UNIVERSITÀ DEGLI STUDI DI UDINE
hic sunt futura

www.uniud.it

Corso di dottorato di ricerca in:

"Scienze e biotecnologie agrarie"

in convenzione con Fondazione Edmund Mach

Ciclo 33°

Titolo della tesi

"**Exploring the role of downy mildew susceptibility genes in *Vitis* by studying the genetic variation and the *in vivo* function**"

in co-tutela con Fondazione Edmund Mach e Scienza Biotechnologies

Dottoranda

Carlotta Pirrello

Supervisore

Prof. Claudio Moser

Co-supervisori

Dr.ssa Silvia Vezzulli
Dr.ssa Giulia Malacarne
Dr. Tieme Zeilmaker

Anno 2021

TABLE OF CONTENTS

SUMMARY

Viticulture is worldly threatened by fungal pathogens. Management of vineyards entails the use of great amounts of fungicides in several applications each year, generating a harmful environmental impact on human health and local biodiversity. In the last decades, resistance (*R*) loci scouting and deployment have been the main strategy to cope with some of the most aggressive grapevine pathogens: *Erysiphe necator*, the causal agent of powdery mildew (PM), and *Plasmopara viticola*, the causal agent of downy mildew (DM). However, *R* loci-based resistance can be easily overcome by pathogens within a few years from their introgression. Recently, susceptibility (*S*) genes have been used as a new source of durable and broad-spectrum resistance in many crops and tree species. In grapevine, S genes like *VvMLO7* were associated with susceptibility to PM and their role has been studied, also using knock down mutants. On the other hand, *S* genes associated to susceptibility to DM are not yet available in grapevine. Loss of function mutations in *AtDMR6* and *AtDLO* provide resistance against DM in Arabidopsis, therefore their putative grapevine orthologs *VvDMR6.1, VvDMR6.2, VvDLO1* and *VvDLO2* are candidate *S* genes, likely associated to DM in grapevine .In this thesis, we explored the role of the aforementioned grapevine *S* genes starting from a broad genetic diversity analysis to a functional characterization study on *dmr6.1* grapevine plants.

Regarding the survey on genetic variability, *VvDMR6.1, VvDMR6.2, VvDLO1* and *VvDLO2* were investigated in 190 grapevine genotypes belonging to *Vitis vinifera* spp., wild species, hybrids and the so-called hybrid/wild species, in order to find *S* genes natural mutants. The scouted genes were deep-sequenced and reads were mapped on PN40024 12× V2 reference genome. A bottleneck analysis was carried out in order to, firstly, identify SNPs (Single Nucleotide Polymorphisms) impacting on the coding sequence, then investigate the potential disrupting role of impacting SNPs on codons and the amino acid sequence and therefore on the protein folding and function. A representative handful of disrupting SNPs were chosen for confirmation by Sanger sequencing. The disrupting impact of amino acid mutations caused by the validated SNPs was then checked on a protein three-dimensional model. DM resistance phenotypic data were collected and compared to the frequency of the reconstructed haplotypes per each gene. Two of them, in *VvDMR6.2,* were found significantly more represented in DM resistant genotypes. *VvMLO7* was sequenced in the 190 grapevine accessions as well, and the resulting data were subjected to the same bottleneck analysis. Once amino acid sequence-disrupting mutations were scouted, we took advantage of the known MLO protein model to identify those mutations that were changing conserved amino acids. Ten mutations were predicted to impact protein function, but no association with phenotypic data was possible since all SNPs were at the heterozygous state. This broad survey

provided a resource for grapevine and plant genetics and could corroborate genomic-assisted breeding programs as well as tailored gene-editing approaches for resistance to biotic stresses.

Functional characterizations of *VvDMR6.1, VvDMR6.2, VvDLO1* and *VvDLO2* were carried out taking advantage of *dmr6.1* grapevine plants previously obtained by the CRISPR/Cas9 genome editing technique. Plants of four different edited lines (each line originated from a separate transformation event) were subjected to *P. viticola* inoculation assay. Leaves were sampled at 0, 24, 96 hours post-inoculation (hpi) and 8 days post-inoculation (dpi). Samples were collected for several purposes. A gene expression analysis of *VvDMR6* and *VvDLO* genes and pathogenesis-related genes was carried out on 0, 24 and 96 hpi samples. Samples taken at 8 dpi were used for symptom assessment via visual, digital, and histological observation. Since the salicylic acid (SA)-inactive forms 2,5-dihydrobenzoic acid (2,5-DHBA) and 2,3-DHBA are products of S5H (SA-5-hydroxylases) and S3H enzymes, putatively encoded by *VvDMR6* and *VvDLO,* these metabolites were quantified through LC-MS in the same samples collected at 0, 24 and 96 hpi.

This overview on the many aspects related to *VvDMR6* and *VvDLO* gene function, made it clear that no uniform effect of *VvDMR6.1* knock-out was detectable among different edited lines and further investigations are needed to define the role of the single genes and the relationship among them.

CHAPTER 1

Introduction

GRAPEVINE SYSTEMATICS AND VITICULTURE OVERVIEW

The Vitaceae family consists of 950 species belonging to 16 genera (Lu et al., 2018). Wen et al. (2018) proposed the classification of Vitaceae members in five tribes according to their phylogenetic studies. The grape genus *Vitis* L., with 75 species, belongs to the Viteae tribe. Members of this tribe are characterized by a type of inflorescence known as thyrse, which can be modified with a fleshy inflorescence axis (lamellate thyrse), a racemose, spirally branching, panicle. The *Vitis* genus is divided in *Muscadinia* (Planch.) Rehder (2n=40 chromosomes) and *Vitis* (2n=38) subgenera, to which belong the *Vitis vinifera* wild subspp. *silvestris* and *sativa*, the latter being the most wildly grown and economically important member of the family.

Grapevine domestication has started at least 8,000 years ago (McGovern et al., 2017) and still plays a fundamental role in the market worldwide. In 2018, seven million hectares were destined to viticulture. Wine making countries are widespread on the planet but mainly located in regions with temperate-humid climates. Europe is still recording the 40% of vineyard areas, the widest in the world, even though these are also expanding in other continents as North America and Asia thanks to the development of surface area (Alston & Sambucci, 2019; Fraga, 2019). Italy is among the first five Countries with the largest area occupied by vineyards, followed by/together with Spain, China, France, and Turkey. In 2018, the Italian territory dedicated to grape cultivation (650,000 hectares) represented the 9% of world vineyards area, 54.8 million hectoliters of wine produced and a considerable growth of +16.3% than 2017 in total grape production (European Union, 2019; OIV, 2019).

Although *V. vinifera* is tolerant to abiotic stresses (e.g. drought, salinity, high temperatures), it is susceptible to many biotic agents (e.g. parasite insects, fungal and bacterial pathogens). Therefore, intensive viticulture takes advantage of many crop protection tools to retain a steady yield, and high productivity is achieved at a high costs for the maintenance of vineyards. Moreover, awareness regarding the negative impact of pesticide/fungicides on environment and human health (Carvalho, 2017) has been rapidly growing. In 2017, over four million tons of pesticides were used globally (FAOSTAT). European Union (EU) regulations are becoming increasingly restrictive in terms of management and approval of new pesticides as is stated in the Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 which establishes a framework for Community action to achieve the sustainable use of pesticides. Clearly, sustainable viticulture is demanding for a reduction of the use of pesticides. This will require both new strategies of vineyards management, as well as genetics and functional approaches.

POWDERY MILDEW

Life cycle

The causative agent of Powdery Mildew (PM) is the biotroph (obligate parasite) *Erysiphe necator* Schw. (asexual morph *Oidium tuckeri* Berk.). PM is recognized by the appearance of a whitish gray dusty layer on the grape which is caused by the spreading of mycelia and conidia onto green tissues (Pearson & Gadoury, 1992) (**Fig.1**).

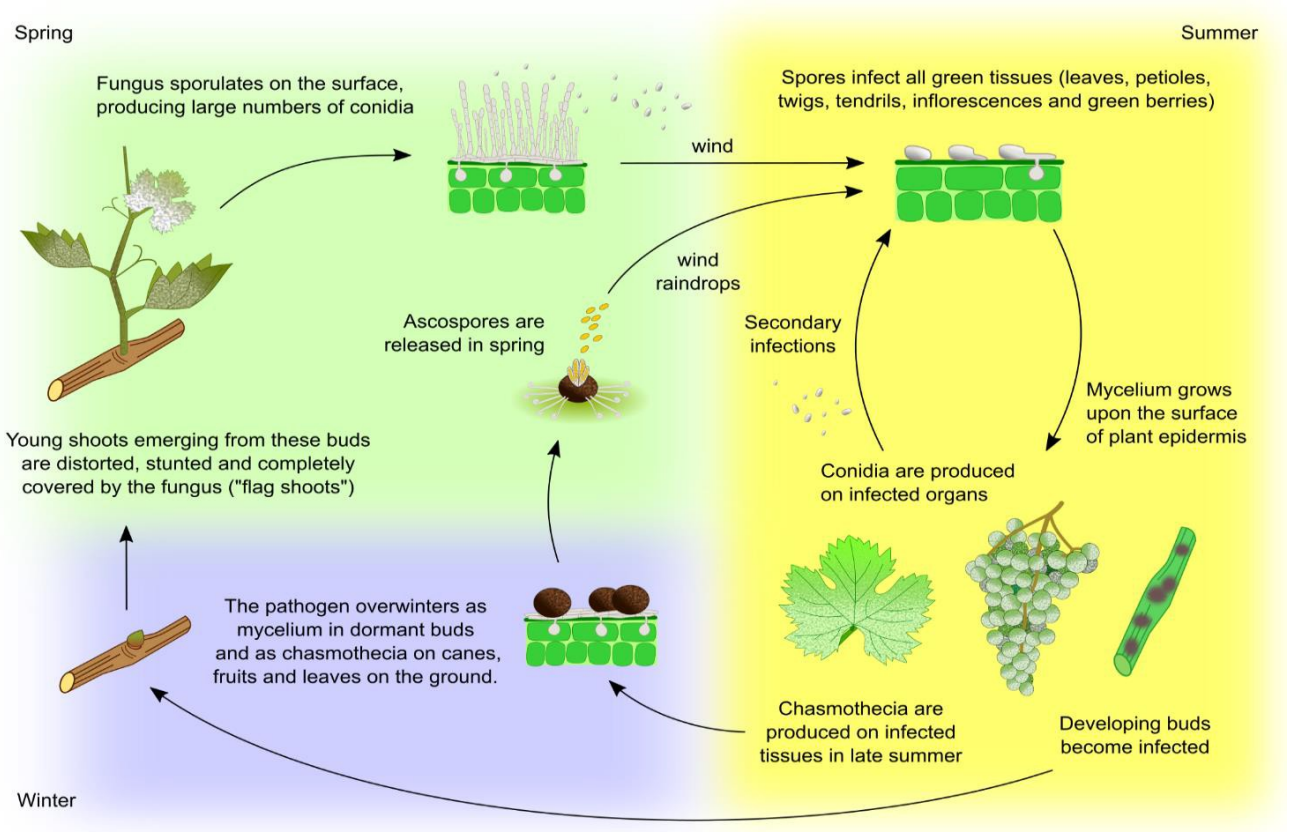


Figure 1. *Erysiphe necator* life cycle (Pirrello et al., 2019).

Two overwintering strategies have been observed in *E. necator*. In areas with relatively mild winters, the fungus commonly overwinters as mycelium on leaf primordia within dormant buds. In the following spring, mycelium activity resumes, resulting in the production of heavily infected and deformed shoots, called “flag shoots”. The fungus sporulates on these shoots, producing a large number of conidia that are carried by the wind to healthy plant tissues. Alternatively, the fungus can overwinter as chasmothecium (syn. cleistothecium, a former term for this structure that is still widely used) in bark, on canes, leftover fruit, and on leaves on the ground. Chasmothecia form on the surface of heavily diseased tissues from mid-summer to autumn. During spring rainfall, the chasmothecia open and release ascospores, which are spread by wind or raindrops to infect the lower leaves near where the chasmothecia have overwintered. Although free water is necessary to release ascospores,

continued wetness is not required for subsequent spore germination and infection. At each new infection site, conidia and ascospores germinate and form an appressorium. From its lower surface a penetration peg develops, piercing the cuticle and entering through an epidermal cell where a haustorium is formed. Mycelium grows upon the surface of the plant epidermis and new conidia are produced within a few days, completing the cycle. Repetition of this cycle continues throughout the growing season resulting in a rapid increase in disease incidence (Wilcox, Gubler, & Uyemoto, 2017).

Disease management

Effective disease control encompasses the combination of sanitary as well as cultivation practices, the use of resistant or at least less susceptible grapevine varieties, the application of fungicides, and decision support systems (e.g. Wilcox, 2003; Hoffman et al., 2004; Molitor & Beyer, 2014). For instance, adequate pruning and removal of leaves covering clusters provide conditions to reduce infections of PM.

Due to the susceptibility of *V. vinifera* cultivars to PM, fungicide applications are necessary to control disease. In particular, thallus of *E. necator* develops almost completely outside of the infected tissues on the leaf and bunch surface, therefore the fungus is susceptible to topical applications of several contact active ingredients (Wilcox et al., 2017). Since the 19th century, sulfur remains the most widely used fungicide, due to its low cost and protectant-curative action (Wilcox et al., 2017). The persistence of sulfur efficacy relies on the lack of resistance development, depending on its multi-site mechanism of action by direct contact and vapor phase: respiration inhibition, chelation of heavy metals needed for biochemical pathways, and disruption of protein function (Oliver & Hewitt, 2014). It causes the damage of cellular membrane followed by loss of water and therefore death of the fungus by dehydration. Other than sulfur, several single-site synthetic fungicides are effective against PM, including contact, translaminar and systemic products, with specifically targeted mechanisms of action. Among them, mitosis and cell division inhibitors (e.g. benzimidazoles) and cell membrane synthesis alteration via ergosterol biosynthesis inhibitors (e.g. triazoles); different mechanisms concern respiration chain inhibition via quinone inhibitors (e.g. strobilurines) or succinate dehydrogenase inhibitors; and signal transduction inhibition (e.g. azanaftalenes) (Oliver & Hewitt, 2014; Wilcox et al., 2017).

Due to the potential negative impacts of fungicide application, non-synthetic chemicals and organic control measures are also used to regulate the disease. For organic viticulture, applications of copper and sulfur are recommended, but generally they are less effective in comparison to the synthetic active compounds (e.g. Loskill et al., 2009; Wilcox et al., 2017). Nowadays, organic management against PM can rely, other than sulfur, on non-toxic substances such as botanical oils and inorganic salts,

acting by contact with the fungal thallus (Wilcox et al., 2017). The application of *Ampelomyces quisqualis* (hyperparasite fungus) at the time of chasmothecia formation can help in reducing the overwintering inoculum of *E. necator* (Pertot et al., 2017).

Among the widely employed fungicides used to control PM are the sterol demethylation inhibitors (DMI) and quinone outside inhibitors (QoI). *E. necator* resistance to DMI was reported in the 80s from California, Portugal and Australia (Ogawa et al., 1988; Gubler et al., 1996; Steva & Cazenave, 1996; Ypema et al., 1997; Savocchia et al., 1999). The DMI resistance is a multigenic trait, but with one major mechanism involving a single mutation in the gene *CYP51* coding for the cytochrome P450 lanosterol C-14a demethylase. Studies on DMI fungicide resistance revealed several possibilities to confer reduced sensitivity: (i) mutation of *CYP51*; (ii) overexpression of *CYP51*; (iii) overexpression of transporter coding for efflux pumps and (iv) other unknown mechanisms able to confer weak resistance (Délye et al., 1997; Délye et al., 1998; Hamamoto et al., 2000; Schnabel & Jones, 2001; Lupetti et al., 2002; Hayashi et al., 2002; Stergiopoulos et al., 2003; Corio-Costet et al., 2003; Wyand & Brown, 2005; Ma et al., 2006; De Waard et al., 2006; Leroux et al., 2007; Luo et al., 2008; Cannon et al., 2009; Kretschmer et al., 2009; Sombardier et al., 2010; Leroux & Walker, 2011; Cools & Fraaije, 2013; Frenkel et al., 2015). *E. necator* is one of the first fungi for which it was demonstrated that a point mutation in *CYP51* is associated with DMI resistance. A mutation in codon 136 converts tyrosine (Y) to phenylalanine (F), reducing the sensitivity to the fungicide (Délye et al., 1997).

Moreover, a nucleotide substitution in position 1119 (A1119C) increases the *CYP51* expression causing a comparable lower sensitivity to the fungicide (Frenkel et al., 2015). QoI fungicides inhibit mitochondrial respiration by binding to the cytochrome bc1 enzyme complex (complex III) at the QoI site, blocking the electron transfer to cytochrome c1, and preventing the synthesis of adenosine-5'-triphosphate (ATP). Several point mutations in the *cytochrome b* (*CYTB*) gene confer QoI resistance (Gisi et al., 2002). *E. necator* resistance to QoI was initially described in the United States (Kennelly et al., 2005; Baudoin et al., 2008; Miles et al., 2012) and it is mainly associated with a point mutation in the codon 143 of *CYTB* that converts glycine (G) to alanine (A) (Bartlett et al., 2002; Ma & Michailides, 2005; Dufour et al., 2011). Recently, the emergence of *E. necator* resistance to other fungicides was reported, such as metrafenone, a benzophenone of which mode of action is still not known, and boscalid, a fungicide that inhibits the activity of the enzyme succinate dehydrogenase (Kunova et al., 2016; Cherrad et al., 2018).

This chapter has been extracted from:

Pirrello, C., Mizzotti, C., Tomazetti, T. C., Colombo, M., Bettinelli, P., Prodorutti, D., … Vezzulli, S. (2019). Emergent Ascomycetes in Viticulture: An Interdisciplinary Overview. Frontiers in Plant Science, 10(November), 1–30. https://doi.org/10.3389/fpls.2019.01394

DOWNY MILDEW

Life cycle

Downy Mildew (DM) is a severe disease caused, in grapevine, by the oomycete *Plasmopara viticola* (Berk. & Curt.) Berl. and de Toni. *P. viticola* is an endemic pathogen of wild species from North America, where it was first observed in 1834. Its accidental arrival in Europe happened in 1878 (Gessler et al., 2011). DM is detectable by the characteristic yellow oil spot-lesions on young leaves adaxial surface and by drier and necrotic lesions on older leaves. In suitable humidity conditions the emergence of white soft sporulation on the abaxial part of the leaves becomes visible (**Fig.2**).

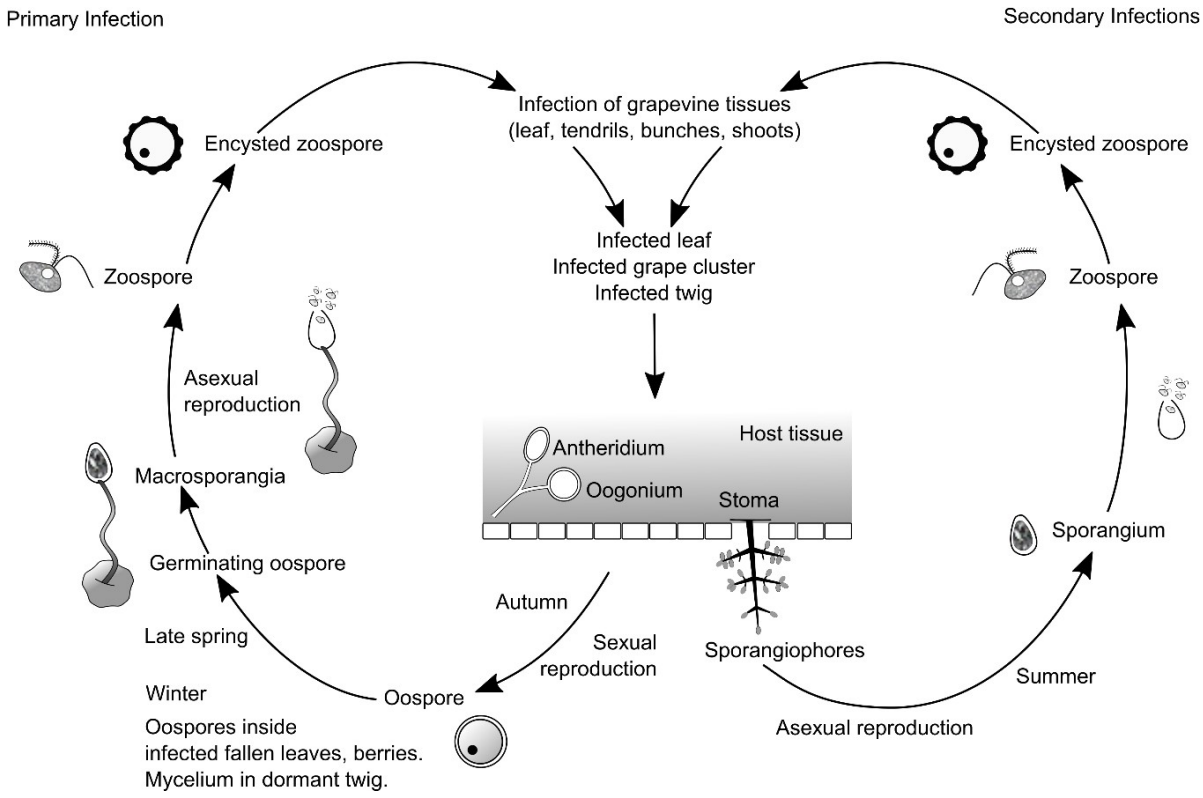


Figure 2. Disease cycle of *P. viticola,* (Berk. & M.A.Curtis) Berl. & De Toni, the causal agent of grapevine downy mildew (Buonassisi et al., 2017).

P. viticola is a diploid and strictly obligate organism whose survival depends on the living host. Its life cycle commences in the Spring when the climate conditions are adequate (12-13°C, moisture) to let the overwintering oospores to germinate and form macrosporangia which, in turn, release zoospores. This asexual phase of reproduction leads to the primary infection. Flagellate zoospores are delivered by rain and wind onto the abaxial face of new leaves. There, an encysted zoospore produces an appressorium and a germinative tube penetrating through the stomata, where a substomatal vesicle is formed giving rise to intercellular mycelium and haustoria. After 5-10 days of incubation at around 25°C, sporangiophores are formed and emerge through the stomata. Sporangia,

carrying asexual zoospores, detach to initiate the secondary infection. The asexual infection repeats itself through cycles until the end of the growing season, when a sexual reproduction phase occurs. Once the two gametangia are formed, antheridia fertilize oogonia originating sexual oospores which overwinter in the host tissues to be the source of primary inoculum of the following year (Burruano, 2000; Gessler et al., 2011; Buonassisi et al., 2017; Fröbel & Zyprian, 2019).

Disease management

High losses in terms of crop yield are the consequences of the uncontrolled spreading of DM in vineyards. Pimentel (2005) reported that in one season DM can bring to devastation up to 75% of a vineyard under optimal weather conditions and in absence of treatments. For this reason, the use of fungicides is still fundamental for DM management in temperate-humid climates (Buonassisi et al., 2017).

Copper-based compounds can be classified in four categories: copper solutions, copper mixtures, copper aqueous suspensions and, dusts. Copper-based fungicides as the "Bordeaux mixture" started to be used since the very first emergence of DM in Europe, at the end of 19th century. During the following years many were the attempts to adjust the product characteristics (liquid or powder consistency, increase or decrease of copper sulfate concentration, number of applications) according to growers and vineyards needs. In a few decades the awareness spread that the success of the treatments depended not so much on the amount of the active principle used but on the climatic and growth conditions of the plant, therefore calendars of preventive applications were established. This finding was particularly important during World War II because of the lack of copper (Gessler et al., 2011). Starting from the half of 20th century, industrial synthetic products were introduced (e.g. dithiocarbamates), then fungicides able of both preventive and curative action (e.g. anilides and cymoxanil) were developed, but soon they showed high toxicity to humans and the ability to favour the development of new resistant pathogen trains (Bavaresco et al., 2019).

Preventive treatments with multi-site-action fungicides as copper and mancozeb still costitutes the 50% of products involved in control strategy on susceptible varieties. In addition to preventive, pre- and post-bloom treatments, the intervention schedule usually requires the spraying/the treatment of vineyards from 3 to 15 times during the vegetative season (on average, every 10-14 days) (Buonassisi et al., 2017; Rienth et al., 2019). Given the EU Council Directive N. 414/91, new products able to provide an alternative to copper were introduced. The mainly commercialized single-site fungicides can be chemically classified in the Quinone outside-inhibitors (QoIs), the phenylamides (PAs), the carboxylic acid amides (CAAs), and the cyano acetamid-oximes (cymoxanil) (Gisi & Sierotzki, 2008). Consequently to the need of discovery of new active principles against *P.viticola*, many new

chemicals with different modes of action were introduced on the market: some are systemic; some have both preventive, with high residual activity, and curative effects; many act directly on sporangia, inhibiting zoospores differentiation and release or germ-tube development; another class is composed by fungicides able to act on mitochondrial respiration; while some others, particularly promising, are able to bind tubulin, therefore inhibiting nuclear division after spore germination (Gessler et al., 2011).

An increasing need to find alternative DM control strategies is perceived in an Integrated Pest Management (IPM) perspective. With this aim, many are the sources that have been tested in the last years: firstly, the use of mathematical models for monitoring field conditions and, secondly, the advantage of biocontrol agents to maintain a low level of the pathogen inoculum in the critical phases of the season. Microbial biocontrol agents can act in different ways: through production of antibiotics or enzymes, competition with the pathogen for nutrients, hyper parasitism, or induction of systemic resistance. Another path that needs to be explored is a different agronomical management to avoid environmental conditions favorable to the pathogen, which could be implemented with the use of substances of natural origins as phosphates, phosphites, microorganisms and plant extracts (Pertot et al., 2017b). Fungitoxic compounds as phytoalexins showed to be induced by the foliar application of potassium phosphite and magnesium phosphite, with activation of Induced Systemic Resistance (ISR) (Bavaresco et al., 2019). Regarding plant essential oils, these are known for their antibacterial, antiviral, antimycotic, antiparasitic and insecticidal properties. Aromatic plants extracts as the ones from sage (Dagostin et al., 2010) and oregano (Rienth et al., 2019) showed to confer reduction of susceptibility to *P. viticola*, on the other hand their high costs are a limit for the use in vineyards. The use of microbial extracts seems to be a valid tool to implement the previous listed control strategies; many have been tested against *P. viticola*: *Trichothecium plasmoparae, Erwinia herbicola, Fusarium proliferatum, Penicillium chrysogenum, Alternaria alternata, Acremonium byssoides* and lately *Trichoderma harzianum* T39 (Gessler et al., 2011). Initially developed for *Botrytis cinerea*, *T. harzianum* T39 showed to induce local and systemic resistance to *P. viticola* as well (Perazzolli et al., 2008; 2012).

PLANT-PATHOGEN INTERACTION

Plants can recognize pathogens and activate an innate immune response (**Fig.3**). Some mechanisms act as a primary defense strategy and their activation is due to the perception of conserved elicitors carried by the invading organism. Plant defense system-eliciting molecules, usually defined microbe-associated molecular patterns (MAMPs), or host-derived damage-associated molecular patterns (DAMPs), or pathogen-associated molecular patterns (PAMPs, as they will be called from now on)**,** can be of different nature: oligosaccharidic (e.g. cyclodextrins, sulfated glucans, fungal chitin), lipidic (e.g. ergosterol, lipopolysaccharides, peptidoglycans), protein molecules (e.g. xylanases, endopolygalacturonases, bacterial flagellin) (Gomès & Coutos-Thévenot, 2009; Héloir et al., 2019). PAMPs are recognized by a trans-membrane reception complex, called pattern recognition receptors (PRRs), composed by receptor-like kinases (RLKs) or receptor-like proteins (RLPs) with an extracellular domain. PAMP-triggered immunity (PTI) consists in the activation of signal cascades involving Ca^{2+} influx, bringing to reactive oxygen species (ROS) production and mitogen-activated protein kinases (MAPKs) activation. If PTI is insufficient to stop the pathogen in the first place, then pathogens release effectors responsible of the so-called effector-triggered susceptibility (ETS). Effectors are directly or indirectly recognized by the plant NBS-LRR proteins with their characteristic nucleotide binding site (NBS) and leucine rich repeat (LRR) domain, resulting in effector-triggered immunity (ETI) (Jones & Dangl, 2006; Héloir et al., 2019). ETI consists in the activation of resistance (R) genes, and usually evolve in a hypersensitive response (HR) (Gomès & Coutos-Thévenot, 2009; Deng et al., 2020; Zhang et al.,2020).

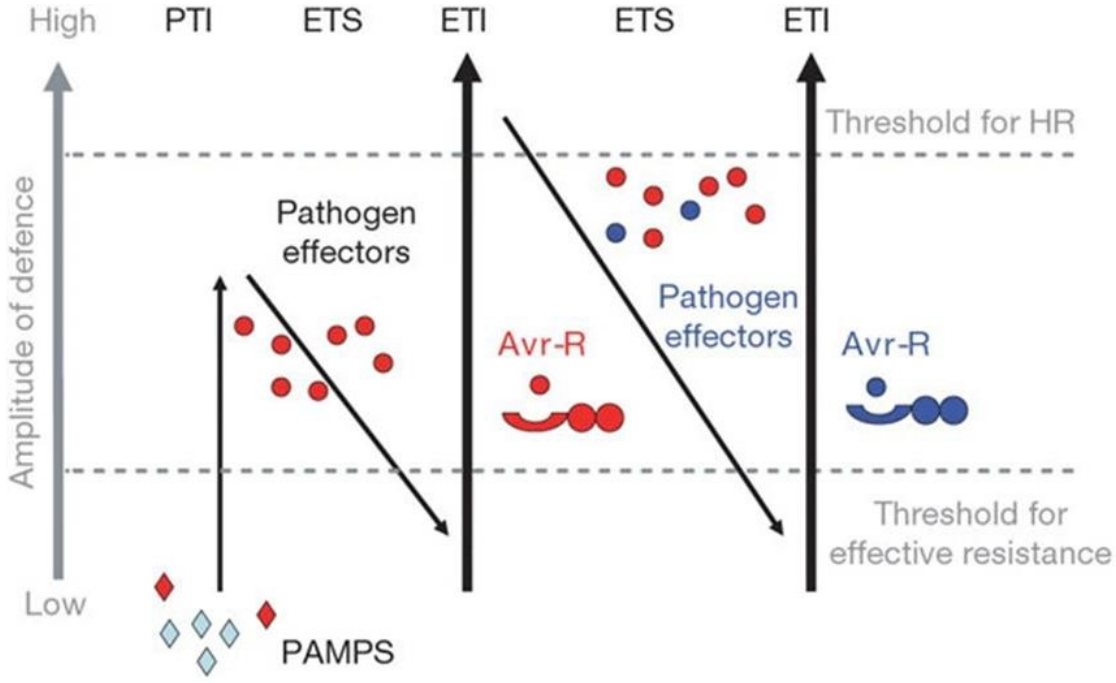


Figure 3. The "zigzag" model of plant immune system activation phases (Jones & Dangl, 2006).

RESISTANCE LOCI

Since 1954, the gene-per-gene model (Flor, 1954) explains the interaction that must be established between the plant dominant *R* gene and the pathogen complementary avirulence (*Avr*) gene to inhibit infection. On the contrary, the disease is able to spread when the compatible interaction does not take place. Over the years many kinds of *R* genes were discovered and they can be classified according to the kind of proteins they encode: (i) cytoplasmic serine/threonine kinases; (ii) LRRs proteins anchored to a transmembrane domain; (iii) RLKs with an extracellular LRR and an intracellular serine/threonine kinase; (iv) proteins with an N-terminal transmembrane anchor and a cytoplasmic coiled-coil (CC) domain; and (v) NBS-LRR proteins which can either be a toll/interleukine-1 receptor (TIR-NBS-LRR, specific to dicotyledonous species) or a coiled-coil domain (CC-NBS-LRR) (Gomès & Coutos-Thévenot, 2009; Fawke et al., 2015). According to Velasco et al., (2007) in Pinot Noir cv 233 entire genes and 112 truncated sequences encoding for NBS-LRR were detected, of which 84 CC-NBS-LRR and 37 TIR-NBS-LRR, interestingly the different categories were found to map in clusters and several linkage groups were identified.

Powdery Mildew

With regards to PM resistance, in the last decades, it emerged that American and Asian *Vitis* represent a valuable source of *R* genes, which are localized within *R*-loci or genomic intervals. *Run1* (*Resistance to Uncinula necator 1*) is a single dominant locus on chromosome 12 known to confer high resistance to *E. necator* detected in *M. rotundifolia* (Bouquet, 1986; Barker et al., 2005). Introgressed into a *V. vinifera* background through marker-assisted selection (MAS) (Pauquet et al., 2001), it was found to co-segregate with the *Rpv1* (*Resistance to Plasmopara viticola 1*) locus and to encode full length and truncated TIR-NBS-LRR (Toll/interleukin-1 receptor nucleotide- binding site-leucine-rich repeat) resistance proteins (Feechan et al., 2013). Surveys on resistant cultivars showed that this locus is involved in the induction of programmed cell death (PCD) within penetrated cells at 24- and 48-hours post-inoculation (hpi) (Dry et al., 2010). Subsequently, the *Run2.1* and *Run2.2* loci variants (haplotypes) were identified on chromosome 18 in *M. rotundifolia* 'Magnolia' (Riaz et al., 2011), while *Ren5* (misnamed, actually *Run3*) was mapped on chromosome 14 in *M. rotundifolia* 'Regale' (Blanc et al., 2012). Resistance to *E. necator* due to PCD was also observed in 'Kishmish vatkana' and 'Dzhandzhal kara'. These related cultivars share the *Ren1* (*Resistance to E. necator 1*) locus carried on the chromosome 13 (Hoffmann et al., 2008; Coleman et al., 2009) and are an exception among the PM resistance donors since they belong to the *V. vinifera proles orientalis*. Very

recently, a genome-wide characterization revealed role of NBS-LRR genes during PM infection in *V. vinifera* (Goyal et al., 2019).

In the same years, several Quantitative Trait Loci (QTL) analyses were carried out with the aim to identify new PM resistance loci. Partial resistance is conferred by major QTLs found on different chromosomes. *Ren2* on chromosome 14 confers race-specific resistance in *V. cinerea* (Dalbó et al., 2001; Cadle-Davidson et al., 2016). *Ren3* on chromosome 15 derived from an undetermined American *Vitis* species was localized in the variety Regent (Welter et al., 2007) and recently found to determine race specific hypersensitive response by two different regions on that chromosome; in fact, Zendler et al. (2017) defined the *Ren3* limit and identified ex novo the distal *Ren9* locus. In addition, *Ren8* was mapped on chromosome 18 although with an uncertain origin (Zyprian et al., 2016). Besides the American sources, the wild Chinese species *V. romanetii* is donor of a non-race-specific and tissue-independent resistance conferred by the dominant locus *Ren4* on chromosome 18 (Riaz et al., 2011; Mahanil et al., 2012); this was introgressed into *V. vinifera* background to obtain vines able to prevent hyphal emergence from the PM agent (Ramming et al., 2011). Moreover, two major R-loci against *E. necator* were discovered in another Chinese species, *V. piasezkii Ren6* and *Ren7* which are respectively localized on chromosome 9 and 19, and they both act in the post-infection stage bringing to PCD. The highest strength of (total) resistance is conferred by *Ren6*, even higher than *Run1*, while *Ren7* is responsible of a weak partial resistance to the pathogen (Pap et al., 2016). Finally, Teh et al. (2017) identified the new *Ren10* locus on chromosome 2 acting moderately against PM sporulation.Nowadays pathogen genetics can inform host genetics and host pathogen interaction mechanisms. For instance, in the Eastern US, where the pathogen co-evolved with many mapped PM resistance genes, the *Ren2* locus has recently fully broken down and is no longer detectable in the vineyard (Cadle-Davidson, 2018). Actually, in North America naturally occurring isolates displaying virulence on vines carrying the *Run* loci were already observed demonstrating that qualitative (vertical) resistance is strong, but since it is race specific can be easily overcome (Feechan et al., 2015). By contrast, partial (horizontal) resistance–which typically is controlled by at least 4-5 QTLs–is usually more durable, particularly when it involves morphological or developmental changes in the plant, although might be prone to gradual loss (erosion) in the long term (Stuthman et al., 2007). Therefore, to achieve long lasting resistance, the combination of both types is needed; this process, named *R* gene pyramiding, relies on genetics built into vines.

This chapter has been extracted from:

Pirrello, C., Mizzotti, C., Tomazetti, T. C., Colombo, M., Bettinelli, P., Prodorutti, D., … Vezzulli, S. (2019). Emergent Ascomycetes in Viticulture: An Interdisciplinary Overview. Frontiers in Plant Science, 10(November), 1–30. https://doi.org/10.3389/fpls.2019.01394

Downy Mildew

Many are the sources of DM resistance employed in the last years. Most of them are North American species, which have co-evolved with *P. viticola* as *V. aestivalis, V. labrusca, V. riparia,* and *Muscadinia rotundifolia* but some Asian individuals as *V. amurensis* should be considered valuable resources, as well.

To date, 31 QTLs associated with resistance against *P. viticola* have been identified in grapevine (Topfer. R, Hausmann L., 2010). Georgian germplasm is the genetic source of the recent *R* loci *Rpv29*, *Rpv30* and *Rpv31* identified by Sargolzaei et al. (2020). The first discovered *R* loci: *Rpv1* (Merdinoglu et al., 2003) and *Rpv2* (Wiedemann-Merdinoglu et al., 2006), respectively located on chr12 and chr18, were found in *Muscadinia rotundifolia*. Interestingly, in a study aimed to confirm the fusion of chromosomes 7 and 20 which led from the 2n=40 in *Muscadinia* to the 2n=38 in *Vitis,* Cochetel et al. (2020), observed that the number of NBS-LRR in *Muscadinia* and Cabernet Sauvignon was similar, but their locations were different. An expansion of the Toll/Interleukin-1 Receptor-like-X (TIR-X) class was detected on *Muscadinia* chromosome 12 at the *Run1/Rpv1* locus, which confers strong dominant resistance to powdery and downy mildew.

On chr18 there are *Rpv15* (Pap et al., unpublished), *Rpv27* (Sapkota et al., 2019) and *Rpv3.* The latter codes a TIR-NB-LRR (Welter et al., 2007; Bellin et al., 2009), it was found in the hybrid Bianca, as well as *Rpv7* and, it is considered to give partial resistance by activating HR two days post inoculation (dpi) and reducing pathogen performance in the following days. Resistance enhancement was observed in a F1 population containing both *Rpv3* and *Rpv10* from *V. amurensis,* a QTL able to induce necrosis, callose deposition and stilbene accumulation (Schwander et al., 2012). Asian *V.amurensis* is the source of many other *R* loci found in the last ten years: the major QTL conferring strong resistance *Rpv8* on chr14 (Blasi et al., 2011); *Rpv12*, coding for a CC-NB-LRR able to induce HR, on chr15 (Venuti et al., 2013); *Rpv25* and *Rpv26* on chr15 (Lin et al., 2019). Recently, new *V. amurensis* accessions were used for scavenging new sources of resistance and three new *R* loci were identified (*Rpv22, Rpv23, Rpv24*; Fu et al., 2020).

Two QTLs related to DM resistance, named *Rpv5* and *Rpv6*, were identified in *V. riparia* by Marguerit et al. (2009). *V. riparia* is the same source also for *Rpv9* and *Rpv13* (Moreira et al., 2011), which showed ability to reduce symptoms emergence and severity. The minor QTL *Rpv14* was identified in *V. cinerea* by Ochssner et al. (2016). *Rpv17, Rpv18, Rpv19, Rpv20* and *Rpv21* were identified by Divilov et al. (2018) in a North American spp. genetic background. *Rpv11* was identified in a couple of resistant hybrids (Fischer et al., 2004; Bellin et al., 2009; Schwander et al., 2012) as well as *Rpv4* in Regent (Welter et al., 2007). Still no data regarding genetic source of resistance and

localization are available for *Rpv16* (Pap et al., unpublished) and *Rpv28* (Bhattarai et al., in preparation).

Particular interest is generated by the creation of resistant varieties, given the advantages they can present: decrease in the use of pesticides and fuel by up to 60-100%; reduction of production costs and the risk of losses due to diseases; lowering of work peaks; less pesticide exposure for workers; less soil compaction because of reduced sprayer movement in vineyards; reduced accumulation of copper residues in soil; enhancement of biodiversity within vineyards, less impacting on flora and fauna (Pertot et al., 2017b). However, it is now clear that monogenetic resistance can not be considered a long-term solution against plant disease since it can be overcome by more virulent strains of the pathogen, as showed for the DM resistant variety Bianca by Peressotti et al. (2010).

Nowadays, taking advantage of the continuous discovery of new resistance QTLs, gene pyramiding is gaining considerable importance as it would improve the efficiency of plant (including grapevine) breeding, leading to the development of genetic stocks and precise development of broad-spectrum resistance capabilities (Joshi & Nayak, 2010). According to the most updated information on MAS (Marker-assisted selection) applications at European level, in France "ResDur" varieties presenting assorted combinations of *Rpv1, Rpv3, Rpv10* (associated with DM) and of *Run1, Ren3* and *Ren3.2* (associated with PM resistance) were obtained by breeding "Bouquet" varieties with American, Asian and wild *Vitis* backgrounds (Delmotte et al., 2018). In Italy, Vezzulli et al. (2019) were able to obtain pyramided genotypes carrying two or three *Run/Ren* loci, up to seven *R* loci in total, while Foria et al. (2018) developed resistant genotypes derived from "élite" cultivars carrying *Rpv1, Rpv12* coupled with *Run1* and *Ren3*. Finally, besides increasing host diversity and complexity, populations of biotrophic pathogens should be regularly monitored for their virulence frequencies and virulence combinations (Miedaner, 2016) in order to improve durability.

In order to indirectly dissect resistance, an alternative approach relies on the biological candidacy of susceptibility (*S*) genes. Unlike *R* genes, *S* genes are required for successful pathogen infection, and thus are considered essential for compatible plant-pathogen interactions (Pirrello et al., 2019).

SUSCEPTIBILITY GENES

Unlike *R* genes, susceptibility (*S*) genes act as negative regulators of defense since they are able to facilitate infection and support compatibility between the plant and the pathogen (Fawke et al., 2015; Deng et al., 2020). First concept of *S* genes was given by Eckardt in 2002 but today an updated list of all *S* genes discovered so far is available in Moniruzzaman et al., 2020. Nowadays *S* genes are considered a promising complementary strategy to *R* loci (*S* genes are key elements of non-host

resistance (NHR), which is defined as "the ability of all genotypes of a plant species to confer resistance to all genotypes of a pathogen species" (Panstruga & Moscou, 2020). In other words, NHR consists into the ability to confer broad-spectrum and durable resistance. Based on the plant-pathogen interactions they promote, three main molecular mechanisms have been associated with *S* genes: (i) basic compatibility, which assists in host recognition and penetration; (ii) sustained compatibility, which is required for pathogen proliferation and spreading; and (iii) negative regulation of immune signals (van Schie & Takken, 2014). *S* gene-mediated resistance can be pathogen-specific when the impaired pathway is implicated in pre-penetration, penetration, or post-penetration requirements of a certain pathogen (Zaidi et al., 2018).

While *R* genes are mostly dominant, the disease resistance provided by manipulation of *S* genes is mostly recessive and associated with some fitness cost. As is often observed, *S* genes are involved in several pathways within the plant and play key physiological roles. Therefore, their mutation is most likely related to pleiotropic effects that can affect the acquired resistance qualities (Engelhardt et al., 2018). *S* genes can belong to several families with different roles within the cell; their peculiarity consists of assisting the pathogen in spreading the infection, and, consequently, to hinder the pathogen activity when mutated in homozygosity (Pirrello et al., 2019). Gene expression studies coupled with transgenic over-expression can be considered a good tool to discover *S* genes as in Toffolatti et al. (2020), as well as complementation studies (Van Damme et al., 2005) and the more popular *S* genes CRISPR/Cas9-mediated knock-out (e.g. Pompili et al., 2020; Wan et al., 2020). Once a *S* gene has been discovered, an interesting and almost unexplored strategy is the scouting of naturally occurring mutated *S* genes in breeding material (e.g.Tegtmeier et al., 2020) as recently suggested by Engelhardt et al. (2018).

Powdery Mildew

Improved efficacy and durability of PM resistance can be enhanced by understanding the genetic basis of resistance and susceptibility. Based on a high-resolution map, Barba et al. (2014) studied the inheritance of *E. necator* resistance and susceptibility of wild *V. rupestris* B38 and cultivated *V. vinifera* 'Chardonnay' finding evidence for quantitative variation. In particular, they identified ten SNPs on chromosome 9 associated with a locus for susceptibility from 'Chardonnay', named *Sen1* (*Susceptibility to E. necator 1*). This finding is a breakthrough towards negative selection among breeding progenies. Initially discovered as a natural mutation in barley (*Hordeum vulgare*; Jorgensen, 1992), *MLO* (*Mildew resistance Locus O*) driven resistance against PM (*Blumeria graminis*) was studied in numerous other plants. Natural *mlo* mutants were identified in cucumber (*Cucumis sativus*), melon (*Cucumis melo*), pea (*Pisum sativum*), tomato (*Solanum lycopersicum*) and tobacco (*Nicotiana*

tabacum) (Kusch & Panstruga, 2017). Unlike wheat (*Triticum aestivum*) which bears the closest orthologs of barley *MLO*, the number of genes belonging to this family varies a lot within dicots: from 10 to 25 members divided in clades where clade V is the one involved in PM susceptibility. In the last decade, grapevine *S* genes were studied in more depth. Feechan et al. (2008) defined the grapevine *VvMLO* gene family with 17 putative members belonging to 6 clades. Studies began in *A. thaliana* with *AtMLO2, AtMLO6* and *AtMLO12* and tomato *SlMLO1* genes, which were found to be required for PM susceptibility, culminating in the identification of 7 *VvMLO* orthologs: *VvMLO1, VvMLO3, VvMLO6, VvMLO7, VvMLO9, VvMLO13* and *VvMLO17*, all belonging to clade V. As a further confirmation for the key role of such genes in grapevine-*E. necator* interaction, different members of *VvMLO* gene family were found to be induced at transcriptional level upon *E. necator* inoculation: *VvMLO3, VvMLO4, VvMLO17* (Feechan et al., 2008) and *VvMLO13, VvMLO7* (Winterhagen et al., 2008). Furthermore, exogenous expression of *VvMLO11* and *VvMLO13* showed partial recovery of susceptibility to *E. cichoracearum* in Arabidopsis *mlo2 mlo6 mlo12* triple mutant (Feechan et al., 2013). In contrast, Pessina et al. (2016) proved that *VvMLO7* and *VvMLO6* RNAi silencing gives the most significant response in terms of resistance to *E. necator* in grapevine, even more than *VvMLO11* and *VvMLO13* knock-down. Members of the MLO protein family show a heptahelical transmembrane structure with three extracellular loops at the N-terminus and three intracellular loops at the C-terminus (Devoto et al., 1999, 2003) next to a calmodulin-binding site, which negatively regulates defence mechanisms by the accumulation of cell wall appositions at the *E. necator* penetration site (Kim et al., 2002; Feechan et al., 2008). Other carried out studies on Arabidopsis showed that MLO protein are involved in a number of physiological aspects, such as as root morphogenesis and architecture (Chen et al., 2009), as well as pollen tube reception by the ovary (Kessler et al., 2010). Given this evidence for their biological function, some *VvMLO* genes are being deep sequenced in a large *Vitis* spp. panel, and thus scouted for their natural variations as novel potential players in grapevine PM resistance breeding (Pirrello et al., 2018). Disrupting *S* genes may interfere with the compatibility between the host and the pathogens and consequently provide broad-spectrum and durable disease resistance. In the past, genetic manipulation of such *S* genes has been shown to confer disease resistance in various economically important crops. Recent studies focused on the use of genome editing to target *S* genes for the development of transgene-free and durable disease-resistant crop varieties (Zaidi et al., 2018). On this trail, an example of *S* gene-mediated approach to induce *E. necator* resistance has recently been presented (Giacomelli et al., 2018). In coming years, it is not excluded that *S* genes associated with the studied ascomycetes will be identified and exploited both in conventional breeding programs andin genome editing strategies.

This chapter has been extracted from:
Pirrello, C., Mizzotti, C., Tomazetti, T. C., Colombo, M., Bettinelli, P., Prodorutti, D., … Vezzulli, S. (2019). Emergent Ascomycetes in Viticulture: An Interdisciplinary Overview. Frontiers in Plant Science, 10(November), 1–30. https://doi.org/10.3389/fpls.2019.01394

Downy Mildew

First evidence of *S* genes to DM was given in Arabidopsis: Van Damme et al. (2005) detected six independent loci, named *DMR* (*Downy Mildew Resistant*), associated with susceptibility to *Hyaloperonospora arabidopsidis* by screening a population of EMS (Ethyl methane sulfonate) mutants. Six genes were investigated through complementation studies and functional analyses: *DMR1, DMR2* and *DMR6* were identified as susceptibility genes. Mutations in these genes were not accompanied by important pleiotropic effects. *DMR1* was found to encode a homoserine kinase (Van Damme et al., 2009), while *DMR6* encodes a 2-oxoglutarate (2OG)-Fe(II) oxygenase (Van Damme et al., 2008), the same as *DLO* (*DMR-like Oxygenases*), later characterized by Zhang et al., (2013). In 2015, Zeilmaker et al. confirmed the similar role of *AtDMR6* and *AtDLO*s and interestingly observed that while *AtDMR6.1* was precisely expressed in sites in direct contact with the pathogen, *AtDLO1* expression was circumscribed around leaf veins and areas close to the sites of infection. Orthologs of DMR6 and DLOs were readily identified in tomato (de Toledo Thomazella et al., 2016) as well as many other crops (e.g. Schouten et al., 2014; Porterfield & Meru, 2017; Sun et al., 2017; Zhang et al., 2018) and fruit trees like grapevine (Zeilmaker et al., 2015). Mutations in *DMR6* confer broad-spectrum resistance: *Sldmr6-1* tomato mutant plants showed resistance against *Phytophthora capsici, Pseudomonas siringae,* and *Xanthomonas* spp. (de Toledo Thomazella et al., 2016). In grapevine, two isoforms of *VvDMR6* were identified (Zeilmaker et al., 2015).

The first proposed function of DMR6 and DLO was defined by Zhang et al. (2013) and Zhang et al. (2017). It was hypothesized that these proteins are involved in salicylic acid (SA) catabolism, more in detail DMR6 as a SA-5-hydroxylase (S5H) and DLO as a SA-5-hydroxylase (S3H). The two enzymes have complementary functions in adding a hydroxylic group in two different positions of the SA aromatic ring, leading to the formation of 2,5-dihydrobenzenic acid (2,5-DHBA) and 2,3-DHBA, respectively (**Fig.4**). Falcone Ferreyra et al. (2015) proposed another mechanism through which DMR6 is able to condition SA biosynthesis, both from the isochorismate and the Phenylalanine Ammonia-Lyase (PAL) pathway (**Fig. 5**).

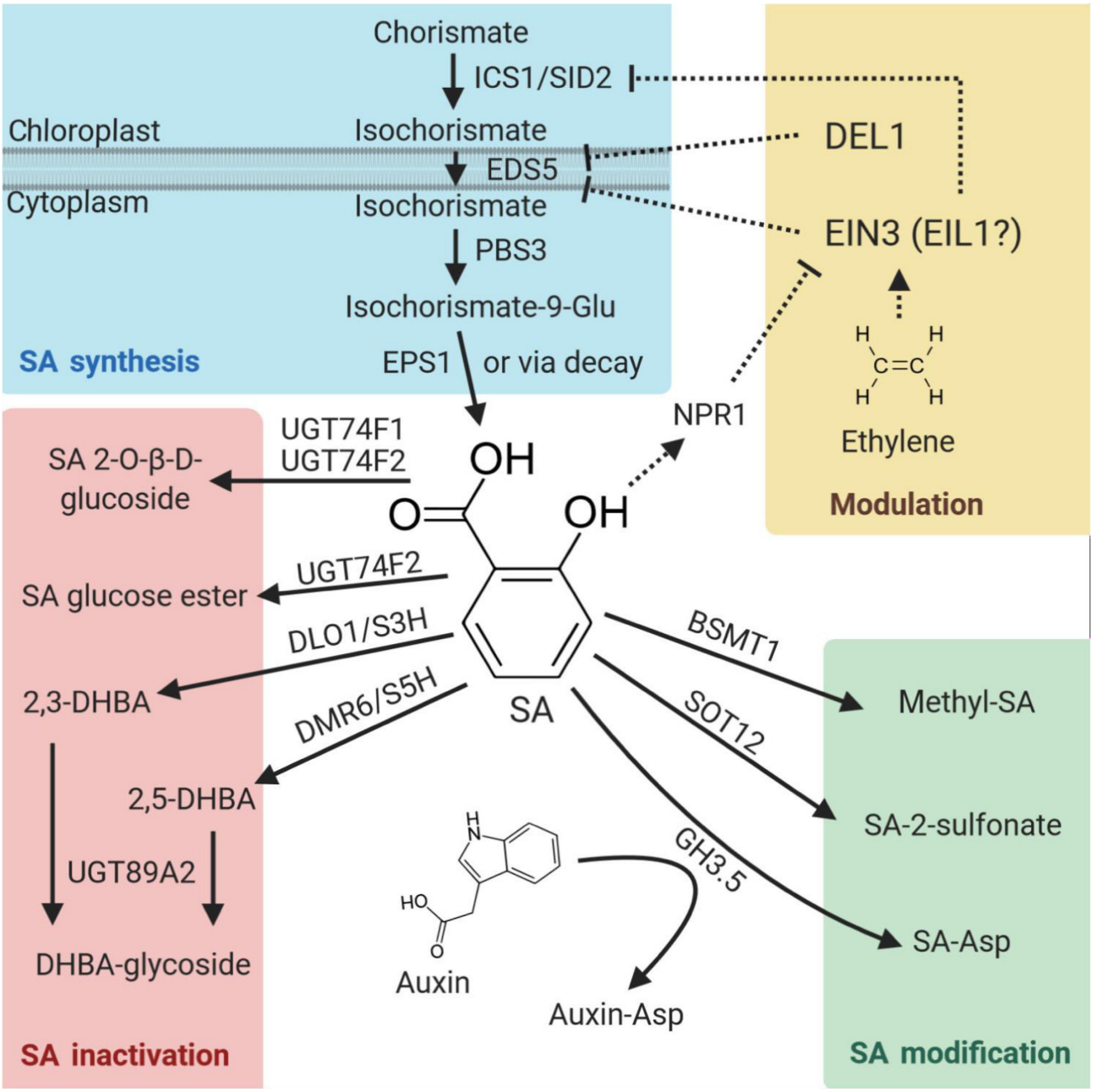


Figure 4. DMR6 and DLO proposed function as catalyzing SA hydroxylation (van Butselaar & Van den Ackerveken, 2020)

In any case, an influence on SA accumulation by DMR6 and DLO is taken for granted. It seems relevant that DMR6 shows the highest affinity for SA as compared to other SA-inactivating enzymes and that DLO1 affinity for SA is lower than that of DMR6 (Zhang et al., 2017)

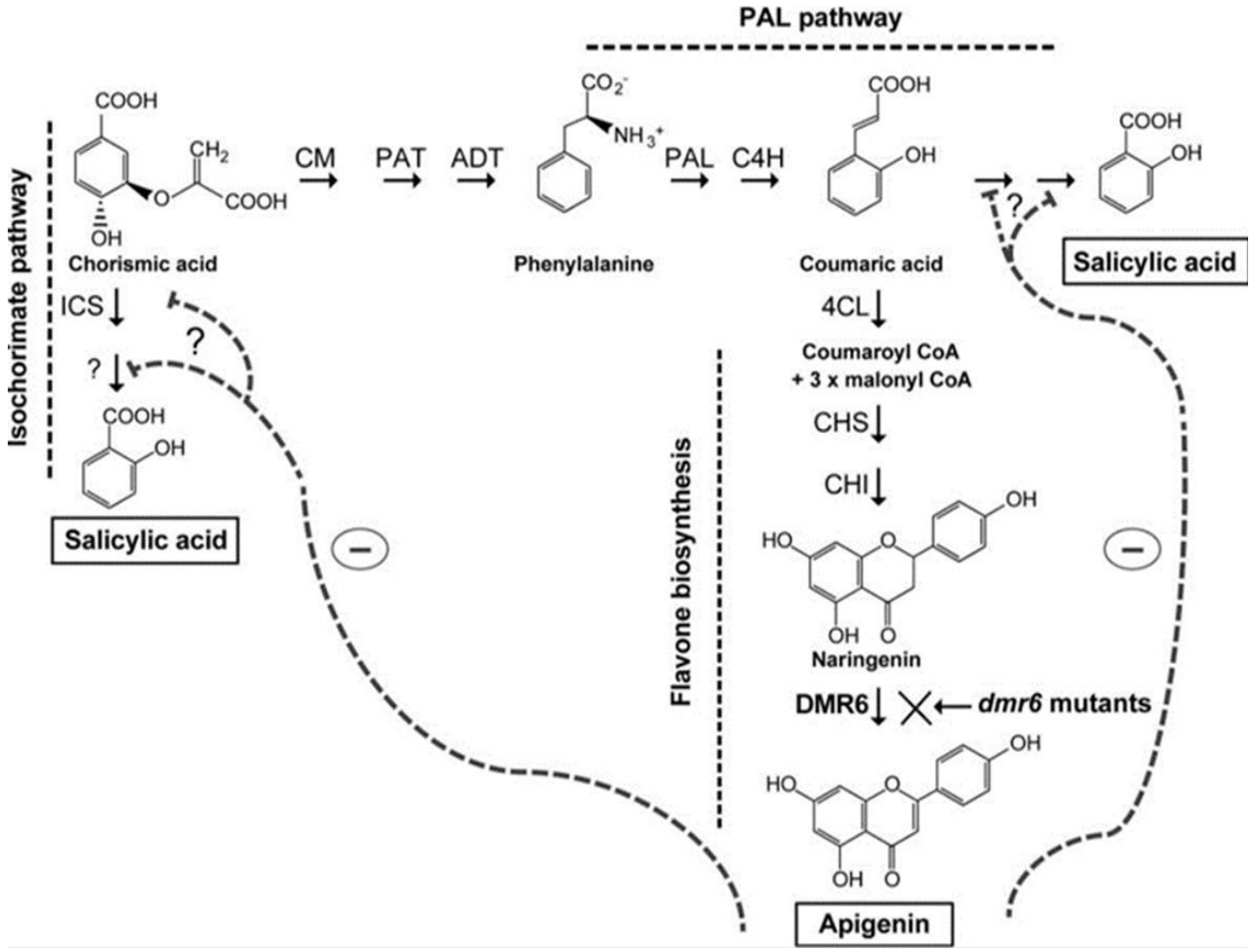


Figure 5. Proposed DMR6 function of covering the metabolic step from Naringenin to Apigenin (Falcone Ferreyra et al., 2015)

SA is an important phyto-hormone, classified as such after having observed its ability to induce resistance and pathogenesis-related (PR) protein expression upon TMV (tobacco mosaic virus) inoculation in tobacco (Raskin, 1992). Many are the roles held by SA in plants: it has an influence on seed germination, cell growth, respiration, response to abiotic stresses, thermogenesis and many other processes (Vlot et al., 2009). When a plant is under pathogen attack, a growth-immunity tradeoff takes place: SA is accumulated to activate the immune response and to suppress development. This mechanism gives an explanation as to why mutants often exhibit growth retardation or developmental disfunctions (Van Damme et al., 2005). One way of controlling SA accumulation, and therefore signaling, is through SA catabolism. Interestingly, SA induces the production of SA-modifying enzymes that synthesize different conjugates: some act as systemic signals moving to other tissues (e.g. methyl-SA), some others result in inactive forms (e.g. SA-glycosylates and DHBAs) (**Fig.4**) (van Schie & Takken, 2014; van Butselaar & Van den Ackerveken, 2020).

