) and mqmscan functions (Multiple QTL Mapping - MQM method described in Jansen, 1993 and implemented in R by Arends et al. 2010), also by using the already identified major QTL as covariates (Broman et al. 2003) to evidence the presence of minor QTL.

Makeqtl and fitqtl functions (model = normal, formula=y~Q1) were used with the most relevant phenotypic data in IM to fit the final QTL models. QTL Bayes credible intervals were determined by bayesint function (α=0.95).

Results of QTL analysis were confirmed with MapQTL 5.0 (Van Ooijen, J.W., 2004) software.

Resistance QTL identified in the cross parents were located on grape reference genome PN40024 (Canaguier et al. 20178) searching the informative recombinants for the loci.

QTL segregation in the population 50041 (Chardonnay x Tskhvedianis tetra) was verified by testing the phenotypic differences between SSR haplotype-derived groups: T-tests (p-value < 0.05) and linear models for rAUDPC data for pathogen sporulation were calculated by software R (R Core Team, 2017).

4 Results

4.1 Populations from controlled crosses

A total of 7 cross combinations were tested in 2018 and 43 bunches were emasculated and pollinated (Table S2). Shavtsitska showed female flowers at phenotypic observations and the absence of fertile pollen was confirmed by two attempts of selfing that yielded very few berries (data not shown). This prevented the use of Shavtsitska as pollen donor. Therefore, our efforts focused on the cross combinations "Shavtsitska x Glera" and "Chardonnay x Tskhvedianis tetra", that produced the populations 50042 and 50041 respectively. The two crosses provided a total of 4551 seeds (Table 3).

In 2019 at INRAE (Colmar, France) 1078 seeds were sown obtaining 851 plantlets (Table 3). The presence of contaminants was checked by SSR markers and only 1 offspring for each cross showed an incompatible genetic profile (presence of alien alleles or self-fertilization) and was discarded.

A total of 277 individuals from the population 50042 (Shavtsitska x Glera) and 280 individuals from the population 50041 (Chardonnay x Tskhvedianis tetra) were potted for the phenotyping bioassays and the mapping study.

Table 3. Results from the two main controlled crosses.

Seed parent	Pollen donor	Bunches	Tot. Seeds	Seedlings			
				Sown	Grown	Genotyped	Retained
Shavtsitska	Glera	14	3136	474	429	420	277
Chardonnay	T. Tetra	10	1415	604	422	318	280

4.2 Phenotypic evaluation of the resistance to *E. necator*

Shavtsitska appeared to have a higher resistance degree to *E. necator* than Tskhvedianis tetra. Due to this preliminary observation it was decided to carried out more resistance bioassays on Shavtsitska and its population. Tskhvedianis tetra and its progeny were studied in a limited way in some complementary experiments. The details on the phenotyping bioassays carried out at INRAE (Colmar, France) were summarized in Table S3.

4.2.2 Studying of early *E. necator* infection by Trypan-Blue staining and SEM

Infection at 1-2-3 dpi was studied by Trypan-Blue staining of pathogen structures. Discs of the grape varieties Shavtsitska and Glera, of 17 individuals of their cross population (50042) and of the control plants (RV1-22-8-78, Kishmish vatkana, Johanniter and Cabernet sauvignon) were observed under the microscope (Table S3).

At 1 dpi, most conidia showed only the appressoria (class 0). Several conidia showed, either only the primary hypha (class 1), or two hyphae (class 2) and very few had a tertiary and/or branched hypha (class 3) as shown in Figure 6. Shavtsitska and RV1-22-8-78 delayed the pathogen growth at 1 dpi and showed a higher number of conidia in classes 0 and 1 compared to the more susceptible genotypes Glera and Cabernet sauvignon (p-value < 0.05, Figure S2a and Table S4). At this stage it was not possible to classify the offspring as resistant and susceptible (Figure 6); moreover, Kishmish vatkana and Johanniter were not differentiated from Glera and Cabernet sauvignon.

At 2 dpi, the pathogen development on the discs became visually different between most of the susceptible and resistant individuals (Figure 7). Shavtsitska and the putative resistant seedlings showed most conidia in classes 0, 1 and 2, and few in class 3. On Glera and putative susceptible offsprings, conidia had longer hyphae (Figure 7b) and most of the conidia were in Class 3. The pairwise comparison between the parents and the controls confirmed the differences between the resistance and susceptible

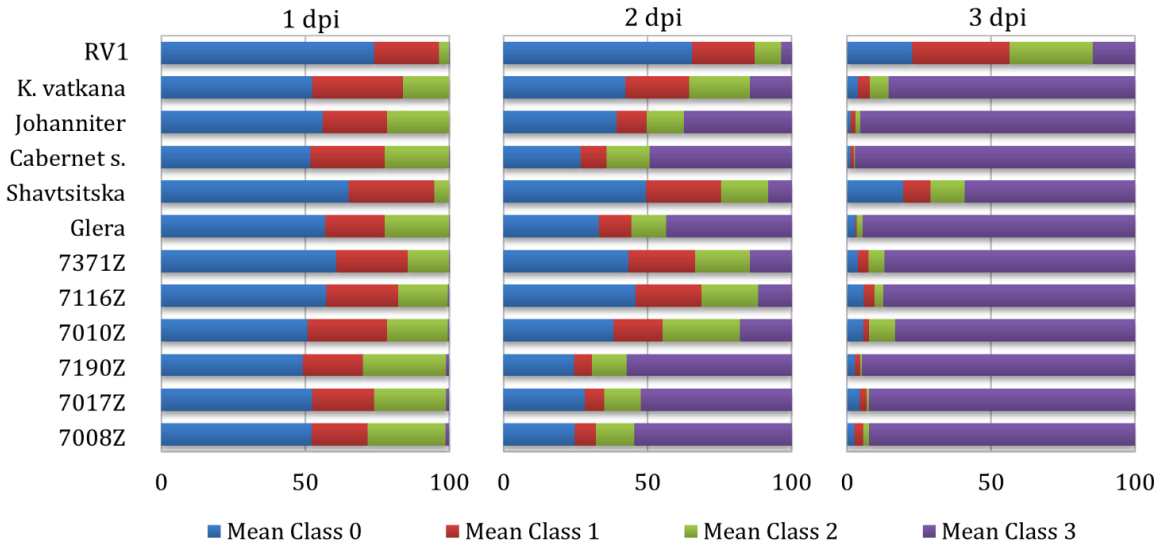

Figure 6. Conidia classification at 1-2-3 dpi following the Trypan-Blue staining. Class 0 identifies conidia showing the appressoria, class 1 the conidia with the primary hypha, class 2 the conidia with the secondary hypha and class 3 the conidia with the tertiary hypha and/or hyphae ramification. Seedlings 7010Z, 7116Z and 7371Z are putative resistant genotypes while 7008Z, 7017Z and 7190Z are putative susceptible ones.

plants but only Shavtsitska, RV1-22-8-78 and Kishmish vatkana significantly delayed the pathogen growth. Johanniter behaviour was not different from Glera and Cabernet sauvignon (p-value < 0.05, Figure S2b and Table S4).

At 3 dpi, all plants but not RV1-22-8-78 showed most conidia in class 3 (Figure 6). On putatively resistant plants, the development of the pathogen was still delayed and limited with short hyphae and few ramifications. On putative susceptible plants conidia produced a wider and more ramified mycelium with very long hyphae and many branches. The colonies also overlapped each other. The classification method identified significant differences, however the observations were not reliable in resolving resistant and susceptible plants as it was for 2 dpi (Figure S2 and Table S4).

In each experiment about 1-3% of the conidia per disc did not germinated (data not shown). Differences between experiment replicates were not detected.

Plant necrosis were few frequent at 1 dpi while they increased at 2 dpi. On resistant plants, the necrotic response was recorded beneath the conidia and hyphae appressoria. On susceptible plants, the necrosis was exhibited only close to the conidia appressoria (Figure 7a).

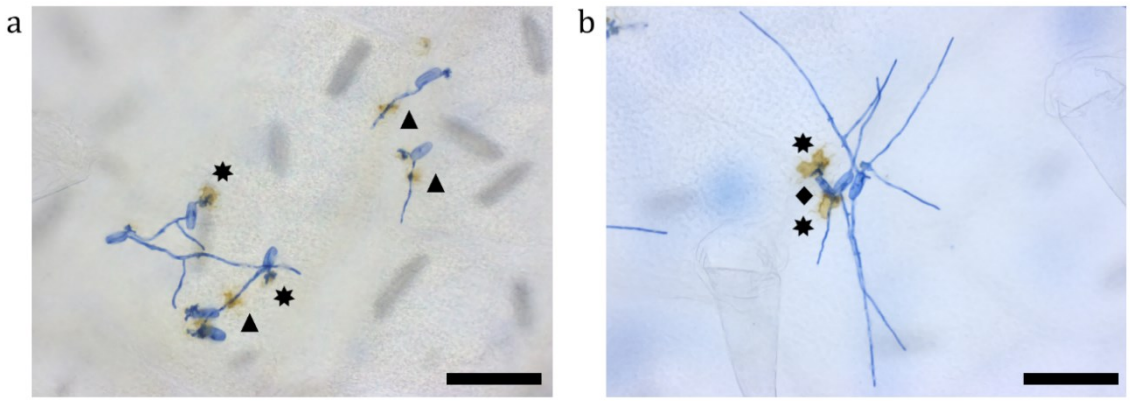

Figure 7. E necator conidia development at 2 days post-infection (dpi) on Shavtsitska (a) and Glera (b) leaf discs. In Shavtsitska, the pathogen growth was delayed and more limited than in Glera, furthermore plant necrotic response (brown area) was present under the conidia appressoria (✵) and under hyphae appressoria (▲). Collapsed conidia were also observed under microscope (◆). Magnification x200. Scale bar 100 µm.

Observations made at SEM largely confirmed the results of the staining bioassays (Figure 8 and Figure S1). The higher magnifications (x600-x2000) and resolution of the instrument permitted to collect several accurate observations of the plant-pathogen interaction:

a- On resistant plants multilobed appressoria were frequently observed, furthermore, more appressoria were early and less regularly produced from short hyphae;

b- *E. necator* was able to establish a successful interaction on straight hairs, while, on prostrate hairs, conidia collapsed if they didn't produce appressoria directly on the leaf surface;

c- Collapsed conidia and hyphae were observed in all plants (more clearly than under brightfield microscope).

Finally, plant necrosis was not visible by SEM but in the area surrounding the conidia appressoria, a depression was often observed on individuals that usually showed necrosis in staining experiments.

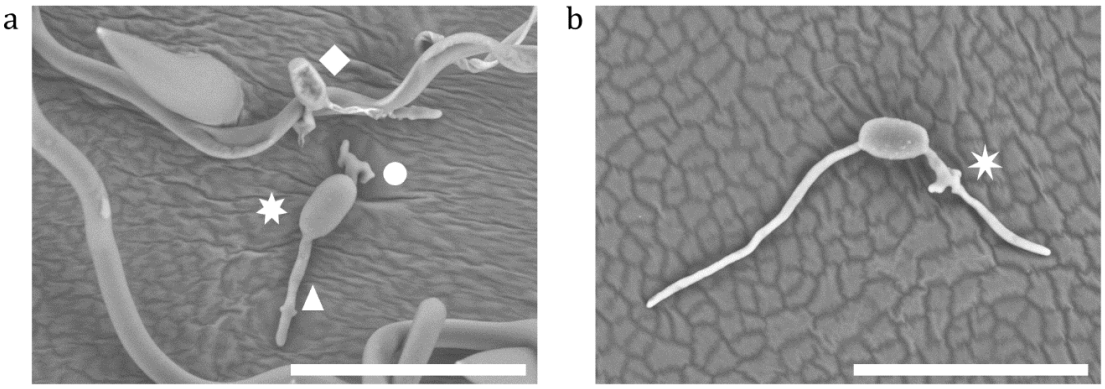

Figure 8. E. necator conidia development at 2 dpi on Shavtsiska (a) and Glera (b). By the scanning electron microscope (SEM) was possible to clearly observe the conidia (✵) and hyphae (▲) appressoria. On Shavtsitska, conidia that fallen on hairs collapsed (◆) and in the area surrounding the conidia appressoria a depression was often observed (●). Magnification x1000. Scale bar 100 um.

4.2.3 Populations phenotyping for resistance to *E. necator*

4.2.3.1 Population 50042 (Shavtsitska x Glera)

Three replicated experiments were carried out for the cross population 50042. In the first experiment only 188 seedlings were studied because part of them showed a reduced growth as a symptom of suboptimal health condition. In the second and third replicate the number of seedlings was raised to 251. Therefore, 251 seedlings were phenotyped, 158 of them three times and 106 twice (Table S3).

The three experiments provided similar results at any observation time (3-5-7-10 dpi) and the progeny revealed different degrees of resistance to *E. necator* (Figure S3). The development of powdery mildew infection on the discs of the seedlings is summarized in the comments that follow.

At 3 dpi, *E. necator* already showed different growth rate on the cross offsprings, in according to the OIV classification: in many discs the mycelium was limited to few hyphae and scored 7; in other cases, the growth was more advanced (patches) and assessed 5; finally, scores 3 (very developed) or 9 (no growth) were very rare (Figure S3). Necrosis was randomly observed on the discs. Lastly, no sporulation was detected.
At 5 dpi, the pathogen mycelium growth reached mainly intermediate or high rate of infection with scores 5, 3 and 1. Only a part of the plants was able to strongly limit the infection (rated 7 or 9). At 5 dpi pathogen sporulation started: it was usually scored 7 (few conidiophores) and sometimes 5 (Figure S3). Conidiophores were produced on discs evaluated 5 or 3 for mycelium growth and only one conidia per conidiophore were counted. Necrosis frequency increased and was more common on plants with delayed pathogen development.
At 7 dpi, mycelium growth achieved mainly classes 3 and 1 and it was difficult to differentiate putative resistant from putative susceptible plants observing this infection feature. Sporulation was rated mostly 5 and 3 (Figure S3), conidia per conidiophore varied from 1 to 4 and different resistance behaviours were easily distinguishable based on these phenotypes. Plant necrosis scores were similar to 5 dpi.
At 10 dpi many samples were very infected with mycelium growth classified 1 (the most frequent class) and 3 and sporulation rated 3 (the most frequent class) and 1 too (Figure S3). Up to 6 conidia per conidiophore was recorded on susceptible plants. Only on few individuals the pathogen had a very reduced growth and sporulation and produced 1-2 conidia per conidiophore.
The parent Shavtsitska displayed a resistant-like phenotype with limited pathogen development and frequent and increasing plant necrotic response: mycelium growth was delayed thorough all experiments (rated up to 5), sporulation was visible from 7 dpi with final scores of 7 and 5 and with 1-2 conidia per conidiophore (Figure 10a). Glera showed a weak and infrequent necrotic response (limited to the beginning of the infection and to the conidia) and was not able to inhibit the *E. necator* development: mycelium grew fast and spread over the whole disc already at 7 dpi (score 1), sporulation was produced at 5 dpi with final scored of 3 and 1 and with up to 6 conidia per conidiophore (Figure 10b).