REFERENCES

Alston, J., & Sambucci, O. (2019). Grapes in the World Economy. In *The Grape Genome* (Compendium, pp. 1–24). https://doi.org/https://doi.org/10.1007/978-3-030-18601-2_1

Barba, P., Cadle-Davidson, L., Harriman, J., Glaubitz, J. C., Brooks, S., Hyma, K., & Reisch, B. (2014). Grapevine powdery mildew resistance and susceptibility loci identified on a high-resolution SNP map. *Theoretical and Applied Genetics*, *127*(1), 73–84. https://doi.org/10.1007/s00122-013-2202-x

Barker, C. L., Donald, T., Pauquet, J., Ratnaparkhe, M. B., Bouquet, A., Adam-Blondon, A. F., … Dry, I. B. (2005). Genetic and physical mapping of the grapevine powdery mildew resistance gene, Run1, using a bacterial artificial chromosome library. *Theoretical and Applied Genetics*, *111*(2), 370–377. https://doi.org/10.1007/s00122-005-2030-8

Bartlett, D. W., Clough, J. M., Godwin, J. R., Hall, A. A., Hamer, M., & Parr-Dobrzanski, B. (2002, July). The strobilurin fungicides. *Pest Management Science*, Vol. 58, pp. 649–662. https://doi.org/10.1002/ps.520

Baudoin, A., Olaya, G., Delmotte, F., Colcol, J. F., & Sierotzki, H. (2008). QoI Resistance of Plasmopara viticola and Erysiphe necator in the Mid-Atlantic United States. *Plant Health Progress*, *9*(1), 25. https://doi.org/10.1094/php-2008-0211-02-rs

Bavaresco, L., Squeri, C., & Vercesi, A. (2019). Field evaluation of new plant protection products against Plasmopara viticola . *BIO Web of Conferences*, *12*, 01007. https://doi.org/10.1051/bioconf/20191201007

Bellin, D., Peressotti, E., Merdinoglu, D., Wiedemann-Merdinoglu, S., Adam-Blondon, A. F., Cipriani, G., … Di Gaspero, G. (2009). Resistance to Plasmopara viticola in grapevine 'Bianca' is controlled by a major dominant gene causing localised necrosis at the infection site. *Theoretical and Applied Genetics*, *120*(1), 163–176. https://doi.org/10.1007/s00122-009-1167-2

Blanc, S., Wiedemann-Merdinoglu, S., Dumas, V., Mestre, P., & Merdinoglu, D. (2012). A reference genetic map of Muscadinia rotundifolia and identification of Ren5, a new major locus for resistance to grapevine powdery mildew. *Theoretical and Applied Genetics*, *125*(8), 1663–1675. https://doi.org/10.1007/s00122-012-1942-3

Blasi, P., Blanc, S., Wiedemann-Merdinoglu, S., Prado, E., Rühl, E. H., Mestre, P., & Merdinoglu, D. (2011). Construction of a reference linkage map of *Vitis* amurensis and genetic mapping of Rpv8, a locus conferring resistance to grapevine downy mildew. *Theoretical and Applied Genetics*, *123*(1), 43–53. https://doi.org/10.1007/s00122-011-1565-0

Bouquet, A. (1986). Introduction dans l'espe`ce *Vitis* vinifera L. d'un caracte`re de re´sistance a`

l'oidium (uncinula necator Schw. Burr.) issu de l'espe`ce Muscadinia rotundifolia (Michx.) Small. *Vignevini*, *12*, 141–146.

Buonassisi, D., Colombo, M., Migliaro, D., Dolzani, C., Peressotti, E., Mizzotti, C., … Vezzulli, S. (2017). Breeding for grapevine downy mildew resistance: a review of "omics" approaches. *Euphytica*, *213*(5), 1–21. https://doi.org/10.1007/s10681-017-1882-8

Burruano, S. (2000). The life-cycle of Plasmopara viticola, cause of downy mildew of vine. *Mycologist*, *14*(4), 179–182. https://doi.org/10.1016/S0269-915X(00)80040-3

Cadle-Davidson, L. (2018). A perspective on breeding and implementing durable powdery mildew resistance. Retrieved from International Conference on Grape Breeding and Genetics XII website: http://gbg2018.u-bordeaux.fr/files/gbg2018/presentation/o60_20180720BordeauxLCD.pdf

Cadle-Davidson, L., Gadoury, D. M., Fresnedo-Ramírez, J., Yang, S., Barba, P., Sun, Q., … Reisch, B. I. (2016). Lessons from a Phenotyping Center Revealed by the Genome-Guided Mapping of Powdery Mildew Resistance Loci. *Phytopathology*, *106*(10), 1159–1169. https://doi.org/10.1094/PHYTO-02-16-0080-FI

Cannon, R. D., Lamping, E., Holmes, A. R., Niimi, K., Baret, P. V., Keniya, M. V., … Monk, B. C. (2009). Efflux-mediated antifungal drug resistance. *Clinical Microbiology Reviews*, *22*(2), 291–321. https://doi.org/10.1128/CMR.00051-08

Carvalho, F. P. (2017). Pesticides, environment, and food safety. *Food and Energy Security*, *6*(2), 48–60. https://doi.org/10.1002/fes3.108

Chen, Z., Noir, S., Kwaaitaal, M., Hartmann, H. A., Wu, M.-J., Mudgil, Y., … Jones, A. M. (2009). Two seven-transmembrane domain MILDEW RESISTANCE LOCUS O proteins cofunction in Arabidopsis root thigmomorphogenesis. *The Plant Cell*, *21*(7), 1972–1991. https://doi.org/10.1105/tpc.108.062653

Cherrad, S., Charnay, A., Hernandez, C., Steva, H., Belbahri, L., & Vacher, S. (2018). Emergence of boscalid-resistant strains of Erysiphe necator in French vineyards. *Microbiological Research*, *216*(August), 79–84. https://doi.org/10.1016/j.micres.2018.08.007

Cochetel, N., Minio, A., Vondras, A. M., Figueroa-Balderas, R., & Cantu, D. (2020). Diploid chromosome-scale assembly of the Muscadinia rotundifolia genome supports chromosome fusion and disease resistance gene expansion during *Vitis* and Muscadinia divergence. *BioRxiv*, (530), 2020.06.02.119792. https://doi.org/10.1101/2020.06.02.119792

Coleman, C., Copetti, D., Cipriani, G., Hoffmann, S., Kozma, P., Kovács, L., … Di Gaspero, G. (2009). The powdery mildew resistance gene REN1 co-segregates with an NBS-LRR gene

cluster in two Central Asian grapevines. *BMC Genetics*, *10*(1), 89. https://doi.org/10.1186/1471-2156-10-89

Cools, H. J., & Fraaije, B. A. (2013). Update on mechanisms of azole resistance in Mycosphaerella graminicola and implications for future control. *Pest Management Science*, *69*(2), 150–155. https://doi.org/10.1002/ps.3348

Corio-Costet, M. F., Bouscaut, J., Delmotte, F., Douence, L., Richart-Cervera, S., & Amrani, L. (2003). Genetic structure of powdery mildew and fungicide resistance: AFLP and molecular tools of detection. *Proceedings 7th ANPP International Conference on Plant Diseases, Tours, France*, 1–8.

Dagostin, S., Formolo, T., Giovannini, O., & Pertot, I. (2010). Salvia officinalis Extract Can Protect Grapevine Against Plasmopara viticola. *Plant Disease*, *94*(5), 575–580. https://doi.org/Doi 10.1094/Pdis-94-5-0575

Dalbó, M. a., Ye, G. N., Weeden, N. F., Wilcox, W. F., & Reisch, B. I. (2001). Marker-assisted Selection for Powdery Mildew Resistance in Grapes. *Journal of the American Society of Horticultural Science*, *126*(1), 83–89. https://doi.org/https://doi.org/10.21273/JASHS.126.1.83

de Toledo Thomazella, D. P., Brail, Q., Dahlbeck, D., & Staskawicz, B. J. (2016). CRISPR-Cas9 mediated mutagenesis of a DMR6 ortholog in tomato confers broad-spectrum disease resistance. *BioRxiv*, (July), 064824. https://doi.org/10.1101/064824

De Waard, M. A., Andrade, A. C., Hayashi, K., Schoonbeek, H. J., Stergiopoulos, I., & Zwiers, L. H. (2006). Impact of fungal drug transporters on fungicide sensitivity, multidrug resistance and virulence. *Pest Management Science*, *62*(3), 195–207. https://doi.org/10.1002/ps.1150

Delmotte, F., Guimier, S., Demeaux, I., Couture, C., Schneider, C., Cailliatte, R., … Deliere, L. (2018). OSCAR, a national observatory to support the deployment of new grapevine disease-resistant varieties in France. In *Book of Abstracts of the XII International Conference on Grapevine Breeding and Genetics, Bordeaux, France, 15-20 July 2018* (p. 29).

Délye, C., Bousset, L., & Corio-Costet, M. F. (1998). PCR cloning and detection of point mutations in the eburicol 14α-demethylase (CYP51) gene from Erysiphe graminis f. Sp. Hordei, a 'recalcitrant' fungus. *Current Genetics*, *34*(5), 399–403. https://doi.org/10.1007/s002940050413

Délye, C., Laigret, F., & Corio-Costet, M. F. (1997). A mutation in the 14α-Demethylase gene of Uncinula necator that correlates with resistance to a sterol biosynthesis inhibitor. *Applied and Environmental Microbiology*, *63*(8), 2966–2970.

Deng, Y., Ning, Y., Yang, D. L., Zhai, K., Wang, G. L., & He, Z. (2020). Molecular Basis of

Disease Resistance and Perspectives on Breeding Strategies for Resistance Improvement in Crops. *Molecular Plant*, *13*(10), 1402–1419. https://doi.org/10.1016/j.molp.2020.09.018

Devoto, A., Hartmann, H. A., Piffanelli, P., Elliott, C., Simmons, C., Taramino, G., … Panstruga, R. (2003). Molecular phylogeny and evolution of the plant-specific seven-transmembrane MLO family. *Journal of Molecular Evolution*, *56*(1), 77–88. https://doi.org/10.1007/s00239-002-2382-5

Devoto, A., Piffanelli, P., Nilsson, I., Wallin, E., Panstruga, R., von Heijne, G., & Schulze-Lefert, P. (1999). Topology, Subcellular Localization, and Sequence Diversity of the Mlo Family in Plants. *Journal of Biological Chemistry*, *274*(49), 34993–35004. https://doi.org/10.1074/jbc.274.49.34993

Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticides. (n.d.). Retrieved from http://data.europa.eu/eli/dir/2009/128/oj

Divilov, K., Barba, P., Cadle-Davidson, L., & Reisch, B. I. (2018). Single and multiple phenotype QTL analyses of downy mildew resistance in interspecific grapevines. *Theoretical and Applied Genetics*, *131*(5), 1133–1143. https://doi.org/10.1007/s00122-018-3065-y

Dry, I. B., Feechan, A., Anderson, C., Jermakow, A. M., Bouquet, A., Adam-Blondon, A. F., & Thomas, M. R. (2010). Molecular strategies to enhance the genetic resistance of grapevines to powdery mildew. *Australian Journal of Grape and Wine Research*, *16*, 94–105. https://doi.org/10.1111/j.1755-0238.2009.00076.x

Dufour, M. C., Fontaine, S., Montarry, J., & Corio-Costet, M. F. (2011). Assessment of fungicide resistance and pathogen diversity in Erysiphe necator using quantitative real-time PCR assays. *Pest Management Science*, *67*(1), 60–69. https://doi.org/10.1002/ps.2032

Eckardt, N. A. (2002). Plant disease susceptibility genes? *Plant Cell*, *14*(9), 1983–1986. https://doi.org/10.1105/tpc.140910

Engelhardt, Stam, R., & Hückelhoven, R. (2018). Good Riddance? Breaking Disease Susceptibility in the Era of New Breeding Technologies. *Agronomy*, *8*(7), 1–16. https://doi.org/10.3390/agronomy8070114

European Union. (2019). Agriculture, forestry and fishery statistics. In *Eurostat*.

Falcone Ferreyra, M. L., Emiliani, J., Rodriguez, E. J., Campos-Bermudez, V. A., Grotewold, E., & Casati, P. (2015). The Identification of Maize and Arabidopsis Type I FLAVONE SYNTHASEs Links Flavones with Hormones and Biotic Interactions. *Plant Physiology*, *169*(2), 1090–1107. https://doi.org/10.1104/pp.15.00515

FAOSTAT. (n.d.). http://www.fao.org/faostat/en/#data/RP. Retrieved from

http://www.fao.org/faostat/en/#data/RP

Fawke, S., Doumane, M., & Schornack, S. (2015). Oomycete Interactions with Plants: Infection Strategies and Resistance Principles. *Microbiology and Molecular Biology Reviews*, *79*(3), 263–280. https://doi.org/10.1128/MMBR.00010-15

Feechan, A., Anderson, C., Torregrosa, L., Jermakow, A., Mestre, P., Wiedemann-Merdinoglu, S., … Dry, I. B. (2013). Genetic dissection of a TIR-NB-LRR locus from the wild North American grapevine species Muscadinia rotundifolia identifies paralogous genes conferring resistance to major fungal and oomycete pathogens in cultivated grapevine. *The Plant Journal*, *76*(4), 661–674. https://doi.org/10.1111/tpj.12327

Feechan, A., Jermakow, A. M., Torregrosa, L., Panstruga, R., & Dry, I. B. (2008). Identification of grapevine MLO gene candidates involved in susceptibility to powdery mildew. *Functional Plant Biology*, *35*(12), 1255. https://doi.org/10.1071/FP08173

Feechan, A., Kocsis, M., Riaz, S., Zhang, W., Gadoury, D. M., Walker, M. A., … Cadle-Davidson, L. (2015). Strategies for RUN1 Deployment Using RUN2 and REN2 to Manage Grapevine Powdery Mildew Informed by Studies of Race Specificity. *Phytopathology*, *105*(8), 1104–1113. https://doi.org/10.1094/PHYTO-09-14-0244-R

Fischer, B. M., Salakhutdinov, I., Akkurt, M., Eibach, R., Edwards, K. J., Töpfer, R., & Zyprian, E. M. (2004). Quantitative trait locus analysis of fungal disease resistance factors on a molecular map of grapevine. *Theoretical and Applied Genetics*, *108*(3), 501–515. https://doi.org/10.1007/s00122-003-1445-3

Flor, H. H. (1954). *Identification of races of flax rust by lines with single rust-conditioning genes*. US Dept. of Agriculture.

Foria, S., Monte, C., Testolin, R., Di Gaspero, G., & Cipriani, G. (2018). Piramydizing resistance genes in grape: a breeding program for the selection of 'elite' cultivars. *Book of Abstracts of the XII International Conference on Grapevine Breeding and Genetics, Bordeaux, France, 15-20 July 2018*. Retrieved from http://gbg2018.u-bordeaux.fr/files/gbg2018/presentation/O17_20180705_GBGB_Bordeaux.pdf

Fraga, H. (2019). Viticulture and winemaking under climate change. *Agronomy*, *9*(12), 2–5. https://doi.org/10.3390/agronomy9120783

Frenkel, O., Cadle-Davidson, L., Wilcox, W. F., & Milgroom, M. G. (2015). Mechanisms of resistance to an azole fungicide in the grapevine powdery mildew fungus, Erysiphe necator. *Phytopathology*, *105*(3), 370–377. https://doi.org/10.1094/PHYTO-07-14-0202-R

Fröbel, S., & Zyprian, E. (2019). Colonization of Different Grapevine Tissues by Plasmopara viticola—A Histological Study. *Frontiers in Plant Science*, *10*(July), 1–13.

https://doi.org/10.3389/fpls.2019.00951

Fu, P., Wu, W., Lai, G., Li, R., Peng, Y., Yang, B., … Lu, J. (2020). Identifying Plasmopara viticola resistance Loci in grapevine (*Vitis* amurensis) via genotyping-by-sequencing-based QTL mapping. *Plant Physiology and Biochemistry*, *154*(May), 75–84. https://doi.org/10.1016/j.plaphy.2020.05.016

Gessler, C., Pertot, I., & Perazzolli, M. (2011). Plasmopara viticola: A review of knowledge on downy mildew of grapevine and effective disease management. *Phytopathologia Mediterranea*, *50*(1), 3–44. https://doi.org/10.14601/Phytopathol_Mediterr-9360

Gisi, U., & Sierotzki, H. (2008). Fungicide modes of action and resistance in downy mildews. *European Journal of Plant Pathology*, *122*(1), 157–167. https://doi.org/10.1007/s10658-008-9290-5

Gisi, U., Sierotzki, H., Cook, A., & McCaffery, A. (2002). Mechanisms influencing the evolution of resistance to Qo inhibitor fungicides. *Pest Management Science*, *58*(9), 859–867. https://doi.org/10.1002/ps.565

Gomès, E., & Coutos-Thévenot, P. (2009). Molecular Aspects of Grapevine-Pathogenic Fungi Interactions. In K. A. Roubelakis-Angelakis (Ed.), *Grapevine Molecular Physiology and Biotechnology: Second Edition* (pp. 407–428). https://doi.org/10.1007/978-90-481-2305-6_15

Goyal, N., Bhatia, G., Sharma, S., Garewal, N., Upadhyay, A., Upadhyay, S. K., & Singh, K. (2019). Genome-wide characterization revealed role of NBS-LRR genes during powdery mildew infection in *Vitis* vinifera. *Genomics*. https://doi.org/10.1016/j.ygeno.2019.02.011

Gubler, W. D., Ypema, H. L., Ouimette, D. G., & Bettiga, L. J. (1996). Occurrence of resistance in Uncinula necator to triadimefon, myclobutanil, and fenarimol in California grapevines. *Plant Disease*, *80*(8), 902–909. https://doi.org/10.1094/PD-80-0902

Hamamoto, H., Hasegawa, K., Nakaune, R., Lee, Y. J., Makizumi, Y., Akutsu, K., & Hibi, T. (2000). Tandem repeat of a transcriptional enhancer upstream of the sterol 14α-demethylase gene (CYP51) in Penicillium digitatum. *Applied and Environmental Microbiology*, *66*(8), 3421–3426. https://doi.org/10.1128/AEM.66.8.3421-3426.2000

Hayashi, K., Schoonbeek, H. J., & De Waard, M. A. (2002). Expression of the ABC transporter BcatrD from Botrytis cinerea reduces sensitivity to sterol demethylation inhibitor fungicides. *Pesticide Biochemistry and Physiology*, *73*(2), 110–121. https://doi.org/10.1016/S0048-3575(02)00015-9

Héloir, M. C., Adrian, M., Brulé, D., Claverie, J., Cordelier, S., Daire, X., … Poinssot, B. (2019). Recognition of Elicitors in Grapevine: From MAMP and DAMP Perception to Induced Resistance. *Frontiers in Plant Science*, *10*(September), 1–17.

https://doi.org/10.3389/fpls.2019.01117

Hoffman, L. E., Wilcox, W. F., Gadoury, D. M., Seem, R. C., & Riegel, D. G. (2004). Integrated Control of Grape Black Rot: Influence of Host Phenology, Inoculum Availability, Sanitation, and Spray Timing. *Phytopathology*, *94*(6), 641–650. https://doi.org/10.1094/PHYTO.2004.94.6.641

Hoffmann, S., Di Gaspero, G., Kovács, L., Howard, S., Kiss, E., Galbács, Z., … Kozma, P. (2008). Resistance to Erysiphe necator in the grapevine 'Kishmish vatkana' is controlled by a single locus through restriction of hyphal growth. *TAG. Theoretical and Applied Genetics. Theoretische Und Angewandte Genetik*, *116*(3), 427–438. https://doi.org/10.1007/s00122-007-0680-4

Jonathan D. G. Jones Jeffery L. Dangl. (2006). The plant immune system. *Nature*, *444*, 323–329.

Jorgensen, J. H. (1992). *Discovery, characterization and exploitation of Mlo powdery mildew*. *66*(Table 1), 141–152.

Joshi, R. K., & Nayak, S. (2010). Gene pyramiding-A broad spectrum technique for developing durable stress resistance in crops. *Biotechnology and Molecular Biology Reviews*, *5*(3), 51–60. Retrieved from http://www.academicjournals.org/BMBR

Kennelly, M. M., Gadoury, D. M., Wilcox, W. F., Magarey, P. A., & Seem, R. C. (2005). Seasonal Development of Ontogenic Resistance to Downy Mildew in Grape Berries and Rachises. *Phytopathology*, *95*(12), 1445–1452. https://doi.org/10.1094/PHYTO-95-1445

Kessler, S. A., Shimosato-Asano, H., Keinath, N. F., Wuest, S. E., Ingram, G., Panstruga, R., & Grossniklaus, U. (2010). Conserved molecular components for pollen tube reception and fungal invasion. *Science (New York, N.Y.)*, *330*(6006), 968–971. https://doi.org/10.1126/science.1195211

Kim, M. C., Panstruga, R., Elliott, C., Müller, J., Devoto, A., Yoon, H. W., … Schulze-Lefert, P. (2002). Calmodulin interacts with MLO protein to regulate defence against mildew in barley. *Nature*, *416*(6879), 447–451. https://doi.org/10.1038/416447a

Kretschmer, M., Leroch, M., Mosbach, A., Walker, A. S., Fillinger, S., Mernke, D., … Hahn, M. (2009). Fungicide-driven evolution and molecular basis of multidrug resistance in field populations of the grey mould fungus Botrytis cinerea. *PLoS Pathogens*, *5*(12). https://doi.org/10.1371/journal.ppat.1000696

Kunova, A., Pizzatti, C., Bonaldi, M., & Cortesi, P. (2016). Metrafenone resistance in a population of Erysiphe necator in northern Italy. *Pest Management Science*, *72*(2), 398–404. https://doi.org/10.1002/ps.4060

Kusch, S., & Panstruga, R. (2017). mlo-Based Resistance: An Apparently Universal 'Weapon' to

Defeat Powdery Mildew Disease. *Molecular Plant-Microbe Interactions : MPMI*, *30*(3), 179–189. https://doi.org/10.1094/MPMI-12-16-0255-CR

L. Giacomelli, T. Zeilmaker, M. Malnoy, J. Rouppe van der Voort, C. M. (2018). Generation of mildew-resistant grapevine clones via genome editing. *ISHS Acta Horticulturae 1248: XII International Conference on Grapevine Breeding and Genetics*. https://doi.org/10.17660/ActaHortic.2019.1248.28

Leroux, P., Albertini, C., Gautier, A., Gredt, M., & Walker, A. (2007). Mutations in the CYP51 gene correlated with changes in sensitivity to sterol 14α-demethylation inhibitors in field isolates of Mycosphaerella graminicola. *Pest Management Science: Formerly Pesticide Science*, *63*(7), 688–698. https://doi.org/10.1002/ps.1390

Leroux, P., & Walker, A. S. (2011). Multiple mechanisms account for resistance to sterol 14α-demethylation inhibitors in field isolates of Mycosphaerella graminicola. *Pest Management Science*, *67*(1), 44–59. https://doi.org/10.1002/ps.2028

Lin, H., Leng, H., Guo, Y., Kondo, S., Zhao, Y., Shi, G., & Guo, X. (2019). QTLs and candidate genes for downy mildew resistance conferred by interspecific grape (V. vinifera L. × V. amurensis Rupr.) crossing. *Scientia Horticulturae*, *244*(June 2018), 200–207. https://doi.org/10.1016/j.scienta.2018.09.045

Loskill, B., Molitor, D., Koch, E., Harms, M., Berkelmann-Löhnertz, B., Hoffmann, C., … Maixner, M. (2009). *Strategien zur Regulation der Schwarzfäule (Guignardia bidwellii) im ökologischen Weinbau*. Retrieved from http://orgprints.org/17072/1/17072-04OE032-jki-maixner-2009-schwarzfaeule.pdf

Lu, L. M., Ickert-Bond, S., & Wen, J. (2018). Recent Advances in Systematics and Evolution of the Grape Family Vitaceae. *Journal of Systematics and Evolution*, *56*(4), 259–261. https://doi.org/10.1111/jse.12449

Luo, C. X., Cox, K. D., Amiri, A., & Schnabel, G. (2008). Occurrence and detection of the DMI resistance-associated genetic element 'Mona' in Monilinia fructicola. *Plant Disease*, *92*(7), 1099–1103. https://doi.org/10.1094/PDIS-92-7-1099

Lupetti, A., Danesi, R., Campa, M., Tacca, M. Del, & Kelly, S. (2002). Molecular basis of resistance to azole antifungals. *Trends in Molecular Medicine*, *8*(2), 76–81. https://doi.org/10.1016/S1471-4914(02)02280-3

Ma, Z., & Michailides, T. J. (2005). Advances in understanding molecular mechanisms of fungicide resistance and molecular detection of resistant genotypes in phytopathogenic fungi. *Crop Protection*, *24*(10), 853–863. https://doi.org/10.1016/j.cropro.2005.01.011

Ma, Z., Proffer, T. J., Jacobs, J. L., & Sundin, G. W. (2006). Overexpression of the 14α-

demethylase target gene (CYP51) mediates fungicide resistance in Blumeriella jaapii. *Applied and Environmental Microbiology*, *72*(4), 2581–2585. https://doi.org/10.1128/AEM.72.4.2581-2585.2006

Mahanil, S., Ramming, D., Cadle-Davidson, M., Owens, C., Garris, A., Myles, S., & Cadle-Davidson, L. (2012). Development of marker sets useful in the early selection of Ren4 powdery mildew resistance and seedlessness for table and raisin grape breeding. *Theoretical and Applied Genetics*, *124*(1), 23–33. https://doi.org/10.1007/s00122-011-1684-7

Marguerit, E., Boury, C., Manicki, A., Donnart, M., Butterlin, G., Némorin, A., … Decroocq, S. (2009). Genetic dissection of sex determinism, inflorescence morphology and downy mildew resistance in grapevine. *Theoretical and Applied Genetics*, *118*(7), 1261–1278. https://doi.org/10.1007/s00122-009-0979-4

McGovern, P., Jalabadze, M., Batiuk, S., Callahan, M. P., Smith, K. E., Hall, G. R., … Lordkipanidze, D. (2017). Early Neolithic wine of Georgia in the South Caucasus. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(48), E10309–E10318. https://doi.org/10.1073/pnas.1714728114

Merdinoglu D., Wiedemann-Merdinoglu S., C. P., & Dumas V., Haetty S., B. G. and G. C. (2003). Genetic analysis of downy mildew resistance derived from Muscadinia rotundifolia. *Acta Horticulturae*, (603), 451–456. https://doi.org/10.17660/ActaHortic. 2003.603.57

Miedaner, T. (2016). Breeding Strategies for Improving Plant Resistance to Diseases. In *Advances in Plant Breeding Strategies: Agronomic, Abiotic and Biotic Stress Traits* (pp. 561–599). https://doi.org/10.1007/978-3-319-22518-0_15

Miles, L. A., Miles, T. D., Kirk, W. W., & Schilder, A. M. C. (2012). Strobilurin (QoI) resistance in populations of Erysiphe necator on grapes in Michigan. *Plant Disease*, *96*(11), 1621–1628. https://doi.org/10.1094/PDIS-01-12-0041-RE

Molitor, D., & Beyer, M. (2014). Epidemiology, identification and disease management of grape black rot and potentially useful metabolites of black rot pathogens for industrial applications - a review. *Annals of Applied Biology*, *165*(3), 305–317. https://doi.org/10.1111/aab.12155

Moniruzzaman, M., Zhong, Y., Yan, H., Yuanda, L., Jiang, B., & Zhong, G. (2020). Exploration of Susceptible Genes with Clustered Regularly Interspaced Short Palindromic Repeats–Tissue-Specific Knockout (CRISPR-TSKO) to Enhance Host Resistance. *Critical Reviews in Plant Sciences*, *39*(5), 387–417. https://doi.org/10.1080/07352689.2020.1810970

Moreira, F. M., Madini, A., Marino, R., Zulini, L., Stefanini, M., Velasco, R., … Grando, M. S. (2011). Genetic linkage maps of two interspecific grape crosses (*Vitis* spp.) used to localize quantitative trait loci for downy mildew resistance. *Tree Genetics and Genomes*, *7*(1), 153–

167. https://doi.org/10.1007/s11295-010-0322-x

Ochssner, I., Hausmann, L., & Töpfer, R. (2016). Rpvl4, a new genetic source for Plasmopara viticola resistance conferred by *Vitis* cinerea. *Vitis - Journal of Grapevine Research*, *55*(2), 79–81. https://doi.org/10.5073/*vitis*.2016.55.79-81

Ogawa, J., Gubler, W., … B. M. by D. B. and M., & 1988, undefined. (1988). Effect of sterol biosynthesis inhibitors on diseases of stone fruits and grapes in California. In D. Berg, M. Plempel, E. Horwood, & Chichester (Eds.), *In Sterol biosynthesis inhibitors—pharmaceutical and agrochemical aspects* (pp. 262–287). Retrieved from http://agris.fao.org/agris-search/search.do?recordID=US201302689034

OIV, & International Organisation of Vine and Wine. (2019). 2019 Statistical Report on World Vitiviniculture. *2019 Statistical Report on World Vitiviniculture*, 23. https://doi.org/64/19/6835 [pii]\n10.1158/0008-5472.CAN-04-1678

Oliver, R. P., & Hewitt, H. G. (2014). Chapter 5: Fungicide performance. In *Fungicides in crop protection* (pp. 71–122). Retrieved from https://espace.curtin.edu.au/handle/20.500.11937/10045

Panstruga, R., & Moscou, M. J. (2020). What is the Molecular Basis of Nonhost Resistance? *Molecular Plant-Microbe Interactions®*, *33*(11), MPMI-06-20-0161. https://doi.org/10.1094/mpmi-06-20-0161-cr

Pap, D., Riaz, S., Dry, I. B., Jermakow, A., Tenscher, A. C., Cantu, D., … Walker, M. A. (2016). Identification of two novel powdery mildew resistance loci, Ren6 and Ren7, from the wild Chinese grape species *Vitis* piasezkii. *BMC Plant Biology*, *16*(1), 170. https://doi.org/10.1186/s12870-016-0855-8

Pauquet, J., Bouquet, A., This, P., & Adam-Blondon, A.-F. (2001). Establishment of a local map of AFLP markers around the powdery mildew resistance gene Run1 in grapevine and assessment of their usefulness for marker assisted selection. *Theoretical and Applied Genetics*, *103*(8), 1201–1210. https://doi.org/10.1007/s001220100664

Pearson, R. C., & Gadoury, D. M. (1992). Powdery Mildew of Grapes. In J. Kumar, H. S. Chaube, U. S. Singh, & A. N. Mukhopadhyay (Eds.), *Plant diseases of international importance. Vol III. Diseases of fruit crops.* (pp. 129–146). Prentice Hall, Englewood Cliffs, USA.

Perazzolli, M., Dagostin, S., Ferrari, A., Elad, Y., & Pertot, I. (2008). Induction of systemic resistance against Plasmopara viticola in grapevine by Trichoderma harzianum T39 and benzothiadiazole. *Biological Control*, *47*(2), 228–234. https://doi.org/10.1016/j.biocontrol.2008.08.008

Perazzolli, M., Moretto, M., Fontana, P., Ferrarini, A., Velasco, R., Moser, C., … Pertot, I. (2012). Downy mildew resistance induced by Trichoderma harzianum T39 in susceptible grapevines partially mimics transcriptional changes of resistant genotypes. *BMC Genomics*, *13*(1), 1–19. https://doi.org/10.1186/1471-2164-13-660

Peressotti, E., Wiedemann-Merdinoglu, S., Delmotte, F., Bellin, D., Di Gaspero, G., Testolin, R., … Mestre, P. (2010). Breakdown of resistance to grapevine downy mildew upon limited deployment of a resistant variety. *BMC Plant Biology*, *10*. https://doi.org/10.1186/1471-2229-10-147

Pertot, I., Caffi, T., Rossi, V., Mugnai, L., Hoffmann, C., Grando, M. S., … Anfora, G. (2017). A critical review of plant protection tools for reducing pesticide use on grapevine and new perspectives for the implementation of IPM in viticulture. *Crop Protection*, *97*, 70–84. https://doi.org/10.1016/j.cropro.2016.11.025

Pessina, S., Lenzi, L., Perazzolli, M., Campa, M., Dalla Costa, L., Urso, S., … Malnoy, M. (2016). Knockdown of MLO genes reduces susceptibility to powdery mildew in grapevine. *Horticulture Research*, *3*(1), 16016. https://doi.org/10.1038/hortres.2016.16

Pimentel, D. (2005). Environmental and economic costs of the application of pesticides primarily in the United States. *Environment, Development and Sustainability*, *7*(2), 229–252. https://doi.org/10.1007/s10668-005-7314-2

Pirrello, C.; Zeilmaker, T.; Giacomelli, L.; Bianco, L.; Moser, C.; Vezzulli, S. (2018). Scouting downy and powdery mildew susceptibility genes: a diversity study in *Vitis* spp. *XII International Conference on Grapevine Breeding and Genetics*. Bordeaux, France.

Pirrello, C., Mizzotti, C., Tomazetti, T. C., Colombo, M., Bettinelli, P., Prodorutti, D., … Vezzulli, S. (2019). Emergent Ascomycetes in Viticulture: An Interdisciplinary Overview. *Frontiers in Plant Science*, *10*(November), 1–30. https://doi.org/10.3389/fpls.2019.01394

Pirrello, C., Zeilmaker, T., Bianco, L., Giacomelli, L., Moser, C., & Vezzulli, S. (2020). *Mining downy mildew susceptibility genes: a diversity study in grapevine*. https://doi.org/10.1101/2020.01.15.898700

Pompili, V., Dalla Costa, L., Piazza, S., Pindo, M., & Malnoy, M. (2020). Reduced fire blight susceptibility in apple cultivars using a high-efficiency CRISPR/Cas9-FLP/FRT-based gene editing system. *Plant Biotechnology Journal*, *18*(3), 845–858. https://doi.org/10.1111/pbi.13253

Porterfield, R., & Meru, G. (2017). Candidate Susceptibility Genes for Powdery and Downy Mildew in Watermelon and Squash. *Journal of Phylogenetics & Evolutionary Biology*, *05*(02). https://doi.org/10.4172/2329-9002.1000186

Ramming, D. W., Gabler, F., Smilanick, J., Cadle-Davidson, M., Barba, P., Mahanil, S., & Cadle-Davidson, L. (2011). A Single Dominant Locus, Ren4 , Confers Rapid Non-Race-Specific Resistance to Grapevine Powdery Mildew. *Phytopathology*, *101*(4), 502–508. https://doi.org/10.1094/PHYTO-09-10-0237

Raskin, I. (1992). Role of Salicylic Acid in Plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, *43*(1), 439–463. https://doi.org/10.1146/annurev.pp.43.060192.002255

Riaz, S., Tenscher, A. C., Ramming, D. W., & Walker, M. A. (2011). Using a limited mapping strategy to identify major QTLs for resistance to grapevine powdery mildew (Erysiphe necator) and their use in marker-assisted breeding. *TAG. Theoretical and Applied Genetics. Theoretische Und Angewandte Genetik*, *122*(6), 1059–1073. https://doi.org/10.1007/s00122-010-1511-6

Rienth, M., Crovadore, J., Ghaffari, S., & Lefort, F. (2019). Oregano essential oil vapour prevents Plasmopara viticola infection in grapevine (*Vitis* Vinifera) and primes plant immunity mechanisms. *PLoS ONE*, *14*(9), 1–29. https://doi.org/10.1371/journal.pone.0222854

Sapkota, S., Chen, L. L., Yang, S., Hyma, K. E., Cadle-Davidson, L., & Hwang, C. F. (2019). Construction of a high-density linkage map and QTL detection of downy mildew resistance in *Vitis* aestivalis-derived 'Norton'. *Theoretical and Applied Genetics*, *132*(1), 137–147. https://doi.org/10.1007/s00122-018-3203-6

Sargolzaei, M., Maddalena, G., Bitsadze, N., Maghradze, D., Bianco, P. A., Failla, O., … Lorenzis, G. De. (2020). *Rpv29 , Rpv30 and Rpv31 : Three Novel Genomic Loci Associated With Resistance to Plasmopara viticola in Vitis vinifera*. *11*(October), 1–16. https://doi.org/10.3389/fpls.2020.562432

Savocchia, S., Stummer, B., Scott, E., & Wicks, T. (1999). Detection of DMI resistance among populations of powdery mildew fungus. *The Australian Grapegrower and Winemaker*, (429), 39–41.

Schnabel, G., & Jones, A. L. (2001). The 14α-Demethylase (CYP51A1) gene is overexpressed in Venturia inaequalis strains resistant to myclobutanil. *Phytopathology*, *91*(1), 102–110. https://doi.org/10.1094/PHYTO.2001.91.1.102

Schouten, H. J., Krauskopf, J., Visser, R. G. F., & Bai, Y. (2014). Identification of candidate genes required for susceptibility to powdery or downy mildew in cucumber. *Euphytica*, *200*(3), 475–486. https://doi.org/10.1007/s10681-014-1216-z

Schwander, F., Eibach, R., Fechter, I., Hausmann, L., Zyprian, E., & Töpfer, R. (2012). Rpv10: A new locus from the Asian *Vitis* gene pool for pyramiding downy mildew resistance loci in grapevine. *Theoretical and Applied Genetics*, *124*(1), 163–176.

https://doi.org/10.1007/s00122-011-1695-4

Sombardier, A., Dufour, M., Blancard, D., & Corio‐Costet, M. (2010). Sensitivity of Podosphaera aphanis isolates to DMI fungicides: distribution and reduced cross‐sensitivity. *Pest Management Science: Formerly Pesticide Science*, *66*(1), 35–43. https://doi.org/https://doi.org/10.1002/ps.1827

Stergiopoulos, I., Van Nistelrooy, J. G. M., Kema, G. H. J., & De Waard, M. A. (2003). Multiple mechanisms account for variation in base-line sensitivity to azole fungicides in field isolates of Mycosphaerella graminicola. *Pest Management Science*, *59*(12), 1333–1343. https://doi.org/10.1002/ps.766

Steva, H., & Cazenave, C. (1996). *Evolution of grape powdery mildew insensitivity to DMI fungicides*.

Stuthman, D. D., Leonard, K. J., & Miller-Garvin, J. (2007). Breeding Crops for Durable Resistance to Disease. *Advances in Agronomy*, Vol. 95, pp. 319–367. https://doi.org/10.1016/S0065-2113(07)95004-X

Sun, K., van Tuinen, A., van Kan, J. A. L., Wolters, A. M. A., Jacobsen, E., Visser, R. G. F., & Bai, Y. (2017). Silencing of DND1 in potato and tomato impedes conidial germination, attachment and hyphal growth of Botrytis cinerea. *BMC Plant Biology*, *17*(1), 235. https://doi.org/10.1186/s12870-017-1184-2

Tegtmeier, R., Pompili, V., Singh, J., Micheletti, D., Silva, K. J. P., Malnoy, M., & Khan, A. (2020). Candidate gene mapping identifies genomic variations in the fire blight susceptibility genes HIPM and DIPM across the Malus germplasm. *Scientific Reports*, *10*(1), 1–12. https://doi.org/10.1038/s41598-020-73284-w

Teh, S. L., Fresnedo-Ramírez, J., Clark, M. D., Gadoury, D. M., Sun, Q., Cadle-Davidson, L., & Luby, J. J. (2017). Genetic dissection of powdery mildew resistance in interspecific half-sib grapevine families using SNP-based maps. *Molecular Breeding : New Strategies in Plant Improvement*, *37*(1), 1. https://doi.org/10.1007/s11032-016-0586-4

Toffolatti, S. L., De Lorenzis, G., Brilli, M., Moser, M., Shariati, V., Tavakol, E., … Quaglino, F. (2020). Novel aspects on the interaction between grapevine and Plasmopara viticola: Dual-RNA-seq analysis highlights gene expression dynamics in the pathogen and the plant during the battle for infection. *Genes*, *11*(3). https://doi.org/10.3390/genes11030261

Topfer.R, Hausmann L., 2010. (2010). Table of Loci for Traits in Grapevine Relevant for Breeding and Genetics. *VIVC - Vitis International Variety Catalogue*, *40024*(2017), 2–5. Retrieved from http://www.genoscope.cns.fr/*vitis*%0Ahttp://www.vivc.de/docs/dataonbreeding/20180122_Ta

ble of Loci for Traits in Grapevine.pdf

van Butselaar, T., & Van den Ackerveken, G. (2020). Salicylic Acid Steers the Growth–Immunity Tradeoff. *Trends in Plant Science*, *25*(6), 566–576. https://doi.org/10.1016/j.tplants.2020.02.002

Van Damme, M., Andel, A., Huibers, R. P., Panstruga, R., Weisbeek, P. J., & Van Den Ackerveken, G. (2005). Identification of Arabidopsis loci required for susceptibility to the downy mildew pathogen Hyaloperonospora parasitica. *Molecular Plant-Microbe Interactions*, *18*(6), 583–592. https://doi.org/10.1094/MPMI-18-0583

Van Damme, M., Huibers, R. P., Elberse, J., & Van Den Ackerveken, G. (2008). Arabidopsis DMR6 encodes a putative 2OG-Fe(II) oxygenase that is defense-associated but required for susceptibility to downy mildew. *Plant Journal*, *54*(5), 785–793. https://doi.org/10.1111/j.1365-313X.2008.03427.x

Van Damme, M., Zeilmaker, T., Elberse, J., Andel, A., De Sain-van Der Velden, M., & Van Den Ackerveken, G. (2009). Downy mildew resistance in arabidopsis by mutation of Homoserine Kinase. *Plant Cell*, *21*(7), 2179–2189. https://doi.org/10.1105/tpc.109.066811

van Schie, C. C. N., & Takken, F. L. W. (2014). Susceptibility Genes 101: How to Be a Good Host. *Annual Review of Phytopathology*, *52*(1), 551–581. https://doi.org/10.1146/annurev-phyto-102313-045854

Velasco, R., Zharkikh, A., Troggio, M., Cartwright, D. A., Cestaro, A., Pruss, D., … Viola, R. (2007). A High Quality Draft Consensus Sequence of the Genome of a Heterozygous Grapevine Variety. *PLoS ONE*, *2*(12), e1326. https://doi.org/10.1371/journal.pone.0001326

Venuti, S., Copetti, D., Foria, S., Falginella, L., Hoffmann, S., Bellin, D., … Di Gaspero, G. (2013). Historical Introgression of the Downy Mildew Resistance Gene Rpv12 from the Asian Species *Vitis* amurensis into Grapevine Varieties. *PLoS ONE*, *8*(4). https://doi.org/10.1371/journal.pone.0061228

Vezzulli, S., Zulini, L., & Stefanini, M. (2019). Genetics-assisted breeding for downy/powdery mildew and phylloxera resistance at FEM. *BIO Web of Conferences*, *12*, 01020. https://doi.org/10.1051/bioconf/20191201020

Vlot, A. C., Dempsey, D. A., & Klessig, D. F. (2009). Salicylic Acid, a Multifaceted Hormone to Combat Disease. *Annual Review of Phytopathology*, *47*(1), 177–206. https://doi.org/10.1146/annurev.phyto.050908.135202

Wan, D. Y., Guo, Y., Cheng, Y., Hu, Y., Xiao, S., Wang, Y., & Wen, Y. Q. (2020). CRISPR/Cas9-mediated mutagenesis of VvMLO3 results in enhanced resistance to powdery mildew in grapevine (*Vitis* vinifera). *Horticulture Research*, *7*(1).

https://doi.org/10.1038/s41438-020-0339-8

Welter, L. J., Göktürk-Baydar, N., Akkurt, M., Maul, E., Eibach, R., Töpfer, R., & Zyprian, E. (2007). Genetic mapping and localization of quantitative trait loci affecting fungal disease resistance and leaf morphology in grapevine (*Vitis* vinifera L). *Molecular Breeding*, *20*(4), 359–374. https://doi.org/10.1007/s11032-007-9097-7

Wen, J., Lu, L. M., Nie, Z. L., Liu, X. Q., Zhang, N., Ickert-Bond, S., … Chen, Z. D. (2018). A new phylogenetic tribal classification of the grape family (Vitaceae). *Journal of Systematics and Evolution*, *56*(4), 262–272. https://doi.org/10.1111/jse.12427

Wiedemann-Merdinoglu S., Prado E., Coste P., D. V., & Butterlin G., B. A. and M. D. (2006). Genetic analysis of resistance to downy mildew from Muscadinia rotundifolia. *Ninth International Conference on Grape Genetics and Breeding*. Udine.

Wilcox, W. F. (2003). *Black rot. Disease Identification Sheet No. 102GFSG-D4*. Retrieved from New York State IPM Program website: https://ecommons.cornell.edu/handle/1813/43076

Wilcox, W. F., Gubler, W. D., & Uyemoto, J. K. (2017). PART I: Diseases Caused by Biotic Factors. In W. F. Wilcox, W. D. Gubler, & J. K. Uyemoto (Eds.), *Compendium of Grape Diseases, Disorders, and Pests, Second Edition, Second Printing* (pp. 17–146). https://doi.org/10.1094/9780890544815.002

Winterhagen, P., Howard, S. F., Qiu, W., & Kovács, L. G. (2008). Transcriptional up-regulation of grapevine MLO genes in response to powdery mildew infection. *American Journal of Enology and Viticulture*, *59*(2), 159–168. Retrieved from http://www.ajevonline.org/content/59/2/159.long

Wyand, R. A., & Brown, J. K. M. (2005). Sequence variation in the CYP51 gene of Blumeria graminis associated with resistance to sterol demethylase inhibiting fungicides. *Fungal Genetics and Biology*, *42*(8), 726–735. https://doi.org/10.1016/j.fgb.2005.04.007

Ypema, H. L., Ypema, M., & Gubler, W. D. (1997). Sensitivity of Uncinula necator to benomyl, triadimefon, myclobutanil, and fenarimol in California. *Plant Disease*, *81*(3), 293–297. https://doi.org/10.1094/PDIS.1997.81.3.293

Zaidi, S. S. e. A., Mukhtar, M. S., & Mansoor, S. (2018, September 1). Genome Editing: Targeting Susceptibility Genes for Plant Disease Resistance. *Trends in Biotechnology*, Vol. 36, pp. 898–906. https://doi.org/10.1016/j.tibtech.2018.04.005

Zeilmaker, T., Ludwig, N. R., Elberse, J., Seidl, M. F., Berke, L., Van Doorn, A., … Van Den Ackerveken, G. (2015). Downy mildew resistant 6 and DMR6-like oxygenase 1 are partially redundant but distinct suppressors of immunity in Arabidopsis. *Plant Journal*, *81*(2), 210–222. https://doi.org/10.1111/tpj.12719

Zendler, D., Schneider, P., Töpfer, R., & Zyprian, E. (2017). Fine mapping of Ren3 reveals two loci mediating hypersensitive response against Erysiphe necator in grapevine. *Euphytica*, *213*(3), 68. https://doi.org/10.1007/s10681-017-1857-9

Zhang, J., Coaker, G., Zhou, J. M., & Dong, X. (2020). Plant Immune Mechanisms: From Reductionistic to Holistic Points of View. *Molecular Plant*, *13*(10), 1358–1378. https://doi.org/10.1016/j.molp.2020.09.007

Zhang, K., Halitschke, R., Yin, C., Liu, C.-J., & Gan, S.-S. (2013). Salicylic acid 3-hydroxylase regulates *Arabidopsis* leaf longevity by mediating salicylic acid catabolism. *Proceedings of the National Academy of Sciences*, *110*(36), 14807–14812. https://doi.org/10.1073/pnas.1302702110

Zhang, W., Mirlohi, S., Li, X., & He, Y. (2018). Identification of functional single-nucleotide polymorphisms affecting leaf hair number in Brassica rapa. *Plant Physiology*, *177*(2), 490–503. https://doi.org/10.1104/pp.18.00025

Zhang, Y. J., Zhao, L., Zhao, J. Z., Li, Y. J., Wang, J. Bin, Guo, R., … Zhanga, K. W. (2017). S5H/DMR6 encodes a salicylic acid 5-hydroxylase that fine-tunes salicylic acid homeostasis. *Plant Physiology*, *175*(3), 1082–1093. https://doi.org/10.1104/pp.17.00695

Zyprian, E., Ochßner, I., Schwander, F., Šimon, S., Hausmann, L., Bonow-Rex, M., … Töpfer, R. (2016). Quantitative trait loci affecting pathogen resistance and ripening of grapevines. *Molecular Genetics and Genomics : MGG*, *291*(4), 1573–1594. https://doi.org/10.1007/s00438-016-1200-5

AIM

In temperate-humid climates, viticulture is heavily threatened by fungal pathogens diseases as DM and PM. Susceptibility *(S)* genes are generating rising interest as sources of broad-spectrum and durable resistance. Their employment in grapevine disease control could represent a novel tool able to integrate and upgrade other defense strategies already widely explored.

Aim of this thesis was to investigate the role of the DM *S* genes *VvDMR6.1*, *VvDMR6.2*, *VvDLO1* and *VvDLO2*, and the PM *S* gene *VvMLO7* in grapevine through two different approaches.

The genetic diversity studies described in Chapters 2 and 3 aimed to scout *S* genes natural mutants in a wide panel of grapevine genotypes. Taking advantage of the identified mutations, a putative role of single genes in susceptibility to DM and PM could be drawn and novel genetic sources for genetic improvement programs could be proposed.

In Chapter 4, the first functional study on *S* genes to DM in grapevine is described. A functional study on *dmr6.1* grapevine plants was carried out with the aim of understanding the weight of *VvDMR6.1* in susceptibility to DM and its relationship with *VvDMR6.2*, *VvDLO1* and *VvDLO2*. Although not conclusive, this is the first information available on the role of *VvDMR6* and *VvDLO* genes in grapevine.

CHAPTER 2

Preliminary study on *VvMLO7* genetic diversity

INTRODUCTION

Since their first discovery in barley (*Hordeum vulgare*; Jorgensen, 1992), mutant *MLO* (*Mildew resistance Locus O*) genes drew the attention of the plant science community. Still many studies are focused on characterizing *MLO*s (e.g. Liyanage et al., 2020; Ramesha et al., 2020) to dissect their physiological role and validate their potential as susceptibility genes (e.g. Consonni et al., 2006; Bai et al., 2008; Humphry et al., 2011; Jiwan et al., 2013; Zheng et al., 2013; Qiu et al., 2015; Fujimura et al., 2016; Pessina et al., 2016 and others reviewed in Kusch & Panstruga, 2017).

Barley MLOs are plasma membrane-localized proteins, characterized by an extracellular N-terminus, seven transmembrane (TM) domains and an intracellular C-terminus. The disordered C-terminus contains a Calmodulin-binding domain, required for full susceptibility to PM (Devoto et al., 1999; Kim et al., 2002; Devoto et al., 2003). Structural integrity of the protein is conferred by four conserved cysteines in two extracellular domains (**Fig.1**) (Kusch et al., 2016).

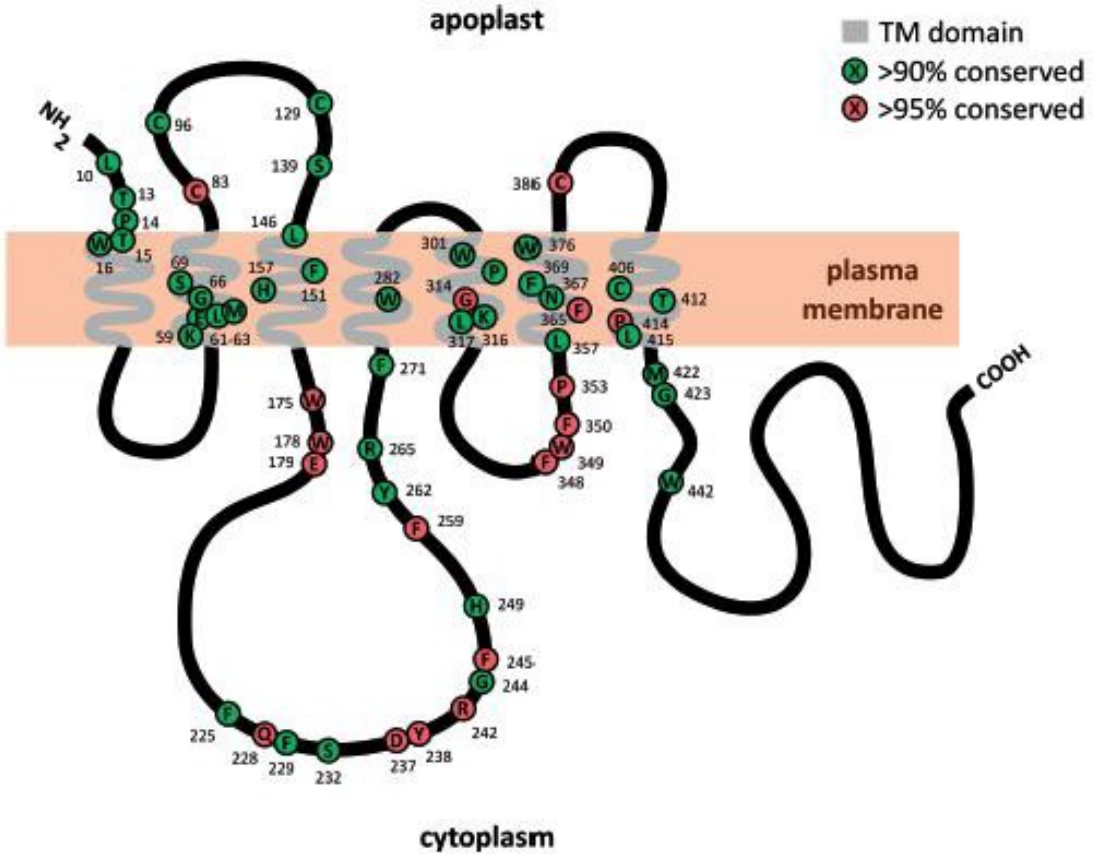


Figure 1. Embriophyt MLO protein with conserved aminoacids and TM domains (Kusch at al., 2016)

Regarding their function, studies on MLO proteins role in Arabidopsis showed their involvement in physiological aspects as root morphogenesis and architecture (Chen et al., 2009). In particular, it seems that the conducive role of MLOs for PM spread is also valid in the regulation of endophytic fungi and arbuscular mycorrhizae and, consequently, MLO inactivation leads to reduced symbiotic fungi colonization (Hilbert et al., 2020; Jacott et al.,, 2020). Moreover, the role of AtMLO5, AtMLO9, AtMLO15 (Meng et al., 2020), and AtMLO10 (Zhang et al., 2020) in pollen tube responses to ovular signals mediated by Ca^{2+} dynamics and interaction with AtCML9 (Calmodulin-like protein 9) has been defined. However, their active role in PM susceptibility finds foundation in many studies aimed to investigate pre-penetration resistance in *mlo* barley mutants: accumulation of callose and β-glucan cell-wall appositions at the penetration site; appositions number and diameter increase; localized cell death due to ROS and defense-related transcripts induction (Kusch & Panstruga, 2017).

Of the six clades identified, clade V is the one that contains members of the *MLO* family involved in PM susceptibility. In grapevine, seven members were found in clade V: *VvMLO1, VvMLO3, VvMLO6, VvMLO7, VvMLO9, VvMLO13* and *VvMLO17* (Feechan et al., 2008). Transcriptional induction by *E. necator* inoculation was observed in different studies: Feechan et al., (2008) noticed induction of *VvMLO3, VvMLO4* and *VvMLO17,* while also *VvMLO13* and *VvMLO7* were induced according to Winterhagen et al., (2008) and *VvMLO11* showed an interesting role in recovering the susceptible phenotype of a resistant mutant Arabidopsis (Feechan et al., 2013)**.** Still, a main role of *VvMLO7* in the susceptibility mechanism to PM was observed through a RNAi silencing experiment by Pessina et al., (2016).

As recently reviewed by Schenke & Cai (2020), genome editing via CRISPR/Cas has become the fastest and more used strategy to generate crops with new resistant traits. However, once new *S* genes are identified, a straightforward procedure consists in a broad-spectrum screening of the species germplasm in order to find natural mutants (Pirrello et al., 2020; Tegtmeier et al., 2020).

In this work, we aimed to investigate genetic diversity in *VvMLO7* throughout a panel of 190 grapevine accessions belonging to different taxons, focusing on SNPs (Single Nucleotide Polymorphisms) to identify potential protein function-disrupting mutations.

MATERIALS AND METHODS

Genetic material and target genes

In the current study, the *VvMLO7* (VIT_13s0019g04060) gene was scouted in 190 grapevine genotypes. Out of these, 139 (73%) are *Vitis* hybrids, 28 (15%) are *V. vinifera* varieties, 12 (6%) belong to wild *Vitis* species and additional 11 (6%) are ascribed as hybrids/wild species.

Amplicon sequencing and read processing

Genomic DNA was extracted from young grapevine leaves using DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany.) according to the manufacturer's protocol, then used to produce amplicons for deep-sequencing. PCR on the templates was performed using Phusion High-Fidelity Polymerase (NEB, Ipswich, Massachusetts, USA) according to the manufacturer's protocol. Primers were specifically designed to amplify 250 bp of the coding regions of target genes and barcoded (**Table 1**). A total of 8 amplicons was sequenced in-house using the Illumina MiSeq platform, covering 70% of the coding sequence.

Amplicon	Illumina forward primer	Illumina reverse primer	Amplicon Position
1	5'-CTCAACTGGGCGCTAGTGTT-3'	3'-CAACCCCGTCAGGAAAATAA-5'	Exon 1
2	5'-TGCAGTGGTTAAAAGGCAGA-3'	3'-AGCATGTGTTCTTAAAATCTTTGG-5'	Exon 2-Exon 3 Junction
3	5'-AAAGGTGTTTCCCACCCTCT-3'	3'-TTGATCCTCCCAAGCCTTC-5'	Exon 4-Exon 5 Junction
4	5'-TGTGTTGTTGCAGATCCAGAG-3'	3'-CAACGTTGTTAACCGATCTGAA-5'	Exon 6-Exon 7 Junction
5	5'-TTTCAGGCACATTTGTCACC-3'	3'-GCTACCAAAGAATGGTATGAGGA-5'	Exon 8-Exon 9 Junction
6	5'-GAGGCCACGAGACTGAGAAA-3'	3'-CCCTTCACCACATCACCTCT-5'	Exon 10-Exon 11 Junction
7	5'-TGCAGAGAGAGGTGATGTGG-3'	3'-AGTCAATCGCTTACCGTGCT-5'	Exon 11-Exon 12 Junction
8	5'-CCCCTCCTCATCTTTGGAGT-3'	3'-CCTGTGTCACCAAGGCATAG-5'	Exon 13-Exon 14 Junction

Table 1. Illumina amplicons and their primer pairs.

Obtained amplicons were then mapped on the PN40024 12X reference genome (Jaillon, 2007) considering the latest V2 gene prediction (Vitulo et al., 2014; Canaguier et al., 2017) through Burrows-Wheeler alignment (BWA; Li & Durbin, 2010), with no filter on mapping quality.

Data mining

Variant calling was performed by BCFtools (H. Li et al., 2009) using the following settings: minimum mapping quality 20; minimum genotype quality 20; minimum base quality 20; maximum per sample depth of coverage 1,000; minimum depth of coverage per site 10; keep read pairs with unexpected insert sizes (for amplicon sequencing). Filtering of results was done with VCFtools (Danecek et al., 2011) to exclude all genotypes with quality below 20 and include only genotypes with read depth ≥ 10. SnpEff was used to further discriminate variants according to their impact (MODIFIER, HIGH, MODERATE or LOW) on gene sequence (Cingolani et al., 2012). Elected-impacting variants were then subjected to SIFT (Sorting Intolerant From Tolerant) (Kumar et al., 2009) analysis to assess the tolerance of aminoacidic variants on the protein primary structure, based on the alignment with sequences in SWISS-PROT/TrEMBL database. Only not tolerated mutations were considered for a last impact evaluation based on variants chemical-physical properties according to Betts & Russel (2003). Both SnpEff and SIFT algorithms were used with default parameters settings.nCCtop web server (Dobson et al., 2015) was used to predict secondary structure of VvMLO7 protein. MUSCLE (Edgar, 2004) was used for protein sequence alignment.

RESULTS AND DISCUSSION

Mutations mapping and genetic diversity estimation

Genetic diversity of *VvMLO7* was assessed in 190 genotype accessions (**Table S1**). Sequencing and mapping on PN40024 12x V2 resulted in 14,622,201 total aligned reads. Of the total 396 mutations detected: 14 were In/Dels (~3.5%), 17 triallelics (~4.3%) and 365 biallelics (~92%). Considering all 365 biallelic mutations 54% were transitions (A↔G, C↔T) and 46% transversions (A↔C, A↔T, C↔G, G↔T), the transitions/transversions ratio of 1.2 remained the same when also triallelic mutations were considered. Of all point mutations detected in the 190 accessions, ~58% were heterozygous and ~42% were homozygous. Heterozygous/homozygous mutations ratio was similar (~1.5) in hybrid and *vinifera* taxons while in wild species and *spp*/hybrids it was closer to 1.

Variants discrimination according to their impact

Variant impact on codon sequence was used as parameter for discriminating the sequence mutations. According to SnpEff, variants can be distinguished in four different classes: “MODIFIER”, falling into intronic regions or upstream/downstream the gene; “LOW”, responsible for synonymous mutations or falling into splice regions; “MODERATE”, bringing to non-synonymous variants; “HIGH” impact, as being responsible for sequence frameshift or premature stop codon occurrence.

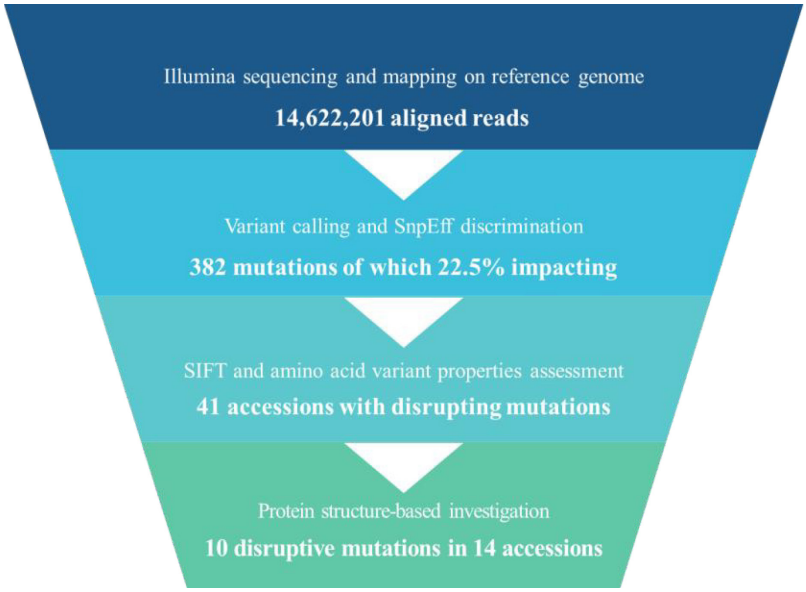


Figure 2. Bottleneck analysis flow with obtained results from every analysis step.

In this study, 49.5% of mutations was classified as "MODIFIER", ~28% as "LOW" and, as expected, a smaller percentage was defined as "MODERATE" (19%) and "HIGH" (~3.5%). The sum of 87 (22.5%) variants showing "HIGH" or "MODERATE" impact was then submitted to a bottleneck analysis flow (**Fig. 2**).

Since SnpEff is usually used to investigate nucleotide variants within the coding region (Amrine et al., 2015; Cardone et al., 2016), low percentages of "MODIFIER" variants are detected. In our study, a significantly high percentage (49,5%) of mutations falling in intronic regions was found in *VvMLO7*. This finding is easily explained by the VvMLO7 gene structure, which is rich of short and shortly interspaced exons, and by the position of the sequenced amplicons (**Table 1**), often including short introns as bridge between two coding regions (**Fig. 3**).

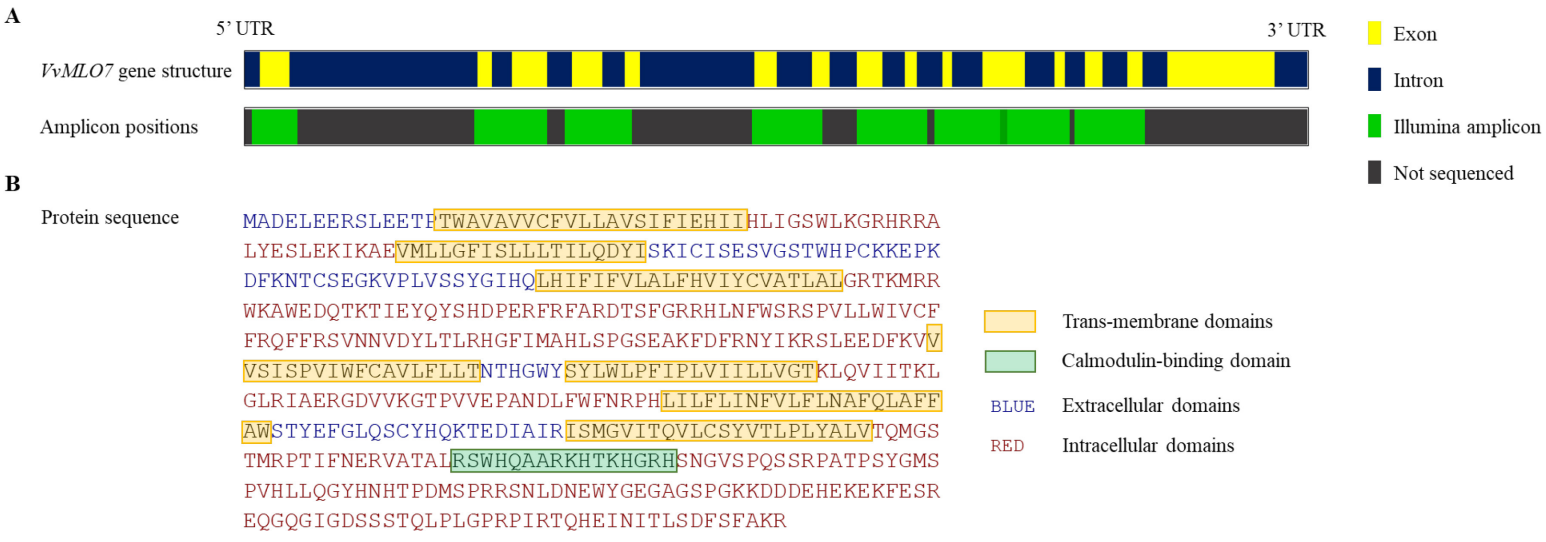


Figure 3. A) *VvMLO7* gene structure with exon and intron positions, amplicons distribution and their coverage with Illumina sequencing. B) VvMLO7 protein sequence with domain positions defined by CCtop (Dobson et al., 2015).

Focusing on the effect of the mutated codons, selected variants were then checked, using the SIFT algorithm, to discover their disruptive potential on VvMLO7 protein secondary structure. Following this analysis, 41 accessions showed at least one disrupting mutation at the heterozygous or homozygous state (**Table 2**). Only two *vinifera* were included in this group, one *spp.*/hybrid, seven wild species and 31 hybrids. Such a high representativeness of hybrid individuals is most likely due to their high genetic variability.

Genotype	**Taxon**	***VvMLO7***
29-2-322	*Vitis* hybrid	HE
30-3-154	*Vitis* hybrid	HO
BS 4825	*Vitis* hybrid	HO
Chancellor	*Vitis* hybrid	HE
Clinton	*Vitis* hybrid	HE
Diamond Muscat	*Vitis* hybrid	HE
F243 Tamiani	*Vitis* hybrid	HE
F9-68	*Vitis* hybrid	HE
FLA BN6-85	*Vitis* hybrid	HE
JS 23-416	*Vitis* hybrid	HE
Kunbaràt	*Vitis* hybrid	HE
Leon Millot	*Vitis* hybrid	HO
Mars	*Vitis* hybrid	HE
MW 1bis	*Vitis* hybrid	HO
MW 38	*Vitis* hybrid	HE
MW 53	*Vitis* hybrid	HE
NY08.0701a	*Vitis* hybrid	HO
NY08.0701b	*Vitis* hybrid	HO
NY65.0562.01	*Vitis* hybrid	HO
NY84.0100.05	*Vitis* hybrid	HO
NY95.0308.02	*Vitis* hybrid	HO
NY97.0503.02	*Vitis* hybrid	HO
NY97.0512.01	*Vitis* hybrid	HO
Petra	*Vitis* hybrid	HE
Poloskei Muskotaly	*Vitis* hybrid	HE
Prior	*Vitis* hybrid	HE
Ribier	*Vitis* hybrid	HE
Roucaneuf	*Vitis* hybrid	HE
Seibel 6339	*Vitis* hybrid	HE
Sheridan	*Vitis* hybrid	HE
V. riparia x V. cordifolia	*Vitis* hybrid	HO
V. aestivalis	*Vitis* spp.	HE
V. berlandieri Texas	*Vitis* spp.	HO
V. cordifolia	*Vitis* spp.	HO
V. rubra	*Vitis* spp.	HE
V. rupestris	*Vitis* spp.	HO
V. rupestris Constantia	*Vitis* spp.	HO
V. smalliana	*Vitis* spp.	HE
Coia11	*Vitis* spp./hybrid	HE
Nosiola	*Vitis vinifera*	HE
Riesling	*Vitis vinifera*	HE

Table 2. List of accessions showing heterozygous (HE) and homozygous (HO) impacting mutations in *VvMLO7*.

Protein structure-based investigation

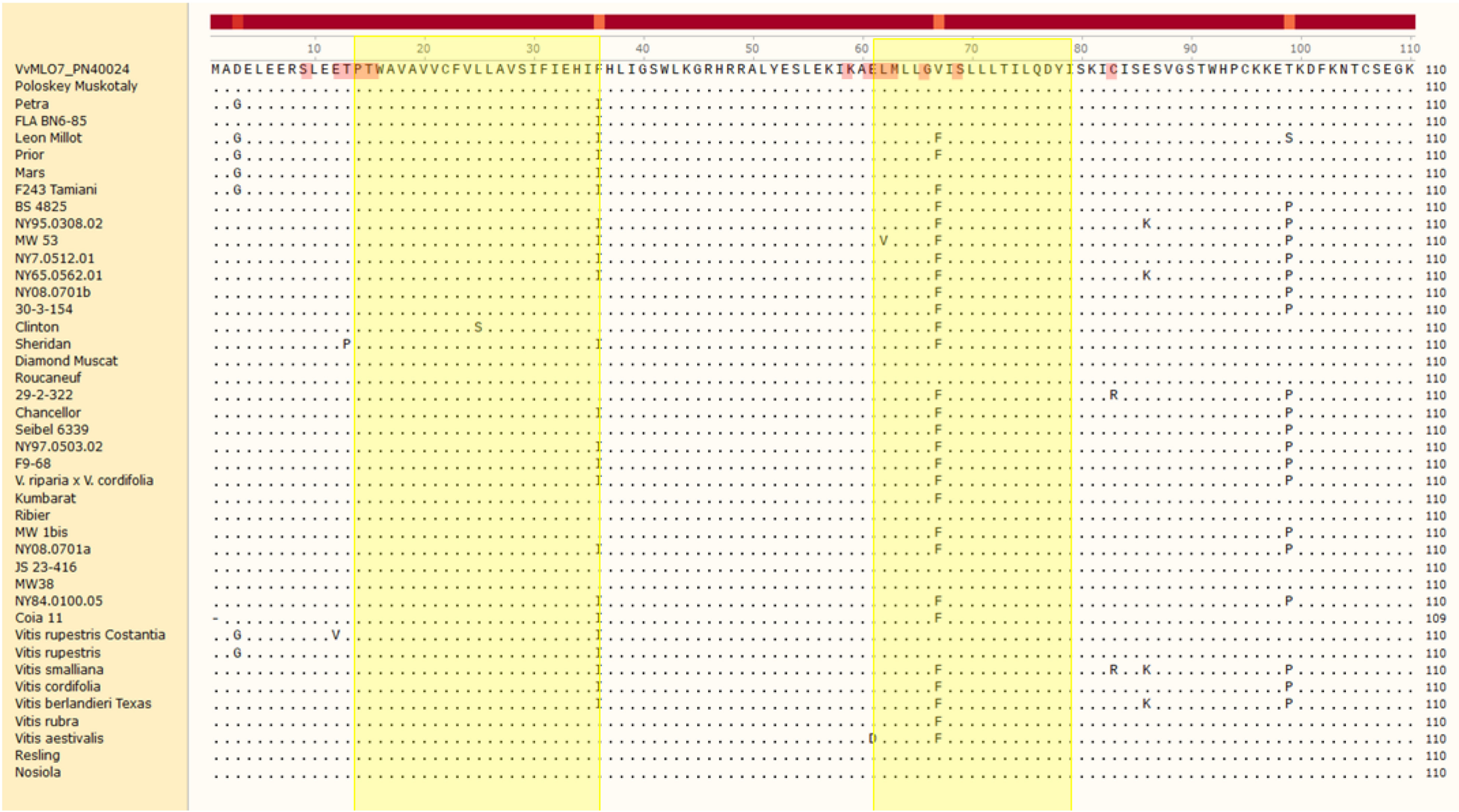
Integral membrane proteins assume a three dimensional conformation which is strongly dictated by the physico-chemical properties of the membrane lipid-bilayer where they reside. Bilayers are composed of two leaflets of amphiphilic phospholipids with the polar heads exposed to the solvent and the acyl chains closely packed and stabilized by hydrophobic interactions. Generally, membrane proteins can be divided into two classes: α-helix bundles and β-barrels, the first class being more abundant than the second one. Integral membrane proteins with α-helical structure contain usually several membrane spanning α-helices that may oligomerize into "bundles" connected by flexible loops, which protrude from the membrane (McKay et al., 2018).

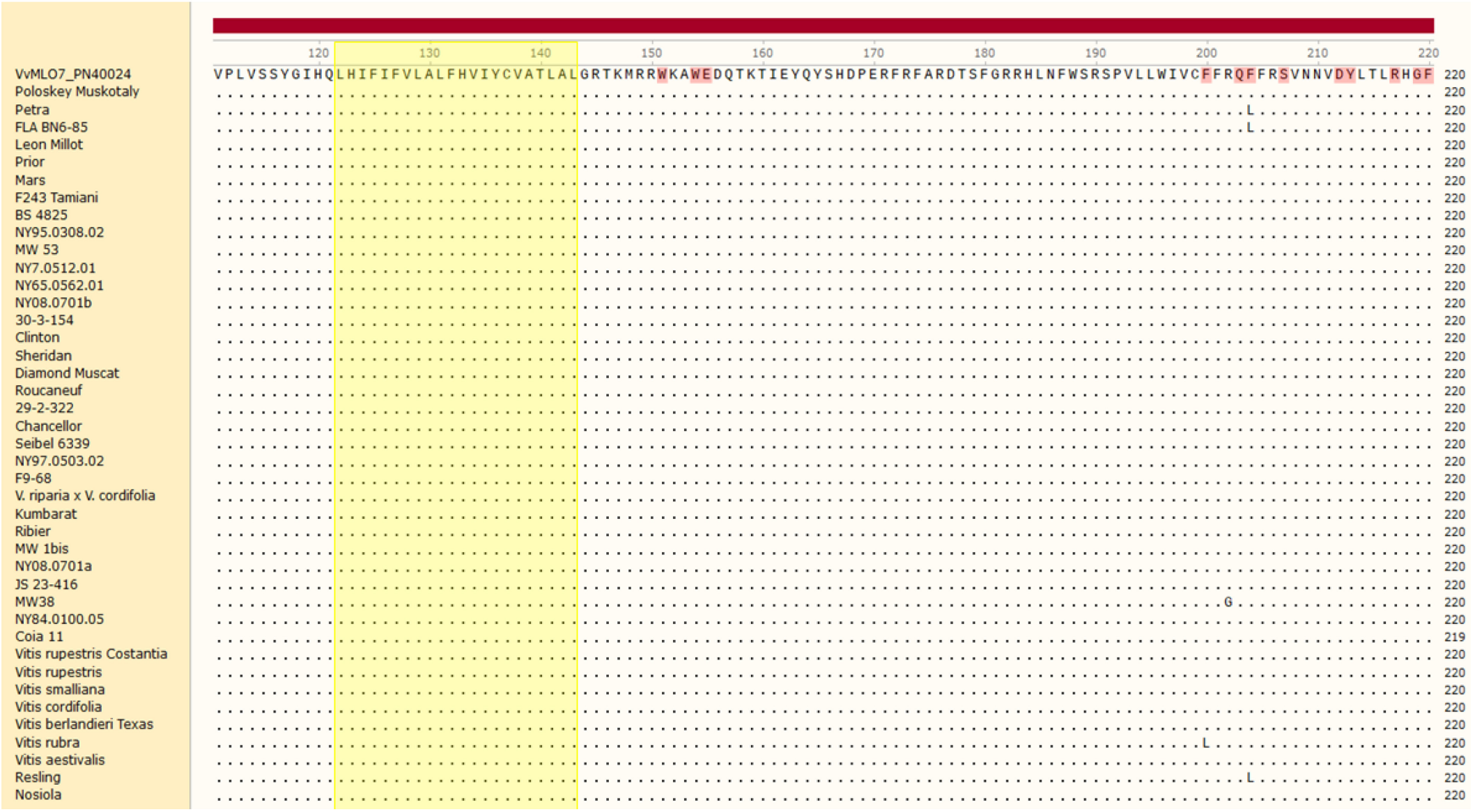
The introduction of polar aminoacids in α-helices can cause severe consequences on protein function, as the V664E oncogenic mutation in a transmembrane domain of a human RTK (Receptor Tyrosine Kinase), observed by Placone et al. (2014). Given our interest in finding disrupting mutations in VvMLO7, α-helical protein domains are thus especially palatable for our scope, together with the conserved residues highlighted by (Kusch et al., 2016) in a comprehensive phylogenetic analysis of the MLO proteins and in the calmodulin-binding site.

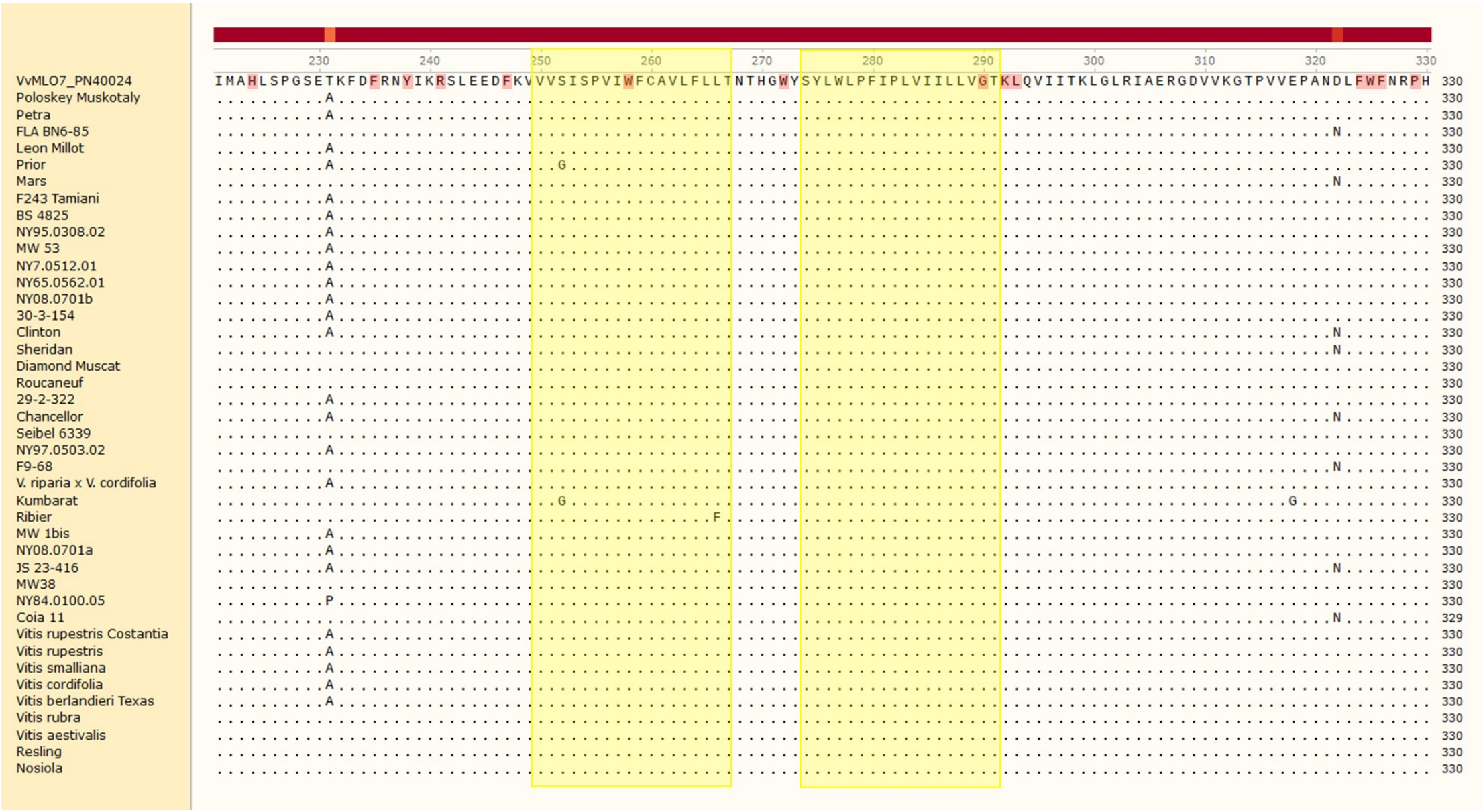
In the specific case of VvMLO7, according to CCtop (Dobson et al., 2015), the seven transmembrane domains were predicted with a reliability of 94.3, in the following positions: TM1 in Thr15-Ile36,TM2in Val62-Ile79, TM3 in Leu122-Leu143, TM4 in Val250-Thr267, TM5 in Ser274-Thr291, TM6 in Leu331-Trp352, TM7 in Ser374-Val395 An alignment of the MLO7 sequence of the 41 selected accessions was carried out with the aim to position the potentially disrupting mutations within the primary sequence. The scouting of these mutations was focused on TM domains and conserved aminoacids as indicated by Kusch et al., (2016), of which TM domains are particularly rich. Mutations were also investigated in the Calmodulin (CaM)-binding site (**Fig.5**), which was spotted in VvMLO7 in position Arg416-His431 through an alignment with AtMLO7 and ZmMLO7 and, according to Kim et al. (2002). CaM must contain a Tryptophan (W) with an aliphatic residue three positions before it and another one four positions after it.

Considering all disrupting mutations occurring in TM domains: a L25S variant was observed in TM1 in Clinton; MW 53 showed a L63V in TM2; both Prior and Kunbaràt had a S252G substitution in TM3; in TM4 Chancellor showed a A351E variant, A344E and Q346R were found in Nosiola TM6; Y386STOP resulted in disrupting TM7 in Poloskei Muskotaly, Diamond Muscat and Roucaneuf; and JS 23-416 showed variants G377E and C384Y. Mutations were also investigated in conserved aminoacid positions indicated by Kusch et al., (2016). No mutations were detected in the CaM-binding domain in the majority of the assessed genotypes but in three cases, the occurrence of a STOP

codon upstream to the calmodulin-binding domain, resulted in a complete loss of this signaling function. All disrupting mutations are reported in **Table 3**.


10
20
30
40
50
60
70
80
90
100
110
VvMLO7_PN40024 MADELEERSLEETPTWAVAVVCFVLLAVSIFIEHIFHLIGSWLKGRHRRALYESLEKIKAELMLLGVISLLLTILQDYISKICISESVGSTWHPCKKETKDFKNTCSEGK 110
Poloskey Muskotaly 110
Petra 110
FLA BN6-85 110
Leon Millot 110
Prior 110
Mars 110
F243 Tamiani 110
BS 4825 110
NY95.0308.02 110
MW 53 110
NY7.0512.01 110
NY65.0562.01 110
NY08.0701b 110
30-3-154 110
Clinton 110
Sheridan 110
Diamond Muscat 110
Roucaneuf 110
29-2-322 110
Chancellor 110
Seibel 6339 110
NY97.0503.02 110
F9-68 110
V. riparia x V. cordifolia 110
Kumbarat 110
Ribier 110
MW 1bis 110
NY08.0701a 110
JS 23-416 110
MW38 110
NY84.0100.05 110
Coia 11 109
Vitis rupestris Costantia 110
Vitis rupestris 110
Vitis smalliana 110
Vitis cordifolia 110
Vitis berlandieri Texas 110
Vitis rubra 110
Vitis aestivalis 110
Resling 110
Nosiola 110


120 130 140 150 160 170 180 190 200 210 220
VvMLO7_PN40024 VPLVSSYGIHQLHIFIFVLALFHVIYCVATLALGRTKMRRWKAWEDQTKTIEYQYSHDPERFRFARDTSFGRRHLNFWSRSPVLLWIVCFFRQFFRSVNNVDYLTLRHGF 220
Poloskey Muskotaly 220
Petra 220
FLA BN6-85 220
Leon Millot 220
Prior 220
Mars 220
F243 Tamiani 220
BS 4825 220
NY95.0308.02 220
MW 53 220
NY7.0512.01 220
NY65.0562.01 220
NY08.0701b 220
30-3-154 220
Clinton 220
Sheridan 220
Diamond Muscat 220
Roucaneuf 220
29-2-322 220
Chancellor 220
Seibel 6339 220
NY97.0503.02 220
F9-68 220
V. riparia x V. cordifolia 220
Kumbarat 220
Ribier 220
MW 1bis 220
NY08.0701a 220
JS 23-416 220
MW38 220
NY84.0100.05 220
Coia 11 219
Vitis rupestris Costantia 220
Vitis rupestris 220
Vitis smalliana 220
Vitis cordifolia 220
Vitis berlandieri Texas 220
Vitis rubra 220
Vitis aestivalis 220
Resling 220
Nosiola 220


230 240 250 260 270 280 290 300 310 320 330
VvMLO7_PN40024 IMAHLSPGSETKFDFRNYIKRSLEEDFKVVVSISPVIWFCAVLFLLTNTHGWYSYLWLPFIPLVIILLVGTKLQVIITKLGLRIAERGDVVKGTPVVEPANDLFWFNRPH 330
Poloskey Muskotaly 330
Petra 330
FLA BN6-85 330
Leon Millot 330
Prior 330
Mars 330
F243 Tamiani 330
BS 4825 330
NY95.0308.02 330
MW 53 330
NY7.0512.01 330
NY65.0562.01 330
NY08.0701b 330
30-3-154 330
Clinton 330
Sheridan 330
Diamond Muscat 330
Roucaneuf 330
29-2-322 330
Chancellor 330
Seibel 6339 330
NY97.0503.02 330
F9-68 330
V. riparia x V. cordifolia 330
Kumbarat 330
Ribier 330
MW 1bis 330
NY08.0701a 330
JS 23-416 330
MW38 330
NY84.0100.05 330
Coia 11 329
Vitis rupestris Costantia 330
Vitis rupestris 330
Vitis smalliana 330
Vitis cordifolia 330
Vitis berlandieri Texas 330
Vitis rubra 330
Vitis aestivalis 330
Resling 330
Nosiola 330

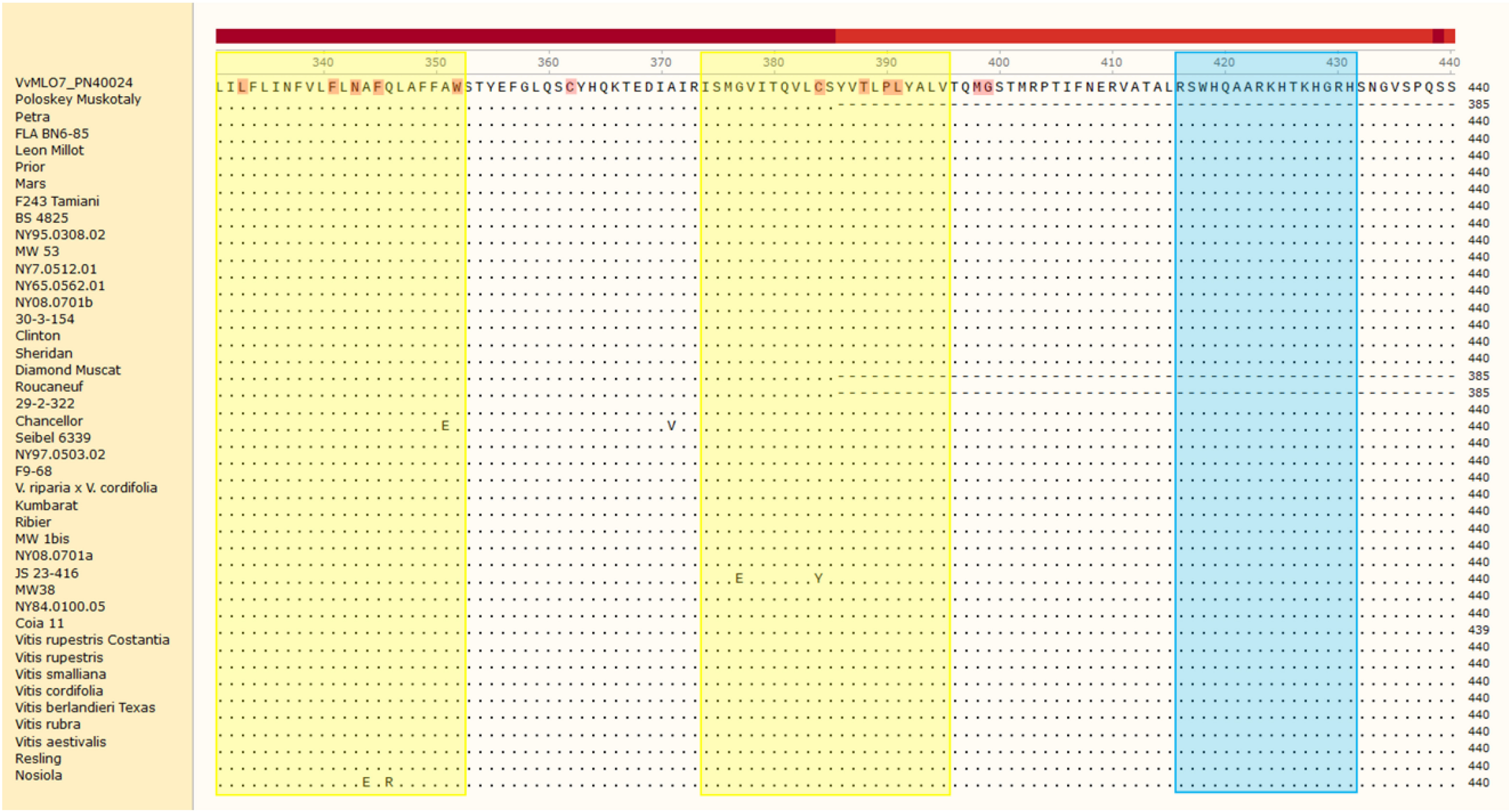

340
350
360
370
380
390
400
410
420
430
440
VvMLO7_PN40024
Poloskey Muskotaly
Petra
FLA BN6-85
Leon Millot
Prior
Mars
F243 Tamiani
BS 4825
NY95.0308.02
MW 53
NY7.0512.01
NY65.0562.01
NY08.0701b
30-3-154
Clinton
Sheridan
Diamond Muscat
Roucaneuf
29-2-322
Chancellor
Seibel 6339
NY97.0503.02
F9-68
V. riparia x V. cordifolia
Kumbarat
Ribier
MW 1bis
NY08.0701a
JS 23-416
MW38
NY84.0100.05
Coia 11
Vitis rupestris Costantia
Vitis rupestris
Vitis smalliana
Vitis cordifolia
Vitis berlandieri Texas
Vitis rubra
Vitis aestivalis
Resling
Nosiola

Figure 5. MLO7 sequence alignment of accessions showing impacting mutations. Accessions are sorted in this order: hybrids, wild species, wild species/hybrid and *vinifera* spp. Yellow squares represent TM domains, blue square represent the CaM-binding site, conserved residues are in pink. The red line represents highly conserved regions in dark red and less conserved regions in lighter red, based on the alignment.

Ten putatively disrupting amino acid substitutions were finally detected throughout 14 accessions and all of them were at the heterozygous state (**Table 3**).

Variant	Accession	OIV 455 (-1)
T13P	Sheridan	6
L25S	Clinton	9
C83R	29-3-322	-
	V. smalliana	9
F204L	Petra	3
	FLA BN6-85	3
	Riesling	2
A344E	Nosiola	-
A351E	Chancellor	4
G377E	JS 23-416	2
C384Y	JS 23-416	2
Y386STOP	Poloskei Muskotaly	-
	Diamond Muscat	-
	Roucaneuf	8
M1-	Coia 11	9

Table 3. List of disrupting mutations and of the accessions where they were detected. Accession OIV 455 (-1) scores (OIV, 2009) were retrieved from www.vivc.de.

Variants obtained downstream this bottleneck analysis were unique, except for the mutation Ala344Glu in Nosiola which was observed also in Chancellor at position 351. In most cases, the mutations substitute neutral and hydrophobic amino acids with polar or charged amino acids, as for example the mutation from the neutral cysteine to the positively charged arginine. These remarkable changes in hydropathy and charge might greatly impact the local secondary structure in a way which is putatively able to disrupt the protein function and consequently affect plant phenotype, especially when they occur within the transmembrane domains of the protein.

Given the aim of this study, ~3% of total point mutations in 7% of accessions were identified as putatively impacting on protein function. Surely, mutations which interrupt protein translation due to premature STOP codon or completely inhibit it with a START codon loss are of particular interest. Their existence in the panel of accessions that we examined is a good omen in view of a spread of this type of analysis in plant science community. Unfortunately, since *VvMLO7* is a recessive gene and all detected mutations were in heterozygosity, no association between genotypes observed and phenotypical information regarding PM susceptibility was possible. However, this kind of resources remain firmly valuable in a breeding-aimed strategy.

REFERENCES

Amrine, K. C. H., Blanco-Ulate, B., Riaz, S., Pap, D., Jones, L., Figueroa-Balderas, R., … Cantu, D. (2015). Comparative transcriptomics of Central Asian *Vitis* vinifera accessions reveals distinct defense strategies against powdery mildew. *Horticulture Research*, *2*(July). https://doi.org/10.1038/hortres.2015.37

Bai, Y., Pavan, S., Zheng, Z., Zappel, N. F., Reinstädler, A., Lotti, C., … Panstruga, R. (2008). Naturally occurring broad-spectrum powdery mildew resistance in a Central American tomato accession is caused by loss of Mlo function. *Molecular Plant-Microbe Interactions*, *21*(1), 30–39. https://doi.org/10.1094/MPMI-21-1-0030

Betts, M. J., & Russel, R. B. (2003). Amino acid properties and consequences of substitutions. In *Bioinformatics for Geneticists* (pp. 289–316). https://doi.org/10.1002/0470867302.ch14

Canaguier, A., Grimplet, J., Di Gaspero, G., Scalabrin, S., Duchêne, E., Choisne, N., … Adam-Blondon, A. F. (2017). A new version of the grapevine reference genome assembly (12X.v2) and of its annotation (VCost.v3). *Genomics Data*, *14*(July), 56–62. https://doi.org/10.1016/j.gdata.2017.09.002

Cardone, M. F., D'Addabbo, P., Alkan, C., Bergamini, C., Catacchio, C. R., Anaclerio, F., … Antonacci, D. (2016). Inter-varietal structural variation in grapevine genomes. *Plant Journal*, *88*(4), 648–661. https://doi.org/10.1111/tpj.13274

Cingolani, P., Platts, A., Wang, L. L., Coon, M., Nguyen, T., Wang, L., … Ruden, D. M. (2012). A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff. *Fly*, *6*(2), 80–92. https://doi.org/10.4161/fly.19695

Consonni, C., Humphry, M. E., Hartmann, H. A., Livaja, M., Durner, J., Westphal, L., … Panstruga, R. (2006). Conserved requirement for a plant host cell protein in powdery mildew pathogenesis. *Nature Genetics*, *38*(6), 716–720. https://doi.org/10.1038/ng1806

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., … Durbin, R. (2011). The variant call format and VCFtools. *Bioinformatics*, *27*(15), 2156–2158. https://doi.org/10.1093/bioinformatics/btr330

Devoto, A., Hartmann, H. A., Piffanelli, P., Elliott, C., Simmons, C., Taramino, G., … Panstruga, R. (2003). Molecular phylogeny and evolution of the plant-specific seven-transmembrane MLO family. *Journal of Molecular Evolution*, *56*(1), 77–88. https://doi.org/10.1007/s00239-002-2382-5

Devoto, A., Piffanelli, P., Nilsson, I., Wallin, E., Panstruga, R., von Heijne, G., & Schulze-Lefert, P. (1999). Topology, Subcellular Localization, and Sequence Diversity of the Mlo Family in Plants. *Journal of Biological Chemistry*, *274*(49), 34993–35004.

https://doi.org/10.1074/jbc.274.49.34993

Dobson, L., Reményi, I., & Tusnády, G. E. (2015). CCTOP: A Consensus Constrained TOPology prediction web server. *Nucleic Acids Research*, *43*(W1), W408–W412. https://doi.org/10.1093/nar/gkv451

Edgar, R. C. (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, *32*(5), 1792–1797. https://doi.org/10.1093/nar/gkh340

Feechan, A., Jermakow, A. M., Ivancevic, A., Godfrey, D., Pak, H., Panstruga, R., & Dry, I. B. (2013). Host Cell Entry of Powdery Mildew Is Correlated with Endosomal Transport of Antagonistically Acting VvPEN1 and VvMLO to the Papilla. *Molecular Plant-Microbe Interactions*, *26*(10), 1138–1150. https://doi.org/10.1094/MPMI-04-13-0091-R

Feechan, A., Jermakow, A. M., Torregrosa, L., Panstruga, R., & Dry, I. B. (2008). Identification of grapevine MLO gene candidates involved in susceptibility to powdery mildew. *Functional Plant Biology*, *35*(12), 1255. https://doi.org/10.1071/FP08173

Fujimura, T., Sato, S., Tajima, T., & Arai, M. (2016). Powdery mildew resistance in the Japanese domestic tobacco cultivar Kokubu is associated with aberrant splicing of MLO orthologues. *Plant Pathology*, *65*(8), 1358–1365. https://doi.org/10.1111/ppa.12498

Hilbert, M., Novero, M., Rovenich, H., Mari, S., Grimm, C., Bonfante, P., & Zuccaro, A. (2020). MLO Differentially Regulates Barley Root Colonization by Beneficial Endophytic and Mycorrhizal Fungi. *Frontiers in Plant Science*, *10*(January), 1–11. https://doi.org/10.3389/fpls.2019.01678

Humphry, M., Reinstädler, A., Ivanov, S., Bisseling, T., & Panstruga, R. (2011). Durable broad-spectrum powdery mildew resistance in pea er1 plants is conferred by natural loss-of-function mutations in PsMLO1. *Molecular Plant Pathology*, *12*(9), 866–878. https://doi.org/10.1111/j.1364-3703.2011.00718.x

Jacott, C. N., Charpentier, M., Murray, J. D., & Ridout, C. J. (2020). Mildew Locus O facilitates colonization by arbuscular mycorrhizal fungi in angiosperms. *New Phytologist*, *227*(2), 343–351. https://doi.org/10.1111/nph.16465

Jaillon. (2007). The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature*. https://doi.org/10.1038/nature06148

Jiwan, D., Roalson, E. H., Main, D., & Dhingra, A. (2013). Antisense expression of peach mildew resistance locus O (PpMlo1) gene confers cross-species resistance to powdery mildew in Fragaria x ananassa. *Transgenic Research*, *22*(6), 1119–1131. https://doi.org/10.1007/s11248-013-9715-6

Jorgensen, J. H. (1992). *Discovery, characterization and exploitation of Mlo powdery mildew*.

66(Table 1), 141–152.

Kim, Min C, Panstruga, R., Elliott, C., Müller, J., Devoto, A., Yoon, H. W., … Schulze-Lefert, P. (2002). Calmodulin interacts with MLO protein to regulate defence against mildew in barley. *Nature*, *416*(6879), 447–451. https://doi.org/10.1038/416447a

Kim, Min Chul, Lee, S. H., Kim, J. K., Chun, H. J., Choi, M. S., Chung, W. S., … Cho, M. J. (2002). Mlo, a modulator of plant defense and cell death, is a novel calmodulin-binding protein. Isolation and characterization of a rice Mlo homologue. *Journal of Biological Chemistry*, *277*(22), 19304–19314. https://doi.org/10.1074/jbc.M108478200

Kumar, P., Henikoff, S., & Ng, P. C. (2009). Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nature Protocols*, *4*(7), 1073–1082. https://doi.org/10.1038/nprot.2009.86

Kusch, S., & Panstruga, R. (2017). mlo-Based Resistance: An Apparently Universal 'Weapon' to Defeat Powdery Mildew Disease. *Molecular Plant-Microbe Interactions : MPMI*, *30*(3), 179–189. https://doi.org/10.1094/MPMI-12-16-0255-CR

Kusch, S., Pesch, L., & Panstruga, R. (2016). Comprehensive phylogenetic analysis sheds light on the diversity and origin of the MLO family of integral membrane proteins. *Genome Biology and Evolution*, *8*(3), 878–895. https://doi.org/10.1093/gbe/evw036

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., … Durbin, R. (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics*, *25*(16), 2078–2079. https://doi.org/10.1093/bioinformatics/btp352

Li, Heng, & Durbin, R. (2010). Fast and accurate long-read alignment with Burrows–Wheeler transform. *Bioinformatics*, *26*(5), 589–595. https://doi.org/10.1093/bioinformatics/btp698

Liyanage, K. K., Khan, S., Herath, V., Brooks, S., Mortimer, P. E., Nadir, S., … Xu, J. (2020). Genome Wide Identification of the MLO Gene Family Associated with Powdery Mildew Resistance in Rubber Trees (Hevea brasiliensis). *Tropical Plant Biology*. https://doi.org/10.1007/s12042-020-09262-3

McKay, M. J., Afrose, F., Koeppe, R. E., & Greathouse, D. V. (2018). Helix formation and stability in membranes. *Biochimica et Biophysica Acta - Biomembranes*, *1860*(10), 2108–2117. https://doi.org/10.1016/j.bbamem.2018.02.010

Meng, J. G., Liang, L., Jia, P. F., Wang, Y. C., Li, H. J., & Yang, W. C. (2020). Integration of ovular signals and exocytosis of a Ca2+ channel by MLOs in pollen tube guidance. *Nature Plants*, *6*(2), 143–153. https://doi.org/10.1038/s41477-020-0599-1

OIV (International Organisation of Vine and Wine). (2009). *OIV descriptor list for grape varieties and Vitis species*. Paris.

Pessina, S., Angeli, D., Martens, S., Visser, R. G. F., Bai, Y., Salamini, F., … Malnoy, M. (2016). The knock-down of the expression of MdMLO19 reduces susceptibility to powdery mildew (Podosphaera leucotricha) in apple (Malus domestica). *Plant Biotechnology Journal*, *14*(10), 2033–2044. https://doi.org/10.1111/pbi.12562

Pessina, S., Lenzi, L., Perazzolli, M., Campa, M., Dalla Costa, L., Urso, S., … Malnoy, M. (2016). Knockdown of MLO genes reduces susceptibility to powdery mildew in grapevine. *Horticulture Research*, *3*(1), 16016. https://doi.org/10.1038/hortres.2016.16

Pirrello, C., Zeilmaker, T., Bianco, L., Giacomelli, L., Moser, C., & Vezzulli, S. (2020). *Mining downy mildew susceptibility genes: a diversity study in grapevine*. https://doi.org/10.1101/2020.01.15.898700

Placone, J., He, L., Del Piccolo, N., & Hristova, K. (2014). Strong dimerization of wild-type ErbB2/Neu transmembrane domain and the oncogenic Val664Glu mutant in mammalian plasma membranes. *Biochimica et Biophysica Acta - Biomembranes*, *1838*(9), 2326–2330. https://doi.org/10.1016/j.bbamem.2014.03.001

Qiu, W., Feechan, A., & Dry, I. (2015). Current understanding of grapevine defense mechanisms against the biotrophic fungus (Erysiphe necator), the causal agent of powdery mildew disease. *Horticulture Research*, *2*(April), 1–9. https://doi.org/10.1038/hortres.2015.20

Ramesha, A., Dubey, H., Vijayan, K., Ponnuvel, K. M., Mishra, R. K., & Suresh, K. (2020). Genome wide characterization revealed MnMLO2 and MnMLO6A as candidate genes involved in powdery mildew susceptibility in mulberry. *Molecular Biology Reports*, *47*(4), 2889–2900. https://doi.org/10.1007/s11033-020-05395-6

Schenke, D., & Cai, D. (2020). Applications of CRISPR/Cas to Improve Crop Disease Resistance: Beyond Inactivation of Susceptibility Factors. *IScience*, *23*(9), 101478. https://doi.org/10.1016/j.isci.2020.101478

Tegtmeier, R., Pompili, V., Singh, J., Micheletti, D., Silva, K. J. P., Malnoy, M., & Khan, A. (2020). Candidate gene mapping identifies genomic variations in the fire blight susceptibility genes HIPM and DIPM across the Malus germplasm. *Scientific Reports*, *10*(1), 1–12. https://doi.org/10.1038/s41598-020-73284-w

Vitulo, N., Forcato, C., Carpinelli, E., Telatin, A., Campagna, D., D'Angelo, M., … Valle, G. (2014). A deep survey of alternative splicing in grape reveals changes in the splicing machinery related to tissue, stress condition and genotype. *BMC Plant Biology*, *14*(1), 99. https://doi.org/10.1186/1471-2229-14-99

Winterhagen, P., Howard, S. F., Qiu, W., & Kovács, L. G. (2008). Transcriptional up-regulation of grapevine MLO genes in response to powdery mildew infection. *American Journal of*

Enology and Viticulture, *59*(2), 159–168. Retrieved from http://www.ajevonline.org/content/59/2/159.long

Zhang, Q., Hou, C., Tian, Y., Tang, M., Feng, C., Ren, Z., … Li, L. (2020). Interaction Between AtCML9 and AtMLO10 Regulates Pollen Tube Development and Seed Setting. *Frontiers in Plant Science*, *11*(July), 1–10. https://doi.org/10.3389/fpls.2020.01119

Zheng, Z., Nonomura, T., Appiano, M., Pavan, S., Matsuda, Y., Toyoda, H., … Bai, Y. (2013). Loss of Function in Mlo Orthologs Reduces Susceptibility of Pepper and Tomato to Powdery Mildew Disease Caused by Leveillula taurica. *PLoS ONE*, *8*(7). https://doi.org/10.1371/journal.pone.0070723

SUPPLEMENTARY MATERIAL

Genotype	Taxon	Breeder/Institute of Provenience	Repository
01-1-768	*Vitis* hybrid	European Institute	Edmund Mach Foundation (IT)
29-02-112	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
29-02-85	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
29-2-133	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
29-2-187	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
29-2-322	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
30-04-154	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
30-3-040	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
30-3-154	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
30-4-190	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
54-2	*Vitis* hybrid	European Institute	Edmund Mach Foundation (IT)
9-16/06	*Vitis* hybrid	European Institute	Edmund Mach Foundation (IT)
94-1-003	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
Alden	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
B87-60	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
BC4	*Vitis* hybrid	European Institute	Edmund Mach Foundation (IT)
Bianca	*Vitis* hybrid	University of Horticulture and Food Industry, Kölyuktetö (HU)	Edmund Mach Foundation (IT)
Black Monukka	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
Blanc du Bois	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Blue Lake	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Bronner	*Vitis* hybrid	Bronner, Johan Philipp	Edmund Mach Foundation (IT)
BS 4825	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Buffalo	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
Cabernet Carbon	*Vitis* hybrid	Becker, Norbert	Edmund Mach Foundation (IT)
Cabernet Cortis	*Vitis* hybrid	Becker, Norbert	Edmund Mach Foundation (IT)
Captivator	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Cardinal	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
Catawba	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
Cayuga White	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)

Chambourcin	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Chancellor	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Chaouch	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
Chardonel	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Clinton	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Columbia	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Concord	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
Conquistador	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Couderc 13	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
D'Arpa	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Daytona	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Diamond	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Diamond Muscat	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
Dunstan 336	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Eger 2	*Vitis* hybrid	Seyve-Villard, Bertille	Edmund Mach Foundation (IT)
Eger 99-11.01	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Eger1	*Vitis* hybrid	Csizmazia, Jozsef; Bereznai, Laszlo	Edmund Mach Foundation (IT)
Esther	*Vitis* hybrid	Szegedi, Sandor	Edmund Mach Foundation (IT)
Exotic	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
F243 Tamiani	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
F272 Everglade	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
F560 Big Brown	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
F9-68	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
Fanny	*Vitis* hybrid	University of Horticulture and Food Industry, Kölyuktetö (HU)	Edmund Mach Foundation (IT)
FLA 449	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
FLA BN6-67	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
FLA BN6-85	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
FLA CB8-1	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
FLA DC1-39	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
FLA W1521	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Flame Tokai	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
Gm6494	*Vitis* hybrid	Geisenheim University (DE)	Edmund Mach Foundation (IT)
Golden Muscat	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)

Helios	*Vitis* hybrid	VSSVVM Research and Breeding Station for Enology and Viticulture (SK)	Edmund Mach Foundation (IT)
Herbert	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Isabella	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Jasmin8/1	*Vitis* hybrid	European Institute	Edmund Mach Foundation (IT)
Johanniter	*Vitis* hybrid	Staatliche Weinbauinstitut Freiburg (CH)	Edmund Mach Foundation (IT)
JS 23-416	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Kunbaràt	*Vitis* hybrid	University of Horticulture and Food Industry (HU)	University of Udine (IT)
Kunleany	*Vitis* hybrid	University of Horticulture and Food Industry (HU)	Edmund Mach Foundation (IT)
Lenoir	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Leon Millot	*Vitis* hybrid	Kuhlmann, Eugène	Edmund Mach Foundation (IT)
Lidi	*Vitis* hybrid	Institute for Viticulture and Enology (HU)	Edmund Mach Foundation (IT)
LU1	*Vitis* hybrid	Mendel University Brno (CZ)	Edmund Mach Foundation (IT)
LU2	*Vitis* hybrid	Mendel University Brno (CZ)	Edmund Mach Foundation (IT)
M11-14/St. George	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Malaga	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
Mantey	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
Mars	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Merzling	*Vitis* hybrid	Staatliche Weinbauinstitut Freiburg (CH)	Edmund Mach Foundation (IT)
Muscaris	*Vitis* hybrid	Staatliche Weinbauinstitut Freiburg (CH)	Edmund Mach Foundation (IT)
MW 1bis	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
MW 38	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
MW 50	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
MW 53	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
MW 54	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
MW 58	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
MW 66	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
MW1	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
MW14	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
Neptune	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Nero	*Vitis* hybrid	University of Horticulture and Food Industry, Kölyuktetö (HU)	Edmund Mach Foundation (IT)
Norris	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)

Norton	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
NY08.0701a	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
NY08.0701b	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
NY09.0807b	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
NY63.1016.01	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
NY65.0562.01	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
NY84.0100.05	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
NY95.0308.02	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
NY97.0503.02	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
NY97.0512.01	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Ontario	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Orlando Seedless	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Palatina	*Vitis* hybrid	University of Horticulture and Food Industries, Szigetcsép (HU)	Edmund Mach Foundation (IT)
Perlette	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
Petra	*Vitis* hybrid	Institute of Viticulture, Arboriculture, Fruit and Horticulture (RS)	Edmund Mach Foundation (IT)
Phoenix	*Vitis* hybrid	Julius Kühn Institute- Geilweilerhof (DE)	Edmund Mach Foundation (IT)
Pixiola	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
Poloskei Muskotaly	*Vitis* hybrid	University for Horticulture and Food Industry (HU)	Edmund Mach Foundation (IT)
Prior	*Vitis* hybrid	Staatliche Weinbauinstitut Freiburg (CH)	Edmund Mach Foundation (IT)
Regent	*Vitis* hybrid	Julius Kühn Institute- Geilweilerhof (DE)	Edmund Mach Foundation (IT)
Ribier	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
Roucaneuf	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
Schuyler	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Seibel 13666	*Vitis* hybrid	Seibel, Albert	Edmund Mach Foundation (IT)
Seibel 2007	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Seibel 6339	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Seibel 880	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Seyval	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Seyve-Villard 5-276	*Vitis* hybrid	Seyve-Villard, Bertille	Edmund Mach Foundation (IT)
Sheridan	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Sirius	*Vitis* hybrid	Julius Kühn Institute- Geilweilerhof (DE)	Edmund Mach Foundation (IT)

Solaris	*Vitis* hybrid	Staatliche Weinbauinstitut Freiburg (CH)	Edmund Mach Foundation (IT)
Souvignier gris	*Vitis* hybrid	Staatliche Weinbauinstitut Freiburg (CH)	Edmund Mach Foundation (IT)
Steuben	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Stover	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Sultana	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
Suwannee	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
SV023	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
Traminette	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
V. riparia x V. cordifolia	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Valvin Muscat	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Venus	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Wayne	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Worden	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Zala Gyoengye	*Vitis* hybrid	University of Horticulture and Food Industry, Kölyuktetö (HU)	Edmund Mach Foundation (IT)
Zarja Severa	*Vitis* hybrid	CGL -Central genetic Laboratory Michurinsk (RU)	Edmund Mach Foundation (IT)
V. aestivalis	*Vitis spp.*	UC Davis (USA)	UC Davis (USA)
V. berlandieri Texas	*Vitis spp.*	Cornell University (USA)	Edmund Mach Foundation (IT)
V. cordifolia	*Vitis spp.*	European Institute	Edmund Mach Foundation (IT)
V. rubra	*Vitis spp.*	European Institute	Edmund Mach Foundation (IT)
V. rufotomentosa	*Vitis spp.*	UC Davis (USA)	UC Davis (USA)
V. rupestris	*Vitis spp.*	Cornell University (USA)	Edmund Mach Foundation (IT)
V. rupestris Constantia	*Vitis spp.*	Cornell University (USA)	Edmund Mach Foundation (IT)
V. rupestris du Lot	*Vitis spp.*	Sijas, M.R.	Edmund Mach Foundation (IT)
V. rupestris Metallique	*Vitis spp.*	European Institute	Edmund Mach Foundation (IT)
V. shuttleworthii	*Vitis spp.*	UC Davis (USA)	UC Davis (USA)
V. simpsonii	*Vitis spp.*	European Institute	Edmund Mach Foundation (IT)
V. smalliana	*Vitis spp.*	UC Davis (USA)	UC Davis (USA)
Coia1	*Vitis spp.*/hybrid	New Jersey - wild coll. (USA)	Edmund Mach Foundation (IT)
Coia10	*Vitis spp.*/hybrid	New Jersey - wild coll. (USA)	Edmund Mach Foundation (IT)
Coia11	*Vitis spp.*/hybrid	New Jersey - wild coll. (USA)	Edmund Mach Foundation (IT)
Coia12	*Vitis spp.*/hybrid	New Jersey - wild coll. (USA)	Edmund Mach Foundation (IT)
Coia4	*Vitis spp.*/hybrid	New Jersey - wild coll. (USA)	Edmund Mach Foundation (IT)

Coia5	*Vitis spp.*/hybrid	New Jersey - wild coll. (USA)	Edmund Mach Foundation (IT)
Coia7	*Vitis spp.*/hybrid	New Jersey - wild coll. (USA)	Edmund Mach Foundation (IT)
Coia9	*Vitis spp.*/hybrid	New Jersey - wild coll. (USA)	Edmund Mach Foundation (IT)
Corella2	*Vitis spp.*/hybrid	New Jersey - wild coll. (USA)	Edmund Mach Foundation (IT)
Corella3	*Vitis spp.*/hybrid	New Jersey - wild coll. (USA)	Edmund Mach Foundation (IT)
Lorenzo1	*Vitis spp.*/hybrid	New Jersey - wild coll. (USA)	Edmund Mach Foundation (IT)
Cabernet franc	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Cabernet Sauvignon	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Chardonnay	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Corvina veronese	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Franconia	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Garganega	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Gewurtztraminer	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
IM6013	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Italia	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Kishmiss Vatkana	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Lagrein	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Malvasia di Candia Aromatica	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Marzemino	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Merlot	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Michele Palieri	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Muller Thurgau	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Muscat Hamburg	*Vitis vinifera*	UC Davis (USA)	UC Davis (USA)
Nosiola	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Pinot blanc	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Pinot gris	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Pinot noir	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
PN40024	*Vitis vinifera*	INRA-Colmar (FR)	Edmund Mach Foundation (IT)
Riesling	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Sauvignon blanc	*Vitis vinifera*	-	Edmund Mach Foundation (IT)

Schiava	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Sultanina	*Vitis vinifera*	-	CRA (IT)
Teroldego	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Zweigelt	*Vitis vinifera*	-	Edmund Mach Foundation (IT)

Table S1. Studied grapevine accession list.