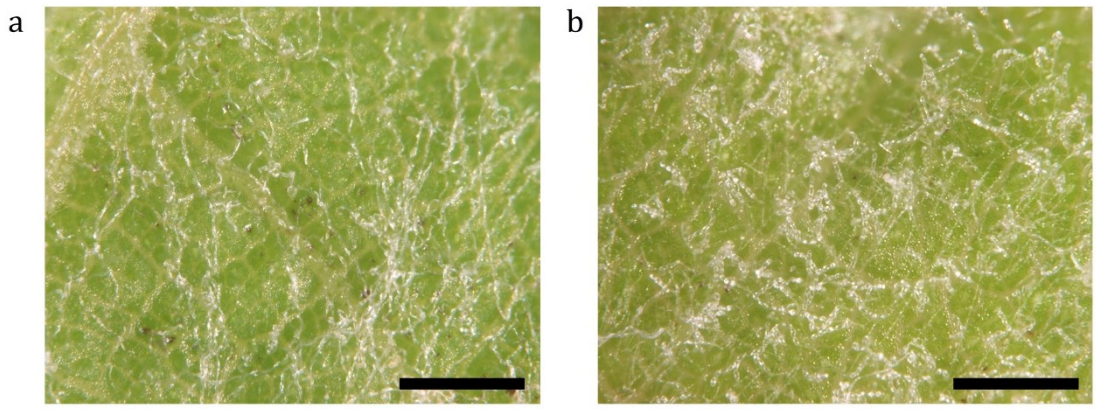

Figure 10. E. necator development on Shavtsitska (a) and Glera (b) discs at 11 dpi. On the resistant plant Shavtsitska the pathogen mycelium growth, sporulation and number of conidia per conidiophores were more limited than on Glera. Furthermore, on Shavtsitska the plant necrotic response was more frequent. Magnification x64. Scale bar 500 µm.

The discs of the control plants showed little symptoms variability within experiments, thus confirming the homogeneity of the inoculations. Shavtsitska showed a degree of resistance to *E. necator* between RV1-22-8-78 (totally resistant with very restricted mycelium growth and no pathogen sporulation) and Kishmish vatkana (partially resistant with delayed and limited mycelium growth and sporulation) while Glera and Cabernet sauvignon displayed the same susceptibility (Figure 9).

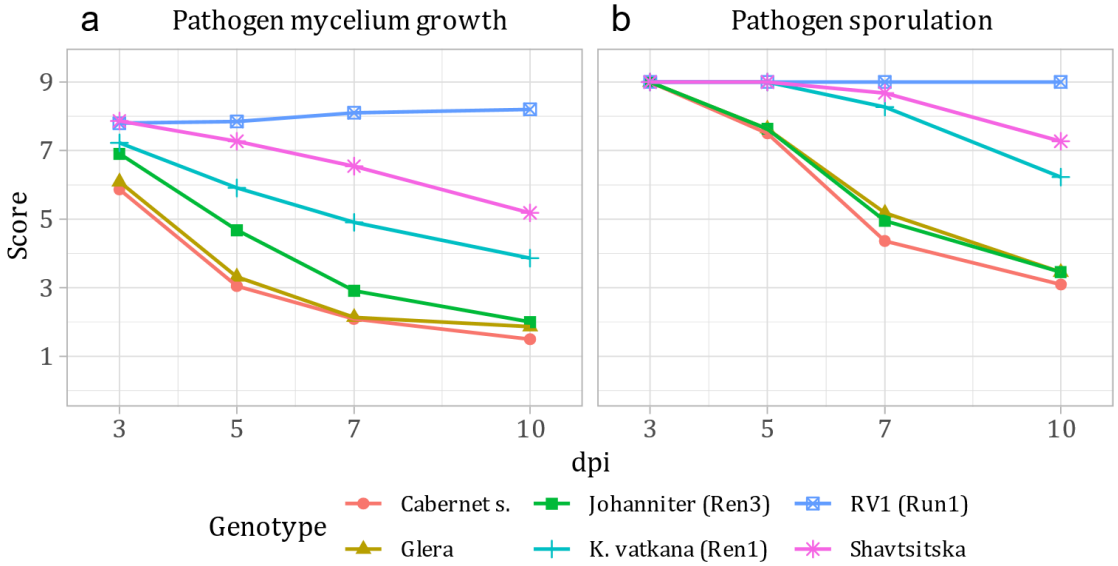

Figure 9. E. necator mycelium growth (a) and sporulation (b) development (mean values from 3 replicated experiments) on Shavtsitska, Glera and control varieties with known degree of resistance to powdery mildew and carrying specific resistance loci to the pathogen (Ren/Run).

Regarding the resistance segregation in the cross population 50042, offsprings usually displayed the same resistance phenotype among replicates. The best Spearman coefficients of correlation between experiments were calculated for pathogen intensity of sporulation at 7 dpi with the following values: 0.59 between experiments replicates

one and two, 0.59 between replicates one and three and 0.69 between replicates two and three. In the first experiment we observed a resistance-like phenotype (similar to Shavtsitska and Kishmish vatkana) in 42% of the offsprings. In the second experiment, *E. necator* grew faster and was more aggressive and only 18% were putatively resistant as Shavtsitska. The third experiment showed about 20% plants with a resistance comparable with that one of the Caucasian parents. Individuals with susceptible-like phenotype (similar to Glera and Cabernet sauvignon) were about 45-50% in all replicates. The remaining progeny displayed intermediate resistance values. As a result of the three experiments, the distribution of offsprings phenotypic data was more or less bimodal depending on the infection feature, on the dpi and on the experiment considered (Figure S3 and Figure 11).

The rAUDPC values for *E. necator* mycelium growth and sporulation were calculated from the averaged scores per dpi per individual. rAUDPC values distributions confirm different degree of resistant to *E. necator* in the progeny and the bimodal distribution of the trait phenotypes (Figure 11). Shavtsiska mean rAUDPC in the three expriment replicates was 0.74 +/- 0.14 for mycelium growth and 0.95 +/- 0.08 for sporulation, while for Glera was 0.33 +/- 0.13 and 0.67 +/- 0.12 respectively.

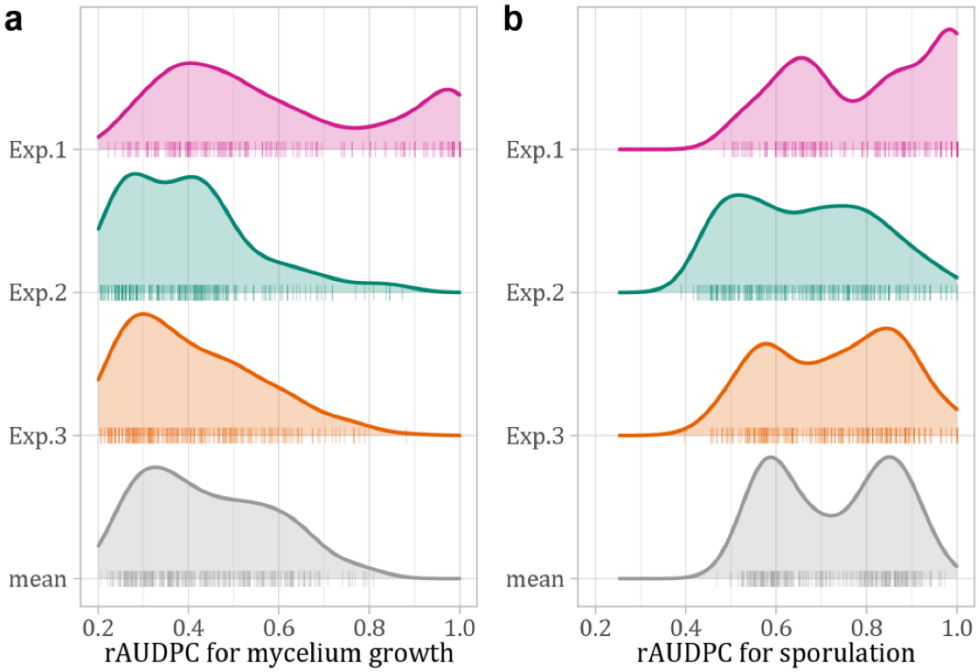

Figure 11. Population 50042 (Shavtsitska x Glera) relative Area Under Disease Pressure Curve (rAUDPC) for pathogen mycelium growth (a) and sporulation (b) values distribution in the three experiments (different colours).

The counts of conidia by Malassez chamber recorded between 0 and 3.25 x 10^5 conidia per ml for the progeny discs. Shavtsitska showed between 0 and 6.5 x 10^4

conidia per ml while Glera between 1.3 x 10^4 and 2.1 x 10^5 conidia per ml. The counts values were square-root transformed to correct the data skewness and normality of residuals. After the transformation the data distribution became bimodal for the first experiment, while normally distributed for the other two replicates. Despite the objectiveness of the data, conidia counts appeared less effective in discriminating possible resistant and susceptible plants.

4.2.3.2 Population 50041 (Chardonnay x Tskhvedianis tetra)

The population 50041 was phenotyped for the resistance to *E. necator* in two experiments (Table S3). A total of 67 seedlings were studied: 58 of them were evaluated twice and 9 once. The progeny displayed different resistant degrees and similar results in the two replicates (Spearman coefficient of correlation of 0.49 for pathogen sporulation intensity at 7 dpi). The powdery mildew infection was evaluated at 2-4-7-9-11 dpi.

At 2 dpi, little (1-2 hyphae with branches) or no pathogen mycelium was detected (marked 7) on the discs but it was not easily observable under stereomicroscope. A necrotic response was recorded on many individuals.

At 4 dpi, *E. necator* developed a widely diffused mycelium, usually scored 3 or 5, and incipient conidiophores without conidia on most of the discs. Plants that limited the pathogen development seemed showing a higher frequency of necrosis.

At 7, 9 and 11 dpi observations on the 50041 offsprings followed those carried out for the population 50042, but the mean degree of infection appeared higher.

Tskhvedianis tetra displayed a partial resistance to *E. necator* (Figure 12) but of weaker strength compared to Shavtsitska. Tskhvedianis tetra delayed the pathogen mycelium and sporulation development up to 7 dpi. At 7 and 11 dpi the infection showed a dense mycelium (scores 1-3) and an intermediate sporulation intensity (score 5) with up to 4 conidia per conidiophore. Tskhvedianis tetra showed a constant necrotic response that increased with the infection. *E. necator* developed well on the susceptible parent Chardonnay (Figure 12).

The resistance trait segregated also observed in the population 50041. The resolution between resistant and susceptible plants was clearer at 5-7 dpi for both mycelium growth and sporulation. At that times, about 60% of the progeny showed a susceptible-like phenotype, while 30% of plants showed variable level of partial resistance to the pathogen.

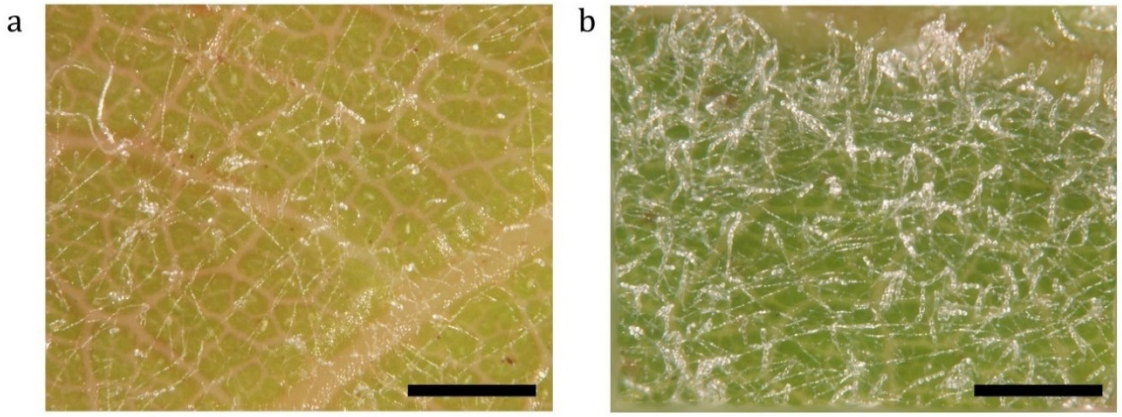

Figure 12. E. necator development on T. tetra (a) and Chardonnay (b) discs at 11 dpi. On T. tetra the mycelium growth, sporulation and number of conidia per conidiophores were more limited than on Chardonnay. Furthermore, on T. tetra the necrotic response (little visible in the photo) was more frequent. Magnification x64. Scale bar 500 μm.

4.3 Sequencing and SNP calling

The improved DNA extraction protocol provided most samples with at least 50 ng/ul DNA had quality ratios 260/280 and 260/230 greater than 1.8. Gel electrophoresis confirmed the high molecular weight of the DNA, the absence of RNA blobs and the suitableness of samples for the GBS analysis.

Together with Shavtsitska and Glera (analysed twice), a total of 184 offsprings were chosen for the genotyping on the basis of the "quality" of phenotyping data: 159 individuals phenotyped three times and 25 individuals phenotyped twice showing stable phenotypes in the experiments (rAUDPC variation for pathogen sporulation < 0.08) were retained.

Two plates of libraries yielded 26.3 ng and 25.8 ng/ul DNA respectively, and generated the expected fragment size distribution. The sequencing produced a total of 498 million reads with an average read pair count per sample of 2.5 million and a coefficient of variation of 36%.

Some 948,472,792 BAM records were verified with Stacks that identified 596,549,705 (62.9%) primary alignments to keep, 151,228,015 (15.9%) alignments with insufficient mapping qualities and 200,695,072 (21.2%) unmapped alignments. Statistics showed a mean of 5,018,374.6 records/sample, of which 40.1% to 67.7% were kept. From the Stacks analysis 695,985 loci were obtained, the mean coverage per-sample was 22.1x, ($\boldsymbol{\sigma}$ = 7.2x, min = 5.1x and max = 44.0x) while the mean number of sites per locus was 129.8, for a total of 90,332,730 sites. Putative SNP were identified, variant sites with minimum allele frequency less than 5% were discarded and one SNP

per locus was retained. Finally, 139,318 SNP variants for 188 samples were exported in the final .vcf file.