CHAPTER 3

Mining Grapevine Downy Mildew Susceptibility Genes: A Resource for Genomics-Based Breeding and Tailored Gene Editing

biomolecules

MDPI

Article

Mining Grapevine Downy Mildew Susceptibility Genes: A Resource for Genomics-Based Breeding and Tailored Gene Editing

Carlotta Pirrello [1,2], Tieme Zeilmaker [3], Luca Bianco [1], Lisa Giacomelli [1,3], Claudio Moser [1] and Silvia Vezzulli [1,*]

[1] Research and Innovation Centre, Edmund Mach Foundation, Via E. Mach 1, 38010 San Michele all'Adige, Italy; carlotta.pirrello@fmach.it (C.P.); luca.bianco@fmach.it (L.B.); giacomelli.scienzagrapes@gmail.com (L.G.); claudio.moser@fmach.it (C.M.)
[2] Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Via delle Scienze 206, 33100 Udine, Italy
[3] SciENZA Biotechnologies B.V., Sciencepark 904, 1098 XH Amsterdam, The Netherlands; T.Zeilmaker@enzazaden.nl
* Correspondence: silvia.vezzulli@fmach.it; Tel.: +39-0461-615387

Abstract: Several pathogens continuously threaten viticulture worldwide. Until now, the investigation on resistance loci has been the main trend to understand the interaction between grapevine and the mildew causal agents. Dominantly inherited gene-based resistance has shown to be race-specific in some cases, to confer partial immunity, and to be potentially overcome within a few years since its introgression. Recently, on the footprint of research conducted in Arabidopsis, putative genes associated with downy mildew susceptibility have been discovered also in the grapevine genome. In this work, we deep-sequenced four putative susceptibility genes—namely *VvDMR6.1*, *VvDMR6.2*, *VvDLO1*, *VvDLO2*—in 190 genetically diverse grapevine genotypes to discover new sources of broad-spectrum and recessively inherited resistance. Identified Single Nucleotide Polymorphisms were screened in a bottleneck analysis from the genetic sequence to their impact on protein structure. Fifty-five genotypes showed at least one impacting mutation in one or more of the scouted genes. Haplotypes were inferred for each gene and two of them at the *VvDMR6.2* gene were found significantly more represented in downy mildew resistant genotypes. The current results provide a resource for grapevine and plant genetics and could corroborate genomic-assisted breeding programs as well as tailored gene editing approaches for resistance to biotic stresses.

Keywords: disease resistance; DLO; DMR; next-gen amplicon sequencing; SNP; susceptibility genes; *Vitis* spp.

Citation: Pirrello, C.; Zeilmaker, T.; Bianco, L.; Giacomelli, L.; Moser, C.; Vezzulli, S. Mining Grapevine Downy Mildew Susceptibility Genes: A Resource for Genomics-Based Breeding and Tailored Gene Editing. *Biomolecules* **2021**, *11*, 181. https://doi.org/10.3390/biom11020181

Academic Editor: Elena Khlestkina
Received: 28 December 2020
Accepted: 26 January 2021
Published: 28 January 2021

1. Introduction

The development of disease-resistant varieties is a convenient alternative to chemical control methods to protect crops from diseases. When it recognizes and invades plant tissues and a plant-pathogen interaction is established, the pathogen is faced with the host response, which involves the activation of signals that translate into a rapid defense response. This immune response helps the host plant to avoid further infection of the pathogen [1]. To suppress this immunity, pathogens produce effector molecules to alter host responses and support compatibility. In turn, plants evolved the ability to recognize these effectors by resistance (*R*) genes. The majority of *R* genes encode nucleotide-binding leucine-rich-repeat (NBS-LRR) proteins. Since *R* genes are specifically directed towards highly polymorphic effector molecules or their derivatives, this kind of immunity is dominantly inherited, mostly race-specific, and rapidly overcome by the capacity of the pathogen to mutate [2]. Analyses of whole-genome sequences have provided and will continue to provide new insights into the dynamics of *R* gene evolution [3].

Besides the established *R* gene model, the susceptibility (*S*) gene model was more recently defined. All plant genes that facilitate infection and support compatibility can be considered *S* genes [4]. They can be classified into the following three groups based on the point at which they act during infection: those involved in early pathogen establishment, those involved in modulation of host defenses, and those involved in pathogen sustenance [5]. The concept of susceptibility genes was first explored in barley by Jorgensen (1992) [6] with the *MLO* (Mildew resistance Locus O) gene involved in susceptibility to powdery mildew. Later, *mlo* mutants were identified also in cucumber, melon, pea, tomato, and tobacco [7]. Other analyzed susceptibility genes are the so called *DMR* (*Downy Mildew Resistant*) genes firstly characterized in Arabidopsis by Van Damme et al. (2005; 2008) [8,9], and *DLO* (*DMR-like Oxygenases*) [10]. *DMR6* and *DLO* are paralogs, their separation occurred prior to the appearance of flowering plants [11]. Both genes encode a 2-oxoglutarate (2OG)-Fe(II) oxygenase [9,10]. The putative functions of *DMR6* and *DLO* were defined by Zhang et al. (2013) and Zhang et al. (2017) [10,12]. DMR6 and DLO are involved in salicylic acid (SA) catabolism. More specifically, DMR6 functions as a SA-5-hydroxylase (S5H) whereas DLO functions as a S3H, converting the active molecule of SA into 2,5-DHBA (dihydrobenzenic acid), and 2,3-DHBA inactive forms, respectively [10,12]. Being involved in SA catabolism, *DMR6* and *DLOs* fall into the category of *S* genes acting in the negative regulation of immune signaling. Their inactivation could improve plant resistance. Initially the *Arabidopsis thaliana dmr6* mutant was isolated from a chemically mutagenized population for its resistance to *Hyaloperonospora arabidopsidis*, the downy mildew (DM) causal agent in this species [8]. Orthologues were readily identified in tomato [13] as well as many other crops [14,15] and fruit trees [11,16]. Mutations in *DMR6* confer broad-spectrum resistance; *Sldmr6-1* tomato mutant plants show resistance against *Phytophthora capsici*, *Pseudomonas siringae*, and *Xanthomonas* spp. [13].

In order to identify mutations and to deepen their impact on plant performance, studies of genetic diversity are essential and have been extensively performed in the plant kingdom, although compared to animals and humans their sequel is still in its infancy. A SNP (Single Nucleotide Polymorphism) provides the ultimate form of molecular marker, based on differences of individual nucleotide bases between DNA sequences [17]. SNPs are more abundant in the genome and more stably inherited than other genetic markers [18] and they can be classified into random, gene targeted, or functional markers according to their localization [19]. The discovery of functional SNPs—that cause phenotype variations—is challenging and scarcely described in the literature. In particular, functional SNPs were used to target flowering time and seed size in lentil [20], midrib color in sorghum [21], leaf hair number in turnip [16], grain length [22], and blast resistance in rice [23].

A variety of approaches have been adopted to identify novel SNPs [24]. In the last decade, computational approaches have dominated SNP discovery methods due to the advent of next-generation sequencing (NGS) [25], followed by third-generation sequencing platforms (TGS) [26], and the consequent ever-increasing sequence information in public databases. Since the first whole plant genome to be sequenced [27], de novo and reference-based SNP discovery and application are now feasible for numerous plant species. Large-scale SNP discovery was performed in almost all sequenced plant genomes such as maize [28], Arabidopsis [29], rice [30], rapeseed [31], potato [32], and pepper [33]. On the method side, Genotyping-By-Sequencing (GBS) has recently emerged as a promising genomic approach to explore plant genetic diversity on a genome-wide scale [34], followed by the more cost-effective Genotyping-in-Thousands by sequencing (GT-seq) [35]. Genetic applications such as linkage mapping, phylogenetics, population structure, association studies, map-based cloning, marker-assisted plant breeding, and functional genomics continue to be enabled by access to large collections of SNPs [36]. In parallel to SNP discovery based on whole genome sequencing, amplicon sequencing has also been successfully applied in plants [37–40] although less frequently than in bacteria [41] or viruses [42].

Recently, as advocated by Gupta et al. (2001) [43], progress has also been made in the development and use of SNPs in woody plants, including some crop and tree species as

apple [44], walnut [45], sweet cherry [46], pear [47], coffee [48], and grapevine [49,50]. This phenomenon is due to the boost in the sequencing of cultivated plant genomes to provide high-density molecular markers for breeding programs aimed to crop improvement as well as to elucidate evolutionary mechanisms through comparative genomics [51,52]. In grapevine a great deal of progress has been made from the first SNP identification in the pre-genomic-era [53] to the sequencing of the whole genome of several *Vitis vinifera* cultivars [54–59], to the very recent report of the genome sequence of *Vitis riparia* [60] and the diploid chromosome-scale assembly of *Muscadinia rotundifolia* [61]. The last two studies represent a turning point on the scavenging of genomes that are donors of disease resistance traits. This issue in *Vitis* spp. is tackled by identifying *R* loci, underlying *R* genes, through quantitative trait loci (QTL) analysis in different genetic backgrounds. Nowadays, 13 *R* loci against powdery mildew and 31 to DM have been identified with different origins, mainly from American and Asian wild species [62,63].

A promising approach to cope with disease resistance is represented by the study of *S* loci. Based on a high-resolution map, Barba et al. (2014) [64] identified on chromosome 9 a locus (*Sen1*) for powdery mildew susceptibility from 'Chardonnay', finding evidence for quantitative variation. Moreover, on the footprint of research conducted on model plants, genes associated with mildew susceptibility have been discovered and dissected also in the grapevine genome. 17 *VvMLO* genes, orthologues of the Arabidopsis *MLOs*, were identified and a few members showed transcriptional induction upon fungal inoculation [65,66]. Lately, more insights in terms of powdery mildew resistance has been achieved by silencing of four *VvMLO* genes through RNAi in grapevine [67].

In this research, we aim to investigate the diversity of the *DMR6* and *DLO* genes in a wide set of *Vitis* spp. to broaden our knowledge about the genetic variation present and about the impact on the protein structure and function. This information will represent a resource to enhance our knowledge of possible alternative or integrative solutions, as compared to the use of *R* loci to be applied in plant molecular breeding strategies.

2. Materials and Methods

2.1. Genetic Material and Target Genes

In the current study, the four *VvDMR6.1*, *VvDMR6.2, VvDLO1*, and *VvDLO2* genes were scouted in 190 grapevine genotypes (Table 1, Table S1).

Table 1. Investigated genes with Illumina amplicon primers and position.

Gene	ID	Amplicon	Illumina Forward Primer 5 -3	Illumina Reverse Primer 3 -5	Amplicon Position
VvDMR6.1	VIT_216s0098g00860	1	CTGCTTAGTAGAGTGGTTAT	CGATGTGTTGGATGAGTTGG	Intron-Exon 1 Junction
		2	ATGTCCCCATAATCGACCTC	GTAGAACTCATCGGCCACCT	Exon 1- Intron Junction
		3	[illegible]	TTGAAGGAAGGAGGATTGGA	Exon 2
		4	TCTCGAACAAATCCTAATTCAAAA	GAAGAATGGTAAGGGCGTTG	Intron-Exon 3 Junction
		5	AACCCGAGCTCACTTATGGA	AAATTTTAAAAACCGGGCAAA	Exon 3-Intron Junction
		6	GGAAATGGGCATGTGCTAATA	TGCCCCAGAACTTCTTGTAA	Intron-Exon 4 Junction
VvDMR6.2	VIT_213s0047g00210	1	TCGGAGTCTTCACTCCCTTT	GCCATAACGGCTACAAGCAT	Exon 1
		2	GGTGTGGATGTGACCAGTGA	CCAAAGGATGGCAATGAAGT	Intron-Exon 2 Junction
		3	AGGAGAAAGTGCACAATTGGA	TCCGAAAAGGAAAAATGATGC	Exon 2-Intron Junction
		4	TCCAAAATGAAGACATAAGAAGGA	TATGTGCTGGCAGTCCGTAA	Intron-Exon 3 Junction
		5	CTTGTCCCGAGCCAGAGTTA	CCTGCATGCAATCATTTGTT	Exon 3-Intron Junction
		6	CCCAGGTGCTTTTGTTGTTA	CCCTTGCTGGACTAATGAGC	Exon 3- Exon 4 Junction
		7	CGATTGCTTCTTTCCTCTGC	CGCATTATGCCTTGTTGAAG	Exon 4
VvDLO1	VIT_215s0048g02430	1	ACAGGCCATCCCTCAGTACA	ATCGACATGTACCCGAAAAA	Exon 1
		2	CCTTGCTTTGACATGATTCTTC	TGAAAGATGGAGGGTTGGAG	Exon 2
		3	CCAACTGGAGAGATTTCCTGA	CGCCTTATCTATGTGGTTCCTC	Exon 2- Exon 3 Junction
		4	CTGGCCATGCTGATCCTAAT	CCTATGGACCGCACTCTTGT	Exon 3- Exon 4 Junction
		5	TTCCTGTAAAGGGCAGGATG	TTCCTGTAAAGGGCAGGATG	Exon 3- Exon 4 Junction
VvDLO2	VIT_202s0025g02970	1	CAACCCCCACTTGTGAATTT	CTTGGCCAATCTGTTTGACA	Intron-Exon 1 Junction
		2	AAGGATGTCCAGGCATCAGA	GAGCCTGACTGGATTGGAAG	Exon 1
		3	AGCTGCCAGAAAGCGAGA	CATGTAACTGCATGTTGGTCAG	Exon 1-Intron Junction
		4	TCTGACCAACATGCAGTTACA	TCTTGGAGAAGAACTGTGATTAAA	Intron-Exon 2 Junction
		5	CTTATGGGTTGCCTGGACAT	TTTTCCTCATTTTTGCAGGTG	Exon 2-Intron Junction

Out of these, 139 (73%) are *Vitis* hybrids, 28 (15%) are *V. vinifera* varieties, 12 (6%) belong to wild *Vitis* species and additional 11 (6%) are ascribed as hybrids/wild species. Phenotypic data about DM resistance degrees were retrieved from literature, public databases, and unpublished information. Pairwise alignment [68] was performed in order to define nucleotide identity between investigated genes.

2.2. Amplicon Sequencing and Read Processing

Genomic DNA was extracted from young grapevine leaves using DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany), according to the manufacturer's protocol, and then used to produce amplicons for deep sequencing. PCR on the templates was performed using Phusion High-Fidelity Polymerase (NEB, Ipswich, MA, USA) according to the manufacturer's protocol. Primers were specifically designed to amplify 250 bp of the coding regions of target genes and barcoded followed by in-house sequencing using the Illumina MiSeq platform (Table 1). A total of 19 amplicons was sequenced including six amplicons for *VvDMR6.1*, seven amplicons for *VvDMR6.2*, four amplicons for *VvDLO1*, and two amplicons for *VvDLO2*. Obtained amplicons were then mapped on the PN40024 12X reference genome [54] considering the latest V2 gene prediction [69,70] through Burrows–Wheeler alignment (BWA) [71] with no filter on mapping quality.

2.3. Sanger Sequencing

Thirteen impacting mutations (six in *VvDMR6.1*, two in *VvDMR6.2*, two in *VvDLO1*, three in *VvDLO2*) in 17 genotypes (12 hybrids, one *V. vinifera*, two wild species, two hybrids/wild species) in 25 combinations (Table S2) were chosen according to their representativeness of the overall results and to the availability of plants in situ. Previously extracted DNA was used to produce 12 targeted Sanger amplicons (six in *VvDMR6.1*, two in *VvDMR6.2*, two in *VvDLO1*, two in *VvDLO2*) by PCR using Phusion High-Fidelity DNA Polymerase (Thermo scientific) according to the manufacturer's protocol. Purification was made enzymatically with ExoSAP-IT PCR Product Cleanup Reagent (Applied Biosystems Inc., Foster City, CA, USA) according to the manufacturer's instructions. 3.2 µM of forward or reverse primer were then added to the sample and sequencing was performed using the BigDye Terminator Cycle Sequencing Ready Reaction Kit v3.1 (Applied Biosystems Inc.) in ten µL final volume. Sequencing reactions were performed using a 2 min initial denaturation step, followed by 25 cycles at 96 °C for 10 s, 50 °C for 5 s, and 60 °C for 4 min and then purified from unincorporated primer and BigDye excess through Multiscreen384SEQ Sequencing reaction Cleanup Plate (Millipore, Carrigtwohill, Co. Cork, Ireland). Capillary electrophoresis of the purified products was performed on a 3730× l DNA Analyzer (Applied Biosystems Inc.). Pregap4/Gap4 from Staden Package software package [72] were used to align DNA sequence electropherograms and scan all polymorphic sites.

2.4. Data Mining and Protein Model

Variant calling was performed by BCFtools [73] using the following settings: minimum mapping quality 20; minimum genotype quality 20; minimum base quality 20; maximum per sample depth of coverage 1000; minimum depth of coverage per site 10; keep read pairs with unexpected insert sizes (for amplicon sequencing). Filtering of results was done with VCFtools [74] to exclude all genotypes with quality below 20 and include only genotypes with read depth ≥10.

SnpEff toolbox was used to further discriminate variants according to their impact (MODIFIER, LOW, MODERATE or HIGH accordingly to the user's manual) on gene sequence [75]. Elected-impacting variants were then subject to SIFT (sorting intolerant from tolerant) [76] analysis to assess the tolerance of amino acid variants on the protein primary structure, based on the alignment with sequences in SWISS-PROT/TrEMBL database. Only not tolerated mutations were considered for a last impact evaluation based on variants chemical-physical properties according to Betts and Russel (2003) [77]. Both SnpEff and SIFT algorithms were used with default parameters settings.

Data obtained from mapping and variant calling were dissected to extrapolate overall genetic information on the studied genotypes. Amplicons were classified according to their level of polymorphism. All the other parameters were calculated considering all genotypes and the various taxon. For each gene, frequencies of occurring mutation arrangement were calculated along with mutation frequency, triallelic variants occurrence, and MAF. PHASE v2.1 software [78] was used for haplotype reconstruction and frequency calculation using PN40024 as the reference genome [54]. The genotypes belonging to specific classes (carried haplotypes) were linked in contingency tables to the phenotypic trait according to OIV 452(-1) [79]. Pearson's Chi-squared Tests for Count Data were performed on each locus separately.

Sequences of bonafide (*) and putative DMR6 and DLO orthologues were collected from literature [11,13,14,80] and available databases (Plaza 3.0) [81] and aligned using ClustalW (https://www.ebi.ac.uk/Tools/msa/clustalo/).

Genes carrying mutations confirmed by Sanger sequencing were subjected to a homology detection and three-dimensional structure prediction using the HHpred tool of MPI Bioinformatic Tools [82] available at https://toolkit.tuebingen.mpg.de/#/tools/hhpred. The algorithm found a Thebaine 6-O-demethylase [83] as the protein sequence with three-dimensional structure available (PDB coordinates: 509W) and highest homology to VvDMR6 and VvDLO and it produced a three-dimensional model carrying the mutations using the MODELLER software [82]. The three-dimensional structure was visualized to better understand the impact of the mutations on the wild type protein structure.

3. Results

3.1. Sequencing and Mapping

VvDMR6.1 and *VvDMR6.2* shared 46.7% nucleotide identity, *VvDMR6.1* and *VvDLO1* 44.8%, *VvDLO1* and *VvDLO2* 38.9%, all other comparisons resulted in a nucleotide identity lower than 40%. In order to identify potentially disrupting mutations, coding sequences of the *VvDMR6.1*, *VvDMR6.2, VvDLO1*, and *VvDLO2* genes (Table 1) from 190 genotypes (Table S1) were deep-sequenced and mapped on the reference genome PN40024 12X V2 (see Section 2). In total, 12,476,502 reads were produced. *VvDMR6.1* was covered by 5,450,614 reads (44%), *VvDMR6.2* by 3,476,587 (28%), *VvDLO1* by 3,270,318 (26%), and *VvDLO2* by 278,983 (2%). The highest coverage was detected in hybrids with a total of 9,357,649 reads (75%), followed by *vinifera* with 1,333,887 (11%), hybrids/wild species with 964,847 (8%) and wild species with 814,225 (6%).

A total of 738 mutations were detected by comparing the aligned reads to the Pinot Noir reference genome; 17 (~2%) short In/Dels and 721 point mutations, including heterozygous (56%) and homozygous (44%) SNPs (Figure 1).

3.2. Genetic Diversity Assessment

Amplicons were classified according to their rate of polymorphism: from the most polymorphic VvDLO2_1 (~13% of the total mutations); to the ones carrying ~8% of mutations VvDMR6.1_3, VvDMR6.1_2, VvDMR6.2_3 gradually decreasing to the lowest rate of polymorphism (less than 3%) in VvDMR6.2_7 and VvDLO1_4. Moreover, out of a total 738 mutations, 25 (~3.4%) triallelic variants were detected of which 13 in hybrids, eight in wild species, nine in *vinifera* varieties and eight in hybrid/wild species. Triallelic mutations were mainly found in *VvDLO2* (~1.6%) followed by *VvDMR6.1* (~1%), *VvDMR6.2* (~0.4%), and *VvDLO1*.

Considering the 696 biallelic mutations in all genotypes, 75% were transitions (A↔G, C↔T) and 25% were transversions (A ↔C, A ↔T, C↔G, G T) with a transition/transversion ratio of three. Both *vinifera* varieties and hybrids show the same assortment with 77% transitions and 23% transversions. In wild species the percentages were 73% and 27% respectively, while 71% and 29% were the values observed in hybrid/wild species.

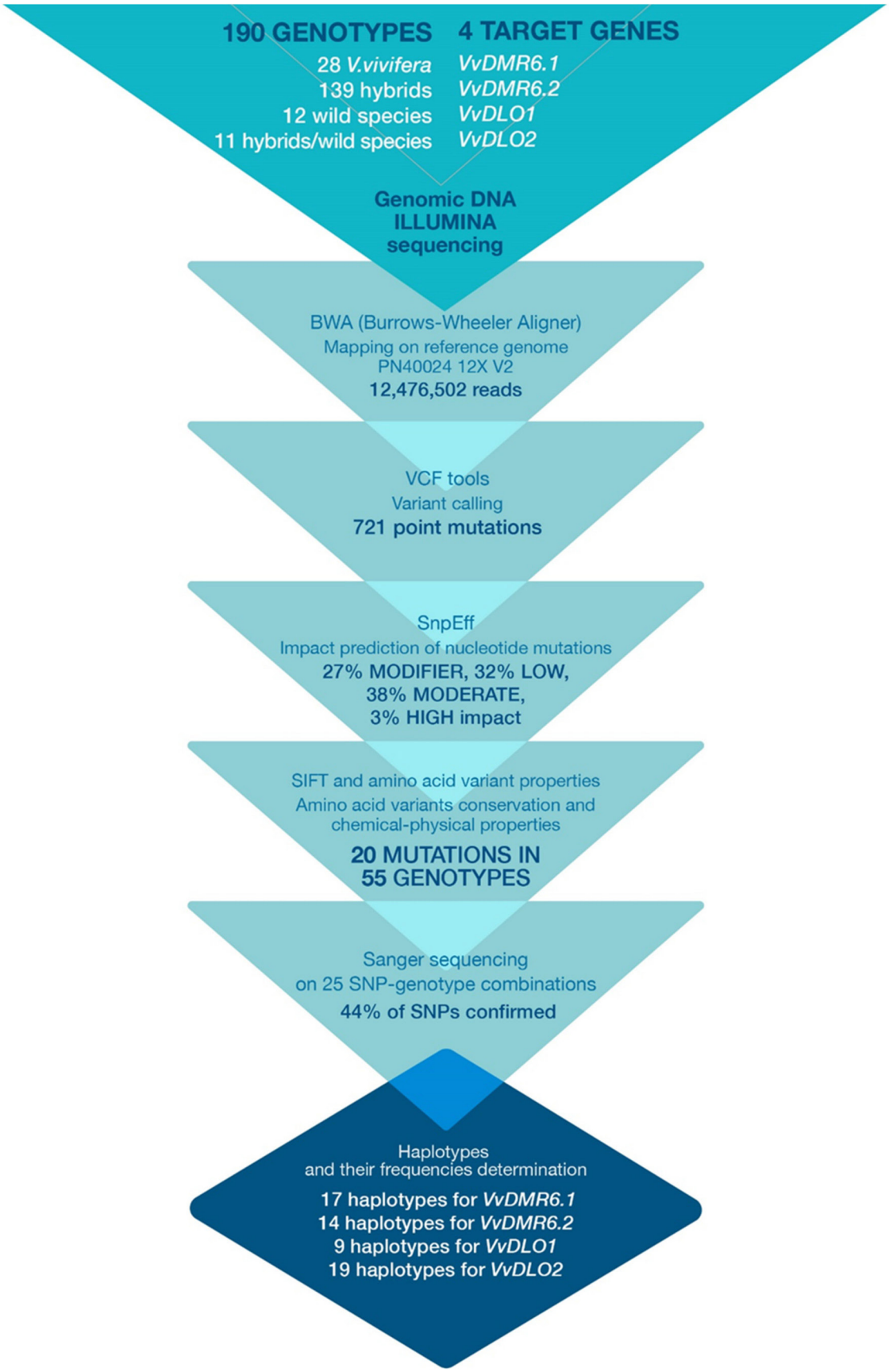


Figure 1. Flow chart of the analysis—tools and criteria—of sequencing data and results obtained downstream of each step.

SNP frequency was calculated both as average across all genes as well as per gene for every taxon. *Vinifera* varieties showed the lowest average frequency (~15 SNPs per Kb) with high differences between the target genes: ~33 SNPs per Kb in *VvDMR6.1*, ~22 SNPs per Kb in *VvDMR6.2*, ~18 SNPs per Kb in *VvDLO1*, and ~7 per Kb in *VvDLO2*. Moreover, the detected average frequency was ~18 SNPs per Kb in both wild species and hybrid/wild species, while they showed respectively ~23 per Kb and ~39 per Kb in *VvDMR6.1* ~20 and ~17.8 SNPs per Kb in *VvDMR6.2*, ~13 and 11 SNPs per Kb in *VvDLO1* and, ~22 and 20 SNPs per Kb in *VvDLO2*. Hybrids showed a higher average frequency (~28 per Kb) due to the dramatically high frequency values in *VvDMR6.1* (~75 per Kb) and in *VvDMR6.2* (~50 per Kb), ~38 SNPs per Kb in *VvDLO1* and 11 per Kb in *VvDLO2*.

In the current work, minor allele frequency (MAF) was calculated for each biallelic mutation. MAF values $0.01 \leq x \leq 0.05$ were represented by the 29% of mutations detected in all genotypes, in particular the 23%, 0%, 2%, and 3% in hybrids, wild species, *vinifera* varieties and hybrids/wild species, respectively. MAF values $0.05 < x \leq 0.1$ were represented by 3% of the mutations in all genotypes as well as in wild species and by 2% in hybrids, *vinifera* varieties and hybrid/wild species. $0.1 < x \leq 0.3$ MAF values were represented by the 5% of mutations in all genotypes as in hybrids; wild species and *vinifera* varieties represented them by the 4% of their mutations and hybrid/wild species by the 2%. A very low percentage of mutations showed MAF $0.3 < x \leq 0.5$: 3% for all genotypes, hybrids and *vinifera*; 2% for wild species and hybrid/wild species.

3.3. Mutation Impact Evaluation

In the current study, upon the variant discrimination performed according to their impact on codon sequence, 27% of total mutations (in particular, 27% in *VvDMR6.1*, 25% in *VvDMR6.2*, 30% in *VvDLO1* and 25% in *VvDLO2*) were classified as "MODIFIER": falling into intronic regions or upstream/downstream the gene. "LOW" impact variants, responsible for synonymous mutations or falling into splice regions, represented the 32% of the total mutations: 36% in *VvDMR6.1*, 32% in *VvDMR6.2*, 32% in *VvDLO1*, and 28% in *VvDLO2*. Of the total mutations, 38% (in particular, 35% in *VvDMR6.1*, 40% in *VvDMR6.2*, 35% in *VvDLO1* and 43% in *VvDLO2*) were non-synonymous variants and therefore classified with "MODERATE" impact. These percentages are partially confirmed in *vinifera* by Amrine et al. (2015) [84], with ~90% of MODIFIER and LOW mutations and ~8% non-synonymous variants in gene sequence. The lowest number of variants (in average 3%: 2% in *VvDMR6.1*, 2% in *VvDMR6.2*, 3% in *VvDLO1* and 4% in *VvDLO2*) was classified with "HIGH" impact as being responsible for sequence frameshifts or premature stop codons. Following the filtering of mutations classified as "MODERATE" and "HIGH" (41%) in order to discriminate amino acid variants according to their conservation, these variants were further checked and mutants carrying different chemical/physical properties from the reference were chosen. Finally, results from both analyses on amino acid sequence were cross-referenced and 20 mutations were elected as potentially affecting the protein structure: 6 in VvDMR6.1, 4 in VvDMR6.2, 4 in VvDLO1, and 6 in VvDLO2 (Table S3, Figure 1).

Twenty-five genotype-SNP combinations were selected for confirmation via Sanger sequencing. 44% of the mutations were confirmed by Sanger sequencing, while 56% were not, indicating a certain discrepancy from Illumina sequencing results. In *VvDMR6.1*, two mutations out of six polymorphisms were validated in one genotype each. The same variant in *VvDMR6.2* was confirmed in three individuals. In *VvDLO1* the confirmed variants were two, both in two different genotypes. Two individuals shared only one mutation in *VvDLO2*. Validated variants spanned among all the scouted genes, and the distribution of genotypes carrying confirmed mutations fairly represented the starting taxon assortment (six hybrids, one wild species, two hybrid/wild species individuals). For each gene, there were mutations that were both confirmed and unconfirmed depending on the genotype, and some individuals carried both confirmed and unconfirmed variants in the same gene. We classified Sanger-investigated variants according to their read coverage

(DP) and to their genotype quality (GQ). Out of the total 25 variants taken into account, 15 showed DP < 100 and 10 mutations with DP > 100 of which only one with DP close to 1000. While within 15 mutations with 10 < DP < 73 only four NGS results (27%) were confirmed, 7 out of the 10 variants (70%) with DP > 100 could be confirmed via Sanger sequencing. Furthermore, seven variants out of 25 (28%) showed a GQ lower than 99, of these only two were confirmed by Sanger sequencing. The remaining 18 mutations (72%) had GQ = 99 and half (nine) of them were confirmed. Considering both DP and GQ values together, six out of the seven variants with GQ < 99 showed DP < 100 but still two of them were Sanger sequencing confirmed. While five out of the nine remaining confirmed mutations showed GQ > 99 and DP > 100, two variants were with 50 < DP < 100. Of all the 20 impacting mutations considered (Table S3), only five were located at less than 60 nucleotides from amplicon or contig edge, and only one at less than 10 nucleotides. All the variants located on boundaries showed DP < 100; 50% of these edge mutations showed GQ < 99 and the other half GQ > 99. All the Sanger-confirmed variants were located far from amplicon ends, while only one was located on a reverse primer.

In order to provide robust results, only the validated mutations, corresponding to 11 genotype-SNP combinations, were selected for haplotype reconstruction and following analyses (Figure 1).

3.4. Mutated DMR and DLO Gene Combinations

Of the 190 studied genotypes, 55 showed at least one of the elected mutations: 37 hybrids, three *vinifera* varieties, six wild species and nine hybrid/wild species. 73% of individuals showed mutations only in one gene: 13% in *VvDMR6.1*, 29% in *VvDMR6.2*, 7% in *VvDLO1* and 24% in *VvDLO2*, while 26% were double mutants within six gene combinations and one genotype was mutant in three genes (Table S4). Haplotypes and their frequencies were determined for *VvDMR6.1*, *VvDMR6.2*, *VvDLO1*, and *VvDLO2* genes. Individuals carrying one impacting mutation per each gene were selected and the gene haplotypes were inferred taking into account all the flanking mutations showing at least MODERATE impact on the gene sequence (Table 2, Table S5). For *VvDMR6.1*, based on 14 SNPs, 17 haplotypes were calculated in 11 genotypes. The reference haplotype was the prominent (18.2% of frequency), all the others were unique, except for two haplotypes respectively shared by two individuals. No particular association between taxon and haplotype occurrence was observed. Regarding *VvDMR6.2*, 14 haplotypes were reconstructed based on 14 SNPs in 27 genotypes. The most shared haplotype (40.7%), showing two impacting mutations, was present in 12 individuals belonging to hybrids and, mainly in homozygous state, to *Vitis spp.*/hybrid individuals. The reference haplotype was the second one mostly represented, and then the third one showed 13% of frequency being shared by six hybrid genotypes. *VvDLO1* showed nine haplotypes based on 11 SNPs in 10 individuals. Besides the most recurrent reference haplotype (30%), the one with 20% of frequency encompassed two impacting mutations in one hybrid and two wild species. Sixteen SNPs in 25 genotypes were taken into account for *VvDLO2*, resulting in 19 haplotypes. Most haplotypes were unique or slightly shared, except for the reference one (34% of frequency) and two other main haplotypes (12% each) respectively shared by only and both hybrids and wild species (Table S5).

Integrating genotypic (haplotypic) data and available phenotypic OIV 452(-1) scores (Table 2), a chi-squared test was performed in order to check that genotypes belonging to specific classes (carried haplotypes) significantly led to the DM resistance trait. Interestingly, in *VvDMR6.2*, significance levels $p = 0.0025$ and $p = 0.018$ were respectively observed for haplotype number 10 and 8.

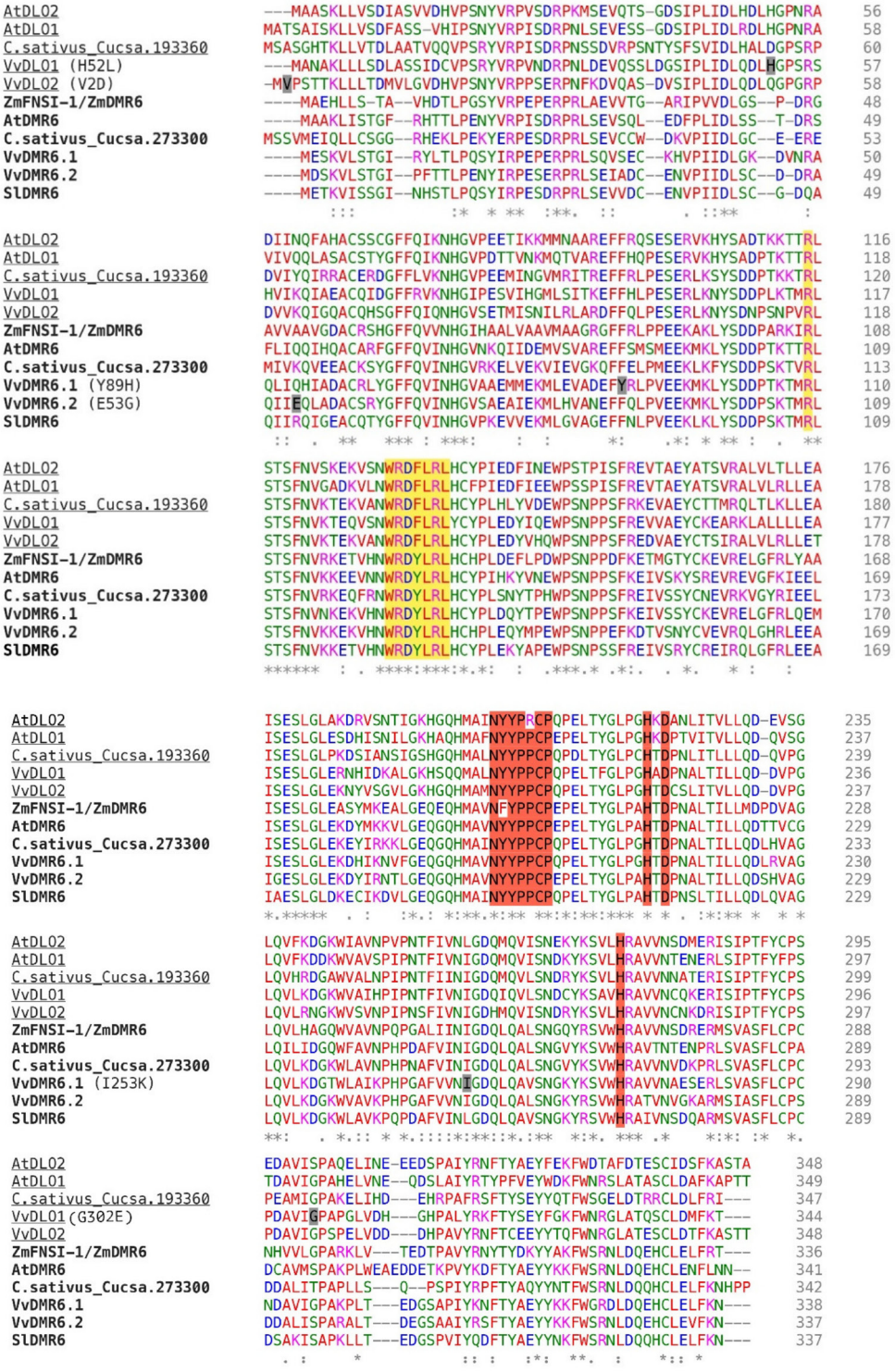
Table 2. Example of the haplotypic structure for each analyzed genotype.

Genotype	Taxon	*VvDMR6.1* Haplotype	*VvDMR6.2* Haplotype	*VvDLO1* Haplotype	*VvDLO2* Haplotype	OIV 452(-1)
PN40024		1,1	1,1	1,1	1,1	
B87-60	*Vitis* hybrid	5,8	-	-	-	
Blanc du Bois	*Vitis* hybrid	15,16	-	-	-	6 †
Blue Lake	*Vitis* hybrid	5,8	8,13	-	-	8 †
Captivator	*Vitis* hybrid	-	7,8	-	-	7 †
Catawba	*Vitis* hybrid	-	1,3	-	-	3 ‡
Chancellor	*Vitis* hybrid	1,17	-	-	1,19	1 ‡
Clinton	*Vitis* hybrid	-	10,10	-	7,14	1 †
D'Arpa	*Vitis* hybrid	-	7,8	-	-	9 †
Diamond	*Vitis* hybrid	-	-	-	1,19	5 ‡
F560 Big Brown	*Vitis* hybrid	-	-	8,9	-	9 †
FLA 449	*Vitis* hybrid	-	1,10	-	-	
FLA W1521	*Vitis* hybrid	-	8,8	-	-	8 †
Golden Muscat	*Vitis* hybrid	-	5,10	-	1,14	2 ‡
Herbert	*Vitis* hybrid	-	5,10	1,3	-	
Kunleany	*Vitis* hybrid	-	-	1,7	-	9 †
Lenoir	*Vitis* hybrid	-	-	9,9	-	8 †
M11-14St. George	*Vitis* hybrid	-	-	-	1,6	9 †
Mantey	*Vitis* hybrid	-	-	2,2	-	
Mars	*Vitis* hybrid	-	1,9	-	-	8 †
MW66	*Vitis* hybrid	2,3	-	-	-	5 †
NY08.0701b	*Vitis* hybrid	12,14	-	-	-	
NY63.1016.01	*Vitis* hybrid	11,13	-	-	-	
NY65.0562.01	*Vitis* hybrid	-	-	-	1,15	
NY84.0100.05	*Vitis* hybrid	1,13	-	-	-	
NY97.0503.02	*Vitis* hybrid	-	-	-	7,14	
NY97.0512.01	*Vitis* hybrid	-	1,4	-	1,17	
Ontario	*Vitis* hybrid	-	-	-	1,4	5 ‡
Petra	*Vitis* hybrid	7,9	1,6	4,5	-	9 †
Pixiola	*Vitis* hybrid	-	-	-	1,18	
Schuyler	*Vitis* hybrid	-	-	-	1,14	5†
Seibel 880	*Vitis* hybrid	-	-	-	1,14	
Sheridan	*Vitis* hybrid	-	10,10	-	-	
Steuben	*Vitis* hybrid	-	-	-	1,5	2 ‡
V. riparia x V. cordifolia	*Vitis* hybrid	-	-	-	11,14	
Venus	*Vitis* hybrid	-	5,8	-	-	7 †
Wayne	*Vitis* hybrid	-	5,10	-	-	
Worden	*Vitis* hybrid	-	10,10	-	1,16	
V. aestivalis	*Vitis spp.*	-	-	-	10,18	9 ‡
V. berlandieri Texas	*Vitis spp.*	-	-	4,4	8,9	9 †
V. cordifolia	*Vitis spp.*	-	-	1,4	8,19	9 †
V. rubra	*Vitis spp.*	-	1,12	-	-	9 †
V. rupestris du Lot	*Vitis spp.*	4,10	-	-	-	9 †
V. smalliana	*Vitis spp.*	-	-	6,1	1,19	
Coia1	*Vitis spp.*/hybrid	-	10,11	-	-	9 †
Coia5	*Vitis spp.*/hybrid	-	10,10	-	-	9 †
Coia7	*Vitis spp.*/hybrid	-	10,14	-	7,19	9 †
Coia9	*Vitis spp.*/hybrid	-	10,1	-	-	9 †
Coia10	*Vitis spp.*/hybrid	-	10,10	-	-	9 †
Coia11	*Vitis spp.*/hybrid	-	10,10	-	-	9 †
Coia12	*Vitis spp.*/hybrid	-	10,10	-	3,19	not available
Corella2	*Vitis spp.*/hybrid	-	-	-	1,18	not available
Lorenzo1	*Vitis spp.*/hybrid	-	-	-	12,13	9 †
Franconia	*Vitis vinifera*	-	-	-	1,2	1 †
Italia	*Vitis vinifera*	-	1,2	-	-	1 †
Pinot gris	*Vitis vinifera*	6,15	-	-	-	1 †

OIV, International Organisation of Vine and Wine; †: unpublished data; ‡: OIV-452(-1) scores provided by Cadle-Davidson (2008) [85].

3.5. Mutation Mapping on Amino Acid Sequences and Protein Structural Model

The amino acid variants corresponding to the mutations confirmed by Sanger sequencing were further investigated: (i) to estimate their conservation at the primary sequence level both within *Vitis* as well as in a larger group comprising other plant species (Figure 2A,B, Figure S1), and (ii) to evaluate their impact on the protein tertiary structure model (Figure 3).



(**A**)

Figure 2. *Cont.*

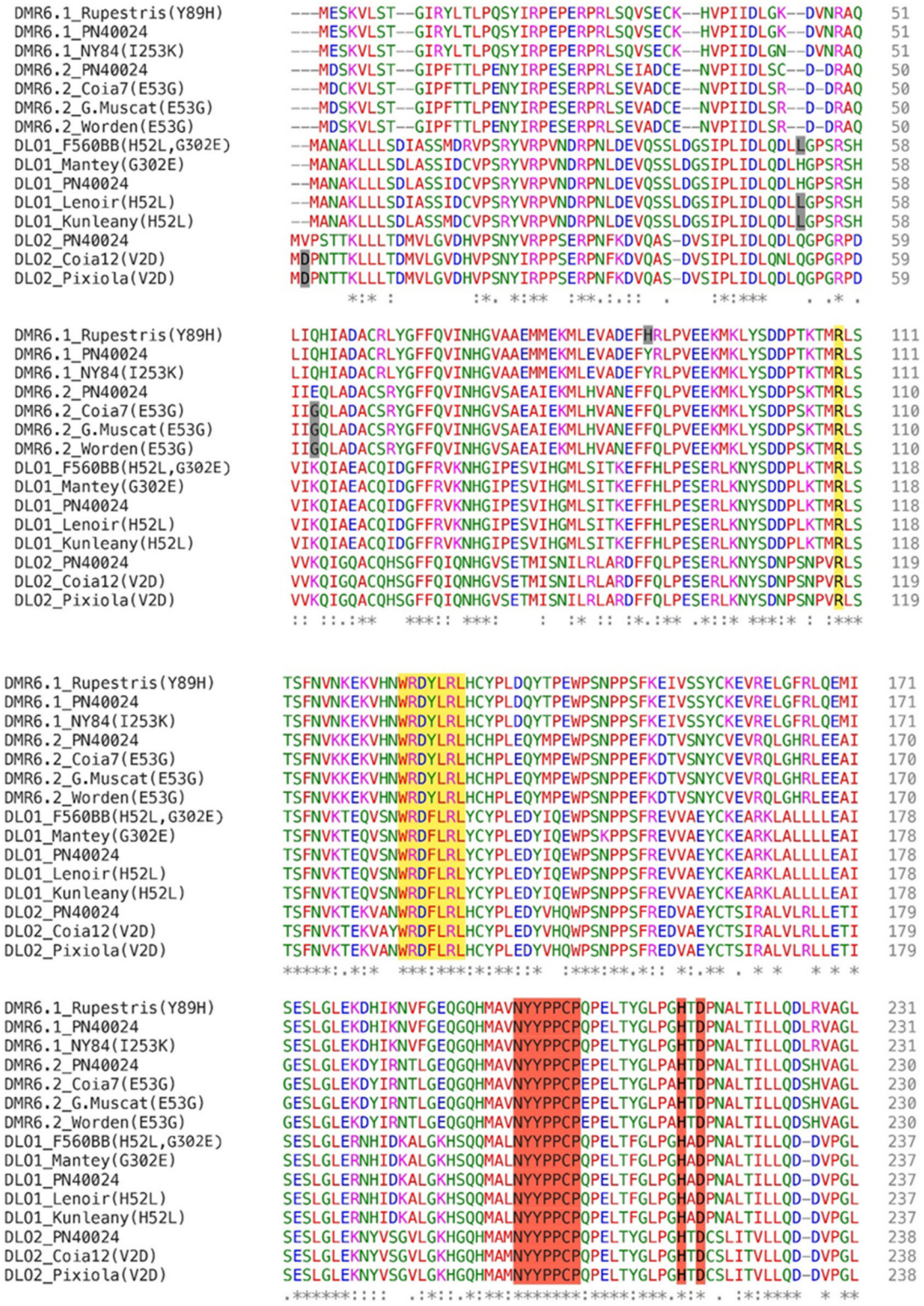
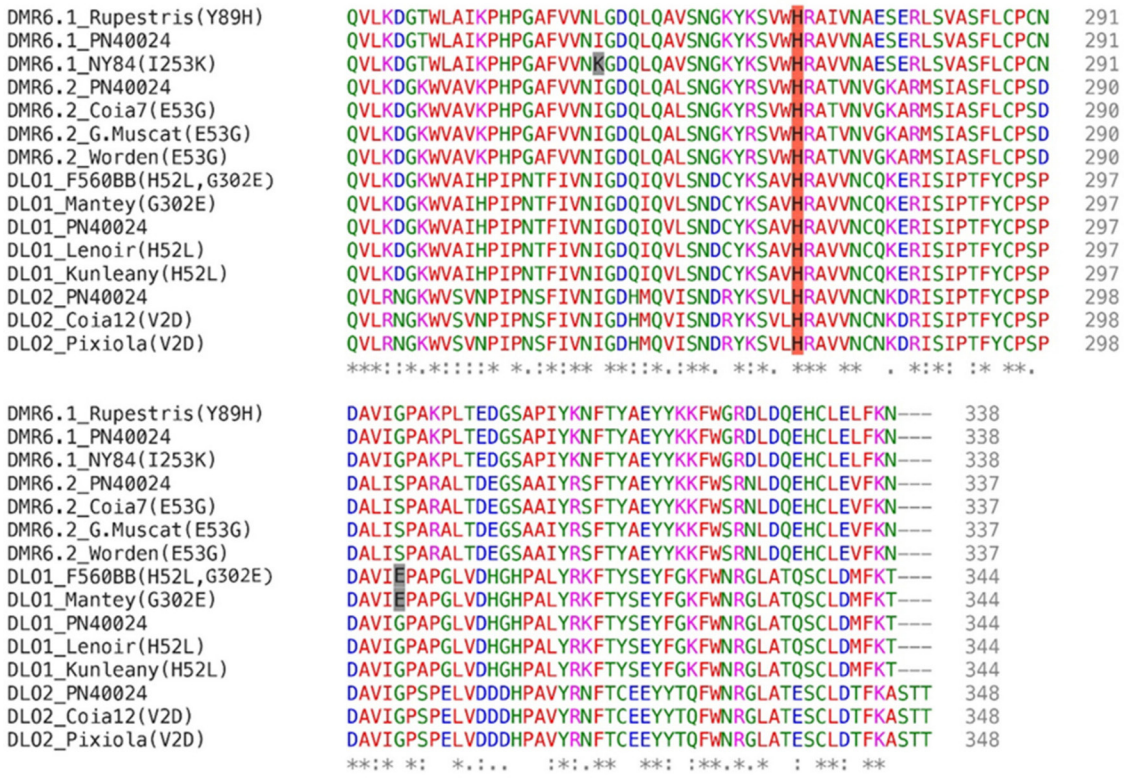


Figure 2. *Cont.*



(**B**)

Figure 2. Amino acid sequence alignments. Amino acids important for the 2-DOG oxidase function (e.g., the NYYPPCP stretch responsible for binding the 2-oxoglutarate substrate and the iron-binding HDH triplet) are highlighted in red. The DLO-DMR6 characterizing motif WRDY/FLRL is highlighted in yellow; R124 within the WRDY/FLRL motif, and R108 of the Arabidopsis thaliana DMR6-1 sequence were shown to be essential for the function and are as well highlighted in yellow [80]. Amino acids that are changing in the different grapevine variants are indicated within parenthesis and their position is highlighted in grey on the sequence. (**A**) CLUSTALW alignment of bonafide DMR6 (in bold) and DLO (underlined) proteins from different species. C.sativus_Cucsa.193360 and C.sativus_Cucsa.273300 were identified as AtDMR6 orthologues in *Cucumis sativus* by Schouten at al. (2014) [14], although no experimental proof is provided. Bonafide DMR and DLO proteins are: *Zea mays* ZmFNSI-1/ZmDMR6, *A. thaliana* AtDMR6, AtDLO1, and AtDLO2; *Solanum lycopersicon* SlDMR6. The grapevine DMR6 and DLO proteins (VvDMR6.1, VvDMR6.2, VvDLO1, and VvDLO2) are those of the PN40024 reference genome. (**B**) CLUSTALW alignment of translated grapevine sequences. Abbreviations: Rupestris: *V. rupestris* du Lot, PN40024: *Pinot noir*-derived near-homozygous line, NY84: NY84.0100.05, F560BB: F560 Big Brown, G.Muscat: Golden Muscat. * (asterisk) indicates positions which have a single, fully conserved residue. : (colon) indicates conservation between groups of strongly similar properties - scoring > 0.5 in the Gonnet PAM 250 matrix. . (period) indicates conservation between groups of weakly similar properties - scoring =< 0.5 in the Gonnet PAM 250 matrix.

Due the high sequence identity among them, the same protein three-dimensional model was used for mapping the mutations of all four proteins. Of the six amino acid substitutions two were found in VvDMR6.1 and VvDLO1 respectively, and one in VvDMR6.2 and VvDLO2 (Figure 3). All these mutations were non-conservative and therefore could potentially determine deep structural changes affecting also on the protein function. As depicted in Figure 3, four mutations appeared to be more exposed to the solvent, while the other two were buried inside the hydrophobic core of the proteins. Changes in the exposed amino acids are often less detrimental on the protein structure/function and this is the case of the V2D and H52L mutations. Although these mutations replaced a hydrophobic residue with a negatively charged one (V > D) and vice versa (H > L), being solvent exposed they do not seem of high impact on the protein structure. G302E and E53G mutations affect both steric hindrance and charge of the amino acid: glycine bearing the smallest side chain and glutamic acid bearing a bulky and negatively charged side chain. Also, for these two

mutations, the location at the protein surface suggests that they may be tolerated and likely do not affect heavily protein function. The remaining mutations Y89H and I253K might instead have a much greater impact on the structure and function of VvDMR6.1, the sequence where they have been found. In this case, amino acids with hydrophobic character (Y and I) and positioned within the hydrophobic core of the globular protein are changed into positively charged amino acids (H and K).

Protein	Variant
VvDMR6.1	Y89H I253K
VvDMR6.2	E53G
VvDLO1	H52L G302E
VvDLO2	V2D

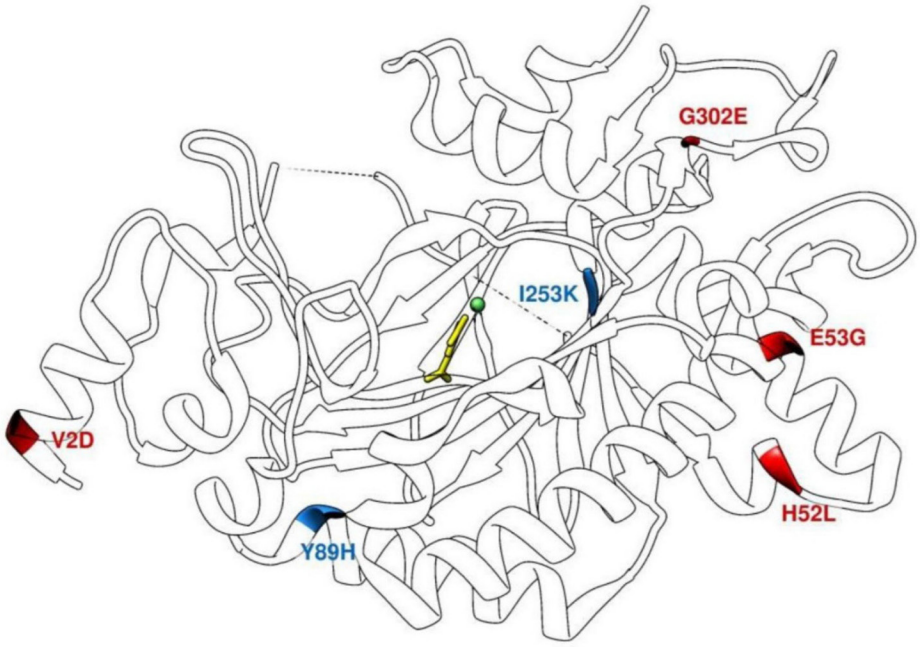


Figure 3. Protein structure model with detected impacting variants. In blue are residues located inside the protein while in red are those more exposed on the surface.

4. Discussion

4.1. Wealth of Genetic Variability

The current survey revealed a high representation of triallelic mutations within our genotype panel, due to the great genetic variability considered. Analogously, the occurrence of triallelism is consistent with previous work in grapevine [86–88]. However, as reported by Bianco et al. (2016) [44] and Marrano et al. (2019) [45], triallelic variants are usually discarded in large scale SNP-based analyses for cost reasons (i.e., they require multiple probes in SNP arrays) and not necessarily because they are less accurate. The obtained results in terms of transitions/transversions slightly diverge from the usual ratio found in grapevine (~1.5 in Salmaso et al., 2004; Lijavetzky et al., 2007; Vezzulli et al., 2008; Vezzulli et al., 2008; ~2 Marrano et al., 2017) [86–90] as well as in beetroot [91], potato [92] and cotton [93], while they are much higher than in soybean [94] and almond [95].

Regarding the detected average of ~15 SNPs per Kb in *vinifera* genotypes, a comparable polymorphism rate (~14.5 SNPs per Kb in coding regions) was found in both cultivated (spp. *sativa*) and non-cultivated (spp. *sylvestris*) *vinifera* species by Lijavetzky et al. (2007) [86]. In contrast, Vezzulli et al. (2008) [87], estimated ~8.5 SNPs per Kb in cultivated *vinifera* and ~6 per Kb in wild *vinifera* individuals coding sequence. Moreover, studying different *Vitis* spp. genotypes, Salmaso et al. (2004) [89] observed an average of ~12 SNPs per Kb in the coding sequence of a set of genes encoding proteins related to sugar metabolism, cell signaling, anthocyanin metabolism, and defense. Based on the first Pinot noir consensus genome sequence, the average SNP frequency was estimated at four SNPs every Kb [55], compatible with the use of such molecular markers for the construction of genetic maps in grapevine [96]. Different polymorphism rates were found in other highly heterozygous

tree species as peach (less than two SNPs per Kb) [97], black cottonwood (~3 per Kb) [98], almond (~9 per Kb) [95], and Tasmanian blue gum tree (~22 per Kb) [99], but all these results have to be carefully taken into account since different SNP calling methods can distort the comparison.

SNP informativeness depends on their reliability among individuals and species and their high transferability rates probably are not consistent with a direct impact on the genetic sequence (when in coding regions). Considering previous studies in grapevine, a larger representativeness of MAF values <0.1 was found in non-*vinifera* genotypes and rootstocks, non-cultivated *vinifera* showed a MAF $0.05 < x < 0.3$ while MAF > 0.1 were severely represented by *vinifera sativa* [86,87,90,100]. As explained by Jones et al. (2007) [101] and Grattapaglia et al. (2011) [102], genotyping studies take advantage of different molecular markers, mostly relying on their informativeness. In this framework, SNPs are informative markers, and this peculiarity is calculated as MAF. SNPs are considered interesting for many goals when MAF values are >0.05 [103,104], but their main usefulness is due to the transferability across genotypes (>0.1) [86]. In the current study, the aim to focus on impacting mutations was achieved, since MAF≤0.05 is a distinguishing mark for rare SNPs which affect the gene sequence and most likely the protein activity.

4.2. Relevance of Mutation Impact

In crops like tomato [105] and *Cucurbita* spp. [106], coding regions and whole genome sequence were scouted to find impacting mutations. A non-synonymous/synonymous mutation ratio of ~1.5 was found in tomato cultivars. In *Cucurbita* spp., the ratio was ~0.8 but only 9% of genetic variants showed HIGH or MODERATE impact in full genomic sequence, suggesting a great presence of intergenic mutations. In the walnut tree genomic sequence, Marrano et al. (2019) [45] identified 2.8% potentially impacting variants, while in the pear genome 55% of mutations were classified as missense and 1% with HIGH impact [107]. In grapevine, a significantly lower presence (0.7%) of HIGH impacting variants was observed in Thompson Seedless cultivar [108] compared to average percentages we observed in all taxa. The present aim to detect potentially disrupting mutations finds support in the great frequency of HIGH- and MODERATE-impact variants compared to the aforementioned research works on grapevine. Particular interest in the current results is given by the occurrence of impacting elected mutations in each one of the four scouted genes. Given the predicted compensative functional role of AtDMR6 and AtDLO in SA catabolism [10,12], obtained data may allow the use of *VvDMR6* and *VvDLO* genes in different combinations to enhance the impact of such homozygous mutations and likely avoid complementary effects.

Regarding the confirmation via Sanger sequencing, a borrowed attempt from clinical studies was tried herein on the overall grapevine Illumina sequencing results. In clinical research, reliability of variant calls is a fundamental precondition that requires the use of Sanger sequencing as gold standard to confirm NGS results and avoid false positives [109–111]. Incidentally, in order to avoid expensive and time-consuming extra analysis, some studies tried to set conditions according to which NGS-based variant calls can be considered definitive [112,113]. Although given the low number of tested samples we cannot draw a definitive conclusion that there is a direct correlation between these conditions and the reliability of Illumina sequencing-based calls, we observed that the most Sanger-confirmed variants (64%) showed DP > 100 and GQ = 99, while all ones were located away from the edges of the amplicons. The latter is in accord to Satya & DiCarlo (2014) [114], who report that variant calling accuracy decreases when SNPs are next to amplicon boundaries.

At this point, it is important to highlight the genetic complexity (high heterozygosity) of the studied genotype panel, which can unpredictably affect the Illumina probe as well as the Sanger sequencing primer annealing. Therefore, in order to provide reliable results, only validated mutations were selected for haplotype reconstruction and subsequent analyzes.

4.3. The Value of Haplotype Consideration

The reported broad genetic survey went back to the haplotype level. In three scouted genes out of four, the prominent haplotype belongs to the reference genotype (PN40024) which is a near-homozygous line [54] derived from the founder *vinifera* variety Pinot (noir) [115]. It is believed that the ancestral haplotype of a gene is the one showing the highest frequency while the rarest ones are the ones showing the most recent mutations occurring on the most shared haplotype [116], this hypothesis is supported by the fact that haplotype frequency is directly related to its age [117,118]. As advocated by Riahi et al. (2013) [119], domestication, hybridization with wild relatives and somatic mutations induced by vegetative propagation are the main reasons for the onset of genetic diversity between and among grapevine taxons.

Considering haplotypic data and available phenotypic OIV 452(-1) scores, two *VvDMR6.2* mutant haplotypes (number 10 and 8) were found more represented in DM resistant genotypes. It is relevant to highlight that none of the scouted target genes are underlying known resistance QTLs and no *R* loci discovered in grapevine so far were detected in the eight genotypes carrying these two haplotypes, except for the partial resistant *Rpv3-3* in three genotypes (Vezzulli S., personal communication). These observations suggest a potential effect of the mutant haplotypes in the defense response to DM. In grapevine, in addition to pursue association studies in large sample panels [120,121], some research works have lately been focusing on the haplotype investigation to dissect the relation between genetic diversity and cis-regulated gene expression in disease-related genes [122,123].

4.4. Scouting of Amino Acid Changes

DMR6 was identified as a putative 2-oxoglutarate (2OG)-Fe(II) oxygenase [9] and it revealed to share the WRD(F/Y)LR motif with DLO in flowering plant species [80]. Interestingly, Zeilmaker et al. (2015) [11] observed that non-conservative mutations in the catalytic sites (H212, H269, D214) of this protein were not able to restore susceptibility in an *Atdmr6.1* mutant background, in a complementation experiment. Unfortunately, no impacting mutation has been observed in any of these positions, but others have been identified that could potentially alter the structure of the protein. In particular, six mutations classified as impacting ones and confirmed by Sanger sequencing were further investigated by mapping on a three-dimensional model of the proteins and by analyzing the amino acid degree of conservation in a sequence alignment.

Drawing conclusions on the actual disrupting impact of the detected mutations will only be possible upon enzymatic assays of wild type and mutant proteins or by indirect functional assays such as the confirmation of the response to DM of the genotypes carrying the different variants. Nevertheless, the in silico analysis on the three-dimensional model of DMR6 and DLO proteins can already provide some insights and guide further investigations. Of the six mutations, two (Y89H and I253K) appeared to have a larger impact than the other four on the protein structure and consequently on the enzymatic activity. These changes occurred in amino acids positioned in the hydrophobic core of the protein. They imply the switch from a hydrophobic character to a hydrophilic character of the side chains, which carry a positive charge in the mutated amino acids. The use of a three-dimensional model to map the impacting mutations helped in inferring with a good approximation the position of the amino acids within the structure, in particular whether they are on the protein surface or buried inside the core of the proteins, and whether they are part of beta-structures or alfa-helices. An additional hint of the importance of the Y89 and I253 residues came from the analysis of DMR6 and DLO sequence alignments both within the *Vitis* species, results from this study, as well on a larger set of species. Y89 corresponds to an extremely conserved phenylalanine in other DMR6 and DLO sequences and this is an indication of the importance of an aromatic residue in that position. Interestingly, the amino acid following phenylalanine in several DLO sequences is a histidine. I253 is even more conserved in the sequence alignments and it is only in a few cases substituted by a leucine or a valine, which bear the same chemical properties. This suggests a structural and

functional role of this amino acid in that specific position, which would be likely disturbed by the mutation into a lysine, as it was observed in one of the studied genotypes.

4.5. Ultimate Application of S Genes

The genetic and protein data observed together with the phenotypic data (Table 2, Figure 2A,B, Figure 3) provide a well-rounded view of the role of the genes scouted here. The *VvDMR6.2* gene arouses a particular interest. The broader genetic analysis allowed us to observe that this gene shows two haplotypes (number 10 and 8) which are more frequently represented in DM resistant genotypes. Through the more focused analysis on the impact of Sanger-confirmed mutations, both haplotypes were found to share the genetic mutation responsible for the amino acid variant E53G. This finding suggests a decisive role of *VvDMR6.2* as *S* gene to grapevine DM and confirms the reliability of the bottleneck analysis here carried out (Figure 1).

Induction of plant defense signaling involves the recognition of specific pathogen effectors by the products of specialized host *R* genes. Numerous plant *R* genes have already been identified and characterized and they are being efficiently used in crop improvement research programs [1]. However, especially in tree species, selection of desirable resistant mutants comes with a cost of lengthy and laborious breeding programs. The effort required to produce resistant plants is often baffled within a few years from the selection because the pathogen evolves mechanisms to circumvent the *R* gene mediated immunity [124,125]. Exploitation of inactive alleles of susceptibility genes seems to be a promising path to introduce effective and durable disease resistance. Since *S* genes' first discovery [6], converting susceptibility genes in resistance factors has become an increasingly complementary strategy to that of breeding for *R* loci [4], and the advent of new reliable genome editing tools has enhanced this trend. The use of genome editing technologies such as CRISPR-Cas9 allow to specifically and rapidly target susceptibility genes to indirectly obtain resistance in a chosen genetic background, which is highly desired in crops like grapevine where the genetic identity is economically important. Recently, the *S* gene *MdDIPM4* was targeted in apple for a genome editing-driven knock out, resulting in edited plants showing reduced susceptibility to the bacterial pathogen *Erwinia amylovora* [126]. A similar approach was carried out by Low et al. (2020) [127] on *Hv2OGO* gene in barley conferring resistance to *Fusarium graminearum*. However, generation of edited plants and testing of their phenotype still requires years [128,129]. *S* genes may play different functions in the plant, thus pleiotropic effects associated with their knockout may entail a certain fitness cost for the plant. Recently, quantitative regulation of gene expression has been achieved with genome editing on *cis*-regulatory elements [125,130,131] and this might be a strategy to limit negative drawbacks associated with a reduced *S* gene function.

5. Conclusions

In this framework, the broad investigation of genetic diversity (until the haplotype level) related to a disease resistance trait presented here has the potential to become a resource in different contexts of plant science, both through the future integration of transcriptomics, proteomics and metabolomics data and as such. The identification of specific homozygous variants in the natural pool can in fact guide genome editing projects in targeting mutations that occur 'naturally'. This "tailored gene editing" that mimics natural polymorphisms has recently been demonstrated by Bastet et al. (2017, 2019) [132,133]. Finally, breeding programs could benefit from information on selected homozygous and heterozygous *S* gene mutations by implementing a next-generation marker-assisted strategy.

Supplementary Materials: The following are available online at https://www.mdpi.com/2218-273X/11/2/181/s1: Figure S1. CLUSTALW alignment of bonafide and putative DMR6 and DLO proteins from different species, Table S1. List of studied grapevine genotypes, Table S2. Selected genotypes for Sanger sequencing of each gene, investigated variants with their physical position, and sequencing primers, Table S3. List of impacting mutations with positions and data in VCF (Variant Call Format), Table S4. List of genotypes showing impacting mutations—heterozygous (He)

or homozygous (Ho) status—in at least one gene, Table S5. Haplotype identification and frequencies determined for the VvDMR6.1, VvDMR6.2, VvDLO1, VvDLO2 genes.

Author Contributions: Conceptualization, S.V.; Methodology, C.P., S.V, and T.Z.; Software, C.P., L.B., and T.Z.; Validation, C.P. and C.M.; Formal analysis, C.P., T.Z., and L.B.; Investigation, C.P., S.V., L.G., and C.M.; Resources, S.V., C.P., and L.G.; Data curation, C.P. and T.Z.; Writing—original draft preparation, C.P. and S.V.; Writing—review and editing, C.M., L.G., and L.B.; Visualization, C.P. and L.G.; Supervision, S.V.; Project administration, C.M.; Funding acquisition, C.M. All authors have read and agreed to the published version of the manuscript.

Funding: C.P. is recipient of a PhD fellowship provided by Fondazione Edmund Mach (FEM) and SciENZA Biotechnologies. She is enrolled at the University of Udine PhD school. The Autonomous Province of Trento (PAT) partially funded this research work.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in Supplementary Materials.

Acknowledgments: The authors are grateful to Andrew Walker and Summaira Riaz (UC Davis), Bruce Reisch (Cornell CALS) and Lorenzo Coia (OCPVA) for grapevine genotype recovery and collection. The authors also thank Bruce Reisch (Cornell CALS) and Luca Zulini (FEM) for providing some unpublished phenotypic data. Moreover, the authors appreciate Guido Van den Ackerveken and Adrien Melquiond (Utrecht University) for providing a PDB model of the DMR6/DLO1 protein, along with Andrea Mattevi (UniPv) for protein modelling and discussion on the impact of the aminoacid variants on the structure. Finally, the authors are thankful to Richard Feron and Quy Dung Peter Dinh (ENZA ZADEN) for Illumina sequencing data analysis, and Luca Cappellin (UniPd) for support on statistical analysis.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Gururani, M.A.; Venkatesh, J.; Upadhyaya, C.P.; Nookaraju, A.; Pandey, S.K.; Park, S.W. Plant disease resistance genes: Current status and future directions. *Physiol. Mol. Plant Pathol.* **2012**, *78*, 51–65. [CrossRef]
2. Jones, J.D.G.; Dangl, J.L. The plant immune system. *Nature* **2006**, *444*, 323–329. [CrossRef] [PubMed]
3. Meyers, B.C.; Kaushik, S.; Nandety, R.S. Evolving disease resistance genes. *Curr. Opin. Plant Biol.* **2005**, *8*, 129–134. [CrossRef] [PubMed]
4. Van Schie, C.C.N.; Takken, F.L.W. Susceptibility genes 101: How to be a good host. *Annu. Rev. Phytopathol.* **2014**, *52*, 551–581. [CrossRef]
5. Fawke, S.; Doumane, M.; Schornack, S. Oomycete Interactions with Plants: Infection Strategies and Resistance Principles. *Microbiol. Mol. Biol. Rev.* **2015**, *79*, 263–280. [CrossRef]
6. Jorgensen, J.H. Discovery, characterization and exploitation of Mlo powdery mildew. *Euphytica* **1992**, *66*, 141–152. [CrossRef]
7. Kusch, S.; Panstruga, R. mlo-Based Resistance: An Apparently Universal "Weapon" to Defeat Powdery Mildew Disease. *Mol. Plant Microbe Interact.* **2017**, *30*, 179–189. [CrossRef]
8. Van Damme, M.; Andel, A.; Huibers, R.P.; Panstruga, R.; Weisbeek, P.J.; Van Den Ackerveken, G. Identification of Arabidopsis loci required for susceptibility to the downy mildew pathogen Hyaloperonospora parasitica. *Mol. Plant Microbe Interact.* **2005**, *18*, 583–592. [CrossRef] [PubMed]
9. Van Damme, M.; Huibers, R.P.; Elberse, J.; Van Den Ackerveken, G. Arabidopsis DMR6 encodes a putative 2OG-Fe(II) oxygenase that is defense-associated but required for susceptibility to downy mildew. *Plant J.* **2008**, *54*, 785–793. [CrossRef] [PubMed]
10. Zhang, K.; Halitschke, R.; Yin, C.; Liu, C.-J.; Gan, S.-S. Salicylic acid 3-hydroxylase regulates *Arabidopsis* leaf longevity by mediating salicylic acid catabolism. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 14807–14812. [CrossRef]
11. Zeilmaker, T.; Ludwig, N.R.; Elberse, J.; Seidl, M.F.; Berke, L.; Van Doorn, A.; Schuurink, R.C.; Snel, B.; Van Den Ackerveken, G. Downy mildew resistant 6 and DMR6-like oxygenase 1 are partially redundant but distinct suppressors of immunity in Arabidopsis. *Plant J.* **2015**, *81*, 210–222. [CrossRef] [PubMed]
12. Zhang, Y.J.; Zhao, L.; Zhao, J.Z.; Li, Y.J.; Wang, J.B.; Guo, R.; Gan, S.S.; Liu, C.J.; Zhanga, K.W. S5H/DMR6 encodes a salicylic acid 5-hydroxylase that fine-tunes salicylic acid homeostasis. *Plant Physiol.* **2017**, *175*, 1082–1093. [CrossRef] [PubMed]
13. De Toledo Thomazella, D.P.; Brail, Q.; Dahlbeck, D.; Staskawicz, B.J. CRISPR-Cas9 mediated mutagenesis of a DMR6 ortholog in tomato confers broad-spectrum disease resistance. *bioRxiv* **2016**, 064824. [CrossRef]
14. Schouten, H.J.; Krauskopf, J.; Visser, R.G.F.; Bai, Y. Identification of candidate genes required for susceptibility to powdery or downy mildew in cucumber. *Euphytica* **2014**, *200*, 475–486. [CrossRef]

15. Sun, K.; van Tuinen, A.; van Kan, J.A.L.; Wolters, A.M.A.; Jacobsen, E.; Visser, R.G.F.; Bai, Y. Silencing of DND1 in potato and tomato impedes conidial germination, attachment and hyphal growth of Botrytis cinerea. *BMC Plant Biol.* **2017**, *17*, 235. [CrossRef]
16. Zhang, W.; Mirlohi, S.; Li, X.; He, Y. Identification of functional single-nucleotide polymorphisms affecting leaf hair number in Brassica rapa. *Plant Physiol.* **2018**, *177*, 490–503. [CrossRef]
17. Ganal, M.W.; Altmann, T.; Röder, M.S. SNP identification in crop plants. *Curr. Opin. Plant Biol.* **2009**, *12*, 211–217. [CrossRef]
18. Brookes, A.J. The essence of SNPs [Review]. *Gene* **1999**, *234*, 177–186. [CrossRef]
19. Andersen, J.R.; Lübberstedt, T. Functional markers in plants. *Trends Plant Sci.* **2003**, *8*, 554–560. [CrossRef]
20. Polanco, C.; Sáenz de Miera, L.E.; González, A.I.; García, P.; Fratini, R.; Vaquero, F.; Javier Vences, F.; De La Vega, M.P. Construction of a high-density interspecific (*Lens culinaris*×*L. Odemensis*) genetic map based on functional markers for mapping morphological and agronomical traits, and QTLs affecting resistance to Ascochyta in lentil. *PLoS ONE* **2019**, *14*, e0214409. [CrossRef]
21. Burow, G.; Chopra, R.; Sattler, S.; Burke, J.; Acosta-Martinez, V.; Xin, Z. Deployment of SNP (CAPS and KASP) markers for allelic discrimination and easy access to functional variants for brown midrib genes bmr6 and bmr12 in Sorghum bicolor. *Mol. Breed.* **2019**, *39*. [CrossRef]
22. Fan, C.; Yu, S.; Wang, C.; Xing, Y. A causal C-A mutation in the second exon of GS3 highly associated with rice grain length and validated as a functional marker. *Theor. Appl. Genet.* **2009**, *118*, 465–472. [CrossRef] [PubMed]
23. Yang, Y.; Zhang, H.; Xuan, N.; Chen, G.; Liu, X.; Yao, F.; Ding, H. Identification of blast resistance genes in 358 rice germplasms (*Oryza sativa* L.) using functional molecular markers. *Eur. J. Plant Pathol.* **2017**, *148*, 567–576. [CrossRef]
24. Edwards, D.; Forster, J.W.; Cogan, N.O.; Batley, J.; Chagné, D. Single nucleotide polymorphism discovery. In *Association Mapping in Plants*; Springer: New York, NY, USA, 2007.
25. Varshney, R.K.; Nayak, S.N.; May, G.D.; Jackson, S.A. Next-generation sequencing technologies and their implications for crop genetics and breeding. *Trends Biotechnol.* **2009**, *27*, 522–530. [CrossRef] [PubMed]
26. Schadt, E.E.; Turner, S.; Kasarskis, A. A window into third-generation sequencing. *Hum. Mol. Genet.* **2010**, *19*, 227–240. [CrossRef]
27. Arabidopsis Genome Initiative. Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. *Nature* **2000**, *408*, 796–815. [CrossRef] [PubMed]
28. Ching, A.; Caldwell, K.S.; Jung, M.; Dolan, M.; Smith, O.S.H.; Tingey, S.; Morgante, M.; Rafalski, A.J. SNP frequency, haplotype structure and linkage disequilibrium in elite maize inbred lines. *BMC Genet.* **2002**, *3*, 1–14. [CrossRef]
29. Atwell, S.; Huang, Y.S.; Vilhjálmsson, B.J.; Willems, G.; Horton, M.; Li, Y.; Meng, D.; Platt, A.; Tarone, A.M.; Hu, T.T.; et al. Genome-wide association study of 107 phenotypes in Arabidopsis thaliana inbred lines. *Nature* **2010**, *465*, 627–631. [CrossRef]
30. Xu, X.; Liu, X.; Ge, S.; Jensen, J.D.; Hu, F.; Li, X.; Dong, Y.; Gutenkunst, R.N.; Fang, L.; Huang, L.; et al. Resequencing 50 accessions of cultivated and wild rice yields markers for identifying agronomically important genes. *Nat. Biotechnol.* **2012**, *30*, 105–111. [CrossRef]
31. Raman, H.; Dalton-Morgan, J.; Diffey, S.; Raman, R.; Alamery, S.; Edwards, D.; Batley, J. SNP markers-based map construction and genome-wide linkage analysis in Brassica napus. *Plant Biotechnol. J.* **2014**, *12*, 851–860. [CrossRef]
32. Vos, P.G.; Uitdewilligen, J.G.A.M.L.; Voorrips, R.E.; Visser, R.G.F.; van Eck, H.J. Development and analysis of a 20K SNP array for potato (Solanum tuberosum): An insight into the breeding history. *Theor. Appl. Genet.* **2015**, *128*, 2387–2401. [CrossRef] [PubMed]
33. Hulse-Kemp, A.M.; Ashrafi, H.; Plieske, J.; Lemm, J.; Stoffel, K.; Hill, T.; Luerssen, H.; Pethiyagoda, C.L.; Lawley, C.T.; Ganal, M.W.; et al. A HapMap leads to a Capsicum annuum SNP infinium array: A new tool for pepper breeding. *Hortic. Res.* **2016**, *3*, 1–10. [CrossRef] [PubMed]
34. Peterson, G.W.; Dong, Y.; Horbach, C.; Fu, Y.B. Genotyping-by-sequencing for plant genetic diversity analysis: A lab guide for SNP genotyping. *Diversity* **2014**, *6*, 665–680. [CrossRef]
35. Campbell, N.R.; Harmon, S.A.; Narum, S.R. Genotyping-in-Thousands by sequencing (GT-seq): A cost effective SNP genotyping method based on custom amplicon sequencing. *Mol. Ecol. Resour.* **2015**, *15*, 855–867. [CrossRef] [PubMed]
36. Kumar, S.; Banks, T.W.; Cloutier, S. SNP discovery through next-generation sequencing and its applications. *Int. J. Plant Genomics* **2012**, *2012*. [CrossRef] [PubMed]
37. Durstewitz, G.; Polley, A.; Plieske, J.; Luerssen, H.; Graner, E.M.; Wieseke, R.; Ganal, M.W. SNP discovery by amplicon sequencing and multiplex SNP genotyping in the allopolyploid species Brassica napus. *Genome* **2010**, *53*, 948–956. [CrossRef]
38. Yang, S.; Fresnedo-Ramírez, J.; Wang, M.; Cote, L.; Schweitzer, P.; Barba, P.; Takacs, E.M.; Clark, M.; Luby, J.; Manns, D.C.; et al. A next-generation marker genotyping platform (AmpSeq) in heterozygous crops: A case study for marker-assisted selection in grapevine. *Hortic. Res.* **2016**, *3*. [CrossRef]
39. Cho, Y.B.; Jones, S.I.; Vodkin, L.O. Mutations in Argonaute5 illuminate epistatic interactions of the K1 and I loci leading to saddle seed color patterns in glycine max. *Plant Cell* **2017**, *29*, 708–725. [CrossRef]
40. Shimray, P.W.; Bajaj, D.; Srivastava, R.; Daware, A.; Upadhyaya, H.D.; Kumar, R.; Bharadwaj, C.; Tyagi, A.K.; Parida, S.K. Identifying Transcription Factor Genes Associated with Yield Traits in Chickpea. *Plant Mol. Biol. Report.* **2017**, *35*, 562–574. [CrossRef]
41. Hong, Y.; Liao, D.; Hu, A.; Wang, H.; Chen, J.; Khan, S.; Su, J.; Li, H. Diversity of endophytic and rhizoplane bacterial communities associated with exotic Spartina alterniflora and native mangrove using Illumina amplicon sequencing. *Can. J. Microbiol.* **2015**, *61*, 723–733. [CrossRef]
42. Kinoti, W.M.; Constable, F.E.; Nancarrow, N.; Plummer, K.M.; Rodoni, B. Analysis of intra-host genetic diversity of Prunus necrotic ringspot virus (PNRSV) using amplicon next generation sequencing. *PLoS ONE* **2017**, *12*, e0179284. [CrossRef] [PubMed]

43. Gupta, P.K.; Roy, J.K.; Prasad, M. Single nucleotide polymorphisms: A new paradigm for molecular marker technology and DNA polymorphism detection with emphasis on their use in plants. *Curr. Sci.* **2001**, *80*, 524–535.
44. Bianco, L.; Cestaro, A.; Linsmith, G.; Muranty, H.; Denancé, C.; Théron, A.; Poncet, C.; Micheletti, D.; Kerschbamer, E.; Di Pierro, E.A.; et al. Development and validation of the Axiom®Apple480K SNP genotyping array. *Plant J.* **2016**, *86*, 62–74. [CrossRef] [PubMed]
45. Marrano, A.; Martínez-García, P.J.; Bianco, L.; Sideli, G.M.; Di Pierro, E.A.; Leslie, C.A.; Stevens, K.A.; Crepeau, M.W.; Troggio, M.; Langley, C.H.; et al. A new genomic tool for walnut (*Juglans regia* L.): Development and validation of the high-density Axiom™ J. regia 700K SNP genotyping array. *Plant Biotechnol. J.* **2019**, *17*, 1027–1036. [CrossRef] [PubMed]
46. Hardner, C.M.; Hayes, B.J.; Kumar, S.; Vanderzande, S.; Cai, L.; Piaskowski, J.; Quero-Garcia, J.; Campoy, J.A.; Barreneche, T.; Giovannini, D.; et al. Prediction of genetic value for sweet cherry fruit maturity among environments using a 6K SNP array. *Hortic. Res.* **2019**, *6*. [CrossRef] [PubMed]
47. Li, X.; Singh, J.; Qin, M.; Li, S.; Zhang, X.; Zhang, M.; Khan, A.; Zhang, S.; Wu, J. Development of an integrated 200K SNP genotyping array and application for genetic mapping, genome assembly improvement and genome wide association studies in pear (Pyrus). *Plant Biotechnol. J.* **2019**, *17*, 1582–1594. [CrossRef] [PubMed]
48. Merot-L'anthoene, V.; Tournebize, R.; Darracq, O.; Rattina, V.; Lepelley, M.; Bellanger, L.; Tranchant-Dubreuil, C.; Coulée, M.; Pégard, M.; Metairon, S.; et al. Development and evaluation of a genome-wide Coffee 8.5K SNP array and its application for high-density genetic mapping and for investigating the origin of *Coffea arabica* L. *Plant Biotechnol. J.* **2019**, *17*, 1418–1430. [CrossRef]
49. Mercati, F.; De Lorenzis, G.; Brancadoro, L.; Lupini, A.; Abenavoli, M.R.; Barbagallo, M.G.; Di Lorenzo, R.; Scienza, A.; Sunseri, F. High-throughput 18K SNP array to assess genetic variability of the main grapevine cultivars from Sicily. *Tree Genet. Genomes* **2016**, *12*. [CrossRef]
50. Laucou, V.; Launay, A.; Bacilieri, R.; Lacombe, T.; Adam-Blondon, A.F.; Bérard, A.; Chauveau, A.; De Andrés, M.T.; Hausmann, L.; Ibáñez, J.; et al. Extended diversity analysis of cultivated grapevine *Vitis vinifera* with 10K genome-wide SNPs. *PLoS ONE* **2018**, *13*, e0192540. [CrossRef]
51. Feuillet, C.; Leach, J.E.; Rogers, J.; Schnable, P.S.; Eversole, K. Crop genome sequencing: Lessons and rationales. *Trends Plant Sci.* **2011**, *16*, 77–88. [CrossRef]
52. Bolger, M.E.; Weisshaar, B.; Scholz, U.; Stein, N.; Usadel, B.; Mayer, K.F.X. Plant genome sequencing—Applications for crop improvement. *Curr. Opin. Biotechnol.* **2014**, *26*, 31–37. [CrossRef] [PubMed]
53. Owens, C.L. SNP detection and genotyping in *Vitis*. *Acta Hortic.* **2003**, *603*, 139–140. [CrossRef]
54. Jaillon, O. The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature* **2007**. [CrossRef]
55. Velasco, R.; Zharkikh, A.; Troggio, M.; Cartwright, D.A.; Cestaro, A.; Pruss, D.; Pindo, M.; FitzGerald, L.M.; Vezzulli, S.; Reid, J.; et al. A High Quality Draft Consensus Sequence of the Genome of a Heterozygous Grapevine Variety. *PLoS ONE* **2007**, *2*, e1326. [CrossRef]
56. Carrier, G.; Le Cunff, L.; Dereeper, A.; Legrand, D.; Sabot, F.; Bouchez, O.; Audeguin, L.; Boursiquot, J.M.; This, P. Transposable elements are a major cause of somatic polymorphism in *Vitis vinifera* L. *PLoS ONE* **2012**, *7*, e32973. [CrossRef]
57. Gambino, G.; Dal Molin, A.; Boccacci, P.; Minio, A.; Chitarra, W.; Avanzato, C.G.; Tononi, P.; Perrone, I.; Raimondi, S.; Schneider, A.; et al. Whole-genome sequencing and SNV genotyping of "Nebbiolo" (*Vitis vinifera* L.) clones. *Sci. Rep.* **2017**, *7*, 1–15. [CrossRef]
58. Roach, M.J.; Johnson, D.L.; Bohlmann, J.; van Vuuren, H.J.J.; Jones, S.J.M.; Pretorius, I.S.; Schmidt, S.A.; Borneman, A.R. Population sequencing reveals clonal diversity and ancestral inbreeding in the grapevine cultivar Chardonnay. *PLoS Genet.* **2018**, *14*. [CrossRef]
59. Minio, A.; Massonnet, M.; Figueroa-Balderas, R.; Castro, A.; Cantu, D. Diploid genome assembly of the wine grape carménère. *Genes Genomes Genet.* **2019**, *9*, 1331–1337. [CrossRef]
60. Girollet, N.; Rubio, B.; Bert, P.-F. De novo phased assembly of the *Vitis* riparia grape genome. *Sci. Data* **2019**, *6*, 1–8. [CrossRef]
61. Cochetel, N.; Minio, A.; Vondras, A.M.; Figueroa-Balderas, R.; Cantu, D. Diploid chromosome-scale assembly of the Muscadinia rotundifolia genome supports chromosome fusion and disease resistance gene expansion during *Vitis* and Muscadinia divergence. *bioRxiv* **2020**. [CrossRef]
62. Topfer, R.; Hausmann, L. Table of Loci for Traits in Grapevine Relevant for Breeding and Genetics. *VIVC Vitis Int. Var. Cat.* **2010**, *40024*, 2–5.
63. Sargolzaei, M.; Maddalena, G.; Bitsadze, N.; Maghradze, D.; Bianco, P.A.; Failla, O.; Toffolatti, S.L.; Lorenzis, G. De Rpv29, Rpv30 and Rpv31: Three Novel Genomic Loci Associated With Resistance to Plasmopara viticola in *Vitis vinifera*. *Front. Plant Sci.* **2020**, *11*, 1–16. [CrossRef] [PubMed]
64. Barba, P.; Cadle-Davidson, L.; Harriman, J.; Glaubitz, J.C.; Brooks, S.; Hyma, K.; Reisch, B. Grapevine powdery mildew resistance and susceptibility loci identified on a high-resolution SNP map. *Theor. Appl. Genet.* **2014**, *127*, 73–84. [CrossRef] [PubMed]
65. Winterhagen, P.; Howard, S.F.; Qiu, W.; Kovács, L.G. Transcriptional up-regulation of grapevine MLO genes in response to powdery mildew infection. *Am. J. Enol. Vitic.* **2008**, *59*, 159–168.
66. Feechan, A.; Jermakow, A.M.; Dry, I.B. Grapevine MLO candidates required for powdery mildew pathogenicity? *Plant Signal. Behav.* **2009**, *4*, 522–523. [CrossRef]
67. Pessina, S.; Lenzi, L.; Perazzolli, M.; Campa, M.; Dalla Costa, L.; Urso, S.; Valè, G.; Salamini, F.; Velasco, R.; Malnoy, M. Knockdown of MLO genes reduces susceptibility to powdery mildew in grapevine. *Hortic. Res.* **2016**, *3*. [CrossRef]

68. Madeira, F.; Park, Y.M.; Lee, J.; Buso, N.; Gur, T.; Madhusoodanan, N.; Basutkar, P.; Tivey, A.R.N.; Potter, S.C.; Finn, R.D.; et al. The EMBL-EBI search and sequence analysis tools APIs in 2019. *Nucleic Acids Res.* **2019**, *47*, W636–W641. [CrossRef]
69. Vitulo, N.; Forcato, C.; Carpinelli, E.; Telatin, A.; Campagna, D.; D'Angelo, M.; Zimbello, R.; Corso, M.; Vannozzi, A.; Bonghi, C.; et al. A deep survey of alternative splicing in grape reveals changes in the splicing machinery related to tissue, stress condition and genotype. *BMC Plant Biol.* **2014**, *14*, 99. [CrossRef]
70. Canaguier, A.; Grimplet, J.; Di Gaspero, G.; Scalabrin, S.; Duchêne, E.; Choisne, N.; Mohellibi, N.; Guichard, C.; Rombauts, S.; Le Clainche, I.; et al. A new version of the grapevine reference genome assembly (12X.v2) and of its annotation (VCost.v3). *Genomics Data* **2017**, *14*, 56–62. [CrossRef]
71. Li, H.; Durbin, R. Fast and accurate long-read alignment with Burrows–Wheeler transform. *Bioinformatics* **2010**, *26*, 589–595. [CrossRef]
72. Staden, R.; Beal, K.F.; Bonfield, J.K. The staden package, 1998. In *Bioinformatics Methods and Protocols*; Humana Press: Totowa, NJ, USA, 2000; pp. 115–130. [CrossRef]
73. Li, H.; Handsaker, B.; Wysoker, A.; Fennell, T.; Ruan, J.; Homer, N.; Marth, G.; Abecasis, G.; Durbin, R. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **2009**, *25*, 2078–2079. [CrossRef] [PubMed]
74. Danecek, P.; Auton, A.; Abecasis, G.; Albers, C.A.; Banks, E.; DePristo, M.A.; Handsaker, R.E.; Lunter, G.; Marth, G.T.; Sherry, S.T.; et al. The variant call format and VCFtools. *Bioinformatics* **2011**, *27*, 2156–2158. [CrossRef] [PubMed]
75. Cingolani, P.; Platts, A.; Wang, L.L.; Coon, M.; Nguyen, T.; Wang, L.; Land, S.J.; Lu, X.; Ruden, D.M. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff. *Fly* **2012**, *6*, 80–92. [CrossRef] [PubMed]
76. Kumar, P.; Henikoff, S.; Ng, P.C. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat. Protoc.* **2009**, *4*, 1073–1082. [CrossRef] [PubMed]
77. Betts, M.J.; Russel, R.B. Amino acid properties and consequences of substitutions. In *Bioinformatics for Geneticists*; John Wiley & Sons, Ltd.: Hoboken, NJ, USA, 2003; pp. 289–316. ISBN 0-470-84393-4.
78. Stephens, M.; Smith, N.J.; Donnelly, P. A new statistical method for haplotype reconstruction from population data. *Am. J. Hum. Genet.* **2001**, *68*, 978–989. [CrossRef]
79. OIV (International Organisation of Vine and Wine). *OIV Descriptor List for Grape Varieties and Vitis Species*; OIV: Paris, France, 2009.
80. Zeilmaker, T. Functional and Applied Aspects of the DOWNY MILDEW RESISTANT 1 and 6 Genes in Arabidopsis. Ph.D. Thesis, Utrecht University, Utrecht, The Netherlands, 2012.
81. Proost, S.; Van Bel, M.; Vaneechoutte, D.; Van De Peer, Y.; Inzé, D.; Mueller-Roeber, B.; Vandepoele, K. PLAZA 3.0: An access point for plant comparative genomics. *Nucleic Acids Res.* **2015**, *43*, D974–D981. [CrossRef]
82. Zimmermann, L.; Stephens, A.; Nam, S.Z.; Rau, D.; Kübler, J.; Lozajic, M.; Gabler, F.; Söding, J.; Lupas, A.N.; Alva, V. A Completely Reimplemented MPI Bioinformatics Toolkit with a New HHpred Server at its Core. *J. Mol. Biol.* **2018**, *430*, 2237–2243. [CrossRef]
83. Kluza, A.; Niedzialkowska, E.; Kurpiewska, K.; Wojdyla, Z.; Quesne, M.; Kot, E.; Porebski, P.J.; Borowski, T. Crystal structure of thebaine 6-O-demethylase from the morphine biosynthesis pathway. *J. Struct. Biol.* **2018**, *202*, 229–235. [CrossRef]
84. Amrine, K.C.H.; Blanco-Ulate, B.; Riaz, S.; Pap, D.; Jones, L.; Figueroa-Balderas, R.; Walker, M.A.; Cantu, D. Comparative transcriptomics of Central Asian *Vitis vinifera* accessions reveals distinct defense strategies against powdery mildew. *Hortic. Res.* **2015**, *2*. [CrossRef]
85. Cadle-Davidson, L. Variation within and between *Vitis* spp. for foliar resistance to the downy mildew pathogen Plasmopara viticola. *Plant Dis.* **2008**, *92*, 1577–1584. [CrossRef]
86. Lijavetzky, D.; Cabezas, J.; Ibáñez, A.; Rodríguez, V.; Martínez-Zapater, J.M. High throughput SNP discovery and genotyping in grapevine (*Vitis vinifera* L.) by combining a re-sequencing approach and SNPlex technology. *BMC Genomics* **2007**, *8*, 1–11. [CrossRef] [PubMed]
87. Vezzulli, S.; Micheletti, D.; Riaz, S.; Pindo, M.; Viola, R.; This, P.; Walker, M.A.; Troggio, M.; Velasco, R. A SNP transferability survey within the genus *Vitis*. *BMC Plant Biol.* **2008**, *8*, 1–10. [CrossRef] [PubMed]
88. Vezzulli, S.; Troggio, M.; Coppola, G.; Jermakow, A.; Cartwright, D.; Zharkikh, A.; Stefanini, M.; Grando, M.S.; Viola, R.; Adam-Blondon, A.F.; et al. A reference integrated map for cultivated grapevine (*Vitis vinifera* L.) from three crosses, based on 283 SSR and 501 SNP-based markers. *Theor. Appl. Genet.* **2008**, *117*, 499–511. [CrossRef]
89. Salmaso, M.; Faes, G.; Segala, C.; Stefanini, M.; Salakhutdinov, I.; Zyprian, E.; Toepfer, R.; Grando, M.S.; Velasco, R. Genome diversity and gene haplotypes in the grapevine (*Vitis*. *Mol. Breed.* **2004**, 385–395. [CrossRef]
90. Marrano, A.; Birolo, G.; Prazzoli, M.L.; Lorenzi, S.; Valle, G.; Grando, M.S. SNP-discovery by RAD-sequencing in a germplasm collection of wild and cultivated grapevines (*V. vinifera* L.). *PLoS ONE* **2017**, *12*, e170655. [CrossRef]
91. Schneider, K.; Weisshaar, B.; Borchardt, D.C.; Salamini, F. SNP frequency and allelic haplotype structure of Beta vulgaris expressed genes. *Mol. Breed.* **2001**, *8*, 63–74. [CrossRef]
92. Simko, I.; Haynes, K.G.; Jones, R.W. Assessment of linkage disequilibrium in potato genome with single nucleotide polymorphism markers. *Genetics* **2006**, *173*, 2237–2245. [CrossRef]
93. Byers, R.L.; Harker, D.B.; Yourstone, S.M.; Maughan, P.J.; Udall, J.A. Development and mapping of SNP assays in allotetraploid cotton. *Theor. Appl. Genet.* **2012**, *124*, 1201–1214. [CrossRef]
94. Zhu, Y.L.; Song, Q.J.; Hyten, D.L.; Van Tassell, C.P.; Matukumalli, L.K.; Grimm, D.R.; Hyatt, S.M.; Fickus, E.W.; Young, N.D.; Cregan, P.B. Single-nucleotide polymorphisms in soybean. *Genetics* **2003**, *163*, 1123–1134. [PubMed]

95. Wu, S.B.; Wirthensohn, M.G.; Hunt, P.; Gibson, J.P.; Sedgley, M. High resolution melting analysis of almond SNPs derived from ESTs. *Theor. Appl. Genet.* **2008**, *118*, 1–14. [CrossRef]
96. Salmaso, M.; Malacarne, G.; Troggio, M.; Faes, G.; Stefanini, M.; Grando, M.S.; Velasco, R. A grapevine (*Vitis vinifera* L.) genetic map integrating the position of 139 expressed genes. *Theor. Appl. Genet.* **2008**, *116*, 1129–1143. [CrossRef] [PubMed]
97. Aranzana, M.J.; Illa, E.; Howad, W.; Arús, P. A first insight into peach [Prunus persica (L.) Batsch] SNP variability. *Tree Genet. Genomes* **2012**, *8*, 1359–1369. [CrossRef]
98. Tuskan, G.A.; Difazio, S.; Jansson, S.; Bohlmann, J.; Grigoriev, I.; Hellsten, U.; Putnam, N.; Ralph, S.; Rombauts, S.; Salamov, A.; et al. The Genome of Black Cottonwood Populus trichocarpa (Torr. & Gray). *Science* **2006**, *313*, 1596–1604. [CrossRef] [PubMed]
99. Thavamanikumar, S.; McManus, L.J.; Tibbits, J.F.G.; Bossinger, G. The significance of single nucleotide polymorphisms (SNPs) in Eucalyptus globulus breeding programs. *Aust. For.* **2011**, *74*, 23–29. [CrossRef]
100. Emanuelli, F.; Lorenzi, S.; Grzeskowiak, L.; Catalano, V.; Stefanini, M.; Troggio, M.; Myles, S.; Martinez-Zapater, J.M.; Zyprian, E.; Moreira, F.M.; et al. Genetic diversity and population structure assessed by SSR and SNP markers in a large germplasm collection of grape. *BMC Plant Biol.* **2013**, *13*, 1–17. [CrossRef]
101. Jones, E.S.; Sullivan, H.; Bhattramakki, D.; Smith, J.S.C. A comparison of simple sequence repeat and single nucleotide polymorphism marker technologies for the genotypic analysis of maize (*Zea mays* L.). *Theor. Appl. Genet.* **2007**, *115*, 361–371. [CrossRef]
102. Grattapaglia, D.; Silva-Junior, O.B.; Kirst, M.; de Lima, B.M.; Faria, D.A.; Pappas, G.J. High-throughput SNP genotyping in the highly heterozygous genome of Eucalyptus: Assay success, polymorphism and transferability across species. *BMC Plant Biol.* **2011**, *11*, 65. [CrossRef]
103. Biswas, C.; Dey, P.; Karmakar, P.G.; Satpathy, S. Discovery of large-scale SNP markers and construction of linkage map in a RIL population of jute (*Corchorus capsularis*). *Mol. Breed.* **2015**, *35*, 1–10. [CrossRef]
104. Cheng, L.; Chen, X.; Jiang, C.; Ma, B.; Ren, M.; Cheng, Y.; Liu, D.; Geng, R.; Yang, A. High-density SNP genetic linkage map construction and quantitative trait locus mapping for resistance to cucumber mosaic virus in tobacco (*Nicotiana tabacum* L.). *Crop J.* **2019**, *7*, 539–547. [CrossRef]
105. Aflitos, S.; Schijlen, E.; De Jong, H.; De Ridder, D.; Smit, S.; Finkers, R.; Wang, J.; Zhang, G.; Li, N.; Mao, L.; et al. Exploring genetic variation in the tomato (Solanum section Lycopersicon) clade by whole-genome sequencing. *Plant J.* **2014**, *80*, 136–148. [CrossRef]
106. Xanthopoulou, A.; Montero-Pau, J.; Mellidou, I.; Kissoudis, C.; Blanca, J.; Picó, B.; Tsaballa, A.; Tsaliki, E.; Dalakouras, A.; Paris, H.S.; et al. Whole-genome resequencing of Cucurbita pepo morphotypes to discover genomic variants associated with morphology and horticulturally valuable traits. *Hortic. Res.* **2019**, *6*. [CrossRef] [PubMed]
107. Dong, X.; Wang, Z.; Tian, L.; Zhang, Y.; Qi, D.; Huo, H.; Xu, J.; Li, Z.; Liao, R.; Shi, M.; et al. De novo assembly of a wild pear (*Pyrus betuleafolia*) genome. *Plant Biotechnol. J.* **2019**, 1–15. [CrossRef] [PubMed]
108. Cardone, M.F.; D'Addabbo, P.; Alkan, C.; Bergamini, C.; Catacchio, C.R.; Anaclerio, F.; Chiatante, G.; Marra, A.; Giannuzzi, G.; Perniola, R.; et al. Inter-varietal structural variation in grapevine genomes. *Plant J.* **2016**, *88*, 648–661. [CrossRef] [PubMed]
109. Baudhuin, L.M.; Lagerstedt, S.A.; Klee, E.W.; Fadra, N.; Oglesbee, D.; Ferber, M.J. Confirming variants in next-generation sequencing panel testing by sanger sequencing. *J. Mol. Diagnostics* **2015**, *17*, 456–461. [CrossRef]
110. Mu, W.; Lu, H.M.; Chen, J.; Li, S.; Elliott, A.M. Sanger Confirmation Is Required to Achieve Optimal Sensitivity and Specificity in Next-Generation Sequencing Panel Testing. *J. Mol. Diagnostics* **2016**, *18*, 923–932. [CrossRef] [PubMed]
111. Quaynor, S.D.; Bosley, M.E.; Duckworth, C.G.; Porter, K.R.; Kim, S.H.; Kim, H.G.; Chorich, L.P.; Sullivan, M.E.; Choi, J.H.; Cameron, R.S.; et al. Targeted next generation sequencing approach identifies eighteen new candidate genes in normosmic hypogonadotropic hypogonadism and Kallmann syndrome. *Mol. Cell. Endocrinol.* **2016**, *437*, 86–96. [CrossRef] [PubMed]
112. Strom, S.P.; Lee, H.; Das, K.; Vilain, E.; Nelson, S.F.; Grody, W.W.; Deignan, J.L. Assessing the necessity of confirmatory testing for exome-sequencing results in a clinical molecular diagnostic laboratory. *Genet. Med.* **2014**, *16*, 510–515. [CrossRef]
113. Zheng, J.; Zhang, H.; Banerjee, S.; Li, Y.; Zhou, J.; Yang, Q.; Tan, X.; Han, P.; Fu, Q.; Cui, X.; et al. A comprehensive assessment of Next-Generation Sequencing variants validation using a secondary technology. *Mol. Genet. Genomic Med.* **2019**, *7*, 1–7. [CrossRef]
114. Satya, R.V.; DiCarlo, J. Edge effects in calling variants from targeted amplicon sequencing. *BMC Genomics* **2014**, *15*, 1–7. [CrossRef]
115. Lacombe, T.; Audeguin, L.; Boselli, M.; Bucchetti, B.; Cabello, F.; Chatelet, P.; Crespan, M.; D'Onofrio, C.; Eiras Dias, J.; Ercisli, S.; et al. Grapevine European Catalogue: Towards a comprehensive list. *Vitis* **2011**, *50*, 65–68.
116. Excoffier, L.; Langaney, A. Origin and Differentiation of Human Mitochondrial DNA. *J. Hum. Genet.* **1989**, *44*, 73. [PubMed]
117. Watterson, G.A.; Guess, H.A. Is the most frequent allele the oldest? *Theor. Popul. Biol.* **1977**, *11*, 141–160. [CrossRef]
118. Donnelly, P.; Tavaré, S. The ages of alleles and a coalescent. *Adv. Appl. Probab.* **1986**, *18*, 1–19. [CrossRef]
119. Riahi, L.; Zoghlami, N.; Dereeper, A.; Laucou, V.; Mliki, A.; This, P. Single nucleotide polymorphism and haplotype diversity of the gene NAC4 in grapevine. *Ind. Crops Prod.* **2013**, *43*, 718–724. [CrossRef]
120. Fernandez, L.; Le Cunff, L.; Tello, J.; Lacombe, T.; Boursiquot, J.M.; Fournier-Level, A.; Bravo, G.; Lalet, S.; Torregrosa, L.; This, P.; et al. Haplotype diversity of VvTFL1A gene and association with cluster traits in grapevine (*V. vinifera*). *BMC Plant Biol.* **2014**, *14*, 1–14. [CrossRef]
121. Nicolas, S.D.; Péros, J.P.; Lacombe, T.; Launay, A.; Le Paslier, M.C.; Bérard, A.; Mangin, B.; Valière, S.; Martins, F.; Le Cunff, L.; et al. Genetic diversity, linkage disequilibrium and power of a large grapevine (*Vitis vinifera* L) diversity panel newly designed for association studies. *BMC Plant Biol.* **2016**, *16*, 1–19. [CrossRef]

122. Magris, G.; Di Gaspero, G.; Marroni, F.; Zenoni, S.; Tornielli, G.B.; Celii, M.; De Paoli, E.; Pezzotti, M.; Conte, F.; Paci, P.; et al. Genetic, epigenetic and genomic effects on variation of gene expression among grape varieties. *Plant J.* **2019**, *99*, 895–909. [CrossRef]
123. Foria, S.; Copetti, D.; Eisenmann, B.; Magris, G.; Vidotto, M.; Scalabrin, S.; Testolin, R.; Cipriani, G.; Wiedemann-Merdinoglu, S.; Bogs, J.; et al. Gene duplication and transposition of mobile elements drive evolution of the Rpv3 resistance locus in grapevine. *Plant J.* **2020**, *101*, 529–542. [CrossRef]
124. Schaart, J.G.; van de Wiel, C.C.M.; Lotz, L.A.P.; Smulders, M.J.M. Opportunities for Products of New Plant Breeding Techniques. *Trends Plant Sci.* **2016**, *21*, 438–449. [CrossRef]
125. Bisht, D.S.; Bhatia, V.; Bhattacharya, R. Improving plant-resistance to insect-pests and pathogens: The new opportunities through targeted genome editing. *Semin. Cell Dev. Biol.* **2019**, 1–12. [CrossRef]
126. Pompili, V.; Dalla Costa, L.; Piazza, S.; Pindo, M.; Malnoy, M. Reduced fire blight susceptibility in apple cultivars using a high-efficiency CRISPR/Cas9-FLP/FRT-based gene editing system. *Plant Biotechnol. J.* **2020**, *18*, 845–858. [CrossRef] [PubMed]
127. Low, Y.C.; Lawton, M.A.; Di, R. Validation of barley 2OGO gene as a functional orthologue of Arabidopsis DMR6 gene in Fusarium head blight susceptibility. *Sci. Rep.* **2020**, 1–13. [CrossRef] [PubMed]
128. Ffrench-Constant, R.H.; Bass, C. Does resistance really carry a fitness cost? *Curr. Opin. Insect Sci.* **2017**, *21*, 39–46. [CrossRef]
129. Zaidi, S.S.E.A.; Mukhtar, M.S.; Mansoor, S. Genome Editing: Targeting Susceptibility Genes for Plant Disease Resistance. *Trends Biotechnol.* **2018**, *36*, 898–906. [CrossRef] [PubMed]
130. Rodríguez-Leal, D.; Lemmon, Z.H.; Man, J.; Bartlett, M.E.; Lippman, Z.B. Engineering Quantitative Trait Variation for Crop Improvement by Genome Editing. *Cell* **2017**, *171*, 470–480.e8. [CrossRef]
131. Wolter, F.; Puchta, H. Application of CRISPR/Cas to Understand Cis- and Trans-Regulatory Elements in Plants. In *Methods in Molecular Biology*; Humana Press: Totowa, NJ, USA, 2018; pp. 23–40.
132. Bastet, A.; Robaglia, C.; Gallois, J.L. eIF4E Resistance: Natural Variation Should Guide Gene Editing. *Trends Plant Sci.* **2017**, *22*, 411–419. [CrossRef]
133. Bastet, A.; Zafirov, D.; Giovinazzo, N.; Guyon-Debast, A.; Nogué, F.; Robaglia, C.; Gallois, J.-L. Mimicking natural polymorphism in eIF4E by CRISPR-Cas9 base editing is associated with resistance to potyviruses. *Plant Biotechnol. J.* **2019**, *17*, 1736–1750. [CrossRef]

SUPPORTING INFORMATION

Table S1. List of studied grapevine genotypes.