4.4 Linkage maps for the cross parents Shavtsitska and Glera

The SNP dataset was initially processed by Perl scripts that filtered 116,214 markers with genotyping rate below 80%, 4256 markers with similar major and minor allele frequency (about 0.5, respectively) and 480 markers with genotyping error rate above 5%. A total of 18,362 putative pseudo-testcross markers were retained.

Shavtsitska and Glera genotypes were verified and 1445 markers were further filtered because were, either homozygous, or heterozygous, or missing, or showed different genotypes in the sample replicates of the parental plants. Of the remaining 16,917 markers, 7,953 were segregating for Shavtsitska and 8,964 for Glera. At this stage, markers resulted well distributed on the chromosomes except for some gaps: six gaps exceeding 2.5 Mb were identified for Shavtsitska in chr 1, 2, 5, 18 and 19, while two regions with low density marker were identified for Glera in chr 2 and 19.

Maternal and paternal maps were built separately. For Shavtsitska map, 1,012 SNP with genotyping rate below 90% and 4,232 co-segregating markers were filtered (together with markers with distorted segregations patterns). The mstmap function confirmed the previously defined 19 linkage groups (LG) and the occurrence of 61 markers that were isolate and/or not clearly attributable to any chromosome. MSTmap algorithm provided a map with inflated distances probably because of problematic markers and genotyping errors: the map length was greater than 3000 cM and linkage groups lengths were often greater than those of former maps. Then, 2,304 LOD-based genotyping errors were corrected to missing data, 344 problematic markers (e.g. with high recombination fraction and low association LOD) and 1 individual (seedling 7067z) with an excess of crossovers were manually filtered. Thus, the final dataset for Shavtsitska map construction was defined by 183 individuals and 2291 markers. The dataset was reanalysed with mstmap function, the corrections were effective for improving the marker order and distances and the MSTmap algorithm provided a trustable map length of 1,475 cM. Final SNP distances were recalculated with est.map function based on Lander-Green algorithm generating the final Shavtsitska map of 1,205 cM length (Figure 14). Finally, the tsp.order function confirmed the MSTmap-defined order: only few close SNP showed alternative positions in the proximal cM.

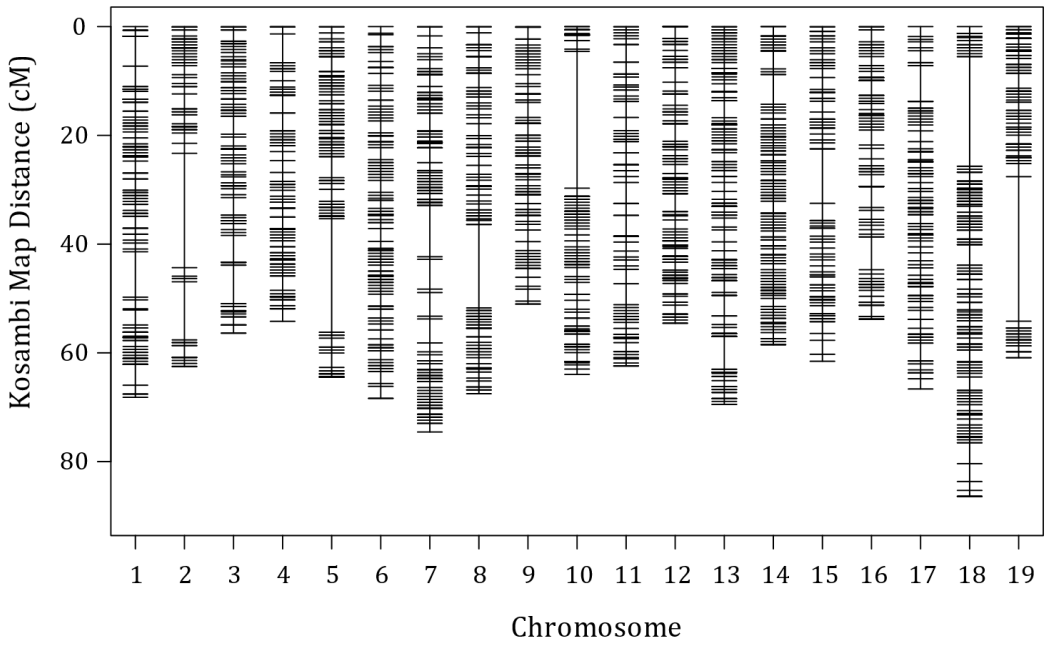

Figure 14. Shavtsitska linkage map.

Glera map was developed following the same custom pipeline. Finally, 183 individuals and 2,627 markers were utilized to calculate the final map that covered a total of 1,315 cM, divided into 19 LG (Figure 13).

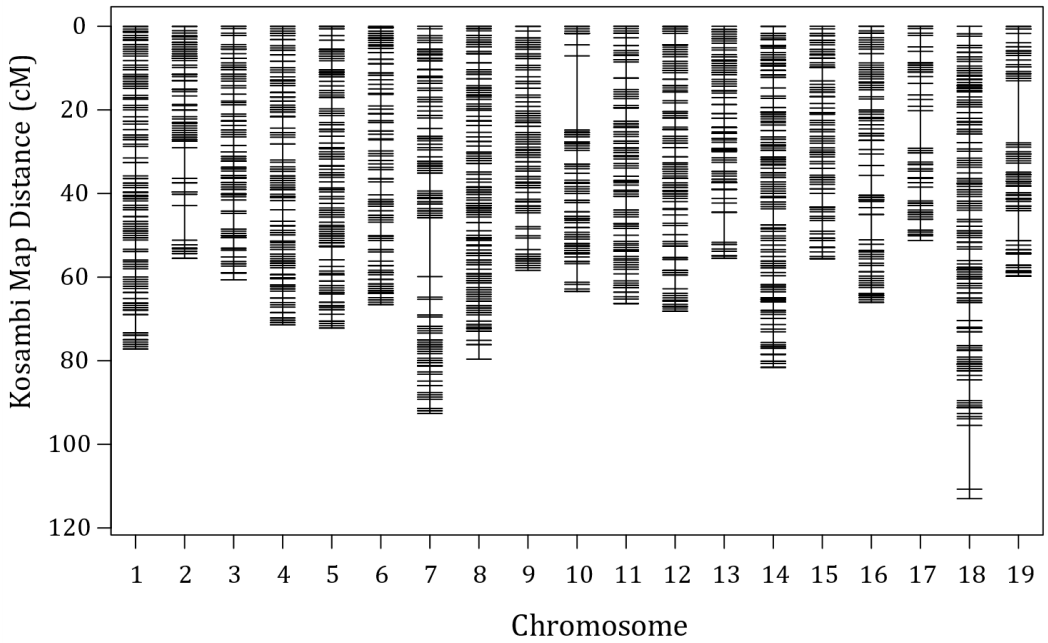

Figure 13. Glera linkage map.

SNP kept a good coverage on most of the LG and only few gaps remained in the parental maps. In particular, Shavtsitska map showed five gaps of about 20 cM in LG 2, 5, 10,18 and 19, while Glera showed a gap of about 18 cM in LG 10. Furthermore, all SNP in Glera LG 13 showed distorted segregations patterns; for such chromosome only the markers not disturbing the linkage order and distances were retained to complete Glera genetic

map. The segregations showed that one end of one chromatid 13 of Glera were not inherited in the offsprings (Figure 15).

Finally, maps showed a good correlation between the genetic order and the physical position of SNP, except for two marker group inversions in chr 3 and 5 (Figure S4).

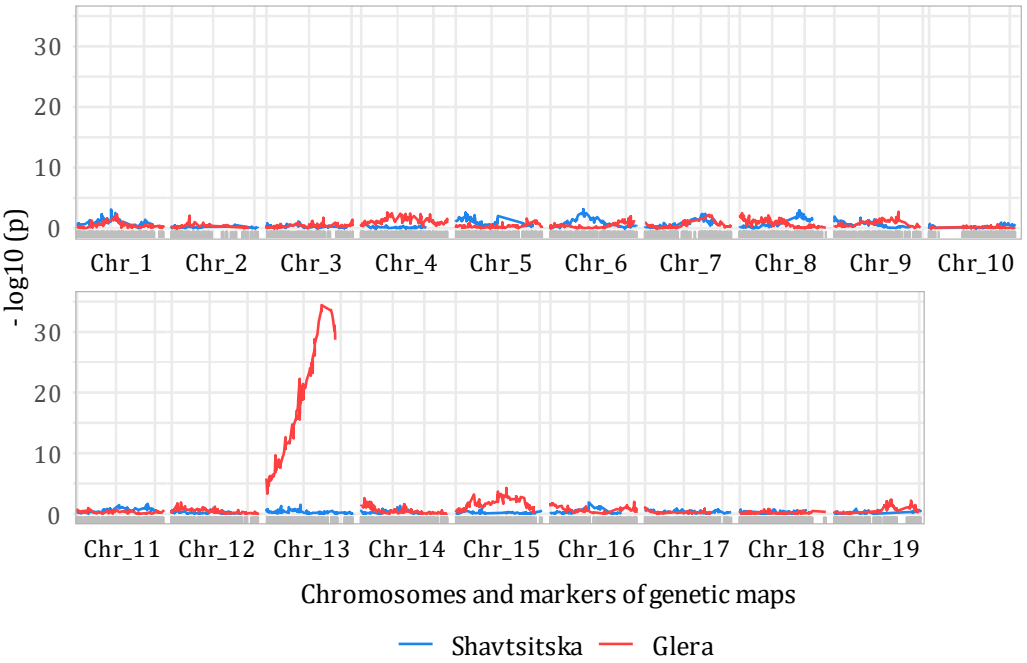

Figure 15 Negative log10 of p-values in chi-square tests (-log10(p)) comparing segregation frequencies of the alleles of the markers retained in the final parental linkage maps (Shavtsitska and Glera). The higher values for Glera chr 13 demonstrates the presence of significant segregation distortions from the expected Mendelian ratio of 0.5. A -log10(p) value of 2 corresponding to a p value of 0.01. Vertical grey breaks are positioned every 30 cM on the genetic maps chromosomes.

Details on the retained markers and individuals after each stage of map construction are summarized in Table S5. The number of markers and the genetic length for each linkage group are reported in (Table 4).

4.5 QTL analysis for the resistance to *E. necator* in Shavtsitska

The QTL analysis identified a strong resistance source to *E. necator* in Shavtsitska, and none in Glera, as expected.

The interval mapping (IM) procedure identified the major QTL in Shavtsitska chr 13 at about 47 cM from the top with all series of phenotypic data obtained for the population 50042 (Figure 16, Figure S5 and Table S6). Generally, the second and the third experiment showed higher LOD values; results for the first experiment suggested that some offsprings had in such replicate a masked susceptibility (probably due to a suboptimal health state) and negatively influence the QTL analysis (Figure S5). Finally, the averaged data from different experiments provided always the highest LOD peaks

for each set of data (Table 5 and Table S6) and were utilized for the subsequent considerations.

Table 4. Shavtsitska and Glera linkage maps details.

Chr.	Shavtsitska map			Glera map		
	Markers	LG length cM	Largest gap cM	Markers	LG length cM	Largest gap cM
1	131	68.2	8.4	159	77.2	4.4
2	72	62.5	21	98	55.5	8.3
3	116	56.4	7.1	139	60.6	3.8
4	112	54.2	5.3	166	71.4	3.8
5	113	64.5	20.9	169	72.2	3.2
6	125	68.4	2.4	131	66.6	3.3
7	157	74.6	9.4	146	92.6	14.1
8	110	67.5	15.3	191	79.6	3.5
9	104	51	2.8	115	58.4	3.3
10	119	64	25.2	102	63.5	17.7
11	108	62.4	3.9	122	66.3	3.9
12	138	54.6	3.3	138	68.2	3.3
13	166	69.5	6	112	55.5	7.2
14	138	58.5	5.5	216	81.6	3.9
15	100	61.5	10	107	55.6	3.3
16	88	53.8	6	122	66.1	6.1
17	131	66.6	6.6	79	51.2	9
18	166	86.4	20.2	198	113	15.3
19	97	60.9	26.6	117	59.8	14.9
Total	2,291	1,205.5	26.6	2,627	1,314.9	17.7

The LOD peaks for mycelium growth varied from 23.78 to 40.17 according to the time of observation (dpi), for sporulation intensity they varied from 36.41 to 60.61 and for plant necrotic response from 5.87 to 32.14 (Figure 16, Figure S5 and Table S6).

The averaged data from count of conidia under microscope well identified the resistance QTL as well as the previous phenotypes (maximum LOD peak of 28.68). The rAUDPC indexes for mycelium growth and sporulation gave similar or better results in term of LOD peaks if compared with the single dpi scores (maximum values of 37.72 and 64.88 LOD respectively) (Figure 16).

The QTL analysis for Shavtsitska showed possible minor QTL (e.g. on chr 14 for IM with sporulation intensity data, Table S6) but the LOD peaks were just above the calculated significance threshold and QTL were not constantly detected in different experiments and with different phenotypic datasets (Table S6).

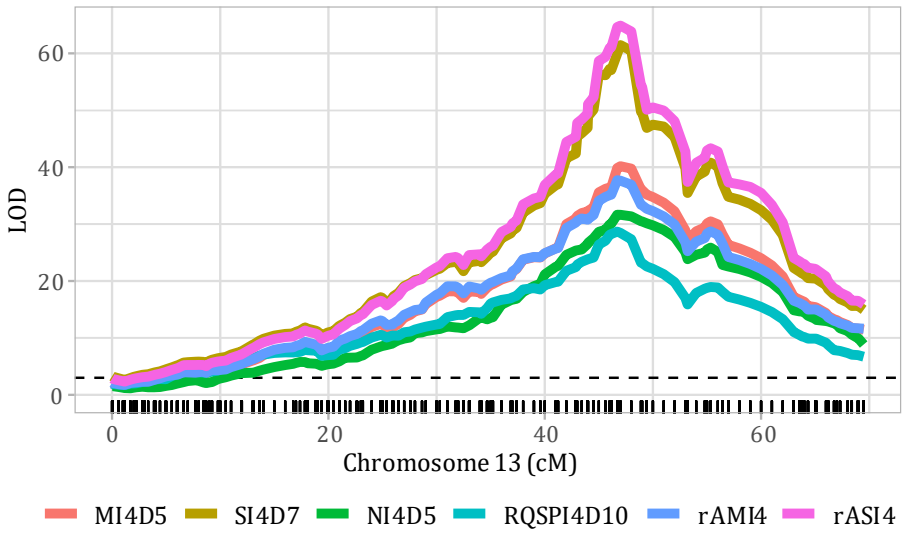

Figure 16. Results of interval mapping for Shavtsitska chr 13. The graph shows the SNP position on Shavtsitska genetic map (x axis) and the LOD values (y axis) for the analysis carried out with the averaged data (calculated from three experiment replicates – I4) for pathogen mycelium growth at 5 dpi (MI4D5), sporulation at 7 dpi (SI4D7), plant necrosis production at 7 dpi (NI4D7), conidia counts at microscope square-root transformed (RQSPI4), relative Area Under Disease Pressure curve for pathogen mycelium growth (rAMI4) and sporulation (rASI4).

Finally, multiple QTL analysis only confirmed the major QTL in Shavtsitska chr 13 and refuted all possible minor QTL.

The most relevant phenotypic data in IM were utilized to fit single QTL models (formula=y~Q1), evaluate the QTL effect (explained variance – R^2) and calculate the locus Bayes credible interval (α=0.95) (Table 5).

Depending on the phenotypic data considered, QTL models explains up to the 80.15% of the observed variance and comprised the locus in an interval of 2.2 cM (Table 5).

Informative recombinants for the resistance locus identified in Shavtsitska were searched among the genotyped progeny: 7 resistant and 5 susceptible plants showed a recombination event between SNP13_ 15078566 and SNP13_ 18998373, that flank the region depicted in Figure 17. Recombination events showed that the locus involved in the resistance to *E. necator* was positioned in a region of 1.4 Mb on the grape reference genome and the region was comprised between the SNP13_16797000 and the SNP13_18213673 markers.

Table 5. Proprieties of the significant QTL associated to *E. necator* resistance determined with the most relevant phenotypic data in interval mapping.

Phenotypic data	Chr	LOD	p-value	Expl. var. %	Pos.	Nearest marker	Bayes conf. Intervals (α=0.95) Pos.	Lower and upper marker
Mycelium growth 5 dpi	13	40.17	<0.001	63.46	47	SNP_13_18102346	46.70 48.89	SNP_13_17909186 SNP_13_18213673
Sporulation intensity 7 dpi	13	61.45	<0.001	77.62	47	SNP_13_18102346	46.70 48.89	SNP_13_18102346 SNP_13_18213673
Plant necrosis 5 dpi	13	31.65	<0.001	54.91	46.7	SNP_13_18102346	46.70 48.89	SNP_13_17909186 SNP_13_18213673
Square root (n conidia)/ml	13	28.68	<0.001	50.68	46.7	SNP_13_18102346	45.61 48.89	SNP_13_15836674 SNP_13_18213673
rAUDPC for mycelium g.	13	37.72	<0.001	61.3	46.7	SNP_13_18102346	46.70 48.89	SNP_13_17909186 SNP_13_18213673
rAUDPC for sporulation i.	13	64.88	<0.001	80.15	47	SNP_13_18102346	46.70 48.89	SNP_13_17909186 SNP_13_18213673

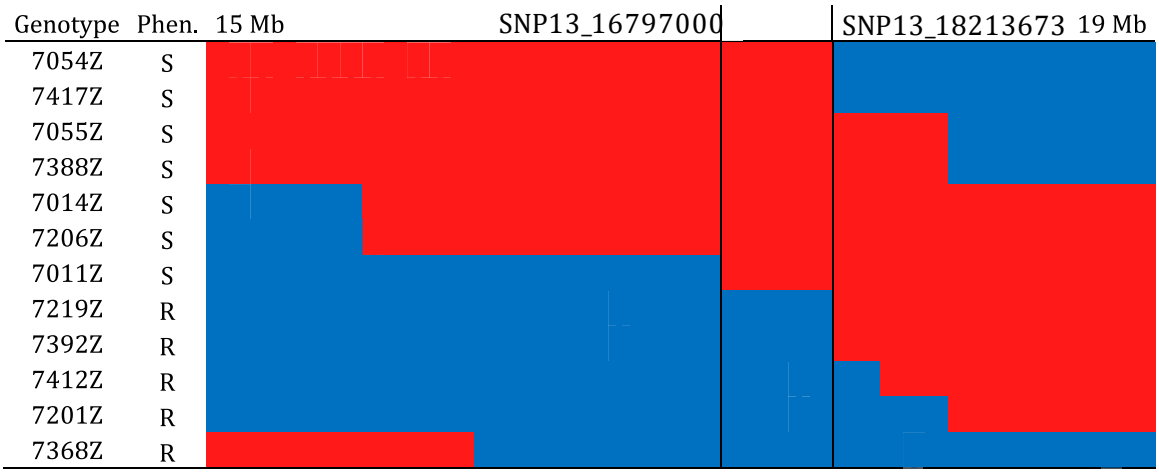

Figure 17. Recombinants for the region associated to the QTL for resistance to *E. necator* and physical position on grape reference genome of the markers flanking the QTL. Susceptible (S) haplotype is in red and resistant (R) haplotype is in blue.

4.6 SSR genotyping of Tskhvedianis tetra population and Caucasian grape germplasm

SC8-0071-014 and Sc47_20 SSR markers (Coleman et al., 2009) resulted on the grape reference genome PN40024 tightly-linked to the position of the resistance QTL identified in Shavtsitska.

Both markers were assayed in a subsample of individuals of the cross populations and in the relative parents. The analysis revealed that the resistant offsprings of population

50042 (Shavtsitska x Glera) and population 50041 (Chardonnay x Tskhvedianis tetra) always inherited from the resistant parents the allele 149 of SC8-0071-014 and the allele 208 of Sc47_20.

Thus, the screening with SC8-0071-014 and Sc47_20 markers was extended to all sixty-seven phenotyped individuals of population 50041. Thirty-five individuals inherited from Tskhvedianis tetra the 149-208 haplotype, thirty-one had the 174-206 haplotype and one individual was recombinant for the two SSR. Highly significant phenotypic differences (p-value < 0.001) between haplotype-derived groups were found with each dataset of rAUDPC values for sporulation intensity tested (Figure 18). Linear models were fitted using haplotypes as fixed factor and the maximum explained phenotypic variance was 74% (R^2). Finally, genetic and statistical analysis confirmed that the same resistance locus segregated in both cross populations and that it was shared from the Caucasian varieties Shavtsitska and Tskhvedianis tetra (Table S7).

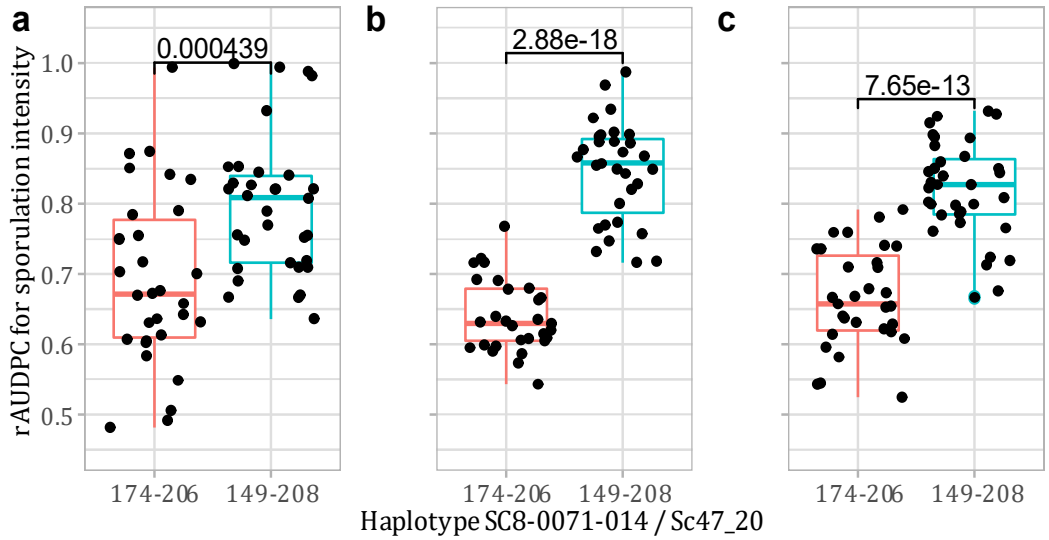

Figure 18. Box-plots for rAUDPC pathogen sporulation data for population 50041 (Chardonnay x Tskhvedianis tetra). Plots identified comparisons for different experiment replicates (a-b) and between calculate averaged data per individual (c). Offsprings are grouped by inherited haplotype. Above the box-plots results (p-values) of T-tests between haplotype-derived groups.

SC8-0071-014 and Sc47_20 markers were finally analysed in further 103 Caucasian varieties stored at the CREA-VE grape germplasm collection (Table S7). For SC8-0071-014 fifteen possible alleles were recorded; the allele 149 was found in twenty-five accessions and was the fourth more frequent allele. For Sc47-20 five alleles were detected and the allele 208 was counted thirty-nine times. The allele pair 149-208 resulted the third more frequent haplotype and it was observed in twenty-four different varieties (Table 6 and Table S7).

SC8-0071-014 and Sc47_20 markers were associate to Ren1 gene (Hoffman et al. 2008; Coleman et al. 2009). In our study, Ren1-associated haplotype was 147-206 and was identified in Kishmish vatkana and Dzhandzhal kara cultivars (Table S7). Both alleles were rare in the Caucasian germplasm and only two accessions displayed the Ren1 haplotype (Table 6 and Table S7).

Table 6. Results from SSR screening in Caucasian grape germplasm conserved at CREA-VE. Alleles 147 and 206 (underlined) are in coupling with the Ren1 resistant haplotype of Kishmish. Alleles 149 and 208 (**bold and underlined**) are in coupling with the QTL resistant haplotype of Shavtsitska and T. tetra. "-" any allele different from those in coupling with Ren1 or the QTL.

SC8-0071-014 alleles	**Times recorded**	**SC8-0071-014 alleles**	**Times recorded**	**Sc47_20 alleles**	**Times recorded**
147	9	170	3	198	15
149	**25**	174	29	202	56
162	3	176	4	204	66
164	26	178	37	206	15
166	1	204	25	**208**	**39**
167	2	206	16		
168	1	210	22		

Sc47_19 / SC8-0071-014	206/-	**208**/-	206/**208**	-/-	**Total**
147/-	2	0	0	6	8
149/-	0	19	4	1	24
147/149	0	1	0	0	1
-/-	8	14	1	50	73
Total	10	34	5	59	105

5 Discussion

Some Caucasian *Vitis vinifera* were recently described to be resistant to *E. necator* (Failla et al., 2016). Preliminary checks on those resistant grapevines did not identify genetic relationship to known resistance sources (loci and grape accessions) and suggested that in the Caucasian germplasm new resistance determinants could be present. With the aim of investigating such a hypothesis, pseudo test-cross populations were developed by using the Caucasian resistant varieties Shavtsitska and Tskhvedianis tetra. Several phenotyping bioassays were carried out on the cross parents and offsprings. Caucasian varieties showed a partial resistance to *E. necator* which segregates in the progeny and resulted being controlled by a major resistance QTL in chromosome 13.

5.1 Caucasian grape varieties show a partial resistance to *E. necator*

The resistance to *E. necator* in Caucasian varieties appeared phenotypically different from the responses deployed by varieties carrying known resistance genes to the pathogen. Shavtsitska early contrasted the pathogen development: at 1-2-3 dpi hyphae growth was strongly delayed on leaf discs compared to susceptible *V. vinifera* Cabernet sauvignon and Glera (Figure 6 and Figure 7). RV1-22-8-78 (carrying the Run1 gene) showed a more intense response to *E. necator* that halt the conidia hyphae development. Kishmish vatkana (carrying the Ren1 gene) had a response similar to Shavtsitska but of weaker intensity although differences between the two varieties were not always statistically significant. The response to the pathogen of Johanniter (carrying the Ren3/Ren9 genes) was similar to that of susceptible genotypes (Figure 6, Figure S2 and Table S4). These results agree with previous studies which reported substantial differences at 2 and 3 dpi in *E. necator* control of *Vitis* accessions carrying different resistance genes (Feechan et al., 2011; Agurto et al., 2017; Hu et al., 2019). Run1 gene was described to early halt *E. necator* conidia penetration and hyphae elongation, reacting at the infection sites through a programmed cell death (PCD) deployment, callose accumulation and ROS generation (Feechan et al., 2011; Pap et al. 2016; Agurto et al., 2017). Kishmish vatkana and other Ren1 carrying varieties were described to have a post-penetration response to *E. necator* that delayed hyphae development (Hoffmann et al., 2008), but with lower intensity (Qiu et al., 2015) and with later reactions in comparison to Run1-mediated resistance (Agurto et al., 2017).

Zendler et al. (2017) observed that Ren3/Ren9 genes do not affect *E. necator* development in the early days of infection. Finally, we speculate that Shavtsitska has a post-penetration reaction to *E. necator* because the variety do not halt *E. necator* growth and shows necrosis (associable to plant PCD) beneath the appressoria of both conidia and hyphae (Figure 7) (Feechan et al. 2011).

According to SEM observations, *E. necator* conidia and hyphae usually produced appressoria characterized by many lobes on RV1-22-8-78, Shavtsitska (Figure 8 and Figure S1) and Kishmish vatkana. Multilobed appressoria for powdery mildews were already observed in resistant *Hordeum* spp. lines (Andersen & Torp, 1986) and resistant *Vitis* spp. accessions (Schnee et al. 2008). Thus, multilobed appressoria would suggest that the pathogen encounters difficulties in establishing effective interactions and in resistant plants functional haustoria are not produced at all penetration sites. SEM images also revealed that the conidia falling on prostrate airs of the leaves do not develop mycelium. In literature, it is reported that leaf hairs can influence pathogen infections acting as a physical barrier or influencing the leaf micro-environmental conditions (Niks & Rubiales, 2002). While a role of trichomes was often proposed in favouring grape resistance to *P. viticola* (Staudt & Kassemeyer, 1995; Kortekamp et al., 1998; Kono et al., 2018), no reference was found about their possible effects on the foliar resistance to *E. necator*. Our conclusions on this theme need to be confirmed because among all studied accessions only Shavtsitska showed a high density of prostrate hairs and an enhanced resistance to *E. necator*.