Genotype	Taxon	Breeder/Institute of Provenience	Repository
01-1-768	*Vitis* hybrid	European Institute	Edmund Mach Foundation (IT)
29-02-112	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
29-02-85	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
29-2-133	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
29-2-187	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
29-2-322	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
30-04-154	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
30-3-040	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
30-3-154	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
30-4-190	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
54-2	*Vitis* hybrid	European Institute	Edmund Mach Foundation (IT)
9-16/06	*Vitis* hybrid	European Institute	Edmund Mach Foundation (IT)
94-1-003	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
Alden	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
B87-60	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
BC4	*Vitis* hybrid	European Institute	Edmund Mach Foundation (IT)
Bianca	*Vitis* hybrid	University of Horticulture and Food Industry, Kölyuktetö (HU)	Edmund Mach Foundation (IT)
Black Monukka	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
Blanc du Bois	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Blue Lake	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Bronner	*Vitis* hybrid	Bronner, Johan Philipp	Edmund Mach Foundation (IT)
BS 4825	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Buffalo	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
Cabernet Carbon	*Vitis* hybrid	Becker, Norbert	Edmund Mach Foundation (IT)
Cabernet Cortis	*Vitis* hybrid	Becker, Norbert	Edmund Mach Foundation (IT)
Captivator	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Cardinal	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
Catawba	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
Cayuga White	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)

Chambourcin	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Chancellor	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Chaouch	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
Chardonel	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Clinton	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Columbia	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Concord	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
Conquistador	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Couderc 13	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
D'Arpa	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Daytona	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Diamond	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Diamond Muscat	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
Dunstan 336	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Eger 2	*Vitis* hybrid	Seyve-Villard, Bertille	Edmund Mach Foundation (IT)
Eger 99-11.01	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Eger1	*Vitis* hybrid	Csizmazia, Jozsef; Bereznai, Laszlo	Edmund Mach Foundation (IT)
Esther	*Vitis* hybrid	Szegedi, Sandor	Edmund Mach Foundation (IT)
Exotic	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
F243 Tamiani	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
F272 Everglade	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
F560 Big Brown	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
F9-68	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
Fanny	*Vitis* hybrid	University of Horticulture and Food Industry, Kölyuktetö (HU)	Edmund Mach Foundation (IT)
FLA 449	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
FLA BN6-67	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
FLA BN6-85	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
FLA CB8-1	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
FLA DC1-39	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
FLA W1521	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Flame Tokai	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
Gm6494	*Vitis* hybrid	Geisenheim University (DE)	Edmund Mach Foundation (IT)
Golden Muscat	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)

Helios	*Vitis* hybrid	VSSVVM Research and Breeding Station for Enology and Viticulture (SK)	Edmund Mach Foundation (IT)
Herbert	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Isabella	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Jasmin8/1	*Vitis* hybrid	European Institute	Edmund Mach Foundation (IT)
Johanniter	*Vitis* hybrid	Staatliche Weinbauinstitut Freiburg (CH)	Edmund Mach Foundation (IT)
JS 23-416	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Kunbaràt	*Vitis* hybrid	University of Horticulture and Food Industry (HU)	University of Udine (IT)
Kunleany	*Vitis* hybrid	University of Horticulture and Food Industry (HU)	Edmund Mach Foundation (IT)
Lenoir	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Leon Millot	*Vitis* hybrid	Kuhlmann, Eugène	Edmund Mach Foundation (IT)
Lidi	*Vitis* hybrid	Institute for Viticulture and Enology (HU)	Edmund Mach Foundation (IT)
LU1	*Vitis* hybrid	Mendel University Brno (CZ)	Edmund Mach Foundation (IT)
LU2	*Vitis* hybrid	Mendel University Brno (CZ)	Edmund Mach Foundation (IT)
M11-14/St. George	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Malaga	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
Mantey	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
Mars	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Merzling	*Vitis* hybrid	Staatliche Weinbauinstitut Freiburg (CH)	Edmund Mach Foundation (IT)
Muscaris	*Vitis* hybrid	Staatliche Weinbauinstitut Freiburg (CH)	Edmund Mach Foundation (IT)
MW 1bis	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
MW 38	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
MW 50	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
MW 53	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
MW 54	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
MW 58	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
MW 66	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
MW1	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
MW14	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
Neptune	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Nero	*Vitis* hybrid	University of Horticulture and Food Industry, Kölyuktetö (HU)	Edmund Mach Foundation (IT)
Norris	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)

Norton	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
NY08.0701a	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
NY08.0701b	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
NY09.0807b	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
NY63.1016.01	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
NY65.0562.01	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
NY84.0100.05	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
NY95.0308.02	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
NY97.0503.02	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
NY97.0512.01	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Ontario	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Orlando Seedless	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Palatina	*Vitis* hybrid	University of Horticulture and Food Industries, Szigetcsép (HU)	Edmund Mach Foundation (IT)
Perlette	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
Petra	*Vitis* hybrid	Institute of Viticulture, Arboriculture, Fruit and Horticulture (RS)	Edmund Mach Foundation (IT)
Phoenix	*Vitis* hybrid	Julius Kühn Institute- Geilweilerhof (DE)	Edmund Mach Foundation (IT)
Pixiola	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
Poloskei Muskotaly	*Vitis* hybrid	University for Horticulture and Food Industry (HU)	Edmund Mach Foundation (IT)
Prior	*Vitis* hybrid	Staatliche Weinbauinstitut Freiburg (CH)	Edmund Mach Foundation (IT)
Regent	*Vitis* hybrid	Julius Kühn Institute- Geilweilerhof (DE)	Edmund Mach Foundation (IT)
Ribier	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
Roucaneuf	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)
Schuyler	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Seibel 13666	*Vitis* hybrid	Seibel, Albert	Edmund Mach Foundation (IT)
Seibel 2007	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Seibel 6339	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Seibel 880	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Seyval	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Seyve-Villard 5-276	*Vitis* hybrid	Seyve-Villard, Bertille	Edmund Mach Foundation (IT)
Sheridan	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Sirius	*Vitis* hybrid	Julius Kühn Institute- Geilweilerhof (DE)	Edmund Mach Foundation (IT)
Solaris	*Vitis* hybrid	Staatliche Weinbauinstitut Freiburg (CH)	Edmund Mach Foundation (IT)
Souvignier gris	*Vitis* hybrid	Staatliche Weinbauinstitut Freiburg (CH)	Edmund Mach Foundation (IT)
Steuben	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Stover	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Sultana	*Vitis* hybrid	UC Davis (USA)	UC Davis (USA)

Suwannee	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
SV023	*Vitis* hybrid	INNO*VITIS*	Edmund Mach Foundation (IT)
Traminette	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
V. riparia x V. cordifolia	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Valvin Muscat	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Venus	*Vitis* hybrid	UC Davis (USA)	Edmund Mach Foundation (IT)
Wayne	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Worden	*Vitis* hybrid	Cornell University (USA)	Cornell University (USA)
Zala Gyoengye	*Vitis* hybrid	University of Horticulture and Food Industry, Kölyuktetö (HU)	Edmund Mach Foundation (IT)
Zarja Severa	*Vitis* hybrid	CGL -Central genetic Laboratory Michurinsk (RU)	Edmund Mach Foundation (IT)
V. aestivalis	*Vitis spp.*	UC Davis (USA)	UC Davis (USA)
V. berlandieri Texas	*Vitis spp.*	Cornell University (USA)	Edmund Mach Foundation (IT)
V. cordifolia	*Vitis spp.*	European Institute	Edmund Mach Foundation (IT)
V. rubra	*Vitis spp.*	European Institute	Edmund Mach Foundation (IT)
V. rufotomentosa	*Vitis spp.*	UC Davis (USA)	UC Davis (USA)
V. rupestris	*Vitis spp.*	Cornell University (USA)	Edmund Mach Foundation (IT)
V. rupestris Constantia	*Vitis spp.*	Cornell University (USA)	Edmund Mach Foundation (IT)
V. rupestris du Lot	*Vitis spp.*	Sijas, M.R.	Edmund Mach Foundation (IT)
V. rupestris Metallique	*Vitis spp.*	European Institute	Edmund Mach Foundation (IT)
V. shuttleworthii	*Vitis spp.*	UC Davis (USA)	UC Davis (USA)
V. simpsonii	*Vitis spp.*	European Institute	Edmund Mach Foundation (IT)
V. smalliana	*Vitis spp.*	UC Davis (USA)	UC Davis (USA)
Coia1	*Vitis spp.*/hybrid	New Jersey - wild coll. (USA)	Edmund Mach Foundation (IT)
Coia10	*Vitis spp.*/hybrid	New Jersey - wild coll. (USA)	Edmund Mach Foundation (IT)
Coia11	*Vitis spp.*/hybrid	New Jersey - wild coll. (USA)	Edmund Mach Foundation (IT)
Coia12	*Vitis spp.*/hybrid	New Jersey - wild coll. (USA)	Edmund Mach Foundation (IT)
Coia4	*Vitis spp.*/hybrid	New Jersey - wild coll. (USA)	Edmund Mach Foundation (IT)
Coia5	*Vitis spp.*/hybrid	New Jersey - wild coll. (USA)	Edmund Mach Foundation (IT)
Coia7	*Vitis spp.*/hybrid	New Jersey - wild coll. (USA)	Edmund Mach Foundation (IT)
Coia9	*Vitis spp.*/hybrid	New Jersey - wild coll. (USA)	Edmund Mach Foundation (IT)
Corella2	*Vitis spp.*/hybrid	New Jersey - wild coll. (USA)	Edmund Mach Foundation (IT)
Corella3	*Vitis spp.*/hybrid	New Jersey - wild coll. (USA)	Edmund Mach Foundation (IT)

Lorenzo1	*Vitis spp.*/hybrid	New Jersey - wild coll. (USA)	Edmund Mach Foundation (IT)
Cabernet franc	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Cabernet Sauvignon	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Chardonnay	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Corvina veronese	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Franconia	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Garganega	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Gewurtztraminer	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
IM6013	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Italia	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Kishmiss Vatkana	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Lagrein	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Malvasia di Candia Aromatica	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Marzemino	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Merlot	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Michele Palieri	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Muller Thurgau	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Muscat Hamburg	*Vitis vinifera*	UC Davis (USA)	UC Davis (USA)
Nosiola	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Pinot blanc	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Pinot gris	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Pinot noir	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
PN40024	*Vitis vinifera*	INRA-Colmar (FR)	Edmund Mach Foundation (IT)
Riesling	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Sauvignon blanc	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Schiava	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Sultanina	*Vitis vinifera*	-	CRA (IT)
Teroldego	*Vitis vinifera*	-	Edmund Mach Foundation (IT)
Zweigelt	*Vitis vinifera*	-	Edmund Mach Foundation (IT)

Table S2. Selected genotypes for Sanger sequencing of each gene, investigated variants with their physical position, and sequencing primers.

Gene	Amplicon	Sanger forward primer 5'-3'	Sanger reverse primer 3'-5'	Variant	Physical position	Selected Accessions
VvDMR6.1	DMR6.1_A	ACTTGACCTTGCCACAAAGT	AGGATGAGGACGAATTAGGCA	T>G	chr16:21186255	Pinot gris
	DMR6.1_A2	TCAATCATGGGGTAGCTGCA	AGGATGAGGACGAATTAGGCA	T>C	chr16:21186384	V. rupestris du Lot
	DMR6.1_A3	TCAATCATGGGGTAGCTGCA	AGGAAGGAGGATTGGAAGGC	T>C	chr16:21186384	Petra
	DMR6.1_A4	TCCAGGAAGCTGCTTAGTAGAG	CTGTTTACGTCCTTGCCGAG	T>C	chr16:21186707	Chancellor
	DMR6.1_B	GGTAGACTCCACTAGAAGCCC	TCCATAAGTGAGCTCGGGTT	T>A	chr16:21183902	Blue Lake
	DMR6.1_C	GATTGCCAGGACACACAGAC	TTGATCCAAGTCCCTGCCC	T>C	chr16:21183413	MW 66
				A>T	chr16:21183675	NY84.0100.05
VvDMR6.2	DMR6.2_A	AGTCTTCACTCCCTTTTCCTTCT	CCTCATTGTCTTTGATGGGTCA	A>G	chr13:15734172	Coia12 , Coia7, Worden
				T>C	chr13:15734275	*V. rubra*
	DMR6.2_A2	GGATTCTAAGGTCCTTTCCACC	CCTCATTGTCTTTGATGGGTCA	A>G	chr13:15734172	Golden Muscat
VvDLO1	DLO1_A	TGTCTGACCTTGCATCCAGT	AAAGATGGAGGGTTGGAGGG	A>T	chr15:16617922	Kunleany, Lenoir, Petra
	DLO1_B	CTTCAAGACGATGTGCCCG	TTTCATGGAGGAATCTGTCGAA	G>A	chr15:16618941	F560 Big Brown, Mantey
VvDLO2	DLO2_A	CCCCACTTGTGAATTTGCAGA	CTGGTGCAATACTCAGCCAC	A>G	chr2:2532165	Coia12
				C>G	chr2:2532133	Coia12
	DLO2_A2	CCCCACTTGTGAATTTGCAGA	CCCCATGGTTTTGAATCTGGA	T>A	chr2:2532551	Coia12 , Coia7, Chancellor, Golden Muscat, Pixiola, Worden

Table S3. List of impacting mutations with positions and data in VCF (Variant Call Format).

Name	Taxon	Gene	Variant	Physical Position	CDS Offset	Aminoacid Offset	GT:PL:DP:AD:GQ	Sanger Seq Confirmation
B87-60	*Vitis* hybrid	*VvDMR6.2*	A>G	chr13:15734172	158/1014	53/337	0/1:141,0,172:23:14,9:99	-
Blanc du Bois	*Vitis* hybrid	*VvDMR6.1*	T>A	chr16:21183902	530/1017	177/338	0/1:255,0,150:14:4,10:99	-
Blue Lake	*Vitis* hybrid	*VvDMR6.1*	T>A	chr16:21183902	530/1017	177/338	0/1:255,0,255:23:11,11:99	NO
		VvDMR6.2	A>G	chr13:15734172	158/1014	53/337	0/1:135,0,175:39:27,12:99	-
Captivator	*Vitis* hybrid	*VvDMR6.2*	A>G	chr13:15734172	158/1014	53/337	0/1:125,0,171:44:32,12:99	-
Catawba	*Vitis* hybrid	*VvDMR6.2*	G>A	chr13:15730387	494/1014	165/337	0/1:78,0,255:11:9,2:56	-
Chancellor	*Vitis* hybrid	*VvDLO2*	T>A	chr2:2532551	5/1047	2/348	0/1:212,0,118:13:4,9:99	NO
		VvDMR6.1	T>C	chr16:21186707	29/1017	10/338	0/1:57,0,255:18:16,2:35	NO
Clinton	*Vitis* hybrid	*VvDLO2*	T>A	chr2:2532551	5/1047	2/348	0/1:237,0,107:13:4,9:99	-
		VvDMR6.2	A>G	chr13:15734172	158/1014	53/337	1/1:255,117,0:39:0,39:99	-
Coia1	*Vitis spp.*/hybrid	*VvDMR6.2*	A>G	chr13:15734172	158/1014	53/337	1/1:255,255,0:158:5,153:99	-
Coia10	*Vitis spp.*/hybrid	*VvDMR6.2*	A>G	chr13:15734172	158/1014	53/337	1/1:255,255,0:252:0,252:99	-
Coia11	*Vitis spp.*/hybrid	*VvDMR6.2*	A>G	chr13:15734172	158/1014	53/337	1/1:255,255,0:111:2,109:99	-
Coia12	*Vitis spp.*/hybrid	*VvDLO2*	T>A	chr2:2532551	5/1047	2/348	0/1:255,0,81:27:4,23:89	YES, He
			A>G	chr2:2532165	391/1047	131/348	0/1:143,0,255,179,255,255:16:12,4,0:99	NO
			C>G	chr2:2532133	423/1047	141/348	0/1:240,0,255:17:9,8:99	NO
		VvDMR6.2	A>G	chr13:15734172	158/1014	53/337	1/1:255,142,0:114:4,110:99	NO
Coia5	*Vitis spp.*/hybrid	*VvDMR6.2*	A>G	chr13:15734172	158/1014	53/337	1/1:255,255,0:95:0,95:99	-

Coia7	*Vitis spp.*/hybrid	*VvDLO2*	T>A	chr2:2532551	5/1047	2/348	0/1:120,0,154:10:6,4:99	NO
		VvDMR6.2	A>G	chr13:15734172	158/1014	53/337	1/1:255,255,0:100:0,100:99	YES, Ho
Coia9	*Vitis spp.*/hybrid	*VvDMR6.2*	A>G	chr13:15734172	158/1014	53/337	1/1:255,255,0:186:1,185:99	-
Corella2	*Vitis spp.*/hybrid	*VvDLO2*	T>A	chr2:2532551	5/1047	2/348	0/1:229,0,125:18:5,12:99	-
D'Arpa	*Vitis* hybrid	*VvDMR6.2*	A>G	chr13:15734172	158/1014	53/337	0/1:121,0,167:82:59,23:99	-
Diamond	*Vitis* hybrid	*VvDLO2*	T>A	chr2:2532551	5/1047	2/348	0/1:214,0,127:14:5,9:99	-
F560 Big Brown	*Vitis* hybrid	*VvDLO1*	G>A	chr15:16618941	905/1035	302/344	0/1:204,0,244:346:187,159:99	YES, He
FLA 449	*Vitis* hybrid	*VvDMR6.2*	A>G	chr13:15734172	158/1014	53/337	0/1:167,0,255:499:367,132:99	-
FLA W1521	*Vitis* hybrid	*VvDMR6.2*	A>G	chr13:15734172	158/1014	53/337	1/1:205,51,0:17:0,17:34	-
Franconia	*Vitis vinifera*	*VvDLO2*	A>T	chr2:2532165	391/1047	131/348	0/2:191,255,255,0,255,255:43:34,0,9:99	-
Golden Muscat	*Vitis* hybrid	*VvDLO2*	T>A	chr2:2532551	5/1047	2/348	0/1:196,0,111:11:4,7:99	NO
		VvDMR6.2	A>G	chr13:15734172	158/1014	53/337	0/1:255,0,255:175:75,100:99	YES, He
Herbert	*Vitis* hybrid	*VvDLO1*	C>A	chr15:16618261	417/1035	139/344	0/1:82,0,255:45:41,4:52	-
		VvDMR6.2	A>G	chr13:15734172	158/1014	53/337	0/1:255,0,255:197:100,97:99	-
Italia	*Vitis vinifera*	*VvDMR6.2*	C>A	chr13:15730243	638/1014	213/337	0/1:157,0,255:108:88,20:99	-
Kunleany	*Vitis* hybrid	*VvDLO1*	A>T	chr15:16617922	155/1035	52/344	0/1:255,0,255:991:508,479:99	YES, He
Lenoir	*Vitis* hybrid	*VvDLO1*	A>T	chr15:16617922	155/1035	52/344	1/1:160,135,0:114:7,106:99	YES, Ho
Lorenzo1	*Vitis spp.*/hybrid	*VvDLO2*	T>A	chr2:2532551	5/1047	2/348	1/1:242,33,0:11:0,11:24	-
M11-14/St. George	*Vitis* hybrid	*VvDLO2*	A>T	chr2:2532398	158/1047	53/348	0/1:100,0,167:127:92,35:89	-
Mantey	*Vitis* hybrid	*VvDLO1*	G>A	chr15:16618941	905/1035	302/344	1/1:255,208,0:69:0,69:99	YES, Ho
Mars	*Vitis* hybrid	*VvDMR6.2*	A>G	chr13:15734172	158/1014	53/337	0/1:135,0,181:118:79,39:99	-
MW 66	*Vitis* hybrid	*VvDMR6.1*	T>C	chr16:21183413	844/1017	282/338	0/1:79,0,164:27:21,6:58	NO

NY08.0701b	*Vitis* hybrid	*VvDMR6.1*	A>T	chr16:21183675	757/1017	253/338	0/1:255,0,255:95:50,45:99	-
NY63.1016.01	*Vitis* hybrid	*VvDMR6.1*	A>T	chr16:21183675	757/1017	253/338	0/1:255,0,192:52:10,42:99	-
NY65.0562.01	*Vitis* hybrid	*VvDLO2*	T>A	chr2:2532551	5/1047	2/348	0/1:230,0,91:13:3,10:99	-
NY84.0100.05	*Vitis* hybrid	*VvDMR6.1*	A>T	chr16:21183675	757/1017	253/338	0/1:255,0,139:73:10,62:99	YES, He
NY97.0503.02	*Vitis* hybrid	*VvDLO2*	T>A	chr2:2532551	5/1047	2/348	0/1:249,0,140:17:5,12:99	-
			A>T	chr2:2532398	158/1047	53/348	0/1:189,0,206:17:9,8:99	-
NY97.0512.01	*Vitis* hybrid	*VvDLO2*	T>A	chr2:2532551	5/1047	2/348	0/1:156,0,188:14:8,6:99	-
Ontario	*Vitis* hybrid	*VvDLO2*	T>A	chr2:2532170	386/1047	129/348	0/1:255,0,255:70:38,32:99	-
Petra	*Vitis* hybrid	*VvDLO1*	A>T	chr15:16617922	155/1035	52/344	0/1:111,0,217:297:197,99:94	NO
		VvDMR6.1	T>C	chr16:21186384	265/1017	89/338	0/1:242,0,214:362:169,193:99	NO
Pinot gris	*Vitis vinifera*	*VvDMR6.1*	T>G	chr16:21186255	394/1017	132/338	1/1:198,39,0:13:0,13:23	NO
Pixiola	*Vitis* hybrid	*VvDLO2*	T>A	chr2:2532551	5/1047	2/348	0/1:239,0,39:15:2,13:47	YES, He
V. rupestris du Lot	*Vitis spp*	*VvDMR6.1*	T>C	chr16:21186384	265/1017	89/338	0/1:240,0,224:448:216,232:99	YES, He
Schuyler	*Vitis* hybrid	*VvDLO2*	T>A	chr2:2532551	5/1047	2/348	0/1:234,0,57:12:2,10:65	-
Seibel 880	*Vitis* hybrid	*VvDLO2*	T>A	chr2:2532551	5/1047	2/348	0/1:199,0,86:12:3,9:94	-
Sheridan	*Vitis* hybrid	*VvDMR6.2*	A>G	chr13:15734172	158/1014	53/337	1/1:255,255,0:358:1,357:99	-
Steuben	*Vitis* hybrid	*VvDLO2*	A>G	chr2:2532176	380/1047	127/348	0/1:126,0,255:20:16,4:99	-
V. aestivalis	*Vitis spp.*	*VvDLO2*	T>A	chr2:2532551	5/1047	2/348	0/1:253,0,86:29:5,24:94	-
V. berlandieri Texas	*Vitis spp.*	*VvDLO1*	A>T	chr15:16617922	155/1035	52/344	1/1:255,255,0:1000:0,998:99	-
		VvDLO2	A>T	chr2:2532398	158/1047	53/348	0/1:255,0,178:18:7,11:99	-
V. cordifolia	*Vitis spp.*	*VvDLO1*	A>T	chr15:16617922	155/1035	52/344	0/1:255,0,255:996:488,507:99	-
		VvDLO2	T>A	chr2:2532551	5/1047	2/348	0/1:223,0,224:23:13,10:99	-
			A>T	chr2:2532398	158/1047	53/348	0/1:180,0,253:23:14,8:99	-
V. riparia x V. cordifolia	*Vitis* hybrid	*VvDLO2*	C>T	chr2:2532230	326/1047	109/348	0/1:60,0,255:17:15,2:35	-
		VvDLO2	T>A	chr2:2532551	5/1047	2/348	0/1:203,0,97:13:3,10:99	-

			A>T	chr2:2532398	158/1047	53/348	0/1:42,0,242:14:12,2:29	-
V. rubra	*Vitis spp.*	*VvDMR6.2*	T>C	chr13:15734275	55/1014	19/337	0/1:72,0,255:13:11,2:50	NO
V. smalliana	*Vitis spp.*	*VvDLO1*	A>T	chr15:16617883	116/1035	39/344	0/1:255,0,255:996:423,573:99	-
		VvDLO2	T>A	chr2:2532551	5/1047	2/348	0/1:204,0,186:16:8,8:99	-
Venus	*Vitis* hybrid	*VvDMR6.2*	A>G	chr13:15734172	158/1014	53/337	0/1:74,0,164:33:27,6:63	-
Wayne	*Vitis* hybrid	*VvDMR6.2*	A>G	chr13:15734172	158/1014	53/337	0/1:255,0,255:120:60,60:99	-
Worden	*Vitis* hybrid	*VvDLO2*	T>A	chr2:2532551	5/1047	2/348	0/1:248,0,107:16:4,12:99	NO
		VvDMR6.2	A>G	chr13:15734172	158/1014	53/337	1/1:255,255,0:301:0,301:99	YES, Ho

Table S4. List of genotypes showing impacting mutations - heterozygous (He) or homozygous (Ho) status - in at least one gene.

Genotype	Taxon	*VvDMR6.1*	*VvDMR6.2*	*VvDLO1*	*VvDLO2*
B87-60	*Vitis* hybrid		He		
Blanc du Bois	*Vitis* hybrid	He			
Blue Lake	*Vitis* hybrid	He	He		
Captivator	*Vitis* hybrid		He		
Catawba	*Vitis* hybrid		He		
Chancellor	*Vitis* hybrid	He			He
Clinton	*Vitis* hybrid		Ho		He
D'Arpa	*Vitis* hybrid		He		
Diamond	*Vitis* hybrid				He
F560 Big Brown	*Vitis* hybrid			He	
FLA 449	*Vitis* hybrid		He		
FLA W1521	*Vitis* hybrid		Ho		
Golden Muscat	*Vitis* hybrid		He		He
Herbert	*Vitis* hybrid		He	He	
Kunleany	*Vitis* hybrid			He	
Lenoir	*Vitis* hybrid			Ho	
M11-14/St. George	*Vitis* hybrid				He
Mantey	*Vitis* hybrid			Ho	
Mars	*Vitis* hybrid		He		
MW 66	*Vitis* hybrid	He			
NY08.0701b	*Vitis* hybrid	He			
NY63.1016.01	*Vitis* hybrid	He			
NY65.0562.01	*Vitis* hybrid				He
NY84.0100.05	*Vitis* hybrid	He			
NY97.0503.02	*Vitis* hybrid	He			He
NY97.0512.01	*Vitis* hybrid		He		He
Ontario	*Vitis* hybrid				He
Petra	*Vitis* hybrid	He	He	He	
Pixiola	*Vitis* hybrid				He
Schuyler	*Vitis* hybrid				He
Seibel 880	*Vitis* hybrid				He
Sheridan	*Vitis* hybrid		Ho		
Steuben	*Vitis* hybrid				He
V. riparia x V. cordifolia	*Vitis* hybrid				He
Venus	*Vitis* hybrid	He	He		
Wayne	*Vitis* hybrid		He		
Worden	*Vitis* hybrid		Ho		He
V. aestivalis	*Vitis spp.*				He
V. berlandieri Texas	*Vitis spp.*			Ho	He
V. cordifolia	*Vitis spp.*			He	He
V. rubra	*Vitis spp.*		He		

V. rupestris du Lot	*Vitis spp.*	He			
V. smalliana	*Vitis spp.*			He	He
Coia1	*Vitis spp.*/hybrid		Ho		
Coia10	*Vitis spp.*/hybrid		Ho		
Coia11	*Vitis spp.*/hybrid		Ho		
Coia12	*Vitis spp.*/hybrid		Ho		He
Coia5	*Vitis spp.*/hybrid		Ho		
Coia7	*Vitis spp.*/hybrid		Ho		He
Coia9	*Vitis spp.*/hybrid		Ho		
Corella2	*Vitis spp.*/hybrid				He
Lorenzo1	*Vitis spp.*/hybrid				Ho
Franconia	*Vitis vinifera*				He
Italia	*Vitis vinifera*		He		
Pinot gris	*Vitis vinifera*	Ho			

Table S5. Haplotype identification and frequencies determined for the *VvDMR6.1, VvDMR6.2, VvDLO1, VvDLO2* genes. Impacting mutations in bold.

Haplotype n.	**Nucleotide position in *VvDMR6.1* gene**														**Genotype**	**Frequency (%)**
	99	131	205	413	422	508	551	2904	2930	3131	3342	3366	3393	3465		
1	T	A	G	G	T	T	T	T	T	A	T	G	T	T	PN40024, Chancellor, NY84.0100.05	18,2
2	T	A	G	G	T	T	T	T	T	A	T	A	T	T	MW66	4,5
3	T	A	G	G	T	T	T	T	T	A	**A**	A	**C**	T	MW66	4,5
4	T	A	G	G	T	T	T	T	T	**T**	T	A	T	T	*V.rupestris* du Lot	4,5
5	T	A	G	G	T	T	T	**A**	T	A	T	G	T	G	Blue Lake	4,5
6	T	A	G	G	T	T	**G**	T	T	A	T	G	T	T	Pinot gris	4,5
7	T	A	G	G	T	G	T	T	C	A	T	G	T	T	Petra	4,5
8	T	A	G	G	T	G	T	**A**	C	A	T	G	T	T	Blue Lake	4,5
9	T	A	G	G	**C**	T	T	T	T	**T**	T	G	T	T	Petra	4,5
10	T	A	G	G	**C**	T	T	T	T	**T**	T	A	T	T	*V.rupestris* du Lot	4,5
11	T	A	C	G	T	T	T	T	T	A	T	G	T	T	NY63.1016.01	4,5
12	T	A	C	G	T	T	T	T	T	A	T	A	T	G	NY08.0701b	4,5
13	T	A	C	G	T	T	T	T	T	**T**	T	G	T	T	NY63.1016.01, NY84.0100.05	9,1
14	T	A	C	G	T	T	T	T	T	**T**	T	A	T	T	NY08.0701b	4,5
15	T	A	C	G	T	T	**G**	T	T	A	T	G	T	T	Blanc du Bois, Pinot gris	9,1
16	T	A	C	A	T	G	T	**A**	C	A	T	G	T	T	Blanc du Bois	4,5
17	C	**G**	C	G	T	T	T	T	T	A	T	G	T	T	Chancellor	4,5

Haplotype n.	Nucleotide position in *VvDMR6.2* gene														Genotype	Frequency (%)
	12	33	60	99	138	143	163	371	540	550	583	3948	4092	4313		
1	T	A	T	A	T	C	A	G	G	T	A	G	C	A	PN40024, Catawba, FLA 449, Mars, NY97.0512.01, Petra, *V.rubra*, Italia	16,7
2	T	A	T	A	T	C	A	G	G	T	A	G	**A**	A	Italia	1,9
3	T	A	T	A	T	C	A	G	G	T	A	**A**	C	A	Catawba	1,9
4	T	A	T	A	T	C	A	G	**A**	**C**	A	G	C	A	NY97.0512.01	1,9
5	T	A	T	G	T	C	A	G	G	T	A	G	C	A	B87-60, Golden Muscat, Herbert, Venus, Wayne	9,3
6	T	A	T	G	T	C	A	G	G	T	A	G	C	**G**	Petra	1,9
7	T	A	T	G	T	A	A	G	G	T	A	G	C	A	Captivator, D'Arpa	3,7
8	T	A	T	G	T	A	**G**	G	G	T	A	G	C	A	B87-60, Blue Lake, Captivator, D'Arpa, FLA W1521, Venus	13,0
9	T	A	T	G	T	A	**G**	C	G	T	A	G	C	A	Mars	1,9
10	T	A	T	G	**C**	C	**G**	G	G	T	A	G	C	A	Clinton, FLA 449, Golden Muscat, Herbert, Sheridan, Wayne, Worden, Coia 1, Coia 10, Coia 11, Coia 12, Coia 5, Coia 7, Coia 9	40,7
11	T	A	T	G	**C**	C	**G**	G	G	T	G	G	C	A	Coia 1	1,9
12	T	A	**C**	G	T	A	A	G	G	T	A	G	C	A	*V.rubra*	1,9
13	T	G	T	A	T	A	A	G	G	T	A	G	C	A	Blue Lake	1,9
14	**C**	A	T	G	**C**	C	**G**	G	G	T	A	G	C	A	Coia 7	1,9

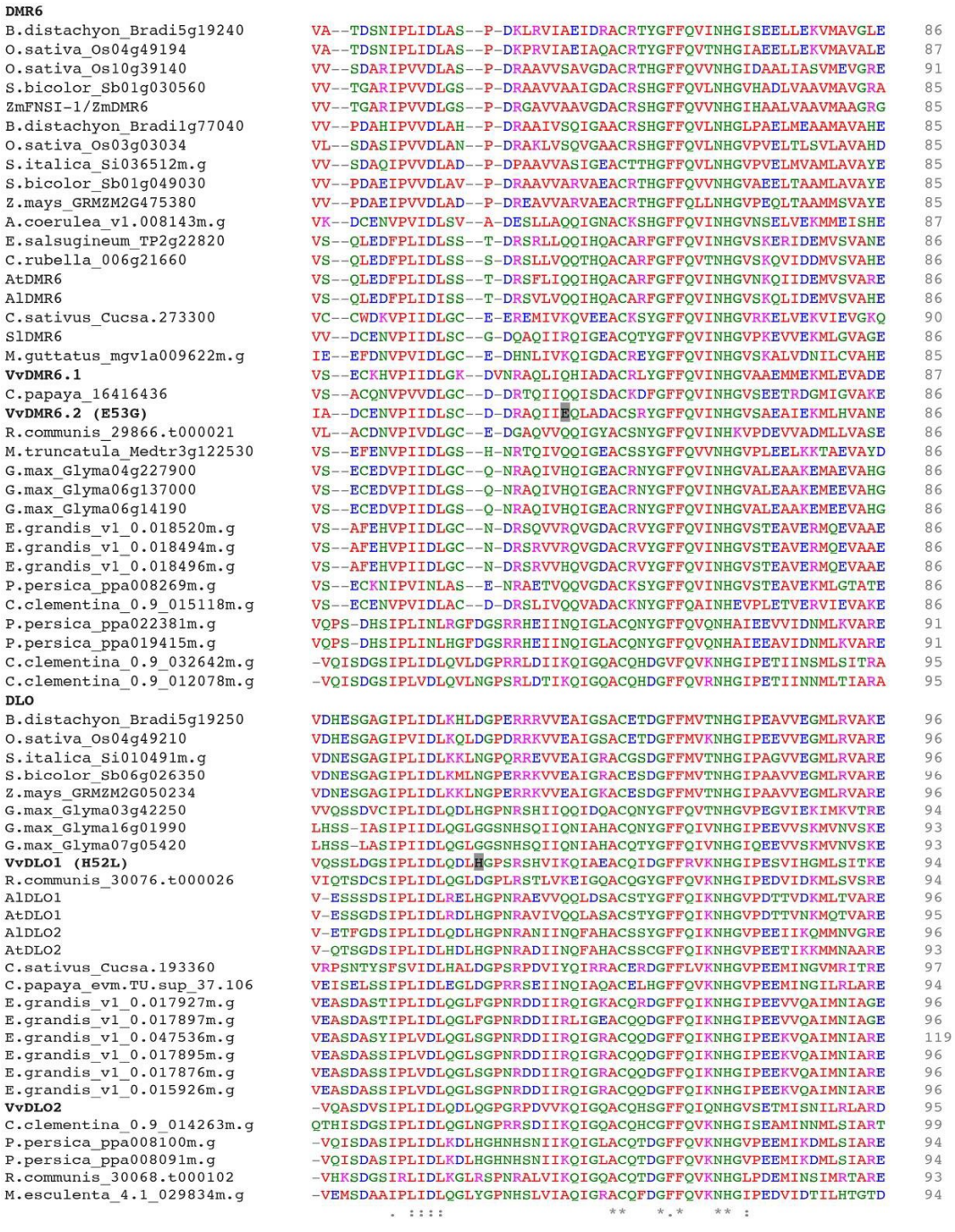
Haplotype n.	**Nucleotide position in *VvDLO1* gene**											**Genotype**	**Frequency (%)**
	49	63	67	68	134	168	173	512	550	699	1192		
1	C	A	T	G	A	C	A	C	A	A	G	PN40024, Herbert, Kunleany, *V.cordifolia, V.smalliana*	30,0
2	C	A	T	G	A	C	A	C	G	A	**A**	Mantey	10,0
3	C	A	T	G	A	C	A	**A**	A	A	G	Herbert	5,0
4	C	A	T	G	**T**	A	**T**	C	A	T	G	Petra, *V.berlandieri* Texas, *V.cordifolia*	20,0
5	C	A	T	C	A	C	A	C	A	A	G	Petra	5,0
6	C	A	T	C	**T**	A	A	C	G	A	G	*V.smalliana*	5,0
7	C	G	T	G	A	C	**T**	C	A	A	G	Kunleany	5,0
8	C	G	C	C	A	C	A	C	A	A	**A**	F560 Big Brown	5,0
9	A	A	T	G	A	C	**T**	C	A	A	G	F560 Big Brown, Lenoir	15,0

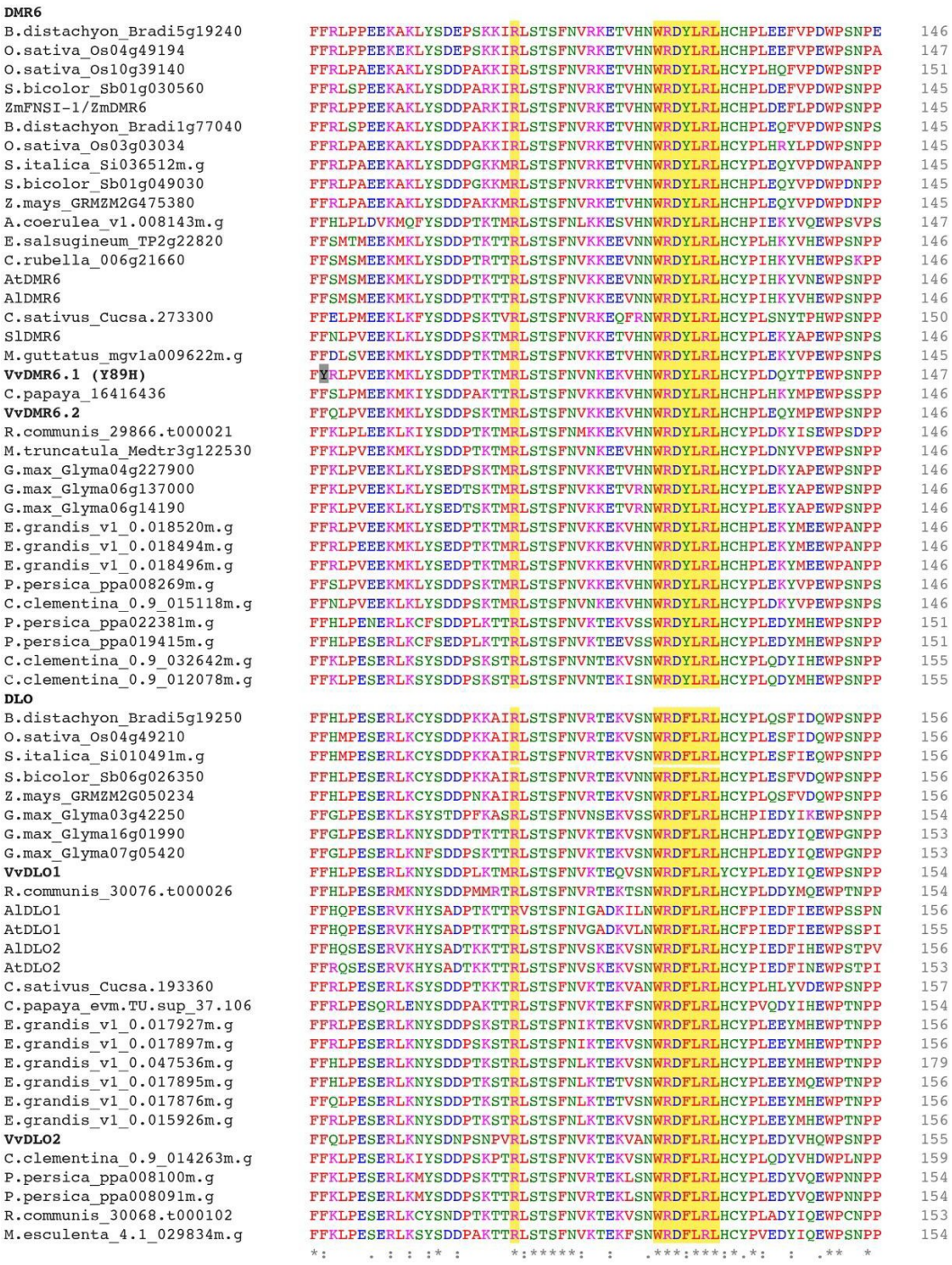
Haplotype n.	Nucleotide position in *VvDLO2* gene																Genotype	Frequency (%)
	4	6	10	18	72	102	150	157	175	325	379	385	390	411	422	472		
1	T	C	G	A	G	A	G	A	A	C	A	T	A	C	C	G	PN40024, Chancellor, Diamond, Golden Muscat, M11-14 St. George, NY65.0562.01, NY97.0512.01, Ontario, Pixiola, Schuyler, Seibel 880, Steuben, Worden, *V.smalliana*, Corella2, Franconia	34,0
2	T	C	G	A	G	A	G	A	A	C	A	T	**G**	C	C	G	Franconia	2,0
3	T	C	G	A	G	A	G	A	A	C	A	T	**G**	C	**G**	G	Coia 12	2,0
4	T	C	G	A	G	A	G	A	A	C	A	**A**	A	C	C	G	Ontario	2,0
5	T	C	G	A	G	A	G	A	A	C	**G**	T	A	C	C	G	Steuben	2,0
6	T	C	G	A	G	A	G	**T**	A	C	A	T	A	C	C	G	M11-14 St.George	2,0
7	T	C	G	A	G	C	G	A	A	C	A	T	A	C	C	G	Clinton, NY97.0503.02, Coia 7	6,0
8	T	C	G	C	G	A	G	**T**	A	C	A	T	A	C	C	G	*V.berlandieri* Texas, *V.cordifolia*	4,0
9	T	C	**A**	A	A	A	G	A	A	C	A	T	A	C	C	G	*V.berlandieri* Texas	2,0
10	T	**G**	G	A	G	A	G	A	A	C	A	T	A	C	C	G	*V.aestivalis*	2,0
11	T	**G**	G	A	G	A	G	**T**	A	**T**	A	T	A	C	C	G	*V. riparia* x *V.cordifolia*	2,0
12	**A**	C	G	A	G	A	G	A	A	C	A	T	A	C	C	G	Lorenzo 1	2,0
13	**A**	C	G	A	A	A	G	A	A	C	A	T	A	C	C	G	Lorenzo 1	2,0
14	**A**	C	G	A	A	A	A	A	A	C	A	T	A	C	C	G	Clinton, Golden Muscat, NY97.0503.02, Schuyler, Seibel 880, *V.riparia* x *V.cordifolia*	12,0
15	**A**	C	G	A	A	A	A	A	A	C	A	T	A	C	C	A	NY65.0562.01	2,0
16	**A**	C	G	A	A	A	A	A	A	C	A	T	A	A	C	G	Worden	2,0
17	**A**	C	G	A	A	A	A	A	**T**	C	A	T	A	C	C	G	NY97.0512.01	2,0
18	**A**	C	**A**	A	A	A	G	A	A	C	A	T	A	C	C	G	Pixiola, *V.aestivalis*, Corella2	6,0
19	**A**	C	**A**	A	A	A	A	A	A	C	A	T	A	C	C	G	Chancellor, Diamond, *V.cordifolia,*	12,0

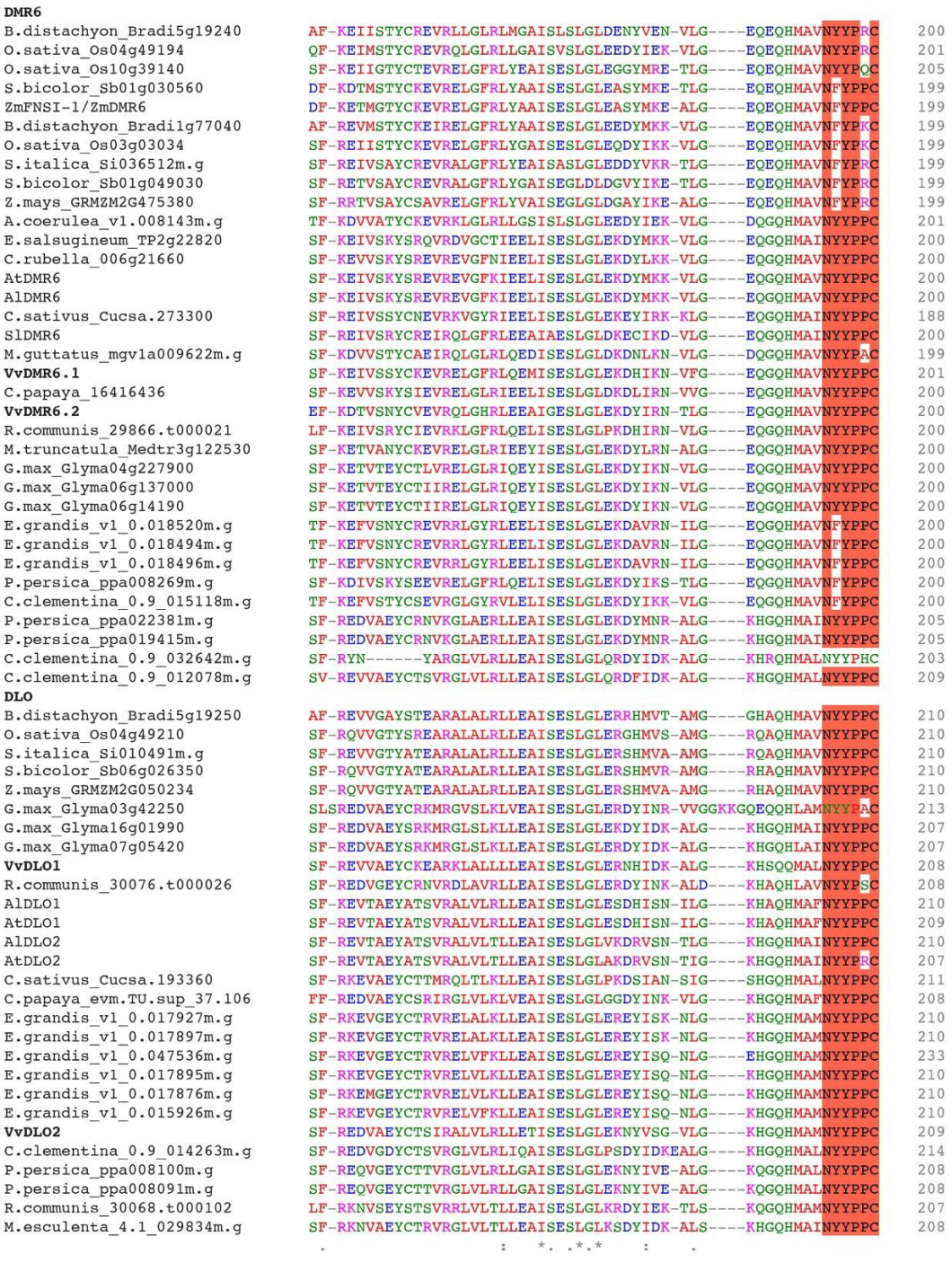
		V.smalliana, Coia 12, Coia 7	

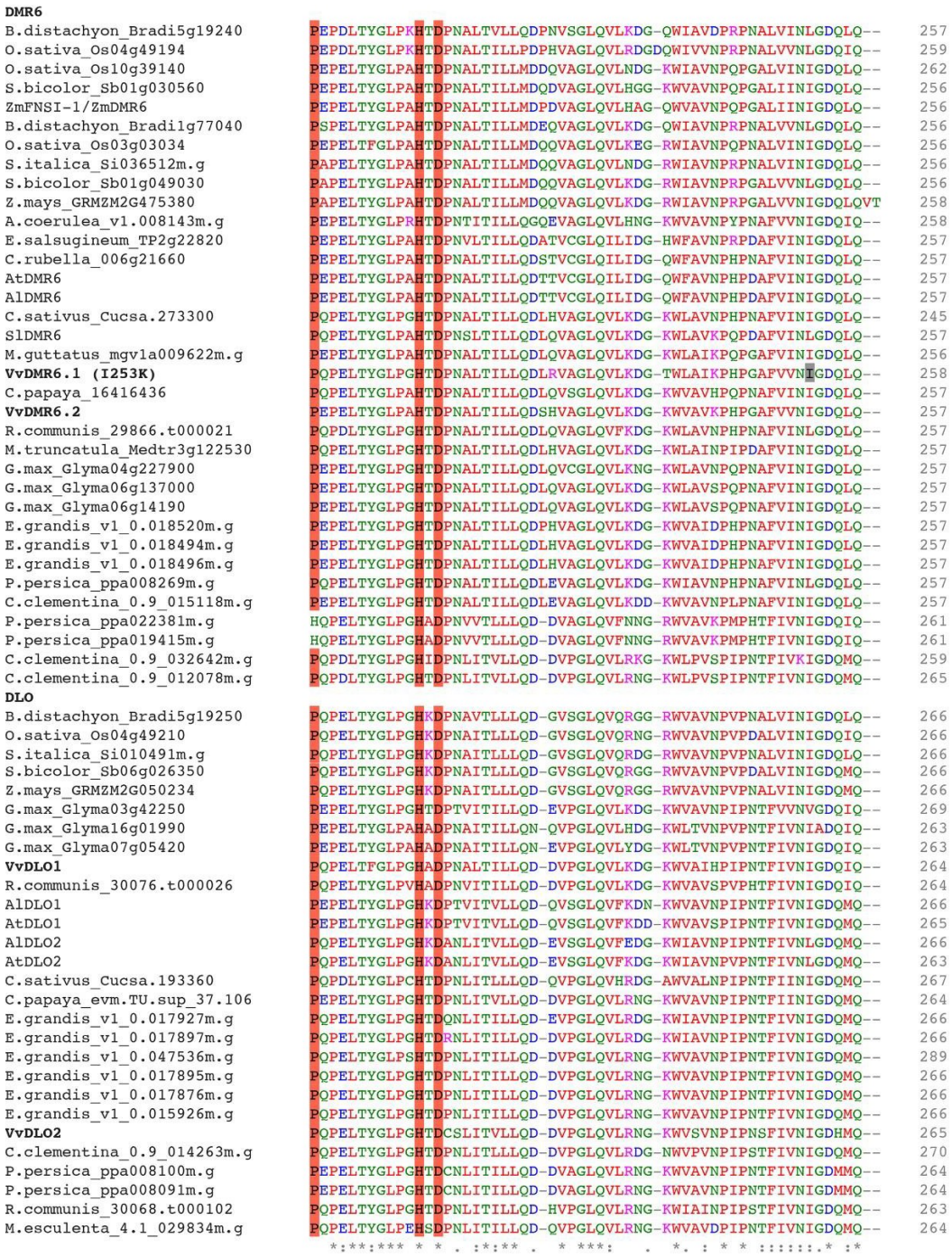
Figure S1. CLUSTALW alignment of bonafide and putative DMR6 and DLO proteins from different species. Amino acids important for the 2-DOG oxidase function (e.g.: the NYYPPCP stretch responsible for binding the 2-oxoglutarate substrate and the iron-binding HDH triplet) are highlighted in red. The DLO-DMR6 characterizing motif WRDY/FLRL is highlighted in yellow; R124 within the WRDY/FLRL motif, and R108 of the *Arabidopsis thaliana* DMR6-1 sequence were shown to be essential for the function and are as well highlighted in yellow [80]. Functional and applied aspects of the DOWNY MILDEW RESISTANT 1 and 6 genes in Arabidopsis. Utrecht University.). Amino acids of grapevine variants are highlighted in grey.
Bonafide DMR6 and DLO proteins are: *Zea mays* ZmFNSI-1/ZmDMR6, *A. thaliana* AtDMR6, AtDLO1 and AtDLO2; *A. lyrata* AlDMR6, AlDLO1 and AlDLO2; *Solanum lycopersicon* SlDMR6. The grapevine PN40024 DMR6 and DLO proteins (VvDMR6.1, VvDMR6.2, VvDLO1, and VvDLO2) are indicated in bold. The amino acid variants in the different grapevine accessions are indicated within parenthesis, and their position onto the PN40024 sequences is highlighted on a grey background.

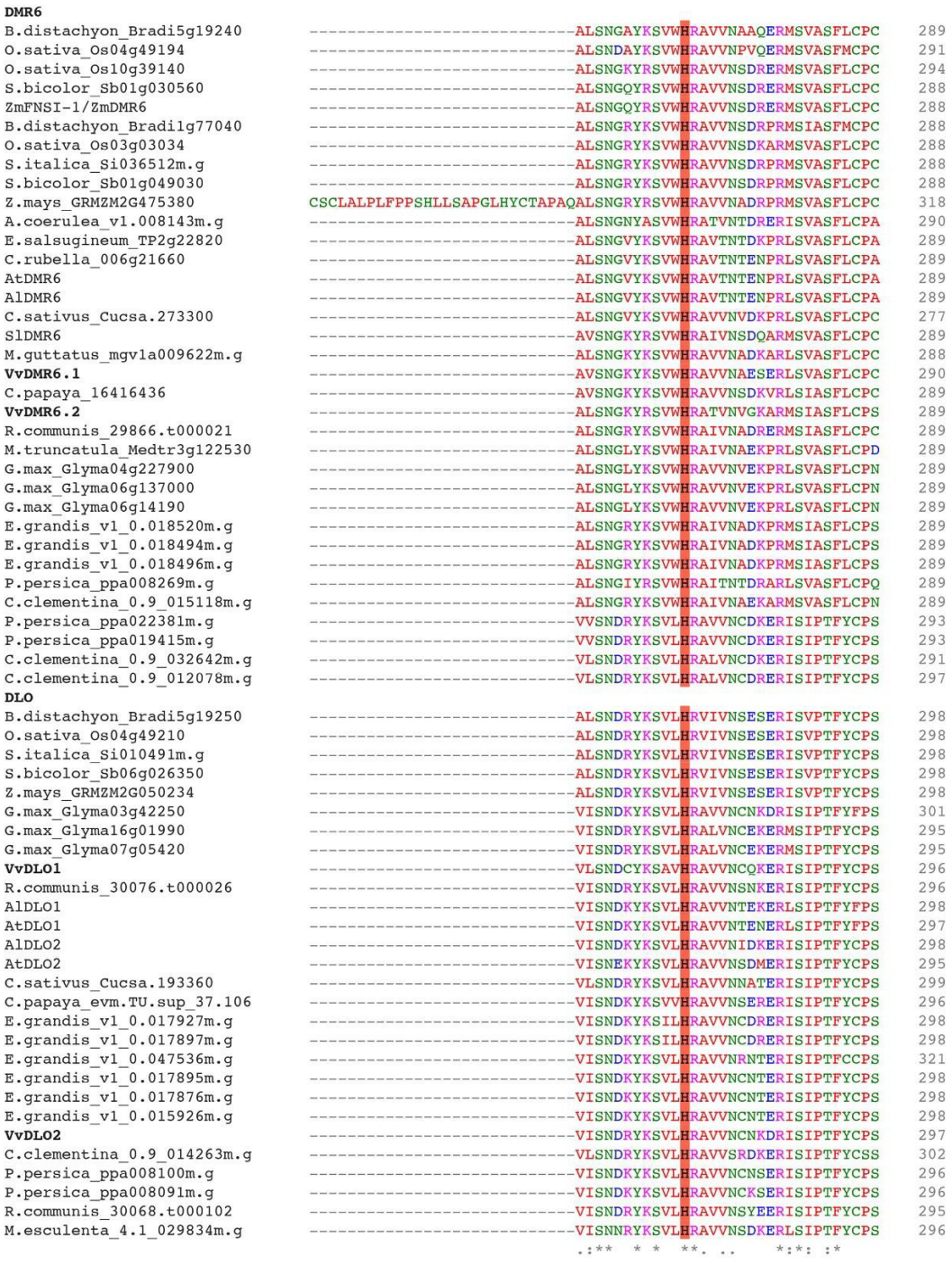

DMR6
B.distachyon_Bradi5g19240 MANQLLST---VSCHDTLPEGYARPESDRPRLAE 31
O.sativa_Os04g49194 MATTQLLST---VEHRETLPEGYARPESDRPRLAE 32
O.sativa_Os10g39140 MAAEAEQQHQLLST----AVHDTMPGKYVRPESQRPRLDL 36
S.bicolor_Sb01g030560 MAEQLLST----AVHDTLPGSYVRPESQRPRLAE 30
ZmFNSI-1/ZmDMR6 MAEHLLST----AVHDTLPGSYVRPEPERPRLAE 30
B.distachyon_Bradi1g77040 MAEQLIST----CAHETLPESYVRDAAERPRLDE 30
O.sativa_Os03g03034 MADQLIST----ADHDTLPGNYVRPEAQRPRLAD 30
S.italica_Si036512m.g MADQLIST----AVHEELPENYVRPEAQRPRLHE 30
S.bicolor_Sb01g049030 MAEQLIST----AEHDTLPDSYVRPETQRPRLRE 30
Z.mays_GRMZM2G475380 MAEHLIST----ADHDRLPDSYVRPETQRPRLRE 30
A.coerulea_v1.008143m.g MESSNVLLT---GTRYSNLPENYVRSVSDRPRLSE 32
E.salsugineum_TP2g22820 MAAKLIST---GFRHTTLPENYVRPHSDRPRLSE 31
C.rubella_006g21660 MAAKLIST---GLRHTTLPGNYVRPISDRPRLSE 31
AtDMR6 MAAKLIST---GFRHTTLPENYVRPISDRPRLSE 31
AlDMR6 MAATLIST---GLRHTTLPENYVRPISDRPRLSE 31
C.sativus_Cucsa.273300 MSSVMEIQLLCS---GGRHEKLPEKYERPESDRPRLSE 35
SlDMR6 METKVISS---GINHSTLPQSYIRPESDRPRLSE 31
M.guttatus_mgv1a009622m.g METKV-IS---GVHFSSLPASYIRPESDRPKLSE 30
VvDMR6.1 MESKVLST---GIRYLTLPQSYIRPEPERPRLSQ 31
C.papaya_16416436 METKVLSS---GVRYENLPENYVRPESERPRLAE 31
VvDMR6.2 MDSKVLST---GIPFTTLPENYIRPESERPRLSE 31
R.communis_29866.t000021 MDTKVLSS---GIRYSNLPESFIRPESERPRLSE 31
M.truncatula_Medtr3g122530 MDTKVLSS---GIHYSKLPESYIRPESDRPCLSQ 31
G.max_Glyma04g227900 MDTKVLSS---GVQYSNLPESYIRPESERPRLSE 31
G.max_Glyma06g137000 MDIKVLSS---GVQYSNLPESYIRPESERPRLSE 31
G.max_Glyma06g14190 MDIKVLSS---GVQYSNLPESYIRPESERPRLSE 31
E.grandis_v1_0.018520m.g MEAKVISG---GTRFTSLPRSYVRPESERPRLSE 31
E.grandis_v1_0.018494m.g MEAKVISG---GTRFTSLPRSYVRPESERPRLFE 31
E.grandis_v1_0.018496m.g MEAKVISG---GTRFTRLPRSYVRPESERPRLSE 31
P.persica_ppa008269m.g METKVLSS---GFKYENLPEGYVRPESERPRLSE 31
C.clementina_0.9_015118m.g MDTKVLSS---GIRYTNLPEGYVRPESERPNLSE 31
P.persica_ppa022381m.g MAAKLLSD-LASGVTCVPSNYVRPVHDRPSLDQ 32
P.persica_ppa019415m.g MAAKLLSD-LASGVTCVPSNYVRPVHDRPSLDQ 32
C.clementina_0.9_032642m.g MAAATTKLLLSD-LASTVKSVPSNYIRPISDRPNLTE 36
C.clementina_0.9_012078m.g MAAATTKLLLSD-LASTVKSVPSNYIRPISDRPNLTE 36
DLO
B.distachyon_Bradi5g19250 MATAIAKPLLSD-LVAESGTVPSSHIRPVGDRPDLAD 36
O.sativa_Os04g49210 MAPAIAKPLLSD-LVAQSGQVPSSHIRPVGDRPDLDN 36
S.italica_Si010491m.g MAPTIAKPLLSD-LVAQIGQVPLSHVRPVGDRPDLAN 36
S.bicolor_Sb06g026350 MAPAISKPLLSD-LVAQIGKVPSSHIRPVGDRPDLAN 36
Z.mays_GRMZM2G050234 MAPAISKPLLTD-LVAQIGKVPSSHIRPVGDRPDLAN 36
G.max_Glyma03g42250 MAEKLVLVSD-MASTMKQVPSNFIRPLGDRPNLQG 34
G.max_Glyma16g01990 MATTKPLLTD-LASTVDRVPSNFIRPIGDRPNLQQ 34
G.max_Glyma07g05420 MAATKPLLTD-LASTIDRVPSNFIRPIGDRPKLHQ 34
VvDLO1 MANAKLLLSD-LASSIDCVPSRYVRPVNDRPNLDE 34
R.communis_30076.t000026 MAIAKPLLSD-LVSTITSVPSNFIRPLSDRPNFNE 34
AlDLO1 MATSATSKLLVSD-FDSSVSHIPSNYVRPILDRPNLSE 37
AtDLO1 MATSAISKLLVSD-FASS-VHIPSNYVRPISDRPNLSE 36
AlDLO2 MAASATSKLLVSD-IASVVDHVPSNYVRPVSDRPNMSE 37
AtDLO2 MAASKLLVSD-IASVVDHVPSNYVRPVSDRPKMSE 34
C.sativus_Cucsa.193360 MSASGHTKLLVTD-LAATVQQVPSRYVRPISDRPNSSD 37
C.papaya_evm.TU.sup_37.106 MAATKLLLAD-LASNLKQVPAKYIQPISDRPNLAD 34
E.grandis_v1_0.017927m.g MATAATKLLLID-RVPGINRVPNSYIRPQADRPNLTE 36
E.grandis_v1_0.017897m.g MATAATKLLLID-RVPGINRVPNSYIRPEADRPNLTE 36
E.grandis_v1_0.047536m.g MLTSAAQINTMTSYSALGRRFGENEKTAAKLLLTD-LASGIDRVPDNYIRSEADRPNLTE 59
E.grandis_v1_0.017895m.g MAPAAAKVLLTD-LASGIDRVPDNYIRSEADRPNLTE 36
E.grandis_v1_0.017876m.g MAPAAAKVLLTD-LASGIDRVPDNYIRSEADRPNLTD 36
E.grandis_v1_0.015926m.g MAPAAAKVLLTD-LASGIDRVPDNYIRSEADRPNLTE 36
VvDLO2 (V2D) MVPSTTKLLLTD-MVLGVDHVPSNYVRPPSERPNFKD 36
C.clementina_0.9_014263m.g MSAAATTATKLLLSD-LAPTLTNVPSDYIRPISDRPSLTD 39
P.persica_ppa008100m.g MATATKLLLTD-LMSGVNHVPSNYVRPISDRPNLSD 35
P.persica_ppa008091m.g MATATKLLLTD-LMSGVNHVPSNYVRPISDRPNLSD 35
R.communis_30068.t000102 MAS-TKLLLADL-ASGVRHVPSKYIRPVSDRPNLSD 34
M.esculenta_4.1_029834m.g MAF-SKPLLADLSSLGVKNVPSSYIRPISDRPNLSD 35

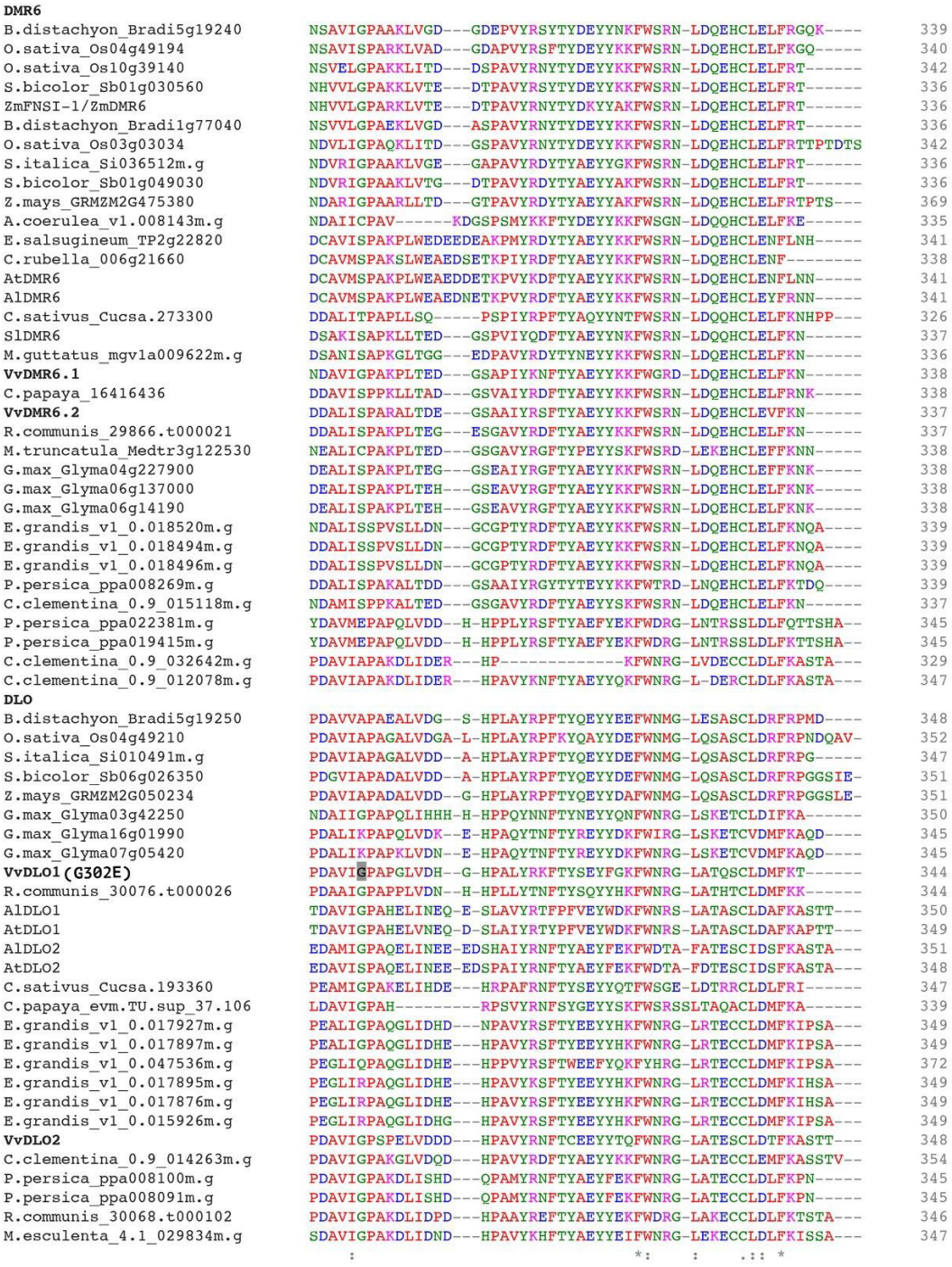

DMR6
B.distachyon_Bradi5g19240 VA--TDSNIPLIDLAS--P-DKLRVIAEIDRACRTYGFFQVINHGISEELLEKVMAVGLE 86
O.sativa_Os04g49194 VA--TDSNIPLIDLAS--P-DKPRVIAEIAQACRTYGFFQVTNHGIAEELLEKVMAVALE 87
O.sativa_Os10g39140 VV--SDARIPVVDLAS--P-DRAAVVSAVGDACRTHGFFQVVNHGIDAALIASVMEVGRE 91
S.bicolor_Sb01g030560 VV--TGARIPVVDLGS--P-DRAAVVAAIGDACRSHGFFQVLNHGVHADLVAAVMAVGRA 85
ZmFNSI-1/ZmDMR6 VV--TGARIPVVDLGS--P-DRGAVVAAVGDACRSHGFFQVVNHGIHAALVAAVMAAGRG 85
B.distachyon_Bradi1g77040 VV--PDAHIPVVDLAH--P-DRAAIVSQIGAACRSHGFFQVLNHGLPAELMEAAMAVAHE 85
O.sativa_Os03g03034 VL--SDASIPVVDLAN--P-DRAKLVSQVGAACRSHGFFQVLNHGVPVELTLSVLAVAHD 85
S.italica_Si036512m.g VV--SDAQIPVVDLAD--P-DPAAVVASIGEACTTHGFFQVLNHGVPVELMVAMLAVAYE 85
S.bicolor_Sb01g049030 VV--PDAEIPVVDLAV--P-DRAAVVARVAEACRTHGFFQVVNHGVAEELTAAMLAVAYE 85
Z.mays_GRMZM2G475380 VV--PDAEIPVVDLAD--P-DREAVVARVAEACRTHGFFQLLNHGVPEQLTAAMMSVAYE 85
A.coerulea_v1.008143m.g VK--DCENVPVIDLSV--A-DESLLAQQIGNACKSHGFFQVINHGVNSELVEKMMEISHE 87
E.salsugineum_TP2g22820 VS--QLEDFPLIDLSS--T-DRSRLLQQIHQACARFGFFQVINHGVSKERIDEMVSVANE 86
C.rubella_006g21660 VS--QLEDFPLIDLSS--S-DRSLLVQQTHQACARFGFFQVTNHGVSKQVIDDMVSVAHE 86
AtDMR6 VS--QLEDFPLIDLSS--T-DRSFLIQQIHQACARFGFFQVINHGVNKQIIDEMVSVARE 86
AlDMR6 VS--QLEDFPLIDISS--T-DRSVLVQQIHQACARFGFFQVINHGVSKQLIDEMVSVAHE 86
C.sativus_Cucsa.273300 VC--CWDKVPIIDLGC--E-EREMIVKQVEEACKSYGFFQVINHGVRKELVEKVIEVGKQ 90
SlDMR6 VV--DCENVPIIDLSC--G-DQAQIIRQIGEACQTYGFFQVINHGVPKEVVEKMLGVAGE 86
M.guttatus_mgv1a009622m.g IE--EFDNVPVIDLGC--E-DHNLIVKQIGDACREYGFFQVINHGVSKALVDNILCVAHE 85
VvDMR6.1 VS--ECKHVPIIDLGK--DVNRAQLIQHIADACRLYGFFQVINHGVAAEMMEKMLEVADE 87
C.papaya_16416436 VS--ACQNVPVVDLGC--D-DRTQIIQQISDACKDFGFFQVINHGVSEETRDGMIGVAKE 86
VvDMR6.2 (E53G) IA--DCENVPIIDLSC--D-DRAQIIEQLADACSRYGFFQVINHGVSAEAIEKMLHVANE 86
R.communis_29866.t000021 VL--ACDNVPIVDLGC--E-DGAQVVQQIGYACSNYGFFQVINHKVPDEVVADMLLVASE 86
M.truncatula_Medtr3g122530 VS--EFENVPIIDLGS--H-NRTQIVQQIGEACSSYGFFQVVNHGVPLEELKKTAEVAYD 86
G.max_Glyma04g227900 VS--ECEDVPIIDLGC--Q-NRAQIVHQIGEACRNYGFFQVINHGVALEAAKEMAEVAHG 86
G.max_Glyma06g137000 VS--ECEDVPIIDLGS--Q-NRAQIVHQIGEACRNYGFFQVINHGVALEAAKEMEEVAHG 86
G.max_Glyma06g14190 VS--ECEDVPIIDLGS--Q-NRAQIVHQIGEACRNYGFFQVINHGVALEAAKEMEEVAHG 86
E.grandis_v1_0.018520m.g VS--AFEHVPIIDLGC--N-DRSQVVRQVGDACRVYGFFQVINHGVSTEAVERMQEVAAE 86
E.grandis_v1_0.018494m.g VS--AFEHVPIIDLGC--N-DRSRVVRQVGDACRVYGFFQVINHGVSTEAVERMQEVAAE 86
E.grandis_v1_0.018496m.g VS--AFEHVPIIDLGC--N-DRSRVVHQVGDACRVYGFFQVINHGVSTEAVERMQEVAAE 86
P.persica_ppa008269m.g VS--ECKNIPVINLAS--E-NRAETVQQVGDACKSYGFFQVINHGVSTEAVEKMLGTATE 86
C.clementina_0.9_015118m.g VS--ECENVPVIDLAC--D-DRSLIVQQVADACKNYGFFQAINHEVPLETVERVIEVAKE 86
P.persica_ppa022381m.g VQPS-DHSIPLINLRGFDGSRRHEIINQIGLACQNYGFFQVQNHAIEEVVIDNMLKVARE 91
P.persica_ppa019415m.g VQPS-DHSIPLINLHGFDGSRRHEIINQIGLACQNYGFFQVQNHAIEEAVIDNMLKVARE 91
C.clementina_0.9_032642m.g -VQISDGSIPLIDLQVLDGPRRLDIIKQIGQACQHDGVFQVKNHGIPETIINSMLSITRA 95
C.clementina_0.9_012078m.g -VQISDGSIPLVDLQVLNGPSRLDTIKQIGQACQHDGFFQVRNHGIPETIINNMLTIARA 95
DLO
B.distachyon_Bradi5g19250 VDHESGAGIPLIDLKHLDGPERRRVVEAIGSACETDGFFMVTNHGIPEAVVEGMLRVAKE 96
O.sativa_Os04g49210 VDHESGAGIPVIDLKQLDGPDRRKVVEAIGSACETDGFFMVKNHGIPEEVVEGMLRVARE 96
S.italica_Si010491m.g VDNESGAGIPLIDLKKLNGPQRREVVEAIGRACGSDGFFMVTNHGIPAGVVEGMLRVARE 96
S.bicolor_Sb06g026350 VDNESGAGIPLIDLKMLNGPERRKVVEAIGRACESDGFFMVTNHGIPAAVVEGMLRVARE 96
Z.mays_GRMZM2G050234 VDNESGAGIPLIDLKKLNGPERRKVVEAIGKACESDGFFMVTNHGIPAAVVEGMLRVARE 96
G.max_Glyma03g42250 VVQSSDVCIPLIDLQDLHGPNRSHIIQQIDQACQNYGFFQVTNHGVPEGVIEKIMKVTRE 94
G.max_Glyma16g01990 LHSS-IASIPIIDLQGLGGSNHSQIIQNIAHACQNYGFFQIVNHGIPEEVVSKMVNVSKE 93
G.max_Glyma07g05420 LHSS-LASIPIIDLQGLGGSNHSQIIQNIAHACQTYGFFQIVNHGIQEEVVSKMVNVSKE 93
VvDLO1 (H52L) VQSSLDGSIPLIDLQDLHGPSRSHVIKQIAEACQIDGFFRVKNHGIPESVIHGMLSITKE 94
R.communis_30076.t000026 VIQTSDCSIPLIDLQGLDGPLRSTLVKEIGQACQGYGFFQVKNHGIPEDVIDKMLSVSRE 94
AlDLO1 V-ESSSDSIPLIDLRELHGPNRAEVVQQLDSACSTYGFFQIKNHGVPDTTVDKMLTVARE 96
AtDLO1 V-ESSGDSIPLIDLRDLHGPNRAVIVQQLASACSTYGFFQIKNHGVPDTTVNKMQTVARE 95
AlDLO2 V-ETFGDSIPLIDLQDLHGPNRANIINQFAHACSSYGFFQIKNHGVPEEIIKQMMNVGRE 96
AtDLO2 V-QTSGDSIPLIDLHDLHGPNRADIINQFAHACSSCGFFQIKNHGVPEETIKKMMNAARE 93
C.sativus_Cucsa.193360 VRPSNTYSFSVIDLHALDGPSRPDVIYQIRRACERDGFFLVKNHGVPEEMINGVMRITRE 97
C.papaya_evm.TU.sup_37.106 VEISELSSIPLIDLEGLDGPRRSEIINQIAQACELHGFFQVKNHGVPEEMINGILRLARE 94
E.grandis_v1_0.017927m.g VEASDASTIPLIDLQGLFGPNRDDIIRQIGKACQRDGFFQIKNHGIPEEVVQAIMNIAGE 96
E.grandis_v1_0.017897m.g VEASDASTIPLIDLQGLFGPNRDDIIRLIGEACQQDGFFQIKNHGIPEEVVQAIMNIAGE 96
E.grandis_v1_0.047536m.g VEASDASYIPLVDLQGLSGPNRDDIIRQIGRACQQDGFFQIKNHGIPEEKVQAIMNIARE 119
E.grandis_v1_0.017895m.g VEASDASSIPLVDLQGLSGPNRDDIIRQIGRACQQDGFFQIKNHGIPEEKVQAIMNIARE 96
E.grandis_v1_0.017876m.g VEASDASSIPLVDLQGLSGPNRDDIIRQIGRACQQDGFFQIKNHGIPEEKVQAIMNIARE 96
E.grandis_v1_0.015926m.g VEASDASSIPLVDLQGLSGPNRDDIIRQIGRACQQDGFFQIKNHGIPEEKVQAIMNIARE 96
VvDLO2 -VQASDVSIPLIDLQDLQGPGRPDVVKQIGQACQHSGFFQIQNHGVSETMISNILRLARD 95
C.clementina_0.9_014263m.g QTHISDGSIPLIDLQGLNGPRRSDIIKQIGQACQHCGFFQVKNHGISEAMINNMLSIART 99
P.persica_ppa008100m.g -VQISDASIPLIDLKDLHGHNHSNIIKQIGLACQTDGFFQVKNHGVPEEMIKDMLSIARE 94
P.persica_ppa008091m.g -VQISDASIPLIDLKDLHGHNHSNIIKQIGLACQTDGFFQVKNHGVPEEMIKDMLSIARE 94
R.communis_30068.t000102 -VHKSDGSIRLIDLKGLRSPNRALVIKQIGQACQTDGFFQVKNHGLPDEMINSIMRTARE 93
M.esculenta_4.1_029834m.g -VEMSDAAIPLIDLQGLYGPNHSLVIAQIGRACQFDGFFQVKNHGIPEDVIDTILHTGTD 94


DMR6
B.distachyon_Bradi5g19240 FFRLPPEEKAKLYSDEPSKKIRLSTSFNVRKETVHNWRDYLRLHCHPLEEFVPDWPSNPE 146
O.sativa_Os04g49194 FFRLPPEEKEKLYSDEPSKKIRLSTSFNVRKETVHNWRDYLRLHCHPLEEFVPEWPSNPA 147
O.sativa_Os10g39140 FFRLPAEEKAKLYSDDPAKKIRLSTSFNVRKETVHNWRDYLRLHCYPLHQFVPDWPSNPP 151
S.bicolor_Sb01g030560 FFRLSPEEKAKLYSDDPARKIRLSTSFNVRKETVHNWRDYLRLHCHPLDEFVPDWPSNPP 145
ZmFNSI-1/ZmDMR6 FFRLPPEEKAKLYSDDPARKIRLSTSFNVRKETVHNWRDYLRLHCHPLDEFLPDWPSNPP 145
B.distachyon_Bradi1g77040 FFRLSPEEKAKLYSDDPAKKIRLSTSFNVRKETVHNWRDYLRLHCHPLEQFVPDWPSNPS 145
O.sativa_Os03g03034 FFRLPAEEKAKLYSDDPAKKIRLSTSFNVRKETVHNWRDYLRLHCYPLHRYLPDWPSNPP 145
S.italica_Si036512m.g FFRLPAEEKAKLYSDDPGKKMRLSTSFNVRKETVHNWRDYLRLHCYPLEQYVPDWPANPP 145
S.bicolor_Sb01g049030 FFRLPAEEKAKLYSDDPGKKMRLSTSFNVRKETVHNWRDYLRLHCHPLEQYVPDWPDNPP 145
Z.mays_GRMZM2G475380 FFRLPAEEKAKLYSDDPAKKMRLSTSFNVRKETVHNWRDYLRLHCHPLEQYVPDWPDNPP 145
A.coerulea_v1.008143m.g FFHLPLDVKMQFYSDDPTKTMRLSTSFNLKKESVHNWRDYLRLHCHPIEKYVQEWPSVPS 147
E.salsugineum_TP2g22820 FFSMTMEEKMKLYSDDPTKTTRLSTSFNVKKEEVNNWRDYLRLHCYPLHKYVHEWPSNPP 146
C.rubella_006g21660 FFSMSMEEKMKLYSDDPTRTTRLSTSFNVKKEEVNNWRDYLRLHCYPIHKYVHEWPSKPP 146
AtDMR6 FFSMSMEEKMKLYSDDPTKTTRLSTSFNVKKEEVNNWRDYLRLHCYPIHKYVNEWPSNPP 146
AlDMR6 FFSMSMEEKMKLYSDDPTKTTRLSTSFNVKKEEVNNWRDYLRLHCYPIHKYVHEWPSNPP 146
C.sativus_Cucsa.273300 FFELPMEEKLKFYSDDPSKTVRLSTSFNVRKEQFRNWRDYLRLHCYPLSNYTPHWPSNPP 150
SlDMR6 FFNLPVEEKLKLYSDDPSKTMRLSTSFNVKKETVHNWRDYLRLHCYPLEKYAPEWPSNPS 146
M.guttatus_mgv1a009622m.g FFDLSVEEKMKLYSDDPTKTMRLSTSFNVKKETVHNWRDYLRLHCYPLEKYVPEWPSNPS 145
VvDMR6.1 (Y89H) FYRLPVEEKMKLYSDDPTKTMRLSTSFNVNKEKVHNWRDYLRLHCYPLDQYTPEWPSNPP 147
C.papaya_16416436 FFSLPMEEKMKIYSDDPAKTTRLSTSFNVKKEKVHNWRDYLRLHCHPLHKYMPEWPSSPP 146
VvDMR6.2 FFQLPVEEKMKLYSDDPSKTMRLSTSFNVKKEKVHNWRDYLRLHCHPLEQYMPEWPSNPP 146
R.communis_29866.t000021 FFKLPLEEKLKIYSDDPTKTMRLSTSFNMKKEKVHNWRDYLRLHCYPLDKYISEWPSDPP 146
M.truncatula_Medtr3g122530 FFKLPVEEKMKLYSDDPTKTMRLSTSFNVNKEEVHNWRDYLRLHCYPLDNYVPEWPSNPP 146
G.max_Glyma04g227900 FFKLPVEEKLKLYSEDPSKTMRLSTSFNVKKETVHNWRDYLRLHCYPLDKYAPEWPSNPP 146
G.max_Glyma06g137000 FFKLPVEEKLKLYSEDTSKTMRLSTSFNVKKETVRNWRDYLRLHCYPLEKYAPEWPSNPP 146
G.max_Glyma06g14190 FFKLPVEEKLKLYSEDTSKTMRLSTSFNVKKETVRNWRDYLRLHCYPLEKYAPEWPSNPP 146
E.grandis_v1_0.018520m.g FFRLPVEEKMKLYSEDPTKTMRLSTSFNVKKEKVHNWRDYLRLHCHPLEKYMEEWPANPP 146
E.grandis_v1_0.018494m.g FFRLPEEEKMKLYSEDPTKTMRLSTSFNVKKEKVHNWRDYLRLHCHPLEKYMEEWPANPP 146
E.grandis_v1_0.018496m.g FFRLPVEEKMKLYSEDPTKTMRLSTSFNVKKEKVHNWRDYLRLHCHPLEKYMEEWPANPP 146
P.persica_ppa008269m.g FFSLPVEEKMKLYSDDPSKTMRLSTSFNVKKEKVHNWRDYLRLHCYPLEKYVPEWPSNPS 146
C.clementina_0.9_015118m.g FFNLPVEEKLKLYSDDPSKTMRLSTSFNVNKEKVHNWRDYLRLHCYPLDKYVPEWPSNPS 146
P.persica_ppa022381m.g FFHLPENERLKCFSDDPLKTTRLSTSFNVKTEKVSSWRDYLRLHCYPLEDYMHEWPSNPP 151
P.persica_ppa019415m.g FFHLPESERLKCFSEDPLKTTRLSTSFNVKTEEVSSWRDYLRLHCYPLEDYMHEWPSNPP 151
C.clementina_0.9_032642m.g FFKLPESERLKSYSDDPSKSTRLSTSFNVNTEKVSNWRDYLRLHCYPLQDYIHEWPSNPP 155
C.clementina_0.9_012078m.g FFKLPESERLKSYSDDPSKSTRLSTSFNVNTEKISNWRDYLRLHCYPLQDYMHEWPSNPP 155
DLO
B.distachyon_Bradi5g19250 FFHLPESERLKCYSDDPKKAIRLSTSFNVRTEKVSNWRDFLRLHCYPLQSFIDQWPSNPP 156
O.sativa_Os04g49210 FFHMPESERLKCYSDDPKKAIRLSTSFNVRTEKVSNWRDFLRLHCYPLESFIDQWPSNPP 156
S.italica_Si010491m.g FFHMPESERLKCYSDDPKKAIRLSTSFNVRTEKVSNWRDFLRLHCYPLESFIEQWPSNPP 156
S.bicolor_Sb06g026350 FFHLPESERLKCYSDDPKKAIRLSTSFNVRTEKVNNWRDFLRLHCYPLESFVDQWPSNPP 156
Z.mays_GRMZM2G050234 FFHLPESERLKCYSDDPNKAIRLSTSFNVRTEKVSNWRDFLRLHCYPLQSFVDQWPSNPP 156
G.max_Glyma03g42250 FFGLPESEKLKSYSTDPFKASRLSTSFNVNSEKVSSWRDFLRLHCHPIEDYIKEWPSNPP 154
G.max_Glyma16g01990 FFGLPESERLKNYSDDPTKTTRLSTSFNVKTEKVSNWRDFLRLHCHPLEDYIQEWPGNPP 153
G.max_Glyma07g05420 FFGLPESERLKNFSDDPSKTTRLSTSFNVKTEKVSNWRDFLRLHCHPLEDYIQEWPGNPP 153
VvDLO1 FFHLPESERLKNYSDDPLKTMRLSTSFNVKTEQVSNWRDFLRLYCYPLEDYIQEWPSNPP 154
R.communis_30076.t000026 FFHLPESERMKNYSDDPMMRTRLSTSFNVRTEKTSNWRDFLRLHCYPLDDYMQEWPTNPP 154
AlDLO1 FFHQPESERVKHYSADPTKTTRVSTSFNIGADKILNWRDFLRLHCFPIEDFIEEWPSSPN 156
AtDLO1 FFHQPESERVKHYSADPTKTTRLSTSFNVGADKVLNWRDFLRLHCFPIEDFIEEWPSSPI 155
AlDLO2 FFHQSESERVKHYSADTKKTTRLSTSFNVSKEKVSNWRDFLRLHCYPIEDFIHEWPSTPV 156
AtDLO2 FFRQSESERVKHYSADTKKTTRLSTSFNVSKEKVSNWRDFLRLHCYPIEDFINEWPSTPI 153
C.sativus_Cucsa.193360 FFRLPESERLKSYSDDPTKKTRLSTSFNVKTEKVANWRDFLRLHCYPLHLYVDEWPSNPP 157
C.papaya_evm.TU.sup_37.106 FFRLPESQRLENYSDDPAKTTRLSTSFNVKTEKFSNWRDFLRLHCYPVQDYIHEWPTNPP 154
E.grandis_v1_0.017927m.g FFRLPESERLKNYSDDPSKSTRLSTSFNIKTEKVSNWRDFLRLHCYPLEEYMHEWPTNPP 156
E.grandis_v1_0.017897m.g FFRLPESERLKNYSDDPSKSTRLSTSFNIKTEKVSNWRDFLRLHCYPLEEYMHEWPTNPP 156
E.grandis_v1_0.047536m.g FFHLPESERLKNYSDDPTKSTRLSTSFNLKTEKVSNWRDFLRLHCYPLEEYMHEWPTNPP 179
E.grandis_v1_0.017895m.g FFHLPESERLKNYSDDPTKSTRLSTSFNLKTETVSNWRDFLRLHCYPLEEYMQEWPTNPP 156
E.grandis_v1_0.017876m.g FFQLPESERLKNYSDDPTKSTRLSTSFNLKTETVSNWRDFLRLHCYPLEEYMHEWPTNPP 156
E.grandis_v1_0.015926m.g FFRLPESERLKNYSDDPTKSTRLSTSFNLKTEKVSNWRDFLRLHCYPLEEYMHEWPTNPP 156
VvDLO2 FFQLPESERLKNYSDNPSNPVRLSTSFNVKTEKVANWRDFLRLHCYPLEDYVHQWPSNPP 155
C.clementina_0.9_014263m.g FFKLPESERLKIYSDDPSKPTRLSTSFNVKTEKVSNWRDFLRLHCYPLQDYVHDWPLNPP 159
P.persica_ppa008100m.g FFKLPESERLKMYSDDPSKTTRLSTSFNVRTEKLSNWRDFLRLHCYPLEDYVQEWPNNPP 154
P.persica_ppa008091m.g FFKLPESERLKMYSDDPSKTTRLSTSFNVRTEKLSNWRDFLRLHCYPLEDYVQEWPNNPP 154
R.communis_30068.t000102 FFKLPESERLKCYSNDPTKTTRLSTSFNVKTEKVSNWRDFLRLHCYPLADYIQEWPCNPP 153
M.esculenta_4.1_029834m.g FFKLPESERLKSYSDDPAKTTRLSTSFNVKTEKFSNWRDFLRLHCYPVEDYIQEWPSNPP 154


DMR6
B.distachyon_Bradi5g19240 AF-KEIISTYCREVRLLGLRLMGAISLSLGLDENYVEN-VLG----EQEQHMAVNYYPRC 200
O.sativa_Os04g49194 QF-KEIMSTYCREVRQLGLRLLGAISVSLGLEEDYIEK-VLG----EQEQHMAVNYYPRC 201
O.sativa_Os10g39140 SF-KEIIGTYCTEVRELGFRLYEAISESLGLEGGYMRE-TLG----EQEQHMAVNYYPQC 205
S.bicolor_Sb01g030560 DF-KDTMSTYCKEVRELGFRLYAAISESLGLEASYMKE-TLG----EQEQHMAVNFYPPC 199
ZmFNSI-1/ZmDMR6 DF-KETMGTYCKEVRELGFRLYAAISESLGLEASYMKE-ALG----EQEQHMAVNFYPPC 199
B.distachyon_Bradi1g77040 AF-REVMSTYCKEIRELGFRLYAAISESLGLEEDYMKK-VLG----EQEQHMAVNFYPKC 199
O.sativa_Os03g03034 SF-REIISTYCKEVRELGFRLYGAISESLGLEQDYIKK-VLG----EQEQHMAVNFYPKC 199
S.italica_Si036512m.g SF-REIVSAYCREVRALGFRLYEAISASLGLEDDYVKR-TLG----EQEQHMAVNFYPRC 199
S.bicolor_Sb01g049030 SF-RETVSAYCREVRALGFRLYGAISEGLDLDGVYIKE-TLG----EQEQHMAVNFYPRC 199
Z.mays_GRMZM2G475380 SF-RRTVSAYCSAVRELGFRLYVAISEGLGLDGAYIKE-ALG----EQEQHMAVNFYPRC 199
A.coerulea_v1.008143m.g TF-KDVVATYCKEVRKLGLRLLGSISLSLGLEEDYIEK-VLG----DQGQHMAVNYYPPC 201
E.salsugineum_TP2g22820 SF-KEIVSKYSRQVRDVGCTIEELISESLGLEKDYMKK-VLG----EQGQHMAINYYPPC 200
C.rubella_006g21660 SF-KEVVSKYSREVREVGFNIEELISESLGLEKDYLKK-VLG----EQGQHMAVNYYPPC 200
AtDMR6 SF-KEIVSKYSREVREVGFKIEELISESLGLEKDYMKK-VLG----EQGQHMAVNYYPPC 200
AlDMR6 SF-KEIVSKYSREVREVGFKIEELISESLGLEKDYMKK-VLG----EQGQHMAVNYYPPC 200
C.sativus_Cucsa.273300 SF-REIVSSYCNEVRKVGYRIEELISESLGLEKEYIRK-KLG----EQGQHMAINYYPPC 188
SlDMR6 SF-REIVSRYCREIRQLGFRLEEAIAESLGLDKECIKD-VLG----EQGQHMAINYYPPC 200
M.guttatus_mgv1a009622m.g SF-KDVVSTYCAEIRQLGLRLQEDISESLGLDKDNLKN-VLG----DQGQHMAVNYYPAC 199
VvDMR6.1 SF-KEIVSSYCKEVRELGFRLQEMISESLGLEKDHIKN-VFG----EQGQHMAVNYYPPC 201
C.papaya_16416436 SF-KEVVSKYSIEVRELGLRIEELISESLGLDKDLIRN-VVG----EQGQHMAVNYYPPC 200
VvDMR6.2 EF-KDTVSNYCVEVRQLGHRLEEAIGESLGLEKDYIRN-TLG----EQGQHMAVNYYPPC 200
R.communis_29866.t000021 LF-KEIVSRYCIEVRKLGFRLQELISESLGLPKDHIRN-VLG----EQGQHMAVNYYPPC 200
M.truncatula_Medtr3g122530 SF-KETVANYCKEVRELGLRIEEYISESLGLEKDYLRN-ALG----EQGQHMAVNYYPPC 200
G.max_Glyma04g227900 SF-KETVTEYCTLVRELGLRIQEYISESLGLEKDYIKN-VLG----EQGQHMAVNYYPPC 200
G.max_Glyma06g137000 SF-KETVTEYCTIIRELGLRIQEYISESLGLEKDYIKN-VLG----EQGQHMAVNYYPPC 200
G.max_Glyma06g14190 SF-KETVTEYCTIIRELGLRIQEYISESLGLEKDYIKN-VLG----EQGQHMAVNYYPPC 200
E.grandis_v1_0.018520m.g TF-KEFVSNYCREVRRLGYRLEELISESLGLEKDAVRN-ILG----EQGQHMAVNFYPPC 200
E.grandis_v1_0.018494m.g TF-KEFVSNYCREVRRLGYRLEELISESLGLEKDAVRN-ILG----EQGQHMAVNFYPPC 200
E.grandis_v1_0.018496m.g TF-KEFVSNYCREVRRLGYRLEELISESLGLEKDAVRN-ILG----EQGQHMAVNFYPPC 200
P.persica_ppa008269m.g SF-KDIVSKYSEEVRELGFRLQELISESLGLEKDYIKS-TLG----EQGQHMAVNFYPPC 200
C.clementina_0.9_015118m.g TF-KEFVSTYCSEVRGLGYRVLELISESLGLEKDYIKK-VLG----EQGQHMAVNFYPPC 200
P.persica_ppa022381m.g SF-REDVAEYCRNVKGLAERLLEAISESLGLEKDYMNR-ALG----KHGQHMAINYYPPC 205
P.persica_ppa019415m.g SF-REDVAEYCRNVKGLAERLLEAISESLGLEKDYMNR-ALG----KHGQHMAINYYPPC 205
C.clementina_0.9_032642m.g SF-RYN------YARGLVLRLLEAISESLGLQRDYIDK-ALG----KHRQHMALNYYPHC 203
C.clementina_0.9_012078m.g SV-REVVAEYCTSVRGLVLRLLEAISESLGLQRDFIDK-ALG----KHGQHMALNYYPPC 209
DLO
B.distachyon_Bradi5g19250 AF-REVVGAYSTEARALALRLLEAISESLGLERRHMVT-AMG----GHAQHMAVNYYPPC 210
O.sativa_Os04g49210 SF-RQVVGTYSREARALALRLLEAISESLGLERGHMVS-AMG----RQAQHMAVNYYPPC 210
S.italica_Si010491m.g SF-REVVGTYATEARALALRLLEAISESLGLERSHMVA-AMG----RQAQHMAVNYYPPC 210
S.bicolor_Sb06g026350 SF-RQVVGTYATEARALALRLLEAISESLGLERSHMVR-AMG----RHAQHMAVNYYPPC 210
Z.mays_GRMZM2G050234 SF-RQVVGTYATEARALALRLLEAISESLGLERSHMVA-AMG----RHAQHMAVNYYPPC 210
G.max_Glyma03g42250 SLSREDVAEYCRKMRGVSLKLVEAISESLGLERDYINR-VVGGKKGQEQQHLAMNYYPAC 213
G.max_Glyma16g01990 SF-REDVAEYSRKMRGLSLKLLEAISESLGLEKDYIDK-ALG----KHGQHMAINYYPPC 207
G.max_Glyma07g05420 SF-REDVAEYSRKMRGLSLKLLEAISESLGLERDYIDK-ALG----KHGQHLAINYYPPC 207
VvDLO1 SF-REVVAEYCKEARKLALLLLEAISESLGLERNHIDK-ALG----KHSQQMALNYYPPC 208
R.communis_30076.t000026 SF-REDVGEYCRNVRDLAVRLLEAISESLGLERDYINK-ALD----KHAQHLAVNYYPSC 208
AlDLO1 SF-KEVTAEYATSVRALVLRLLEAISESLGLESDHISN-ILG----KHAQHMAFNYYPPC 210
AtDLO1 SF-REVTAEYATSVRALVLRLLEAISESLGLESDHISN-ILG----KHAQHMAFNYYPPC 209
AlDLO2 SF-REVTAEYATSVRALVLTLLEAISESLGLVKDRVSN-TLG----KHGQHMAINYYPPC 210
AtDLO2 SF-REVTAEYATSVRALVLTLLEAISESLGLAKDRVSN-TIG----KHGQHMAINYYPRC 207
C.sativus_Cucsa.193360 SF-RKEVAEYCTTMRQLTLKLLEAISESLGLPKDSIAN-SIG----SHGQHMALNYYPPC 211
C.papaya_evm.TU.sup_37.106 FF-REDVAEYCSRIRGLVLKLVEAISESLGLGGDYINK-VLG----KHGQHMAFNYYPPC 208
E.grandis_v1_0.017927m.g SF-RKEVGEYCTRVRELALKLLEAISESLGLEREYISK-NLG----KHGQHMAMNYYPPC 210
E.grandis_v1_0.017897m.g SF-RKEVGEYCTRVRELALKLLEAISESLGLEREYISK-NLG----KHGQHMAMNYYPPC 210
E.grandis_v1_0.047536m.g SF-RKEVGEYCTRVRELVFKLLEAISESLGLEREYISQ-NLG----EHGQHMAMNYYPPC 233
E.grandis_v1_0.017895m.g SF-RKEVGEYCTRVRELVLKLLEAISESLGLEREYISQ-NLG----KHGQHMAMNYYPPC 210
E.grandis_v1_0.017876m.g SF-RKEMGEYCTRVRELVLKLLEAISESLGLEREYISQ-NLG----KHGQHMAMNYYPPC 210
E.grandis_v1_0.015926m.g SF-RKEVGEYCTRVRELVFKLLEAISESLGLEREYISQ-NLG----KHGQHMAMNYYPPC 210
VvDLO2 SF-REDVAEYCTSIRALVLRLLETISESLGLEKNYVSG-VLG----KHGQHMAMNYYPPC 209
C.clementina_0.9_014263m.g SF-REDVGDYCTSVRGLVLRLIQAISESLGLPSDYIDKEALG----KHGQHMALNYYPPC 214
P.persica_ppa008100m.g SF-REQVGEYCTTVRGLVLRLLGAISESLGLEKNYIVE-ALG----KQGQHMALNYYPPC 208
P.persica_ppa008091m.g SF-REQVGEYCTTVRGLVLRLLGAISESLGLEKNYIVE-ALG----KQGQHMALNYYPPC 208
R.communis_30068.t000102 LF-RKNVSEYSTSVRRLVLTLLEAISESLGLKRDYIEK-TLS----KQGQHMAMNYYPPC 207
M.esculenta_4.1_029834m.g SF-RKNVAEYCTRVRGLVLTLLEAISESLGLKSDYIDK-ALS----KHGQHMAINYYPPC 208


DMR6
B.distachyon_Bradi5g19240 PEPDLTYGLPKHTDPNALTVLLQDPNVSGLQVLKDG-QWIAVDPRPNALVINLGDQLQ-- 257
O.sativa_Os04g49194 PEPDLTYGLPKHTDPNALTILLPDPHVAGLQVLRDGDQWIVVNPRPNALVVNLGDQIQ-- 259
O.sativa_Os10g39140 PEPELTYGLPAHTDPNALTILLMDDQVAGLQVLNDG-KWIAVNPQPGALVINIGDQLQ-- 262
S.bicolor_Sb01g030560 PEPELTYGLPAHTDPNALTILLMDQDVAGLQVLHGG-KWVAVNPQPGALIINIGDQLQ-- 256
ZmFNSI-1/ZmDMR6 PEPELTYGLPAHTDPNALTILLMDPDVAGLQVLHAG-QWVAVNPQPGALIINIGDQLQ-- 256
B.distachyon_Bradi1g77040 PSPELTYGLPAHTDPNALTILLMDEQVAGLQVLKDG-QWIAVNPRPNALVVNLGDQLQ-- 256
O.sativa_Os03g03034 PEPELTFGLPAHTDPNALTILLMDQQVAGLQVLKEG-RWIAVNPQPNALVINIGDQLQ-- 256
S.italica_Si036512m.g PAPELTYGLPAHTDPNALTILLMDQQVAGLQVLNDG-RWIAVNPRPNALVINIGDQLQ-- 256
S.bicolor_Sb01g049030 PAPELTYGLPAHTDPNALTILLMDQQVAGLQVLKDG-RWIAVNPRPGALVVNLGDQLQ-- 256
Z.mays_GRMZM2G475380 PAPELTYGLPAHTDPNALTILLMDQQVAGLQVLKDG-RWIAVNPRPGALVVNIGDQLQVT 258
A.coerulea_v1.008143m.g PEPELTYGLPRHTDPNTITILLQGQEVAGLQVLHNG-KWVAVNPYPNAFVVNIGDQIQ-- 258
E.salsugineum_TP2g22820 PEPELTYGLPAHTDPNVLTILLQDATVCGLQILIDG-HWFAVNPRPDAFVINIGDQLQ-- 257
C.rubella_006g21660 PEPELTYGLPAHTDPNALTILLQDSTVCGLQILIDG-QWFAVNPHPNAFVINIGDQLQ-- 257
AtDMR6 PEPELTYGLPAHTDPNALTILLQDTTVCGLQILIDG-QWFAVNPHPDAFVINIGDQLQ-- 257
AlDMR6 PEPELTYGLPAHTDPNALTILLQDTTVCGLQILIDG-QWFAVNPHPDAFVINIGDQLQ-- 257
C.sativus_Cucsa.273300 PQPELTYGLPGHTDPNALTILLQDLHVAGLQVLKDG-KWLAVNPHPNAFVINIGDQLQ-- 245
SlDMR6 PQPELTYGLPAHTDPNSLTILLQDLQVAGLQVLKDG-KWLAVKPQPDAFVINLGDQLQ-- 257
M.guttatus_mgv1a009622m.g PEPELTYGLPAHTDPNALTILLQDLQVAGLQVLKDG-KWLAIKPQPGAFVINIGDQLQ-- 256
VvDMR6.1 (I253K) PQPELTYGLPGHTDPNALTILLQDLRVAGLQVLKDG-TWLAIKPHPGAFVVNIGDQLQ-- 258
C.papaya_16416436 PQPELTYGLPAHTDPNALTILLQDLQVSGLQVLKDG-KWVAVHPQPNAFVINIGDQLQ-- 257
VvDMR6.2 PEPELTYGLPAHTDPNALTILLQDSHVAGLQVLKDG-KWVAVKPHPGAFVVNIGDQLQ-- 257
R.communis_29866.t000021 PQPDLTYGLPGHTDPNALTILLQDLQVAGLQVFKDG-KWLAVNPHPNAFVINLGDQLQ-- 257
M.truncatula_Medtr3g122530 PQPELTYGLPGHTDPNALTILLQDLHVAGLQVLKDG-KWLAINPIPDAFVINIGDQLQ-- 257
G.max_Glyma04g227900 PEPELTYGLPGHTDPNALTILLQDLQVCGLQVLKNG-KWLAVNPQPNAFVINIGDQLQ-- 257
G.max_Glyma06g137000 PEPELTYGLPGHTDPNALTILLQDLQVAGLQVLKDG-KWLAVSPQPNAFVINIGDQLQ-- 257
G.max_Glyma06g14190 PEPELTYGLPGHTDPNALTILLQDLQVAGLQVLKDG-KWLAVSPQPNAFVINIGDQLQ-- 257
E.grandis_v1_0.018520m.g PEPELTYGLPGHTDPNALTILLQDPHVAGLQVLKDG-KWVAIDPHPNAFVINIGDQLQ-- 257
E.grandis_v1_0.018494m.g PEPELTYGLPGHTDPNALTILLQDLHVAGLQVLKDG-KWVAIDPHPNAFVINIGDQLQ-- 257
E.grandis_v1_0.018496m.g PEPELTYGLPGHTDPNALTILLQDLHVAGLQVLKDG-KWVAIDPHPNAFVINIGDQLQ-- 257
P.persica_ppa008269m.g PQPELTYGLPGHTDPNALTILLQDLEVAGLQVLKDG-KWIAVNPHPNAFVINLGDQLQ-- 257
C.clementina_0.9_015118m.g PEPELTYGLPGHTDPNALTILLQDLEVAGLQVLKDD-KWVAVNPLPNAFVINIGDQLQ-- 257
P.persica_ppa022381m.g HQPELTYGLPGHADPNVVTLLLQD-DVAGLQVFNNG-RWVAVKPMPHTFIVNIGDQIQ-- 261
P.persica_ppa019415m.g HQPELTYGLPGHADPNVVTLLLQD-DVAGLQVFNNG-RWVAVKPMPHTFIVNIGDQIQ-- 261
C.clementina_0.9_032642m.g PQPDLTYGLPGHIDPNLITVLLQD-DVPGLQVLRKG-KWLPVSPIPNTFIVKIGDQMQ-- 259
C.clementina_0.9_012078m.g PQPDLTYGLPGHTDPNLITVLLQD-DVPGLQVLRNG-KWLPVSPIPNTFIVNIGDQMQ-- 265
DLO
B.distachyon_Bradi5g19250 PQPELTYGLPGHKDPNAVTLLLQD-GVSGLQVQRGG-RWVAVNPVPNALVINIGDQLQ-- 266
O.sativa_Os04g49210 PQPELTYGLPGHKDPNAITLLLQD-GVSGLQVQRNG-RWVAVNPVPDALVINIGDQIQ-- 266
S.italica_Si010491m.g PQPELTYGLPGHKDPNAITLLLQD-GVSGLQVQRDG-RWVAVNPVPNALVINIGDQLQ-- 266
S.bicolor_Sb06g026350 PQPELTYGLPGHKDPNAITLLLQD-GVSGLQVQRGG-RWVAVNPVPDALVINIGDQMQ-- 266
Z.mays_GRMZM2G050234 PQPELTYGLPGHKDPNAITLLLQD-GVSGLQVQRGG-RWVAVNPVPNALVINIGDQMQ-- 266
G.max_Glyma03g42250 PEPELTYGLPGHTDPTVITILLQD-EVPGLQVLKDG-KWVAVNPIPNTFVVNVGDQIQ-- 269
G.max_Glyma16g01990 PEPELTYGLPAHADPNAITILLQN-QVPGLQVLHDG-KWLTVNPVPNTFIVNIADQIQ-- 263
G.max_Glyma07g05420 PEPELTYGLPAHADPNAITILLQN-EVPGLQVLYDG-KWLTVNPVPNTFIVNIGDQIQ-- 263
VvDLO1 PQPELTFGLPGHADPNALTILLQD-DVPGLQVLKDG-KWVAIHPIPNTFIVNIGDQIQ-- 264
R.communis_30076.t000026 PQPELTYGLPVHADPNVITILLQD-DVPGLQVLKDG-KWVAVSPVPHTFIVNIGDQIQ-- 264
AlDLO1 PEPELTYGLPGHKDPTVITVLLQD-QVSGLQVFKDN-KWVAVNPIPNTFIVNIGDQMQ-- 266
AtDLO1 PEPELTYGLPGHKDPTVITVLLQD-QVSGLQVFKDD-KWVAVSPIPNTFIVNIGDQMQ-- 265
AlDLO2 PQPELTYGLPGHKDANLITVLLQD-EVSGLQVFEDG-KWIAVNPIPNTFIVNLGDQMQ-- 266
AtDLO2 PQPELTYGLPGHKDANLITVLLQD-EVSGLQVFKDG-KWIAVNPVPNTFIVNLGDQMQ-- 263
C.sativus_Cucsa.193360 PQPDLTYGLPCHTDPNLITLLLQD-QVPGLQVHRDG-AWVALNPIPNTFIINIGDQMQ-- 267
C.papaya_evm.TU.sup_37.106 PEPELTYGLPGHTDPNLITVLLQD-DVPGLQVLRNG-KWVAVNPIPNTFIINIGDQMQ-- 264
E.grandis_v1_0.017927m.g PQPELTYGLPGHTDQNLITILLQD-EVPGLQVLRDG-KWIAVNPIPNTFIVNIGDQMQ-- 266
E.grandis_v1_0.017897m.g PQPELTYGLPGHTDRNLITILLQD-DVPGLQVLRDG-KWIAVNPIPNTFIVNIGDQMQ-- 266
E.grandis_v1_0.047536m.g PQPELTYGLPSHTDPNLITLLLQD-DVPGLQVLRNG-KWVAVNPIPNTFIVNIGDQMQ-- 289
E.grandis_v1_0.017895m.g PQPELTYGLPGHTDPNLITILLQD-DVPGLQVLRNG-KWVAVNPIPNTFIVNIGDQMQ-- 266
E.grandis_v1_0.017876m.g PQPELTYGLPGHTDPNLITILLQD-DVPGLQVLRNG-KWVAVNPIPNTFIVNIGDQMQ-- 266
E.grandis_v1_0.015926m.g PQPELTYGLPGHTDPNLITILLQD-DVPGLQVLRNG-KWVAVNPIPNTFIVNIGDQMQ-- 266
VvDLO2 PQPELTYGLPGHTDCSLITVLLQD-DVPGLQVLRNG-KWVSVNPIPNSFIVNIGDHMQ-- 265
C.clementina_0.9_014263m.g PQPELTYGLPGHTDPNLITLLLQD-DVPGLQVLRDG-NWVPVNPIPSTFIVNIGDQMQ-- 270
P.persica_ppa008100m.g PEPELTYGLPGHTDCNLITILLQD-DVAGLQVLRNG-KWVAVNPIPNTFIVNIGDMMQ-- 264
P.persica_ppa008091m.g PEPELTYGLPGHTDCNLITILLQD-DVAGLQVLRNG-KWVAVNPIPNTFIVNIGDMMQ-- 264
R.communis_30068.t000102 PQPELTYGLPGHTDPNLITILLQD-HVPGLQVLRNG-KWIAINPIPSTFIVNIGDQMQ-- 263
M.esculenta_4.1_029834m.g PQPELTYGLPEHSDPNLITILLQD-QVPGLQVLRNG-KWVAVDPIPNTFIVNIGDQMQ-- 264


DMR6
B.distachyon_Bradi5g19240 ----------ALSNGAYKSVWHRAVVNAAQERMSVASFLCPC 289
O.sativa_Os04g49194 ----------ALSNDAYKSVWHRAVVNPVQERMSVASFMCPC 291
O.sativa_Os10g39140 ----------ALSNGKYRSVWHRAVVNSDRERMSVASFLCPC 294
S.bicolor_Sb01g030560 ----------ALSNGQYRSVWHRAVVNSDRERMSVASFLCPC 288
ZmFNSI-1/ZmDMR6 ----------ALSNGQYRSVWHRAVVNSDRERMSVASFLCPC 288
B.distachyon_Bradi1g77040 ----------ALSNGRYKSVWHRAVVNSDRPRMSIASFMCPC 288
O.sativa_Os03g03034 ----------ALSNGRYKSVWHRAVVNSDKARMSVASFLCPC 288
S.italica_Si036512m.g ----------ALSNGRYKSVWHRAVVNSDRPRMSVASFLCPC 288
S.bicolor_Sb01g049030 ----------ALSNGRYKSVWHRAVVNSDRPRMSVASFLCPC 288
Z.mays_GRMZM2G475380 CSCLALPLFPPSHLLSAPGLHYCTAPAQALSNGRYRSVWHRAVVNADRPRMSVASFLCPC 318
A.coerulea_v1.008143m.g ----------ALSNGNYASVWHRATVNTDRERISVASFLCPA 290
E.salsugineum_TP2g22820 ----------ALSNGVYKSVWHRAVTNTDKPRLSVASFLCPA 289
C.rubella_006g21660 ----------ALSNGVYKSVWHRAVTNTENPRLSVASFLCPA 289
AtDMR6 ----------ALSNGVYKSVWHRAVTNTENPRLSVASFLCPA 289
AlDMR6 ----------ALSNGVYKSVWHRAVTNTENPRLSVASFLCPA 289
C.sativus_Cucsa.273300 ----------ALSNGVYKSVWHRAVVNVDKPRLSVASFLCPC 277
SlDMR6 ----------AVSNGKYRSVWHRAIVNSDQARMSVASFLCPC 289
M.guttatus_mgv1a009622m.g ----------ALSNGKYKSVWHRAVVNADKARLSVASFLCPC 288
VvDMR6.1 ----------AVSNGKYKSVWHRAVVNAESERLSVASFLCPC 290
C.papaya_16416436 ----------AVSNGKYKSVWHRAVVNSDKVRLSIASFLCPC 289
VvDMR6.2 ----------ALSNGKYRSVWHRATVNVGKARMSIASFLCPS 289
R.communis_29866.t000021 ----------ALSNGRYKSVWHRAIVNADRERMSIASFLCPC 289
M.truncatula_Medtr3g122530 ----------ALSNGLYKSVWHRAIVNAEKPRLSVASFLCPD 289
G.max_Glyma04g227900 ----------ALSNGLYKSVWHRAVVNVEKPRLSVASFLCPN 289
G.max_Glyma06g137000 ----------ALSNGLYKSVWHRAVVNVEKPRLSVASFLCPN 289
G.max_Glyma06g14190 ----------ALSNGLYKSVWHRAVVNVEKPRLSVASFLCPN 289
E.grandis_v1_0.018520m.g ----------ALSNGRYKSVWHRAIVNADKPRMSIASFLCPS 289
E.grandis_v1_0.018494m.g ----------ALSNGRYKSVWHRAIVNADKPRMSIASFLCPS 289
E.grandis_v1_0.018496m.g ----------ALSNGRYKSVWHRAIVNADKPRMSIASFLCPS 289
P.persica_ppa008269m.g ----------ALSNGIYRSVWHRAITNTDRARLSVASFLCPQ 289
C.clementina_0.9_015118m.g ----------ALSNGRYKSVWHRAIVNAEKARMSVASFLCPN 289
P.persica_ppa022381m.g ----------VVSNDRYKSVLHRAVVNCDKERISIPTFYCPS 293
P.persica_ppa019415m.g ----------VVSNDRYKSVLHRAVVNCDKERISIPTFYCPS 293
C.clementina_0.9_032642m.g ----------VLSNDRYKSVLHRALVNCDKERISIPTFYCPS 291
C.clementina_0.9_012078m.g ----------VLSNDRYKSVLHRALVNCDRERISIPTFYCPS 297
DLO
B.distachyon_Bradi5g19250 ----------ALSNDRYKSVLHRVIVNSESERISVPTFYCPS 298
O.sativa_Os04g49210 ----------ALSNDRYKSVLHRVIVNSESERISVPTFYCPS 298
S.italica_Si010491m.g ----------ALSNDRYKSVLHRVIVNSESERISVPTFYCPS 298
S.bicolor_Sb06g026350 ----------ALSNDRYKSVLHRVIVNSESERISVPTFYCPS 298
Z.mays_GRMZM2G050234 ----------ALSNDRYKSVLHRVIVNSESERISVPTFYCPS 298
G.max_Glyma03g42250 ----------VISNDKYKSVLHRAVVNCNKDRISIPTFYFPS 301
G.max_Glyma16g01990 ----------VISNDRYKSVLHRALVNCEKERMSIPTFYCPS 295
G.max_Glyma07g05420 ----------VISNDRYKSVLHRALVNCEKERMSIPTFYCPS 295
VvDLO1 ----------VLSNDCYKSAVHRAVVNCQKERISIPTFYCPS 296
R.communis_30076.t000026 ----------VISNDRYKSVLHRAVVNSNKERISIPTFYCPS 296
AlDLO1 ----------VISNDKYKSVLHRAVVNTEKERLSIPTFYFPS 298
AtDLO1 ----------VISNDKYKSVLHRAVVNTENERLSIPTFYFPS 297
AlDLO2 ----------VISNDKYKSVLHRAVVNIDKERISIPTFYCPS 298
AtDLO2 ----------VISNEKYKSVLHRAVVNSDMERISIPTFYCPS 295
C.sativus_Cucsa.193360 ----------VLSNDRYKSVLHRAVVNNATERISIPTFYCPS 299
C.papaya_evm.TU.sup_37.106 ----------VISNDKYKSVVHRAVVNSERERISIPTFYCPS 296
E.grandis_v1_0.017927m.g ----------VISNDKYKSILHRAVVNCDRERISIPTFYCPS 298
E.grandis_v1_0.017897m.g ----------VISNDKYKSILHRAVVNCDRERISIPTFYCPS 298
E.grandis_v1_0.047536m.g ----------VISNDKYKSVLHRAVVNRNTERISIPTFCCPS 321
E.grandis_v1_0.017895m.g ----------VISNDKYKSVLHRAVVNCNTERISIPTFYCPS 298
E.grandis_v1_0.017876m.g ----------VISNDKYKSVLHRAVVNCNTERISIPTFYCPS 298
E.grandis_v1_0.015926m.g ----------VISNDKYKSVLHRAVVNCNTERISIPTFYCPS 298
VvDLO2 ----------VISNDRYKSVLHRAVVNCNKDRISIPTFYCPS 297
C.clementina_0.9_014263m.g ----------VLSNDRYKSVLHRAVVSRDKERISIPTFYCSS 302
P.persica_ppa008100m.g ----------VISNDKYKSVLHRAVVNCNSERISIPTFYCPS 296
P.persica_ppa008091m.g ----------VISNDKYKSVLHRAVVNCKSERISIPTFYCPS 296
R.communis_30068.t000102 ----------VISNDRYKSVLHRAVVNSYEERISIPTFYCPS 295
M.esculenta_4.1_029834m.g ----------VISNNRYKSVLHRAVVNSDKERLSIPTFYCPS 296