Shavtsitska, Tskhvedianis tetra and their resistance offsprings showed, a partial resistance to *E. necator*. It means that the pathogen was able to complete his lifecycle but its development was contrasted by the host: *E. necator* mycelium growth was early slowed down and restricted, sporulation started at 6-7 dpi and was limited to 2-4 conidia per conidiophore at 10-11 dpi. In comparison, susceptible genotypes did not influence mycelium growth, conidiophores appear at 4-5 dpi (Gao et al., 2016), reaching 5-6 conidia per conidiophore and producing, on average, 2.6 times more conidia at 10-11 dpi. The resistance observed in the Caucasian accessions was not effective as the response of genotypes carrying Run1 or Ren6 genes, that halt pathogen hyphal growth and sporulation (total resistance effects) (Feechan et al. 2013; 2015; Pap et al. 2016). The partial resistance in Caucasian accessions described in our study

has similar effects to that observed in genotypes carrying the Ren1 and Ren7 genes (Hoffman et al. 2008; Coleman et al. 2009; Amrine et al., 2015; Pap et al. 2016).
In our populations the resistance segregation was intermediate between the qualitative inheritance of Run1, Ren4 and Ren6 loci (Pauquet et al., 2001; Ramming et al., 2011; Pap et al. 2016) and the quantitative segregation observed for *V. rupestris* (Barba et al., 2014, 2015). The bimodal distribution of phenotypic scores suggests the presence of a major determinant for the trait under observation; while the occurrence of more resistance degrees would suggest the presence of further minor genetic determinant for the resistance.

5.2 A major QTL control the resistance to *E. necator* in Caucasian grapevines

The genotyping by sequencing (GBS) technique (Elshire et al., 2011) performed very well in our study despite the challenges represented by highly heterozygous species, that might generate erroneous SNP calling, high-percentages of missing data and heterozygote under-calling (Spindel et al., 2013; Barba et al., 2014; Cadle-Davidson et al., 2016). We developed the two parental maps, each of about 1,190 cM and 2500 markers, divided in 19 LG (Figure 13, Figure 14 and Table 4) and with marker order consistent with grape reference genome (Figure S4) (Canaguier et al. 2017). Our map lengths and markers density agree with previous GBS-derived linkage maps (Barba et al., 2014; Hyma et al., 2015; Delame et al., 2019; Sapkota et al., 2019; Vezzulli et al. 2019a). Unexpectedly, Glera chr 13 had all markers with distorted segregations patterns. Group of markers with distorted segregations may be common in interspecific crosses (e.g. Myburg et al., 2004; Riaz et al., 2008; Delame et al. 2019), but were also observed in crosses between *V. vinifera* cultivars (e.g. Riaz et al., 2004; Troggio et al. 2007). Distorted segregations may be unpredictable and occur because of post-zygotic lethal combinations between alleles of cross parents, that influence the viability of zygotes, the germination rate of seeds and the seedling survival (Myburg et al., 2004; Delame et al. 2019). In our case one allele on one end of chr 13 of Glera was defective and not inherited in the offsprings. These evidences may suggest the presence of a new locus responsible for the gamete selection in *V. vinifera* in addition to the ones described for Riaz et al. (2004; 2008) on chr 14. Markers with distorted segregations are usually not retained in linkage maps because they can determine spurious linkage, erroneous marker order and imprecise QTL analysis (Xian-Liang et

al., 2006). To complete Glera chr 13 we maintained only SNP not influencing the marker order and distances compared to grape reference genome. Finally, the QTL analysis for the studied trait was not influenced from the segregation event.

All approaches adopted in QTL analysis identified a single major locus for the resistance to *E. necator* on chr 13 of Shavtsitska (Figure 16 and Figure S5), that was further confirmed by the investigations on the Tskhvedianis tetra cross population. The phenotyping data showed different performance in recognising the QTL: the LOD scores were always significant, but the higher LOD values and differences between susceptible and resistant offsprings were observed at 5 or 7 dpi (as reported from Blanc et al., 2012), in particular for the pathogen sporulation (Figure 16 and Figure S5). The rAUDPC indexes, that summarize the infection progress, resulted being the most informative and reproducible data and explained up to 80% of the phenotypic variance (Table 5). In genetic mapping, the methods of phenotypic data collection, that comprise standardized sampling, handling, infection processing and rating, are as important as it is the genetic design and analysis. Our reproducible results confirmed the effectiveness of the phenotyping strategy and underlined the need, in resistance mapping studies, to utilize data that effectively summarize the biology as well as the genetics of the trait (Cadle-Davidson et al., 2016).The SNP flanking the QTL of resistance to *E. necator* of Shavtsitska were positioned in chromosome 13 at 16.8 and 18.2 Mb on the grape reference genome (Figure 17). In the same region, Hoffman et al. (2008) identified the locus Ren1 that was mapped starting from the Kishmish vatkana SSR-based genetic map. The locus was further saturated with SSR markers and Ren1 was delimited to an area of 1.4 Mb on the PN40024 reference genome (Coleman et al., 2009). In our study, we reported the first high-density genetic map based on SNP for a *V. vinifera* varieties resistant to *E. necator* and demonstrated the power of the GBS approaches for quickly narrowing the region of interest and identifying candidate genes (Cadle-Davidson et al., 2016).

Our work showed that the resistance to *E. necator* of Caucasian grapevines is coded by a mayor and effective gene. On contrary, the resistance to *P. viticola* in such germplasm appeared controlled by three different minor loci (Sargolzaei et al., 2020). Therefore, our results strongly increased the interest in Caucasian grape accessions for breeding programs to produce new resistant grape varieties (Sargolzaei et al., 2021). Both the introduction/pyramiding of mayor and minor resistance source are important to

define promising and durable traits (e.g. Eibach et al. 2007; Feechan et al. 2015). For this reason, Caucasian varieties carrying both resistances to *P. viticola* and *E. necator* are valuable germplasm and need to be investigated at a larger extent. The cross-checking of the results of our paper and that one of Sargolzaei et al., (2020) did not show varieties carrying the resistance to both pathogens, but a more exhaustive analysis will be carry out.

5.2 Origin of the resistance to *E. necator* in the Caucasian grapevines

The screening of Shavtsitska and Tskhvedianis tetra populations with SC8-0071-014 and Sc47_20 markers (Coleman et al., 2009), revealed that the allele 149 of SC8-0071-014 and the allele 208 of Sc47_20 are in coupling and present in both Caucasian parents and their resistant offsprings (Table 6 and Table S7). We extended the SSR analysis to 103 Caucasian *V. vinifera* subsp. *vinifera* accession conserved at CREA-VE discovering that the haplotype 149-208 was shared by 24 varieties. These results suggest that the resistance to *E. necator* could be very frequent in Caucasian germplasm. Eleven Caucasian grapevines were phenotypically characterized as partial resistant to *E. necator* from Failla et al. (2016). For six of those grapevines our molecular analysis showed the presence of the Caucasian resistant haplotype in chr 13. Other five phenotypically resistant accessions did not share the same haplotype and would suggest a more complex genetic landscape behind the resistant trait. Our genetic findings are consistent with Riaz et al. (2020) study, that identified in many Caucasian *V. vinifera* subsp. *sylvestris* a resistance QTL in chr 13.

Our research in addition to other studies identified the resistance to *E. necator* in many *V. vinifera* grapevines of different geographic area (Caucasus and Central Asia) and collected evidences of its inheritance shared by wild and cultivated *V. vinifera* subspecies (Coleman et al., 2009; Hoffmann et al., 2008; Riaz et al., 2013, 2020). This information and the long history of grapes isolation in Caucasian region (Imazio et al., 2013; De Lorenzis et al., 2015) suggests that the resistance trait might have been inherited from a *V. vinifera* progenitor thousands of years ago and conserved in Caucasian cultivars until today. In the ancestor/s, probably, the region evolved to fight different fungi-caused diseases, conserving with the time an array of R-genes (Coleman et al. 2009). The maintenance of the trait in *V. vinifera* through the domestication until today was probably not intentional because there is no literature reporting powdery

mildew disease in Europe and Asia before the 19th century. However, literature suggested that both natural and intentional grapevines selection took place in the last two centuries in the region (Ocete et al., 2012; Maghradze et al., 2020), when the pressure of *E. necator* on grape cultivation became evident due to pathogen introduction from North America. A recent selection could explain the high frequency of resistance haplotype 149-208 within the Caucasian cultivars.

5.4 Genetic basis of resistance to *E. necator* in Caucasian and Central Asia grape germplasm

The mapping of co-located QTL for the resistance to *E. necator* in many and unrelated *V. vinifera* reveal a high complexity of the investigated region in chromosome 13, that encompasses some megabase from upstream to downstream of the mapped loci (Coleman et al 2009). This would suggest a question: are the Caucasian and Central Asia resistant *V. vinifera* grapevines, that carry different marker haplotypes, sharing the same resistance genes or are we coping with different resistance sources developed starting from a common ancestor?

Phenotypic information collected in our research often show distinct responses to *E. necator* between Caucasian grapevines (in particular Shavtsitska) and Kishmish vatkana. However, resistance variation within cross populations of the study and within Caucasus and Central Asia grape resistant accessions (Riaz et al., 2013; 2020; Amrine et al., 2015), does not allow to confirm whether the genetic basis of resistances are different or not. Genetic studies would suggest that multiple resistance loci may existed for genetic regions enriched in R-genes. For instance, on chr 18 Ren4, from *V. romanetii* (Ramming et al. 2011), and Run2, from *V. rotundifolia* (Riaz et al., 2011), loci map in the same position of grape reference genome; furthermore, Run2 is associated to two resistant haplotypes (Run2.1 and Run2.2) that originate from different *V. rotundifolia* accessions (Riaz et al., 2011). Ren1 region in chr 13 contains numerous genes encoding NBS-LRR proteins and appears prone to produce genetic variation (Velasco et al. 2007; Coleman et al., 2009). The natural selection and the evolution mechanisms at the basis of R-genes (Hammond-Kosack & Jones, 1997; Leister, 2004; Mcdowell & Simon, 2006; Marone et al., 2013; Panchy et al., 2016) could have developed in Caucasian and Central Asia *V. vinifera* accessions different resistance genes and/or unique combinations of resistance factors.

Genetic information collected until now cannot determine whether grapevines from Central Asia and Caucasus share or not the same resistance genes. Further narrowing of the genetic region of chromosome 13 explored up to now, as well as comparative sequence analysis and deep transcriptomic study would allow to focus the attention on precise genetic differences. Phenotyping and histochemical observations could also provide new insights on the origin of the resistance variation and on the mechanism behind the trait.

7. Conclusion

The mapping study on the two grape varieties Shavtsitska and Tskhvedianis tetra (*Vitis vinifera* L. subsp. *vinifera*) native to Caucasus revealed the possible presence of new locus of resistance to *E. necator* that mapped in the chromosome 13, nearby the region where Ren1 gene of Central Asia grapevines is located. The genomic region surrounding Ren1, in grape reference genome, resulted being very rich in NBS-LRR resistant genes and prone to produce genetic variation. The Caucasian resistant accessions have a different allelic profile for the locus compared to Ren1 carrying-genotypes from Central Asia. We speculate that Eurasian *V. vinifera* grapes could have developed multiple and independent resistant genes located on chromosome 13 around Ren1 genetic region.

Shavtsitska, Tskhvedianis tetra and resistant seedlings are characterized by a partial resistance to *E. necator* able to delay and limit the pathogen mycelium growth and sporulation in laboratory conditions. As a result of the genetic screening of Caucasian grape germplasm, the resistance trait appears to be widely diffused in such grapevines. Caucasian accessions might therefore be interesting for grape breeding programs because they are also cultivated varieties with a "vinifera" genetic background and pleasant agronomic characteristics. The new investigated source of resistance to *E. necator* can be introduced in breeding programs in one or limited cross generations, in the perspective of producing new elite cultivars with pyramided resistance genes for a more sustainable viticulture.

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7 Supplementary material

- Supplementary Figure 1: SEM images for early *Erysiphe necator* infection on Shavtsitska and Glera
- Supplementary Figure 2: Pairwise comparisons with Trypan-Blue experiments data
- Supplementary Figure 3: 50042 population (Shavtsitska x Glera) phenotyping scores distribution
- Supplementary Figure 4: Linkage maps markers dot-plot: physical position vs genetic order and distances
- Supplementary Figure 5: LOD profile from interval mapping for resistance to *Erysiphe necator*
- Supplementary Table 1: Caucasian grape germplasm conserved at CREA-VE
- Supplementary Table 2: Grapevines controlled crosses and seed yields
- Supplementary Table 3: Phenotyping experiments carried-out to study the resistance to *Erysiphe necator*
- Supplementary Table 4: Summaries of linear models fitted with Trypan-Blue experiments data
- Supplementary Table 5: Script and filtering stage for the construction of parental linkage maps
- Supplementary Table 6: Significant LOD peaks data from interval mapping for resistance to *Erysiphe necator*
- Supplementary Table 7: SSR recorded alleles for the Caucasian grape germplasm

Supplementary Figure 1: SEM images for early *Erysiphe necator* infection on Shavtsitska and Glera

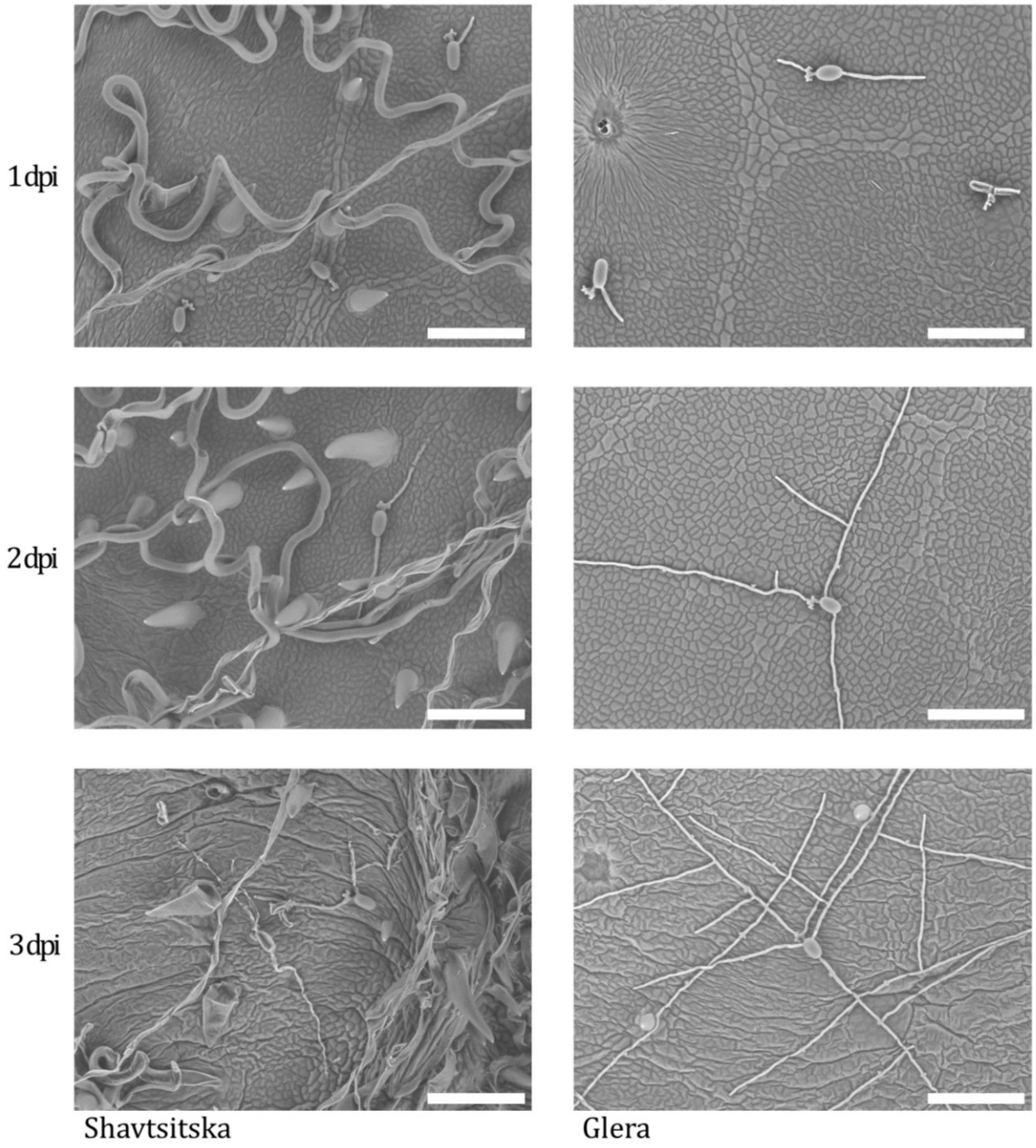
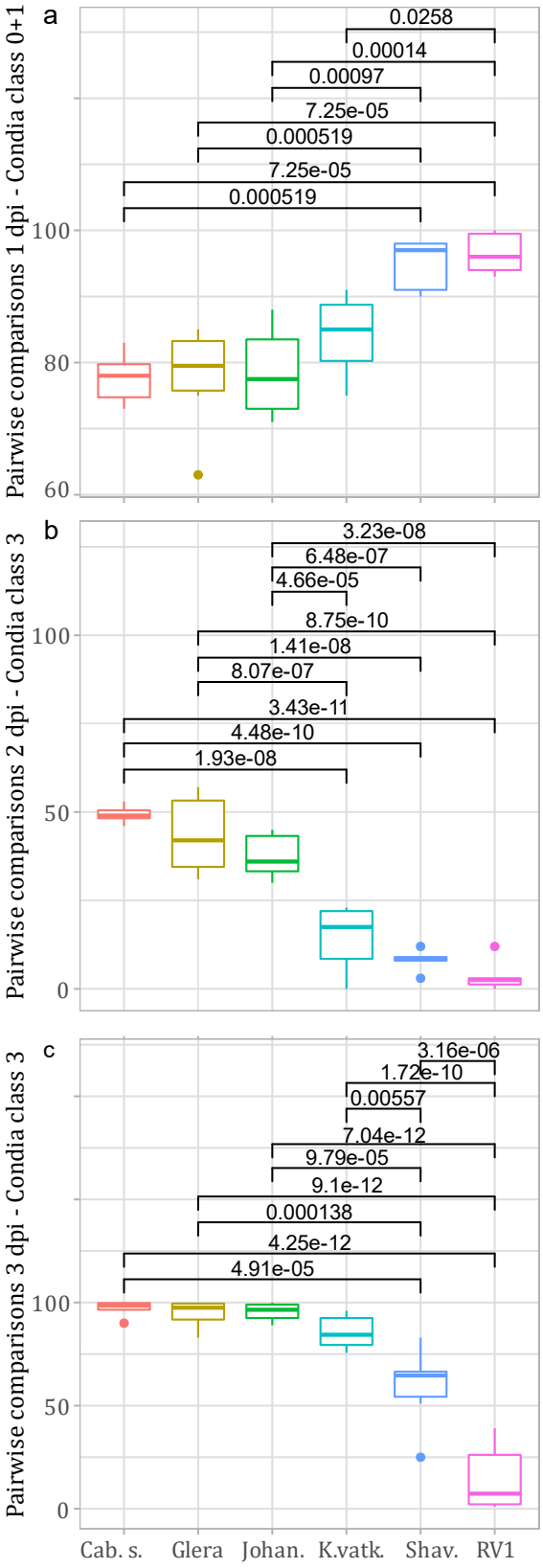


Figure S1. Scanning-electron-microscope (SEM) images for Shavtsitska and Glera discs infected with *E. necator* at 1-2-3 days post-infection (dpi). Magnification x400. Scale bars 100 µm.

Supplementary Figure 2: Pairwise comparisons with Trypan-Blue experiments data



PWC_dpi	Conidia_class	Genotype_1	Genotype_2	p.value
1	0+1	Cabernet s.	Glera	1.000
1	0+1	Cabernet s.	Johanniter	1.000
1	0+1	Cabernet s.	K. vatkana	0.544
1	0+1	Glera	Johanniter	1.000
1	0+1	Glera	K. vatkana	0.544
1	0+1	Johanniter	K. vatkana	0.682
1	0+1	K. vatkana	Shavtsitska	0.090
1	0+1	Shavtsitska	RV1_22_8_78	0.996
2	3	Cabernet s.	Glera	0.702
2	3	Cabernet s.	Johanniter	0.065
2	3	Glera	Johanniter	0.678
2	3	K. vatkana	Shavtsitska	0.627
2	3	K. vatkana	RV1_22_8_78	0.101
2	3	Shavtsitska	RV1_22_8_78	0.855
3	3	Cabernet s.	Glera	0.999
3	3	Cabernet s.	Johanniter	1.000
3	3	Cabernet s.	K. vatkana	0.523
3	3	Glera	Johanniter	1.000
3	3	Glera	K. vatkana	0.751
3	3	Johanniter	K. vatkana	0.677

Figure S2. Box-plots for the most discriminant Trypan-Blue experiments data. *Erysiphe necator* conidia were classified based on the number of formed hyphae: class 0+1 contains conidia with only the appressoria plus conidia with the primary hyphae, while class 3 contained conidia with three hyphae or branched hyphae. Above boxplots significant results of pairwise comparisons (p-value from Tuckey HSD tests below 0.05) between the varieties Shavtsitska, Glera and control plants (Cabernet sauvignon, Johanniter, Kishmish vatkana and RV1-22-8-78). Not significant differences (p-value above 0.05) are listed in the table on the right.

Supplementary Figure 3: 50042 population (Shavtsitska x Glera) phenotyping scores distribution

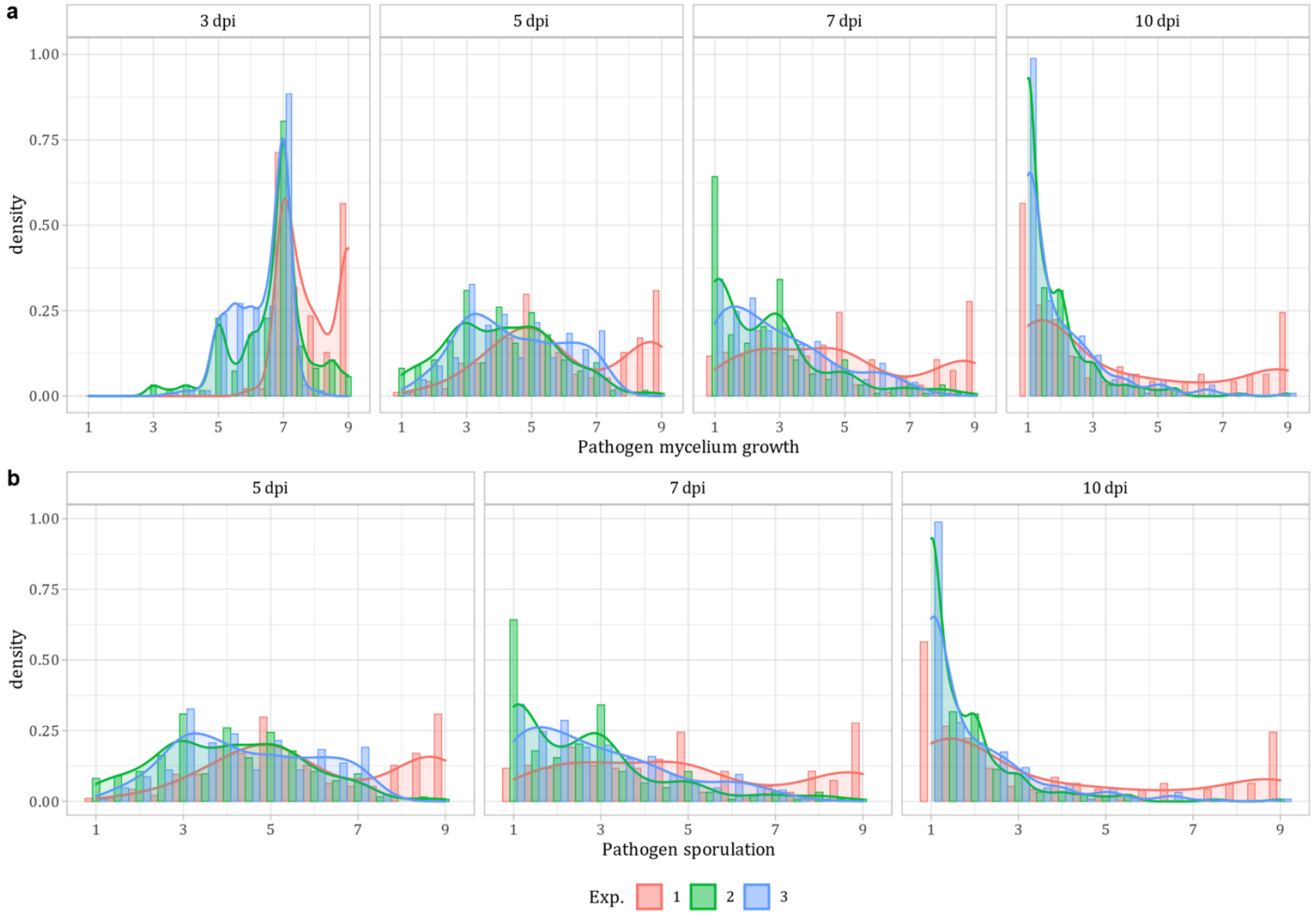


Figure S3. Phenotyping scores distribution for *E. necator* mycelium growth (a) and sporulation intensity (b) assessed for population 50042 (Shavtsitska x Glera) at 3-5-7-10 days post-infection. Colours identified the different replicates of the experiment.

Supplementary Figure 4: Linkage maps markers dot-plot: physical position vs genetic order and distances

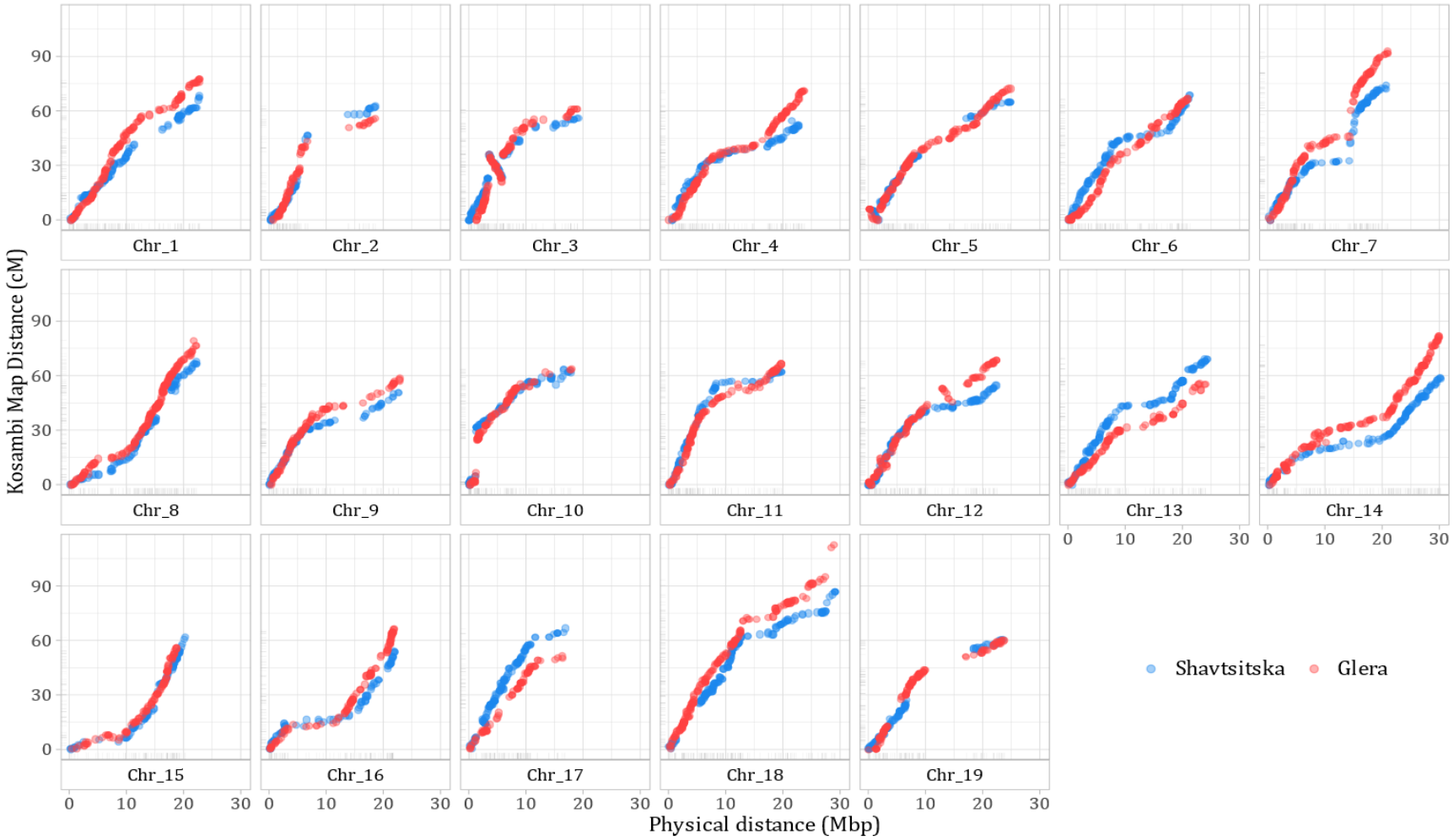


Figure S4. Dot plot for SNP markers utilized for the development of the parental linkage maps of Shavtsitska (blue) and Glera (red). On the x axis the SNP physical distances while on y axis SNP genetic distances.

Supplementary Figure 5: LOD profile from interval mapping for resistance to *Erysiphe necator*

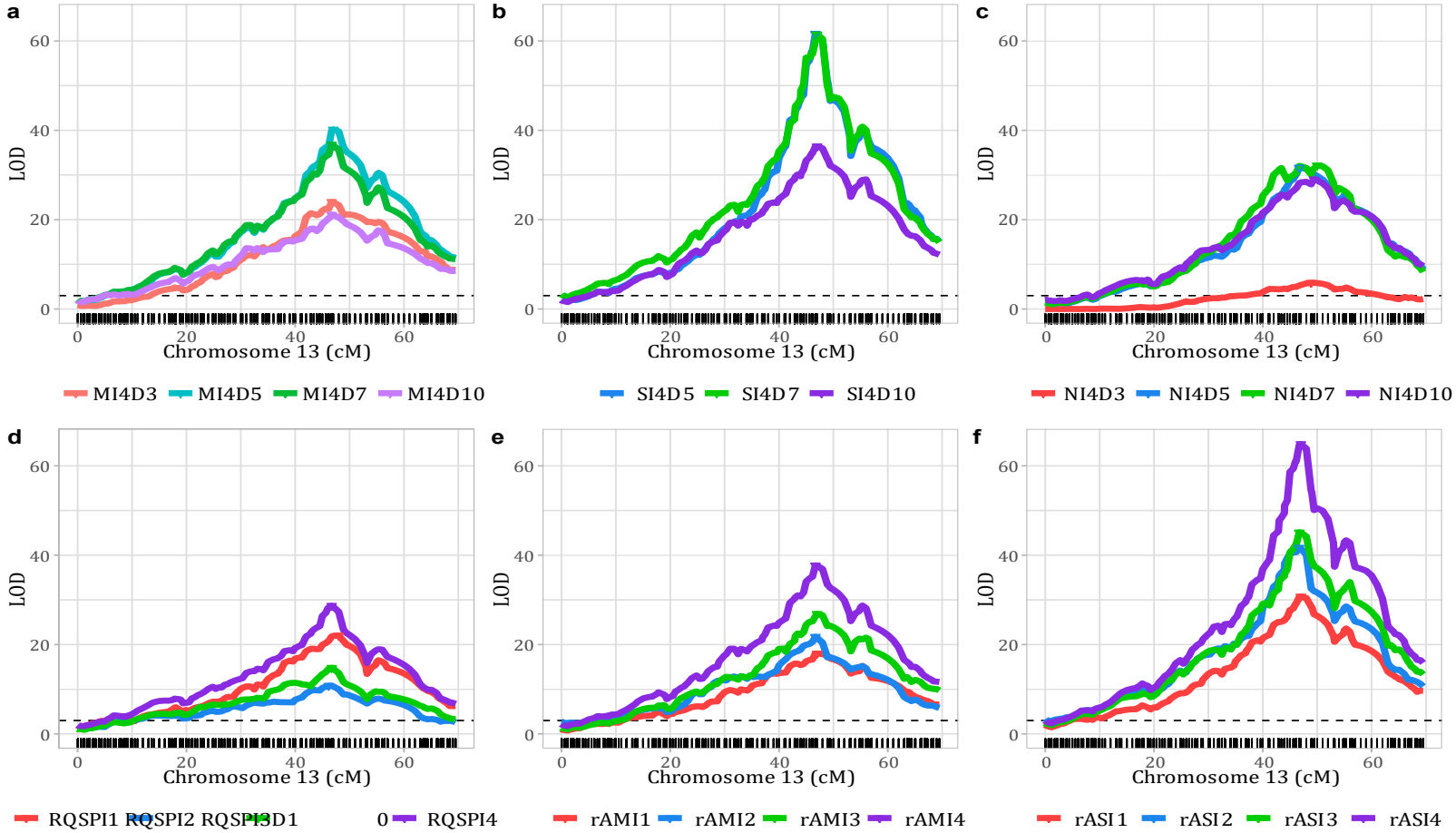


Figure S5. Results from interval mapping for resistance to *E. necator* identified in Shavtsitska (only chr 13 is shown). Graphs "a", "b" and "c" show the LOD for the analysis carry out with the averaged data (from three experiments - I4) for pathogen mycelium growth (M - a), sporulation (S - b) and plant necrosis production (N - c) assessed at 3 (red) – 5 (blue) – 7 (green) – 10 (violet) days post-infection (D). Graphs "d", "e" and "f" show the LOD for the analysis carry out with conidia counts square-root transformed (RQSP - d), relative Area Under Disease Pressure curve for pathogen mycelium growth (rAM - e) and sporulation (rAS - f) for experiment replicates I1 (red) – I2 (blue) – I3 (green) and the final averaged data (I4 - violet).

Supplementary Table 1: Caucasian grape germplasm conserved at CREA-VE

Table S1. Caucasian *Vitis vinifera* subsp. *vinifera* conserved at CREA-VE (Susegana, Italy). The names/identities of the accessions are defined/verified according to the prime names in VIVC database (https://www.vivc.de/). Varieties 1 and 2 come from Central Asia and carry Ren1 gene (Hoffmann et al., 2008; Coleman et al., 2009), while varieties 3 and 4 are Caucasian cross parents resistant to *Erysiphe necator* studied in the project.

N	Accession name	Variety identity according to VIVC
1	Kishmish vatkana	Confirmed
2	Dzhandzhal kara	Confirmed
3	Shavtsitska	No SSR
4	Tskhvedianis tetra	No SSR
5	Absciluri	Zerdagi
6	Adjaruli tetri	Confirmed
7	Adreuli tkhelkana	Confirmed
8	Akhmetis shavi	Confirmed
9	Akomsctali	Confirmed
10	Aladasturi	Confirmed
11	Alexandrouli	No SSR
12	Almura tetri	Bakhtiori
13	Amlakhu	Confirmed
14	Ananura	Danakharuli
15	Argvetuli sapere	Asuretuli shavi
16	Aspindzura	Confirmed
17	Badagi	Confirmed
18	Batomura	No SSR
19	Bazaleturi	Confirmed
20	Boglarka	No SSR
21	Brola	Confirmed
22	Budescuri tetri	Budai shuli
23	Buera	Confirmed
24	Chapscira	No SSR
25	Charistvala sciavi	No SSR
26	Chinuri	Confirmed
27	Chitistvala kachuri	Chitistvala bodburi
28	Cichaveri	Khushia shavi
29	Citiskverzcha meschuri	No SSR
30	Danacharuli	Confirmed
31	Dondghlabi shavi	Confirmed
32	Dzvelshavi obchuri	Confirmed
33	Endeladzis shavi	Confirmed
34	Gabechouri tsiteli	Confirmed
35	Ghrubela kartlis	Confirmed
36	Gorula	Confirmed
37	Goruli mtsvane	Confirmed
38	Ikaltos tsiteli	Confirmed

N	Accession name	Variety name from VIVC
39	Jani	Confirmed
40	Jani bakhvis	Confirmed
41	Jineshi	Confirmed
42	Jmeruli sciavi	No SSR
43	Jvari	Confirmed
44	Kakhis tetri	Confirmed
45	Kamuri sciavi	Confirmed
46	Katchitchi	Confirmed
47	Kharistvala tetri	Confirmed
48	Khikhvi	Confirmed
49	Kistauris saghvine	Confirmed
50	Klardzhuli	Confirmed
51	Krakhuna	Confirmed
52	Ktsia	Confirmed
53	Kumsi tetri	No SSR
54	Kumsmtevana	Confirmed
55	Kuprascviliseuli	No SSR
56	Kurkena	Confirmed
57	Kvelouri	Confirmed
58	Maghlari shavi	Confirmed
59	Maghlari tvrina	Confirmed
60	Mamukas sapere	Mamukas vasi
61	Mekrencichi	No SSR
62	Mgalobliscvili	Shavbarda
63	Mrgvali vardisperi qurdzeni	Confirmed
64	Mskhviltvala tetri	Confirmed
65	Mtevandidi	Usakheluri
66	Mtsuane avrechi	Confirmed
67	Mtsvivani mschvilmartsvala	No SSR
68	Mtzvane kachuri	Confirmed
69	Ochtoura	No SSR
70	Odjalesci	No SSR
71	Orona	No SSR
72	Otskhanuri sapere	Confirmed
73	Portoka	Durif
74	Rkaziteli	Confirmed
75	Rko shavi	Confirmed
76	Samarkhi	Confirmed
77	Sapena	Confirmed
78	Saperavi atenis	Confirmed
79	Saperavi grdzelmtevana	Confirmed
80	Schilatubani	Confirmed
81	Seura	Confirmed
82	Shavkapito	Confirmed
83	Shavtkhila	Confirmed

N	Accession name	Variety name from VIVC
84	Sirgula	Confirmed
85	Tamaris vasi	Borchalo
86	Tavkara	Confirmed
87	Tavkveri	Confirmed
88	Tchvitiluri	Confirmed
89	Tciodi salchinosi	No SSR
90	Teumuta	No SSR
91	Tita kartlis	Confirmed
92	Tkvalapa sciavi	Confirmed
93	Tshoris tetra	No SSR
94	Tsirkvalis tetri	Confirmed
95	Tsitelouri	Confirmed
96	Tsitska	Confirmed
97	Tsitska sacicheris	No SSR
98	Tskobila	Confirmed
99	Tsolikouri	Confirmed
100	Tsulukidzis tetra	Confirmed
101	Ubakluri	Confirmed
102	Usachelouri	No SSR
103	Vardaguyn yerevani	Not confirmed
104	Vazisubnis tsiteli	Confirmed
105	Vertkvitchalis shavi	Confirmed
106	Vertkvitchalis tetri	Confirmed
107	Zveli alexandrouli	No SSR

Supplementary Table 2: Grapevines controlled crosses and seed yield

Table S2. Results of grape controlled crosses between the resistant Caucasian *Vitis vinifera* Shavtsitska and Tskhvedianis tetra and the susceptible varieties Glera and Chardonnay.

Cross	Seed parent	Pollen donor	Crossed bunches	Ripened bunches	Collected berries	Collected seeds	Stored seeds	Seeds per berry	Discarded seeds %
1	Shavtsitska	Glera	14	14	1946	3165	3136	1.63	0.01
2	Chardonnay	T. tetra	13	10	1175	1533	1415	1.30	0.08
3	Glera	Shavtsitska	7	7	399	462	175	1.16	0.62
4	Chardonnay	Shavtsitska	3	2	142	126	86	0.89	0.32
5	Glera	T. tetra	2	2	82	103	90	1.26	0.13
6	T. tetra	Chardonnay	2	2	178	366	354	2.06	0.03
7	T. tetra	Glera	2	2	281	662	646	2.36	0.02
8	Shavtsitska	Shavtsitska	2	1	6	6	6	1.00	0.00
		Total	**45**	**40**	**4209**	**6423**	**5908**		

Supplementary Table 3: Phenotyping experiments carried-out to study the resistance to *Erysiphe necator*

Table S3. Bioassays list with the number of assessed offsprings, parents, controls plants, discs per genotype and recorded phenotypic data.

Experiment				Vegetal material					
				Progeny		Parents - controls		n total	Recorded phenotypic data
n	ID	Rep.	Pop.	n	disc/gen.	n	disc/gen.	discs	
1	Exp19PM03	1	50042	188	1	8	3	213	Pathogen mycelium growth, sporulation intensity, number of conidia per conidiophore and plant necrosis presence at 3-5-7-10 dpi for four disc areas. Conidia count by Malassez chamber. rAUDPC calculation.
2	Exp19PM03	2	50042	247	1	9	4 or 1	284	
3	Exp19PM03	3	50042	251	1	8	4	284	
4	Exp19PM04	1	50041	64	1	7	1	71	Pathogen mycelium growth, sporulation intensity, number of conidia per conidiophore and plant necrosis presence at 2-4-7-9-11 dpi for four discs areas. rAUDPC calculation.
5	Exp19PM04	2	50041	61	1	8	1 or 2	71	
6	Exp19PM12	1	50042	17	6	6	6	138	Conidia classification based on the number of formed hyphae after pathogen Trypan-Blue staining for 1-2 dpi
7	Exp19PM12	2	50042	17	6	6	6	138	
8	Exp19PM13	1	50042	10	1	6	2 or 3	69	Images collection by SEM for 1-2-3 dpi
9	Exp20PM04	1	50042	17	3	6	3	69	Conidia classification based on the number of formed hyphae after pathogen Trypan-Blue staining for 3 dpi
10	Exp20PM04	2	50042	17	3	6	3	69	

Supplementary Table 4: Summaries of linear models fitted with Trypan-Blue experiments data

Table S4. Statistics tables for linear models (LM) fitted for the most discriminant conidia classification data identified for Trypan-Blue experiments: sum of conidia in Class 0 (conidia showing only the appressoria) and conidia in Class 1 (conidia showing the primary hypha) for 1-day post-infection (dpi); number of conidia in Class 3 (conidia showing three hyphae and/or hyphae ramification) for 2 and 3 dpi. As intercept is defined the susceptible control Cabernet sauvignon. Confidence intervals α=0.95.

	Linear model for 1 dpi Class 0+1			**Linear model for 2 dpi Class 3**			**Linear model for 3 dpi Class 3**		
Predictors	**Est. means**	**Std. errors**	**Conf. intervals**	**Est. means**	**Std. errors**	**Conf. intervals**	**Est. means**	**Std. errors**	**Conf. intervals**
Intercept - (Cabernet s.)	77.67	2.36	72.83 82.50	49.33	2.86	43.49 55.18	97.17	4.74	87.49 106.84
Glera	0.00	3.33	-6.84 6.84	-5.83	4.05	-14.10 2.43	-2.50	6.70	-16.18 11.18
Shavtsitska	17.13	3.50	9.96 24.31	-41.17	4.05	-49.43 -32.90	-37.95	6.70	-51.64 -24.27
Johanniter	0.33	3.33	-6.01 7.67	-11.83	4.05	-20.10 -3.57	-1.67	6.70	-15.35 12.02
K. vatkana	6.33	3.73	-1.32 13.98	-34.83	4.05	-43.10 -26.57	-11.60	6.70	-25.28 2.08
RV1-22-8-78	18.83	3.33	11.99 25.67	-45.83	4.05	-54.10 -37.57	-82.58	6.70	-96.26 -68.89
Residual std. err.	5.76			7.01			11.60		
ANOVA	p-value <0.001			p-value <0.001			p-value <0.001		

Supplementary Table 5: Script and filtering stage for the construction of parental linkage maps

Table S5. Detailed on the pipeline utilized for the parental linkage maps development with number of SNP and individuals filtered at each stage

Software	Reference	Description	Shavtsitska	Glera
Perl	Hyma et al. 2015	**Initial SNP dataset**	**139,318**	
		SNP with genotyping rates < 80%	107,054	
		Monomorphic SNP	6	
		SNP with similar major and minor allele frequencies of 0.5	4,256	
		SNP with genotyping rates < 80% after genotyping error correction based on genotype quality (GQ)	9,160	
		SNP with genotyping error rate > 5%	480	
		Markers retained after Perl elaboration	**18,362**	
R	Custom analysis	SNP with homozygous, heterozygous, missing and different genotypes in one or both parents sample replicates	1,445	
		Pseudo-testcross markers obtained	**7,953**	**8,964**
R	Package qtl (Broman et al. 2003) and ASMap (Taylor & Butler. 2017) Functions in (Hyma et al. 2015) and custom functions	SNP with genotyping rate < 90%	1,012	1,227
		SNP co-segregating	4,232	4,772
		SNP not associated to linkage groups and/or isolate	61	75
		SNP manually filtered based on association LOD values, recombination fractions, numbers of crossover and double crossover and interval distances	357	263
		Genotyping error correction based on genotypes LOD	2,304	2,536
		Final markers	**2,291**	**2,627**
		Individuals of population 50042 genotyped	**184**	
		Individuals with many crossover or double crossover	1	
		Final individuals	**183**	

Supplementary Table 6: Significant LOD peaks data from interval mapping for resistance to *Erysiphe necator*

Table S6. Significant QTL identified by interval mapping (parametric models, EM method (Lander & Botstein, 1989) and LOD significance thresholds determined by 1,000 permutation tests) carried out by different phenotypic data related to *E. necator* infection for cross population 50042 (Shavtsitska x Glera).

Phenotipic data	Exp. replicate	dpi	Code	Chr	Bayes conf. intervals (α=0.95)			LOD	p-value
					Peak position cM	Lower limit cM	Upper limit cM		
E. necator mycelium growth	1	3	MI1D3	13	42.87	35.21	55.33	7.02	<0.001
	2	3	MI2D3	13	46.70	42.87	48.89	9.82	<0.001
	3	3	MI3D3	13	47.00	45.61	48.89	21.60	<0.001
	averaged data	3	MI4D3	13	46.70	46.70	48.89	23.98	<0.001
	1	5	MI1D5	13	48.00	46.70	49.43	15.46	<0.001
	2	5	MI2D5	13	46.70	46.70	48.89	22.43	<0.001
	3	5	MI3D5	13	47.00	46.70	48.89	32.57	<0.001
	averaged data	5	MI4D5	13	47.00	46.70	48.89	40.17	<0.001
	1	7	MI1D7	13	46.70	45.61	49.43	19.34	<0.001
	2	7	MI2D7	13	46.70	44.51	48.89	17.82	<0.001
	3	7	MI3D7	13	47.00	46.15	48.89	22.04	<0.001
	averaged data	7	MI4D7	13	46.70	46.70	48.89	36.80	<0.001
	1	10	MI1D10	13	48.00	45.61	53.21	14.98	<0.001
	2	10	MI2D10	13	46.70	28.66	53.21	8.63	<0.001
	3	10	MI3D10	13	46.70	37.39	69.48	7.25	<0.001
	averaged data	10	MI4D10	13	46.70	45.61	48.89	21.12	<0.001
E. necator sporulation intensity	1	5	SI1D5	13	46.15	42.87	48.89	14.23	<0.001
	1	5	SI1D5	14	4.47	0.00	14.28	3.93	0.008
	2	5	SI2D5	13	46.70	45.61	48.89	37.73	<0.001
	3	5	SI3D5	13	46.70	46.70	48.89	40.32	<0.001
	averaged data	5	SI4D5	13	46.70	46.70	48.89	61.61	<0.001
	averaged data	5	SI4D5	14	1.69	0.00	14.28	3.46	0.020
	1	7	SI1D7	13	47.00	46.70	48.89	31.51	<0.001
	1	7	SI1D7	14	4.47	0.00	14.80	3.84	0.009
	2	7	SI2D7	13	46.70	44.51	48.89	37.70	<0.001
	3	7	SI3D7	13	47.00	46.70	48.89	41.61	<0.001
	averaged data	7	SI4D7	13	47.00	46.70	48.89	61.45	<0.001
	1	10	SI1D10	13	48.00	46.70	49.43	20.38	<0.001
	2	10	SI2D10	13	46.70	42.87	48.89	13.29	<0.001

	3	10	SI3D10	13	47.00	45.61	56.43	20.46	<0.001
	averaged data	10	SI4D10	13	47.00	46.70	48.89	36.41	<0.001
Presence of plant necrosis	3	3	NI3D3	2	61.94	6.09	62.54	3.12	0.045
	3	3	NI3D3	13	50.00	46.70	56.97	5.98	<0.001
	averaged data	3	NI4D3	7	25.02	8.82	42.32	2.96	0.048
	averaged data	3	NI4D3	13	48.89	43.97	56.43	5.87	<0.001
	1	5	NI1D5	13	48.00	42.87	53.21	9.23	<0.001
	2	5	NI2D5	13	46.70	42.87	48.89	17.18	<0.001
	3	5	NI3D5	13	50.00	46.70	55.33	19.25	<0.001
	averaged data	5	NI4D5	13	46.70	46.70	48.89	31.65	<0.001
	1	7	NI1D7	13	42.87	39.59	53.21	8.26	<0.001
	2	7	NI2D7	13	42.87	41.23	48.89	17.21	<0.001
	3	7	NI3D7	13	50.00	46.70	53.21	27.76	<0.001
	averaged data	7	NI4D7	13	50.00	42.87	53.21	32.14	<0.001
	1	10	NI1D10	13	49.43	42.87	53.21	10.35	<0.001
	2	10	NI2D10	13	46.70	41.23	53.21	11.31	<0.001
	3	10	NI3D10	13	49.43	46.70	53.21	22.73	<0.001
	averaged data	10	NI4D10	13	49.43	46.70	53.21	29.01	<0.001
Square root of conidia/ml	1	10	RQSPI1	13	48.00	46.15	48.89	22.01	<0.001
	2	10	RQSPI2	13	46.15	42.87	48.89	10.78	<0.001
	3	10	RQSPI3	13	46.70	45.61	48.89	14.80	<0.001
	averaged data	10	RQSPI4	13	46.70	45.61	48.89	28.68	<0.001
rAUDPC for mycelium growth	1		rAMI1	13	47.00	46.70	53.21	17.98	<0.001
	2		rAMI2	13	46.70	45.61	48.89	21.74	<0.001
	3		rAMI3	13	47.00	46.70	48.89	26.86	<0.001
	averaged data		rAMI4	13	46.70	46.70	48.89	37.72	<0.001
rAUDPC for sporulation intensity	1		rASI1	13	47.00	46.70	48.89	30.74	<0.001
	1		rASI1	14	8.31	0.00	15.89	3.40	0.026
	2		rASI2	13	46.70	44.51	48.89	41.68	<0.001
	3		rASI3	13	46.70	46.70	48.89	45.11	<0.001
	averaged data		rASI4	13	47.00	46.70	48.89	64.88	<0.001

Supplementary Table 7: SSR recorded alleles for the Caucasian grape germplasm

Table S7 Markers alleles recorded for Caucasian *Vitis vinifera* L. subsp. *vinifera* conserved at CREA-VE (Susegana, Italy). SC8-0071-014 and Sc47_20 are tightly-linked SSR on grape reference genome to the position of the QTL of resistance to *Erysiphe necator* identified in Shavtsitska and to Ren1 gene (Hoffman et al. 2008; Coleman et al., 2009). The variety names are defined according to VIVC database (https://www.vivc.de/) for true-to-type plants while for the remaining accessions are defined according to CREA-VE database. Ren1 associated alleles are underlined. Alleles associated to the QTL identified in Shavtsitska are **bold and underlined**. "md" is used when the marker shows only one allele (the variety could be either or homozygous or have a null allele).

N	Variety name from VIVC/ CREA-VE	True-to-type	SSR			
			SC8-0071-014		Sc47_20	
1	Kishmish vatkana	Yes	147	164	204	206
2	Dzhandzhal kara	Yes	147	180	202	206
3	Shavtsitska	No	**149**	178	204	**208**
4	Tskhvedianis tetra	No	**149**	174	206	**208**
5	Zerdagi	Yes	178	204	202	204
6	Adjaruli tetri	Yes	**149**	174	**208**	md
7	Adreuli tkhelkana	Yes	178	204	202	204
8	Akhmetis shavi	Yes	164	178	202	204
9	Akomsctali	Yes	**149**	210	204	**208**
10	Aladasturi	Yes	**149**	174	**208**	md
11	Alexandrouli	No	**149**	174	206	**208**
12	Bakhtiori	Yes	164	178	204	md
13	Amlakhu	Yes	206	210	202	204
14	Danakharuli	Yes	**149**	204	202	**208**
15	Asuretuli shavi	Yes	**149**	164	204	**208**
16	Aspindzura	Yes	178	210	198	204
17	Badagi	Yes	147	**149**	202	**208**
18	Batomura	No	164	206	202	204
19	Bazaleturi	Yes	147	206	202	md
20	Boglarka	No	167	174	204	**208**
21	Brola	Yes	164	174	204	206
22	Budai shuli	Yes	204	210	198	202
23	Buera	Yes	164	178	202	204
24	Chapscira	No	**149**	164	204	**208**
25	Charistvala sciavi	No	164	178	202	204
26	Chinuri	Yes	164	210	198	202
27	Chitistvala bodburi	Yes	178	204	202	204
28	Khushia shavi	Yes	**149**	204	202	**208**
29	Citiskverzcha meschuri	No	164	210	198	204
30	Danacharuli	Yes	**149**	204	202	**208**
31	Dondghlabi shavi	Yes	164	206	202	204
32	Dzvelshavi obchuri	Yes	174	178	204	206

N	Variety name from VIVC/ CREA-VE	True-to-type	SSR			
			SC8-0071-014		Sc47_20	
33	Endeladzis shavi	Yes	174	206	202	**208**
34	Gabechouri tsiteli	Yes	162	174	**208**	md
35	Ghrubela kartlis	Yes	164	178	202	204
36	Gorula	Yes	204	md	202	md
37	Goruli mtsvane	Yes	178	204	202	204
38	Ikaltos tsiteli	Yes	147	178	202	206
39	Jani	Yes	**149**	164	204	**208**
40	Jani bakhvis	Yes	174	210	204	**208**
41	Jineshi	Yes	174	210	204	**208**
42	Jmeruli sciavi	No	**149**	174	206	**208**
43	Jvari	Yes	164	178	202	204
44	Kakhis tetri	Yes	174	178	204	md
45	Kamuri sciavi	Yes	206	210	202	204
46	Katchitchi	Yes	**149**	174	206	**208**
47	Kharistvala tetri	Yes	164	178	202	204
48	Khikhvi	Yes	176	204	202	204
49	Kistauris saghvine	Yes	178	210	198	204
50	Klardzhuli	Yes	164	md	204	md
51	Krakhuna	Yes	174	206	202	**208**
52	Ktsia	Yes	178	md	204	md
53	Kumsi tetri	No	176	204	202	204
54	Kumsmtevana	Yes	178	210	198	204
55	Kuprascviliseuli	No	**149**	md	204	**208**
56	Kurkena	Yes	178	210	198	204
57	Kvelouri	Yes	**149**	210	198	**208**
58	Maghlari shavi	Yes	178	210	202	204
59	Maghlari tvrina	Yes	**149**	206	202	**208**
60	Mamukas vasi	Yes	**149**	206	202	**208**
61	Mekrencichi	No	147	164	202	204
62	Shavbarda	Yes	204	206	202	md
63	Mrgvali vardisperi qurdzeni	Yes	174	204	202	**208**
64	Mskhviltvala tetri	Yes	164	204	202	md
65	Usakheluri	Yes	170	174	204	**208**
66	Mtsuane avrechi	Yes	170	178	204	206
67	Mtsvivani mschvilmartsvala	No	**149**	178	202	204
68	Mtzvane kachuri	Yes	176	210	198	204
69	Ochtoura	No	174	210	198	**208**
70	Odjalesci	No	174	206	202	**208**
71	Orona	No	174	206	202	206
72	Otskhanuri sapere	Yes	147	168	202	204
73	Durif	Yes	174	md	206	md
74	Rkaziteli	Yes	166	210	198	202
75	Rko shavi	Yes	174	178	204	206
76	Samarkhi	Yes	**149**	178	204	**208**

N	Variety name from VIVC/ CREA-VE	True-to-type	SSR			
			SC8-0071-014		Sc47_20	
77	Sapena	Yes	178	204	202	204
78	Saperavi atenis	Yes	178	210	198	204
79	Saperavi grdzelmtevana	Yes	**149**	178	204	**208**
80	Schilatubani	Yes	170	174	204	**208**
81	Seura	Yes	**149**	164	204	**208**
82	Shavkapito	Yes	178	204	202	204
83	Shavtkhila	Yes	178	204	202	204
84	Sirgula	Yes	204	210	198	202
85	Borchalo	Yes	164	178	204	md
86	Tavkara	Yes	174	210	198	**208**
87	Tavkveri	Yes	164	178	204	206
88	Tchvitiluri	Yes	174	md	206	md
89	Tciodi salchinosi	No	174	206	**208**	md
90	Teumuta	No	147	174	202	206
91	Tita kartlis	Yes	164	204	202	204
92	Tkvalapa sciavi	Yes	178	204	202	204
93	Tshoris tetra	No	178	210	198	204
94	Tsirkvalis tetri	Yes	174	md	204	**208**
95	Tsitelouri	Yes	162	174	206	**208**
96	Tsitska	Yes	204	206	202	md
97	Tsitska sacicheris	No	164	204	202	204
98	Tskobila	Yes	178	204	202	204
99	Tsolikouri	Yes	147	210	202	204
100	Tsulukidzis tetra	Yes	176	204	202	204
101	Ubakluri	Yes	147	164	202	md
102	Usachelouri	No	178	206	202	204
103	Vardaguyn yerevani	No	164	167	202	204
104	Vazisubnis tsiteli	Yes	**149**	162	204	**208**
105	Vertkvitchalis shavi	Yes	164	178	204	md
106	Vertkvitchalis tetri	Yes	147	206	202	md
107	Zveli alexandrouli	No	**149**	204	202	**208**