DMR6
B.distachyon_Bradi5g19240 NSAVIGPAAKLVGD---GDEPVYRSYTYDEYYNKFWSRN-LDQEHCLELFRGQK---- 339
O.sativa_Os04g49194 NSAVISPARKLVAD---GDAPVYRSFTYDEYYKKFWSRN-LDQEHCLELFKGQ----- 340
O.sativa_Os10g39140 NSVELGPAKKLITD---DSPAVYRNYTYDEYYKKFWSRN-LDQEHCLELFRT------ 342
S.bicolor_Sb01g030560 NHVVLGPAKKLVTE---DTPAVYRSYTYDEYYKKFWSRN-LDQEHCLELFRT------ 336
ZmFNSI-1/ZmDMR6 NHVVLGPARKLVTE---DTPAVYRNYTYDKYYAKFWSRN-LDQEHCLELFRT------ 336
B.distachyon_Bradi1g77040 NSVVLGPAEKLVGD---ASPAVYRNYTYDEYYKKFWSRN-LDQEHCLELFRT------ 336
O.sativa_Os03g03034 NDVLIGPAQKLITD---GSPAVYRNYTYDEYYKKFWSRN-LDQEHCLELFRTTPTDTS 342
S.italica_Si036512m.g NDVRIGPAAKLVGE---GAPAVYRDYTYAEYYGKFWSRN-LDQEHCLELFRT------ 336
S.bicolor_Sb01g049030 NDVRIGPAAKLVTG---DTPAVYRDYTYAEYYAKFWSRN-LDQEHCLELFRT------ 336
Z.mays_GRMZM2G475380 NDARIGPAARLLTD---GTPAVYRDYTYAEYYAKFWSRN-LDQEHCLELFRTPTS--- 369
A.coerulea_v1.008143m.g NDAIICPAV------KDGSPSMYKKFTYDEYYKKFWSGN-LDQQHCLELFKE------ 335
E.salsugineum_TP2g22820 DCAVISPAKPLWEDEEDEAKPMYRDYTYAEYYKKFWSRN-LDQEHCLENFLNH----- 341
C.rubella_006g21660 DCAVMSPAKSLWEAEDSETKPIYRDFTYAEYYKKFWSRN-LDQEHCLENF-------- 338
AtDMR6 DCAVMSPAKPLWEAEDDETKPVYKDFTYAEYYKKFWSRN-LDQEHCLENFLNN----- 341
AlDMR6 DCAVMSPAKPLWEAEDNETKPVYRDFTYAEYYKKFWSRN-LDQEHCLEYFRNN----- 341
C.sativus_Cucsa.273300 DDALITPAPLLSQ-----PSPIYRPFTYAQYYNTFWSRN-LDQQHCLELFKNHPP--- 326
SlDMR6 DSAKISAPKLLTED---GSPVIYQDFTYAEYYNKFWSRN-LDQQHCLELFKN------ 337
M.guttatus_mgv1a009622m.g DSANISAPKGLTGG---EDPAVYRDYTYNEYYKKFWSRN-LDQEHCLELFKN------ 336
VvDMR6.1 NDAVIGPAKPLTED---GSAPIYKNFTYAEYYKKFWGRD-LDQEHCLELFKN------ 338
C.papaya_16416436 DDAVISPPKLLTAD---GSVAIYRDFTYAEYYKKFWSRN-LDQEHCLELFRNK----- 338
VvDMR6.2 DDALISPARALTDE---GSAAIYRSFTYAEYYKKFWSRN-LDQEHCLEVFKN------ 337
R.communis_29866.t000021 DDALISPAKPLTEG---ESGAVYRDFTYAEYYKKFWSRN-LDQEHCLELFKN------ 337
M.truncatula_Medtr3g122530 NEALICPAKPLTED---GSGAVYRGFTYPEYYSKFWSRD-LEKEHCLEFFKNN----- 338
G.max_Glyma04g227900 DEALISPAKPLTEG---GSEAIYRGFTYAEYYKKFWSRN-LDQEHCLEFFKNK----- 338
G.max_Glyma06g137000 DEALISPAKPLTEH---GSEAVYRGFTYAEYYKKFWSRN-LDQEHCLELFKNK----- 338
G.max_Glyma06g14190 DEALISPAKPLTEH---GSEAVYRGFTYAEYYKKFWSRN-LDQEHCLELFKNK----- 338
E.grandis_v1_0.018520m.g NDALISSPVSLLDN---GCGPTYRDFTYAEYYKKFWSRN-LDQEHCLELFKNQA---- 339
E.grandis_v1_0.018494m.g DDALISSPVSLLDN---GCGPTYRDFTYAEYYKKFWSRN-LDQEHCLELFKNQA---- 339
E.grandis_v1_0.018496m.g DDALISSPVSLLDN---GCGPTYRDFTYAEYYKKFWSRN-LDQEHCLELFKNQA---- 339
P.persica_ppa008269m.g DDALISPAKALTDD---GSAAIYRGYTYTEYYKKFWTRD-LNQEHCLELFKTDQ---- 339
C.clementina_0.9_015118m.g NDAMISPPKALTED---GSGAVYRDFTYAEYYSKFWSRN-LDQEHCLELFKN------ 337
P.persica_ppa022381m.g YDAVMEPAPQLVDD--H-HPPLYRSFTYAEFYEKFWDRG-LNTRSSLDLFQTTSHA-- 345
P.persica_ppa019415m.g YDAVMEPAPQLVDD--H-HPPLYRSFTYAEFYEKFWDRG-LNTRSSLDLFKTTSHA-- 345
C.clementina_0.9_032642m.g PDAVIAPAKDLIDER---HP-------------KFWNRG-LVDECCLDLFKASTA--- 329
C.clementina_0.9_012078m.g PDAVIAPAKDLIDER---HPAVYKNFTYAEYYQKFWNRG-L-DERCLDLFKASTA--- 347
DLO
B.distachyon_Bradi5g19250 PDAVVAPAEALVDG--S-HPLAYRPFTYQEYYEEFWNMG-LESASCLDRFRPMD---- 348
O.sativa_Os04g49210 PDAVIAPAGALVDGA-L-HPLAYRPFKYQAYYDEFWNMG-LQSASCLDRFRPNDQAV- 352
S.italica_Si010491m.g PDAVIAPAGALVDD--A-HPLAYRPFTYQEYYDEFWNMG-LQSASCLDRFRPG------ 347
S.bicolor_Sb06g026350 PDGVIAPADALVDD--A-HPLAYRPFTYQEYYDEFWNMG-LQSASCLDRFRPGGSIE- 351
Z.mays_GRMZM2G050234 PDAVIAPADALVDD--G-HPLAYRPFTYQEYYDAFWNMG-LQSASCLDRFRPGGSLE- 351
G.max_Glyma03g42250 NDAIIGPAPQLIHHH-H-HPPQYNNFTYNEYYQNFWNRG-LSKETCLDIFKA------ 350
G.max_Glyma16g01990 PDALIKPAPQLVDK--E-HPAQYTNFTYREYYDKFWIRG-LSKETCVDMFKAQD---- 345
G.max_Glyma07g05420 PDALIKPAPKLVDN--E-HPAQYTNFTYREYYDKFWNRG-LSKETCVDMFKAQD---- 345
VvDLO1(G302E) PDAVIGPAPGLVDH--G-HPALYRKFTYSEYFGKFWNRG-LATQSCLDMFKT------ 344
R.communis_30076.t000026 PDAAIGPAPPLVDN--H-HPLLYTNFTYSQYYHKFWNRG-LATHTCLDMFKK------ 344
AlDLO1 TDAVIGPAHELINEQ-E-SLAVYRTFPFVEYWDKFWNRS-LATASCLDAFKASTT--- 350
AtDLO1 TDAVIGPAHELVNEQ-D-SLAIYRTYPFVEYWDKFWNRS-LATASCLDAFKAPTT--- 349
AlDLO2 EDAMIGPAQELINEE-EDSHAIYRNFTYAEYFEKFWDTA-FATESCIDSFKASTA--- 351
AtDLO2 EDAVISPAQELINEE-EDSPAIYRNFTYAEYFEKFWDTA-FDTESCIDSFKASTA--- 348
C.sativus_Cucsa.193360 PEAMIGPAKELIHDE---HRPAFRNFTYSEYYQTFWSGE-LDTRRCLDLFRI------ 347
C.papaya_evm.TU.sup_37.106 LDAVIGPAH---------RPSVYRNFSYGEYYSKFWSRSSLTAQACLDMFKA------ 339
E.grandis_v1_0.017927m.g PEALIGPAQGLIDHD---NPAVYRSFTYEEYYHKFWNRG-LRTECCLDMFKIPSA--- 349
E.grandis_v1_0.017897m.g PEALIGPAQGLIDHE---HPAVYRSFTYEEYYHKFWNRG-LRTECCLDMFKIPSA--- 349
E.grandis_v1_0.047536m.g PEGLIQPAQGLIDHE---HPPVYRSFTWEEFYQKFYHRG-LRTECCLDMFKIPSA--- 372
E.grandis_v1_0.017895m.g PEGLIRPAQGLIDHE---HPAVYKSFTYEEYYHKFWNRG-LRTECCLDMFKIHSA--- 349
E.grandis_v1_0.017876m.g PEGLIRPAQGLIDHE---HPAVYRSFTYEEYYHKFWNRG-LRTECCLDMFKIHSA--- 349
E.grandis_v1_0.015926m.g PEGLIRPAQGLIDHG---HPAVYRSFTYEEYYHKFWNRG-LRTECCLDMFKIPSA--- 349
VvDLO2 PDAVIGPSPELVDDD---HPAVYRNFTCEEYYTQFWNRG-LATESCLDTFKASTT--- 348
C.clementina_0.9_014263m.g PDAVIGPAKGLVDQD---HPAVYRDFTYAEYYKKFWNRG-LATECCLEMFKASSTV-- 354
P.persica_ppa008100m.g PDAVIGPAKDLISHD---QPAMYRNFTYAEYFEKFWNRG-LATECCLDLFKPN----- 345
P.persica_ppa008091m.g PDAVIGPAKDLISHD---QPAMYRNFTYAEYFEKFWNRG-LATECCLDLFKPN----- 345
R.communis_30068.t000102 PDAVIGPAKDLIDPD---HPAAYREFTYAEYYEKFWDRG-LAKECCLDLFKTSTA--- 346
M.esculenta_4.1_029834m.g SDAVIGPAKDLIDND---HPAVYKHFTYAEYYEIFWNRG-LEKECCLDLFKISSA--- 347

CHAPTER 4

Functional characterization of *VvDMR6* and *VvDLO* genes

INTRODUCTION

The new breeding techniques (NBT) such as TALEN (Transcription-Activator Like Effector Nucleases) and CRISPR/Cas9 systems are nowadays the principal available resources for cutting edge targeted mutagenesis (Wang et al., 2014). In particular the CRISPR/Cas9 technology takes the lead as genome editing technique for obtaining new mutated crops either by gene knock-out through Non-Homologous End Joining (NHEJ) or by genome rewriting via Homology Directed Repair (HDR) (Schenke & Cai, 2020).

The first CRISPR/Cas9-based work on grapevine is that one of Ren et al. (2016). This was followed by a few others with different purposes including improvement of the technology (e.g. Malnoy et al. (2016), or to functionally characterize specific genes such as *VvPDS* (Nakajima et al., 2017), *VvWRKY52* (X. Wang et al., 2018), *VvCCD8* (Ren et al., 2020) and *VvPR4b* (Li et al., 2020).

In a perspective of obtaining durable resistance to pathogens, loss of function mutation of susceptibility (*S*) genes has been proposed as a very promising strategy and CRISPR/Cas9 mediated genome editing is a very efficient technique to obtain gene knock-out by introducing small insertions or deletions. Given their important role for host recognition, pathogen survival and spread in plant host, a loss-of-function mutation in *S* genes can induce host defense responses, providing long-lasting and broad-spectrum resistance (van Schie & Takken, 2014). In 2013, Jiang et al. were the first to target rice *OsSWEET11* and *OsSWEET14 S* genes to engineer plant disease resistance via CRISPR/Cas9. Many studies followed focusing on gene disruption and obtained complete or increased resistance to pathogens (Moniruzzaman et al., 2020). In grapevine, *S* gene knock-out has been applied to the *VvMLO* (*Mildew resistance Locus O*) gene family in order to increase resistance to powdery mildew (PM) (e.g. Pessina et al., 2016; Wan et al., 2020).

Downy mildew (DM) is one of the most destructive grapevine diseases (Pimentel, 2005) caused by the oomycete *Plasmopara viticola* (Berk. & Curt.) Berl. and de Toni. The growing interest in conferring durable resistance to this pathogen, which is basically not assured on the long range by resistance (*R*) genes, paved the way to the exploitation of the *DMR* (*Downy Mildew Resistant*) *S* genes, which is the matter of investigation of this PhD work. Since their first discovery and functional characterization in Arabidopsis, passing through the discovery of *DLO* (*DMR-like Oxygenase*) genes (Van Damme et al., 2005; Van Damme et al., 2008; K. Zhang et al., 2013; Zeilmaker et al., 2015), orthologues of *DMR* genes were then searched and identified in different crops and tree species (Schouten at el., 2014; Zeilmaker et al., 2015; Porterfield & Meru, 2017; Sun et al., 2017; W. Zhang et al., 2018). The first attempt of genome-editing based knock-out of a *DMR6* gene was made in tomato by de Toledo Thomazella et al. (2016), which obtained resistance against the oomycete and

other bacterial pathogens, with no pleiotropic effects. Two copies of the *DMR6* gene and three of the *DLO* gene are present in the grapevine genome and are named *VvDMR6.1* and *VvDMR6.2* (Zeilmaker, et al., 2015), and *VvDLO1, VvDLO2* and *VvDLO3,* respectively.

Even though different hypothesis on their function were proposed (K. Zhang et al., 2013; Falcone Ferreyra et al., 2015; Y. J. Zhang et al., 2017), a role of *DMR6* and *DLO* in a regulatory mechanism leading to salicylic acid (SA) accumulation has been accepted. Of particular interest is the role of DMR6 as a SA-5-hydroxylase (S5H) and DLO as a SA-3-hydroxylase (S3H) (K. Zhang et al., 2013; Y. J. Zhang et al., 2017). The products of these enzymes are SA inactive forms: 2,5-dihydrobenzenic acid (2,5-DHBA) and 2,3-dihydrobenzenic acid (2,3-DHBA), respectively, which can become active again via glycosylation (Ding & Ding, 2020).

The role of SA in induction of immunity and in SAR (Systemic Acquired Resistance) is now well-established (Raskin, 1992; Vlot et al., 2009; Klessig et al., 2018). A key player in SA-mediated resistance is *NPR1* (*Non-expressor of Pathogenesis-Related gene 1*), identified also in grapevine by Le Henanff et al. (2009). The translocation of *NPR1* into the nucleus, where it acts as transcriptional co-activator, enhances the binding of transcription factors (TFs) to SA-responsive promoter elements (Pieterse et al., 2009). This transcriptional reprogramming induces increased lignin and callose production for cell wall strengthening, synthesis of metabolites as phytoalexins and of proteins directly interfering with the pathogen (van Butselaar & Van den Ackerveken, 2020) including the pathogenesis related proteins (PR proteins). Two members of the *VvPR10 (Pathogenesis-Related 10*) gene family, *VvPR10.1* and *VvPR10.3,* are known to show enhanced transcription in response to fungal attack (Polesani et al., 2010; Haile et al., 2017). Transcriptional activation in response to *P. viticola* was also observed for *VvPR2* (encoding a β-1,3-glucanase) (Malacarne et al., 2011). Moreover, Merz et al. (2015) indicated a significant correlation between the expression of *VvPR10.1* and the transcription factor *VvWRKY33* during grape berry development and fungal infection, in particular *VvWRKY33* induction is simultaneous with that of *VvPR10* in response to DM.

In grapevine, stilbenes, a group of molecules based on the *trans*-resveratrol moiety, are the major phytoalexins. Several studies have reported on stilbenes induction upon DM infection (e.g., (Langcake & Pryce, 1976; Alonso-Villaverde et al., 2011). A more rapid and extensive accumulation of stilbenes in DM-resistant genotypes in comparison to DM-susceptible genotypes was observed (Chitarrini et al., 2017), and stilbenes antifungal activity against *P. viticola* and other fungi was demonstrated in a few studies (e.g., Pezet et al., 2004; Adrian & Jeandet, 2012; Gabaston et al., 2017). Stilbenes are phenylpropanoids produced from the precursors coumaroyl CoA and malonyl CoA, which are converted to resveratrol by the action of the stilbene synthase (STS) enzymes. Forty-eight putative *STS* gene sequences were identified in grapevine (Vannozzi et al., 2012). Transcriptional

regulation of *STS*s was deciphered by Höll et al. (2013), who found the regulatory factors *VvMYB14* and *VvMYB15* strongly co-expressed with *VvSTSs* in grapevine leaves upon biotic and abiotic stresses, including *P. viticola* infection. Later on, MYB14 and MYB15 were demonstrated to bind the *VvSTS29* and *VvSTS41* promoters and thereby regulate their transcription (Fang et al., 2014; Vannozzi et al., 2018).

Taking advantage of existing genome-edited *dmr6.1* grapevine plants, aim of this work was to investigate the role of *VvDMR6.1* in *Vitis vinifera* plants, to characterize its function and its co-regulation with the *VvDMR6.2* and *VvDLO* genes and investigate its role in defense response involving PR genes, stilbenes metabolism and SA metabolism.

MATERIALS AND METHODS

Selection of edited plants

Four *V. vinifera* cv. Sugraone *dmr6.1* lines, previously obtained by knocking out the *DMR6.1* gene with the CRISPR/Cas9 machinery (Giacomelli et al., 2018), were selected for this study. Preliminarily to this study, the editing was targeted on the first exon, the type of editing and the frequency of mutations were also assessed by high coverage deep-sequencing, and analysis of the reads via the CRISPResso platform (Pinello et al., 2016) using standard settings regarding filtering low-quality reads and trimming of adapter sequences.

Plant growth

Wild type cv. Sugraone plants were regenerated from the same non-transformed callus that was also used for the transformation experiments that generated the edited plants. All plants were propagated and grown *in vitro* in growth chamber under controlled conditions (16h/8h light/dark photoperiod, 23°C, and 60% relative humidity (RH)), and then acclimatized, in the same conditions, in rooting soil with low percentage of pumice. Plants were then transferred to 2 liters pots, grown from 3 to 9 months in a greenhouse and then transferred to controlled growth chamber prior artificial inoculation assay.

P. viticola inoculation assay

P. viticola [(Berk. et Curt.) Berl. et de Toni] was collected on a local vineyard and propagated on grapevine plants. In order to obtain sporangia for the inoculation assay, symptomatic plants were placed overnight in the dark at 100% RH. Leaves showing fresh sporulation were collected, soaked in cold (4°C) distilled water to release zoospores and stirred for 1 hour. The solution was finally filtered to remove leaf residues.

For the *P. viticola* inoculation assay one wild type line together with four lines of edited plants produced by propagation of selected edited lines were used. To ensure that a visible phenotype could be considered the consequence of *VvDMR6.1 knock-out,* rather than an unwanted effect of the transformation, we tested edited lines generated by independent transformation events. Plants used for the inoculation were of similar size, bearing about 10-20 leaves. For each line, half of the plants was sprayed with the sporangia suspension on the abaxial side of leaves, and the other half with distilled water (control). The inoculation was performed outside the growth chamber, then each plant was covered with a plastic bag, transferred inside the growth chamber, and kept in the dark for the first 24h to ensure high humidity and to allow the penetration of the pathogen. Plants were then uncovered and maintained under controlled conditions (a 16h/8h light/dark photoperiod, 23°C, 60% RH) for 6 days until symptom assessment. To induce sporulation for phenotypic analysis each plant was covered again with a plastic bag at 7 dpi (days post infection). *V. riparia* were used as negative controls.

Symptom assessment

Symptoms were evaluated at 8 dpi through visual estimation of two parameters defined by EPPO (OEPP/EPPO, 2001): i) the percentage area of sporulation (Disease severity, DS) on the lower leaf surface of all infected leaves, and ii) the number of leaves showing sporulation on the total number of inoculated leaves (Disease incidence; DI). Plants were also classified using the OIV 452(-1) descriptor, recommended by the Office International de la Vigne et du Vin (OIV, 2009), where classes are numbered from 1 to 9 from the most susceptible vine to the totally resistant one, characterized by absence of sporulation. DS was also assessed by digital analysis of pictures through ImageJ (Rasband, 1997; Collins, 2007) as in Pessina et al., (2016).

Staining and microscopy

Leaf samples at 24 and 96 hpi (hours post-infection) and 8 dpi were used for fluorescence microscopic examination: the *P. viticola* infection structures were visualized by staining with aniline blue according to Díez-Navajas et al. (2007). For the purpose, a Zeiss Axio Imager Z2 Fluorescence Microscope was used with a DAPI filter (excitation wavelength 352-402 nm, emission wavelength 417-477 nm) and 10x and 40x objectives.

Gene expression analysis

Plant leaves were sampled at 0, 24 and 96 hpi. Total RNA was isolated from samples using the Spectrum Plant Total RNA Kit (Merck KGaA, Darmstadt, Germany) according to the manufacturer's

instructions. Total RNA was treated with DNAse I (Thermo Fisher Scientific) to remove contaminating genomic DNA, and then quantified with an ND-8000 nanodrop spectrophotometer. RNA integrity was checked using the Agilent 4200 TapeStation system. cDNA was then synthesized using the SuperscriptVILO™ cDNA Synthesis Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. qRT-PCR analyses were carried out using the KAPA SYBR FAST qPCR Kit (Merck KGaA, Darmstadt, Germany) in a ViiA™ 7 thermocycler (Thermo Fisher Scientific). Plates in the 384-well format were set up according to the sample maximization strategy proposed in Hellemans et al. (2007) with three technical replicates for each reaction. Amplification conditions included an initial enzyme activation step at 95°C for 20s, followed by 40 cycles of 95°C for 1 sec plus 60°C for 20 sec. The LinReg software was used to calculate reaction efficiencies from non-baseline-corrected data (Ruijter et al., 2009). Two reference genes were chosen according to results given by the GeNorm software (Vandesompele et al., 2002): *VvACTIN* (VIT_04s0044g00580) and *VvATP16* (VIT_03s0038g00790). Normalized relative quantities (NRQs) of the investigated genes (**Table 1**) were then calculated by dividing the RQ (relative quantification) by a normalization factor, based on the expression of the two reference genes (Reid et al., 2006). Primer pairs specific for each selected gene were designed as reported in **Table 1**.

Metabolic analysis

Leaf samples harvested at 0, 24 and 96 hpi were freeze-dried, and then used for SA, 2,5-DHBA and 2,3-DHBA extraction and analysis according to Zeilmaker et al., (2015). 5-fluorosalicylic acid was used as internal standard, while SA, 2,3-DHBA and 2,5-DHBA were used to create calibration curves for quantitative analysis.

Statistical analysis and investigation tools

Different factors (time, genotypes with different editing, control and treated plants, different biological replicates) were taken into consideration when analyzing the symptom. Statistical analysis was performed with the R software (R Core Team, 2013). Robust PCA (principal component analysis) and a univariate general linear model analysis (Winkler et al., 2014) were applied in order to identify the most impacting factors and to assess the presence of significant expression patterns. The output of the univariate analysis was expressed in terms of confidence intervals with a given reference value (intercept). Central points represented expected values (the most probable estimated values with non-parametric distribution), bars represented confidence intervals.

A preliminary gene expression investigation through the GREAT database (https://great.colmar.inrae.fr) and the Grape eFP Browser (http://bar.utoronto.ca/efp_grape/cgi-bin/efpWeb.cgi; Fasoli et al., 2012) was also performed.

Gene name	Gene ID	Forward primer	Reverse primer	Reference
VvACT	VIT_04s0044g00580	5'-ATGTGCCTGCCATGTATGTTGCC-3'	3'-AGCTGCTCTTTGCAGTTTCCAGC-5'	Bézier et al., 2002
VvATP16	VIT_03s0038g00790	5'-CTTCTCCTGTATGGGAGCTG-3'	3'-CCATAACAACTGGTACAATCGAC-5'	Gamm et al., 2011
VvDMR6.1	VIT_16s0098g00860	5'-AGAGAGTGAAAGGCTATCAG-3'	3'-GATCCAAGTCCCTGCCCCAG-5'	This study
VvDMR6.2	VIT_13s0047g00210	5'-ATGTCGGTAAGGCAAGGATG-3'	3'-CCAGAACTTTTTGTAATACTC-5'	This study
VvDLO1	VIT_15s0048g02430	5'-GCTACAAGAGTGCGGTCCAT-3'	3'-CTGTAAAGGGCAGGATGACC-5'	This study
VvDLO2	VIT_02s0025g02970	5'-GCGAGGAATACTACACTCAG-3'	3'-TCAAGTGGTAGATGCTTTG-5'	This study
VvPR2	VIT_08s0007g06060	5'-GGCTATGTTTGATTCCACTGT-3'	3'-GGCAAGTTGTCACCCTCCATT-5'	Malacarne et al., 2011
VvPR10.1	VIT_05s0077g01530	5'-GCACATCCCGATGCCTATTAAG-3'	3'-ACTTACTGAGACTGATAGATGCAATGAATA-5'	Merz et al., 2015
VvMYB14	VIT_07s0005g03340	5'-TCTGAGGCCGGATATCAAAC-3'	3'-GGGACGCATCAAGAGAGTGT-5'	Höll et al., 2013
VvMYB15	VIT_05s0049g01020	5'-CAAGAATGAACAGATGGAGGAG-3'	3'-TCTGCGACTGCTGGGAAA-5'	Höll et al., 2013
VvSTS29	VIT_16s0100g01010	5'-GGTTTTGGACCAGGCTTGACT -3'	3'-GAGATAAATACCTTACTCCTATTCAAC-5'	Höll et al., 2013
VvSTS41	VIT_16s0100g01130	5'-GAGTACTATTTGGTTTTGGACCT -3'	3'-AACTCCTATTTGATACAAAACAACGT-5'	Vannozzi et al., 2012
VvWRKY33	VIT_08s0058g00690	5'-ATTCAAGCACTAGTATGAACAGAGCAG-3'	3'-CCTTGTTGCCTTGGCATGA-5'	Merz et al., 2015

Table 1. List of qPCR gene specific primer pairs used in this study.

RESULTS and DISCUSSION

Selection of edited lines and plant growth

Four edited lines (ELs) out of several ones available and bearing a *VvDMR6.1* gene knock-out, were considered for phenotyping experiments: gene expression analysis of 11 genes related to defense, downy mildew symptoms assessment by visual and microscopic analysis, SA and SA-derivatives metabolic analysis. The editing target sequence was designed in the first exon in order to obtain a truncated protein, and therefore loss of function (**Table 2**). The high coverage sequence analysis highlighted that EL1 and 2 carry a one-nucleotide (nt) insertion in 100% and 96% of the analyzed reads, respectively, with 4% of the reads showing a 4-nt deletion in EL2, EL3 is 100% edited with 2- or 3-nt deletion, and EL4 has 2-nt deletion or one-nt insertion as observed in 98,4% of the reads.

Edited Line	Editing	Truncated protein lenght
Edited Line 1	1-nt insertion	88 aa
Edited Line 2	1-nt insertion	87 aa
	4-nt deletion	46 aa
Edited Line 3	2-nt deletion	87 aa
	3-nt deletion	85 aa
Edited Line 4	2-nt deletion	87 aa
	1-nt insertion	88 aa

Table 2. Edited lines with detected mutations and the truncated protein length.

According to Charrier et al. (2019), mutants obtained through CRISPR/Cas9 can be classified as “homozygous” when the same mutation is present on both alleles of the mutated gene, “heterozygous” when the mutation occurs in only one, “biallelic” when both alleles are mutated but they carry different mutations, and “chimeric” when different cell lineages, originated from different cells during embryogenesis, give raise to the whole plant and, as a result, more alleles are detected. In this study EL1 was homozygous, EL2 could probably be defined biallelic, and EL3 and EL4 presented a chimeric pattern according to Charrier.

Edited plants grown *in vitro* showed a variety of phenotypes, depending on the line and even on the single plant. In particular, ELs 2 and 3 showed a bushy architecture with short internodes and yellow leaf tips. Van Damme et al. (2005) observed dwarf phenotype in *dmr3, dmr4* and *dmr5* but not in *dmr6* Arabidopsis. Based on these evidences and on de Toledo Thomazella et al., (2016), no pleiotropic effects were detected in our *dmr6* plants, since the *in vitro* phenotype was completely restored after acclimatization *to vivo*, as leaves generated *in vitro* were lost and new leaves were grown. For the experiments performed in this study, approximately between 25 and 40 plants of each line were grown.

Symptom assessment following *Plasmopara* infection

Leaves inoculated with *P. viticola* were sampled at 24, 96 hpi and 8 dpi and stained according to (Díez-Navajas et al., 2007) in order to analyze disease progression throughout the plant tissues (**Fig. 1A**). At 24 hpi, *P. viticola* infection structures were detected in WT, EL1, EL2, and EL3 plants but not in the EL4 plants. The mycelium development observed at 24 hpi in our experiment compared to that obtained by Polesani et al. (2010) on leaf disks of a different *V. vinifera* cultivar, appeared more similar to that observed at 12 hpi than at 24 hpi in this former study. Moreover, the number of germinating spores relative to the number of stomata seemed to suggest a lower degree of infection both in WT and ELs with compared to previous observations (Polesani et al., 2010; Milli et al., 2012). Clearly, this can be ascribed to several parameters which in the actual experiment differ from the previous ones: the different cultivar, the different *P. viticola* inoculum and the infection on whole leaf rather than leaf disks, just to mention some relevant ones.

At 96 hpi, highly diffused colonization of leaf tissues was detected in the WT, whereas in the ELs the mycelium growth was still limited to small patches around the penetration site, similarly to what was observed by Polesani et al. (2010) at the same time point in the resistant *V. riparia* cv. Gloire de Montpellier. In this PhD work, *V. riparia* was also inoculated and considered as a negative control, given its resistance to DM due to resistance QTL in linkage groups 9 and 12 (Marguerit et al., 2009), but no infection structures or mycelium were detected in its leaves at any time point neither upon visual inspection nor upon histological analysis.

At 8 dpi, the observed pattern was very similar between WT and EL 1 and EL 3 with a large and diffused spreading of the mycelium. In EL2 and EL4 leaf tissue instead, the mycelium growth was less dense than that observed on WT and other lines. The occurrence of fan-shaped hyphae observed in this study was reported also in susceptible Riesling and Müller-Thurgau cvs. at 6 dpi, in a recent study by Fröbel & Zyprian (2019), and considered as a characteristic of a well spread mycelium. In summary, the epifluorescence microscopic analysis of the four edited lines suggested a partial inhibition of mycelial growth between 0 and 96 hpi in *dmr6.1* edited plants, which was not sufficient to stop sporulation at later times points post inoculation.

In order to obtain disease severity (DS) and disease incidence (DI) OEPP/EPPO (OEPP/EPPO, 2001), a DM symptoms assessment was carried out on six plants per line through visual and digital estimation of the sporulation area on each leaf (**Fig. 1B**). Through visual estimation we observed similar DS and DI scores both in the WT and the edited plants. DS values ranged between a minimum of 10.3% in EL3 and a maximum of 14.5% in EL4, while DI showed a mean value of 76.8%, with a minimum of 73.2% and a maximum of 80.4%, indicating that the majority of the leaves showed signs

of sporulation. WT plants scored 11.8% for DS and 76.8% for DI. DS scores were also obtained through digital analysis of images of the leaves surface, by ImageJ (**Fig. 1C**). As expected, DS scores captured by image analysis resulted slightly higher than those obtained by visual inspection, given the higher sensitivity of the image analysis method. In ELs, DS scores ranged between 20% (in EL4) and 29% (in EL3) and was lowest in the WT (18.5%). Interestingly, slightly divergent results were obtained from the two analyses, especially in the case of the EL3 DS scores. Nonetheless, both estimation methods confirmed that there were no significant differences between the ELs and the WT plants in terms of disease severity and disease incidence. DS and DI parameters were also converted into OIV 452(-1) scores according to Vezzulli et al., (2018): all edited lines fell between scores six and four, whereas the WT fell between scores five and six.

In conclusion, no relevant phenotypic effect on DM resistance was observed in the *dmr6.1* edited lines as compared to the WT, in contrast to what previously observed in Arabidopsis (Van Damme et al., 2008) and tomato (de Toledo Thomazella et al., 2016), although a slightly slower mycelium growth was observed within the first 96 hours after inoculation.

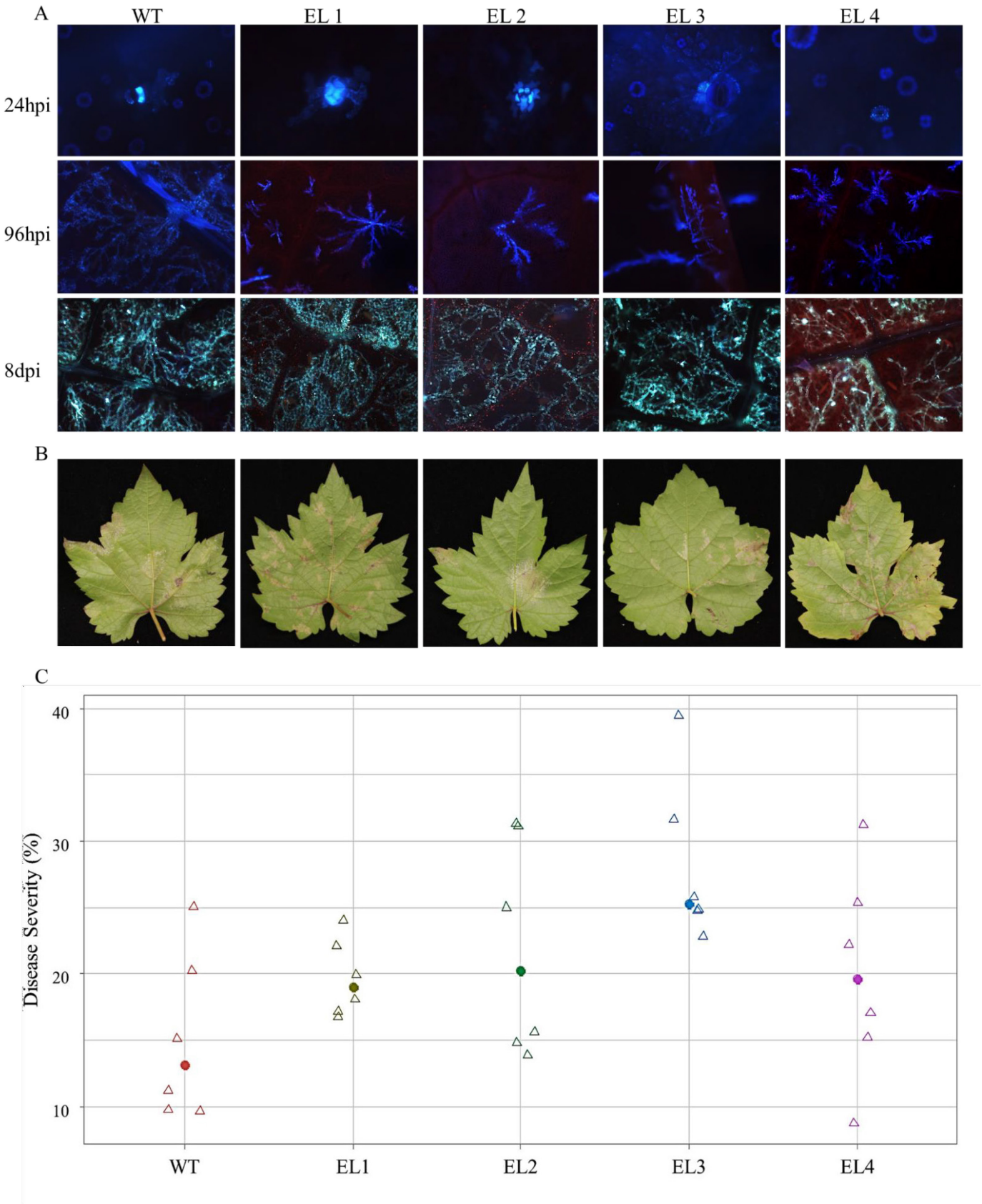


Figure 1. A) Histological analysis of *P. viticola* development in infected grapevine leaves at 24, 96 hpi and 8 dpi. Images at 24 hpi were observed with 400x magnification, while at 96 hpi and 8 dpi with 100x magnification. B) Images of representative infected leaves at 8 dpi. C) Disease severity data obtained by analysis with ImageJ, triangles are single plant scores, dots are medians.

Gene expression analysis of *VvDMR6*, *VvDLOs* and pathogenesis-related genes in the *dmr6.1* background

Available grapevine transcriptomic data were investigated by *in silico* analysis, to characterize the gene expression of *VvDMR6* and *VvDLO* in various grapevine tissues at different developmental stages and upon biotic and abiotic stresses. *VvDLO2* showed the highest expression level in mature and senescent leaves, and in tendrils, whereas *VvDLO1* was mostly expressed in the flower. *VvDMR6.1,* on the other hand, was particularly expressed in roots, while *VvDMR6.2* was especially expressed in mature stems, leaves and tendrils (**Fig. 2A**). Although *VvDMR6.1* seems to be specifically expressed in roots, other experiments indicated that the gene is highly induced in other organs such as leaves and shoots, in response to biotic stresses, at least in susceptible varieties, corroborating its role as a susceptibility gene (**Fig. 2B**). *VvDMR6.1*-*VvDLO1* co-induction takes place in response to the ascomycete *Lasiodiplodia theobromae* at 24hpi. *P. viticola* inoculation strongly enhances *VvDMR6.1* and *VvDMR6.2* transcription at 24 hpi in young plants, but an upregulation is seen at almost all the time points available in the *in silico* analysis. *VvDLO1* shows high expression differences between young and adult plants, since the highest induction is observed at 24 hpi in 2-year-old plants. *VvDLO2* expression is high at intermediate time points (48 and 72 hpi) and in resistant plants. Interestingly, its transcription profile seems to be opposite to that of *VvDMR6.1*: when *VvDMR6.1* is highly expressed, *VvDLO2* is expressed at low level and vice versa. (**Fig. 2C**).

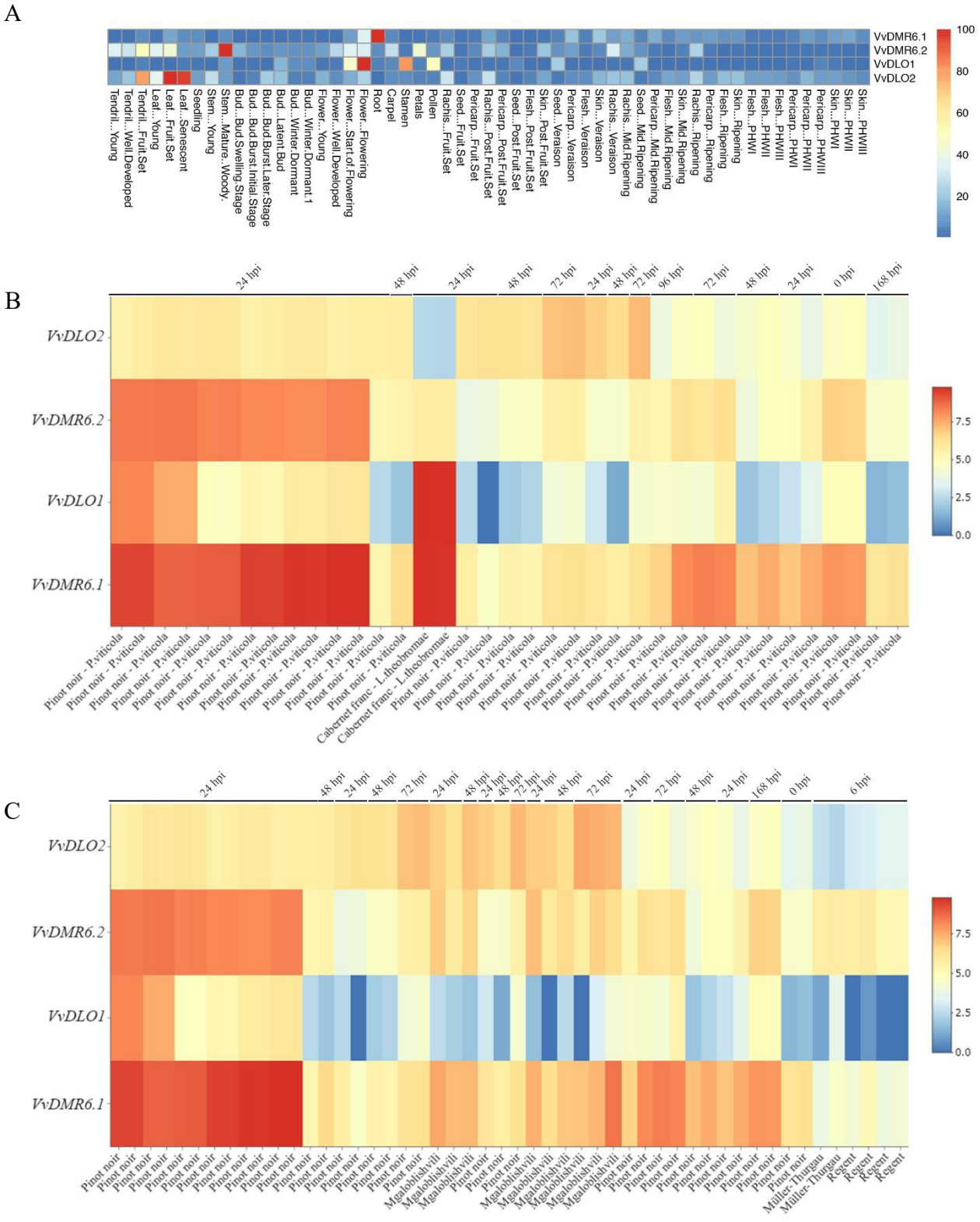


Figure 2. A) Heatmap of *VvDMR6.1, VvDMR6.2, VvDLO1* and *VvDLO2* expression in tissues at different developmental stages. Absolute RPKM values were downloaded from Grape eFP Browser (Fasoli et al., 2012) and normalized across conditions. B) Heatmap of *VvDMR6* and *VvDLO* expression in response to biotic stress (GREAT; https://great.colmar.inrae.fr). C) Heatmap of *VvDMR6* and *VvDLO* expression in response to *P. viticola* (GREAT; https://great.colmar.inrae.fr). Log RPKM values are showed.

In this study, the expression of *VvDMR6*, *VvDLOs* and of selected pathogenesis related genes was measured in the *dmr6.1* background of two edited lines. EL1, EL2 and the WT were used for gene expression analysis by RT-qPCR. In general, a very high variability in terms of gene expression was observed among replicates, which complicated the data analysis.

Despite the high variability observed in the qPCR results, a multivariate statistical determined that time (timepoint) was the most influencing factor (**Fig. 3A**). Moreover, a measure of the variability in terms of confidence intervals was given through a univariate analysis. Considering the WT control line in the mock conditions, no significant *VvDMR6* and *VvDLO* gene regulation effects were observed in response to basic experimental conditions as mock spraying, plastic covering, and high RH, at least at later time points (**Fig. 3B**). In order to further investigate significant differences in gene expression, the timepoint factor was not considered in the following univariate analyses (**Fig. 3C**). Interestingly, a significant downregulation of *VvDMR6.1* expression was observed in inoculated EL1 (**Fig. 3C**), which is in line with what happens in the *dmr6.1* background. This may either indicate that the edited *VvDMR6.1* transcript is less stable than the wild type form, or that the presence of the mutated transcript activates a negative feedback control on its transcription. This result was not confirmed however in EL2. Moreover, no increase in *VvDLO1* transcript was detected in the *dmr6.1* background of the EL1, as was instead previously reported in the *A. thaliana dmr6* mutant where a compensative induction of *AtDLO1* was demonstrated by Zeilmaker et al. (2015).

The pathogenesis-related genes *PR2* and *PR 10.1* together with the transcription factor *WRKY33* were also investigated. *VvWRKY33* expression in control WT plants showed a significant enhancement at 24hpi compared to 0 hpi (**Fig. 3B**). Enhanced transcription of *VvWRKY33* at 24 hpi was found in previous studies in infected plants but, in our case, it was observed under mock conditions. However, a very early upregulation of *VvWRKY33*, within a few hours, was detected in the resistant cv Regent by Merz et al. (2015). This might be the reason why in this study we could not see enhanced expression in the inoculated plants at 24 hpi, while its putative target *VvPR10.1* showed induction from 48 hpi.

No upregulation of *VvPR2* was detected in our samples, although this gene represents a marker of defense response to several pathogens including *Plasmopara viticola*. In grapevine, *VvPR2* enhanced transcription was shown in resistant Merzling x Teroldego offspring compared to the susceptible genotypes upon *P. viticola* inoculation (Malacarne et al., 2011). In addition, it was reported that the loss of a functional *DMR6* gene in Arabidopsis is responsible for enhanced expression levels of several defense-associated genes, including *PR* genes such as *PR1*, *PR2* and *PR5* (Van Damme et al., 2008).

In summary, we should consider the gene expression data obtained within the PhD work as preliminary indications to be confirmed with more experiments. The low disease severity observed in the symptom assessment is likely the consequence of a rather low infection rate of the foliar tissue. The low infection reflected in a low defense response also at the molecular level as suggested by the not significant modulation of known defense responsive genes such as the *PR* genes and the *STS* genes.

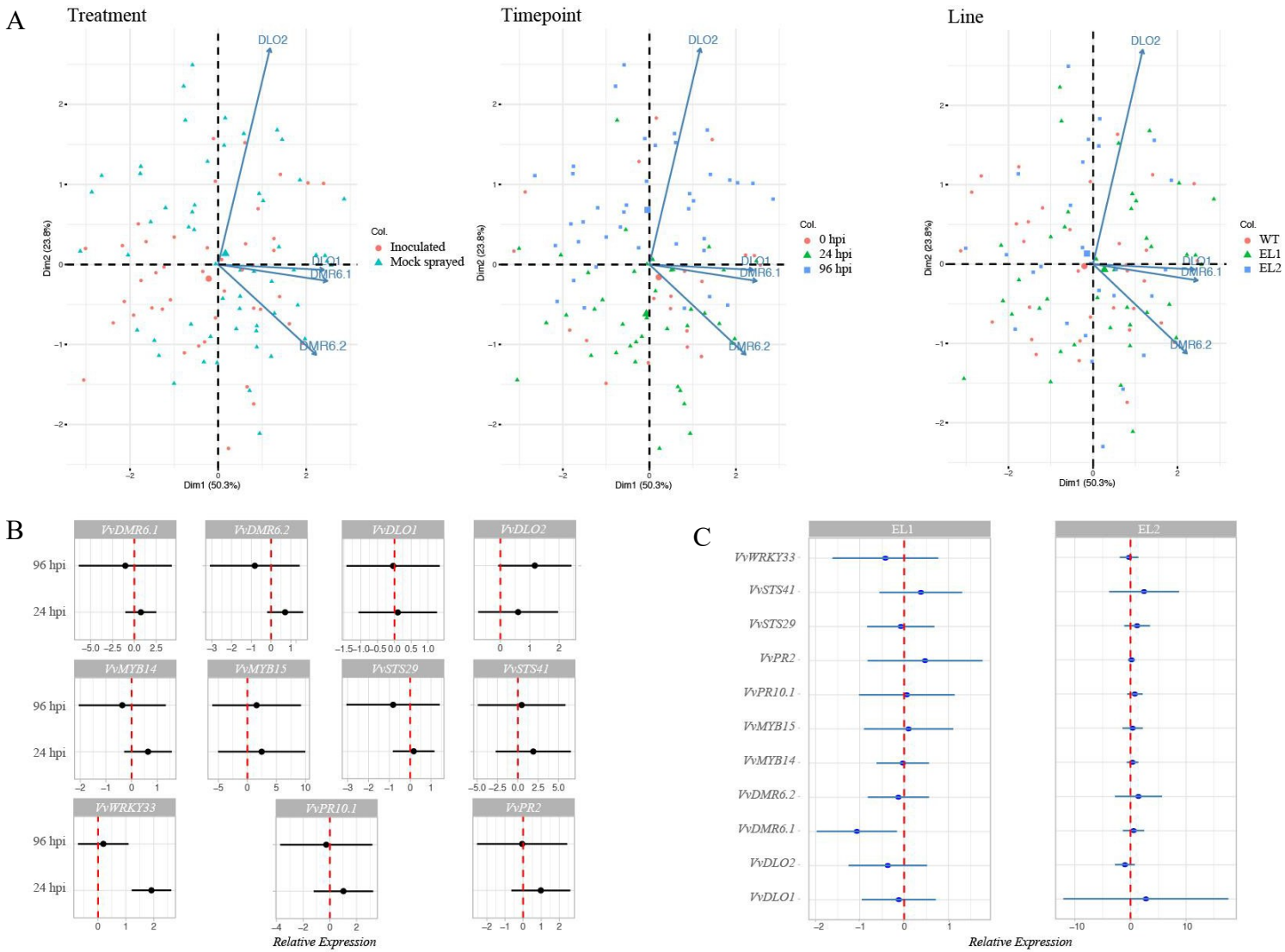


Figure 3. A) PCA of gene expression data shows timepoint as the most influencing factor among the factors considered (treatment, timepoint and line). B) Representation of differences in terms of confidence intervals for gene expression at 24 hpi and 96 hpi in WT control plants. The intercept (red line) represents the gene expression in WT line at 0 hpi (*p-value=0.05*). C) Representation of differences in terms of confidence intervals for gene expression obtained for each gene in inoculated samples for each line, not considering the time factor. The intercept (red line) represents the gene expression in control plants (*p-value=0.05*).

Salicylic acid, 2,5-DHBA and 2,3-DHBA quantification

Total SA, 2,5-DHBA and 2,3-DHBA were quantified in WT and *dmr6.1* ELs plants at 0, 24 and 96 hpi. SA and DHBAs levels were detected in a similar range as in other analyzed crops (unpublished data), namely in the order of nanograms per mg of dry weight, with SA being an order of magnitude more abundant than 2,5-DHBA and 2,3-DHBA. No evident differences in the metabolite content was observed between WT and ELs plants according to treatment, timepoint or genotype as ascertained from a multivariate statistical analysis (data not shown). In general, the SA content was higher than the amount of DHBAs, and 2,3-DHBA was less represented than 2,5-DHBA in each line. Overall, the data showed a high variability between replicates. A statistical attempt to reduce data variability was made by focusing on metabolite correlations (**Fig. 4A**). SA/DHBAs patterns showed rather similar trends comparing different lines. A wide distribution of data was visible in WT, EL2 and EL4, suggesting no particular correlation between metabolite amounts. Interestingly, SA/DHBAs showed some correlation in EL1 and EL3 at 24 hpi and to a lesser extent at 96 hpi. A more defined correlation between data was observed in the 2,5-DHBA/2,3-DHBA ratios in EL1, EL2, EL3 and EL4, but was less defined in the WT plants. These differences in DHBAs correlation in the ELs as compared to WT suggest an effect of the *VvDMR6.1* knock-out in the metabolites accumulation, even though this effect does not reflect heavily on the plant phenotype. In general, all three analyzed metabolites were equal or slightly less abundant in treated plants as compared to control ones (**Fig. 4B, 4C)**. Indeed, no significant differences in metabolite concentration were observed in treated plants compared to control ones at any time point, with the exception of a lower amount of SA in EL4 at 96 hpi and an important, but not significant, decrease of 2,5-DHBA in EL2 and WT inoculated plants compared to the mock ones at 96 hpi and 24 hpi, respectively (**Fig. 4B, 4C**).

A significant increase of the SA/2,5-DHBA ratio was observed in WT inoculated plants at 24 hpi, due to a significant reduction of 2,5-DHBA levels in WT plants but not in the ELs. This is not in line with Zhang et al. (2017), where a reduction in 2,5-DHBA concentration was found in *A. thaliana s5h* mutants compared to WT plants. The same study (Zhang et al., 2017) reported no differences in SA amount between WT and the *s5h (DMR6)* single mutant, but it reported a high increase in SA level in the *s3h (DLO)* and *s5h_s3h* lines. A significant effect on the SA amount by knocking out *DMR6* and *DLO* genes was previously indicated also in Arabidopsis during infection experiments at 4 dpi with *Hyaloperonospora parasitica*. At 4 dpi, SA levels were higher in *dmr6* plants than in *dlo* plants, but even higher in the *dmr6_dlo* double mutants (Zeilmaker et al., 2015). These evidences could explain our results with no significant modulation of SA, and the necessity to knock out both *VvDMR6* and *VvDLO* genes in grapevine in order to obtain effects on SA accumulation.

Another factor to take into consideration in interpreting our results is plant age: the plant used in the study were about one-year old. Indeed, in the model plant Arabidopsis, K. Zhang et al. (2013), highlighted higher amounts of free and glycosylated SA in *s3h* young plants, and higher levels of DHBAs in senescent leaves. For this reason, it would be interesting to perform the same metabolic evaluations on *dmr6.1* plants of different ages.

In grapevine, SA increase in response to *P. viticola* infection and to the defence response elicitor LAM (β-glucan laminarin) was reported between 0 to 48 hours post treatment, with larger differences in SA accumulation at 12 and 36 hpi (Gauthier et al., 2014; Guerreiro et al., 2016). In our case, no particular increase in SA accumulation was observed in infected plants compared to mock inoculated, even in WT line**.**

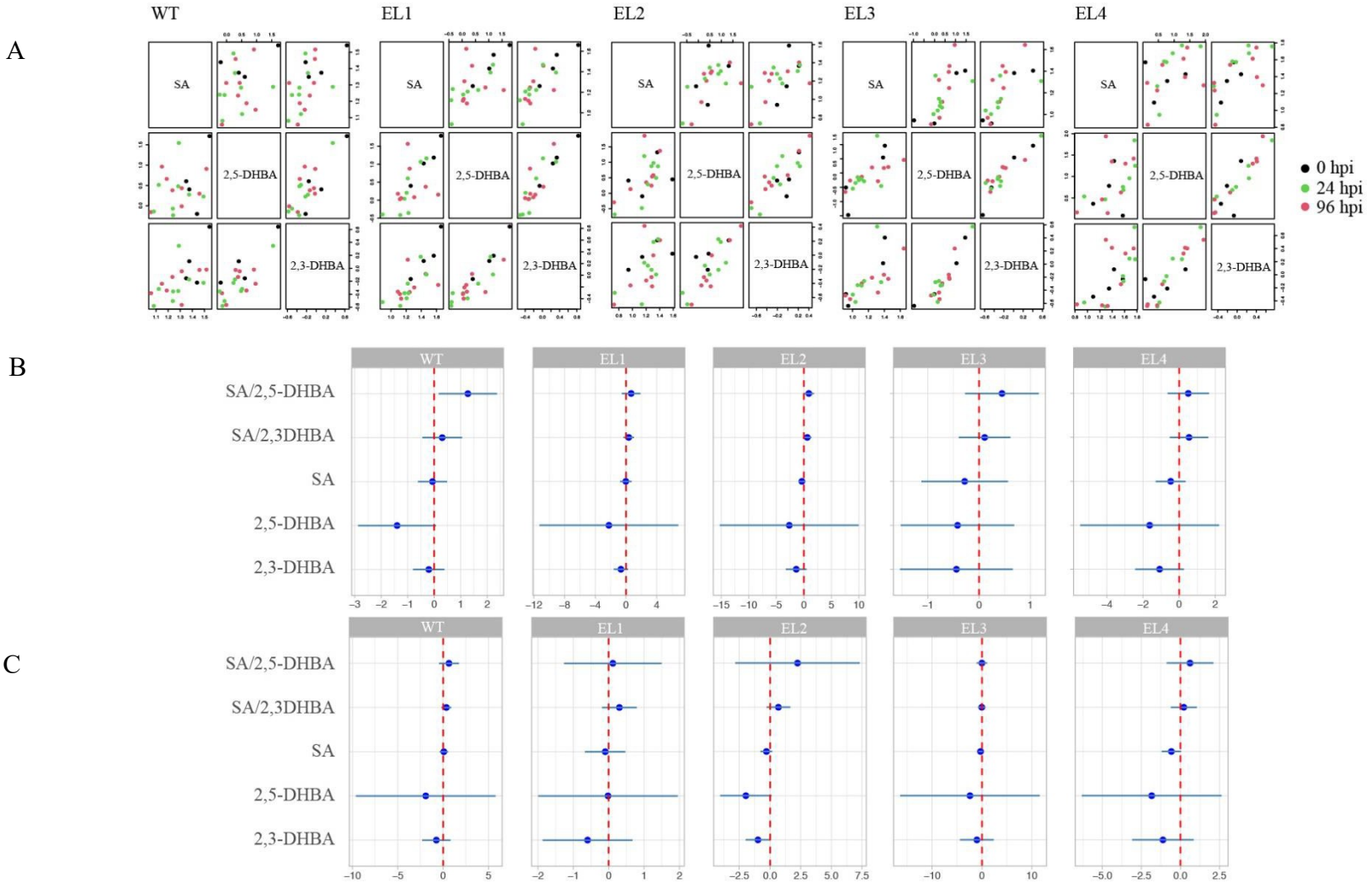


Figure 4. A) Representation of the correlation between metabolites (SA, 2,5-DHBA, 2,3-DHBA) for each line. B) Representation of differences in terms of confidence intervals of relative quantity (log2 values) in inoculated samples at 24 hpi. The intercept (red line) represents relative quantities in mock sprayed samples at 24 hpi (*p-value=0.05*). C) Representation of differences in terms of confidence intervals of relative quantity (log2 values) in inoculated samples at 96hpi. The intercept (red line) represents relative quantities in mock sprayed samples at 96 hpi (*p-value=0.05*).

CONCLUSIONS

In grapevine, two *VvDMR6* isoforms and three *VvDLO* genes were identified as the *AtDMR6* and *AtDLO* orthologues. In the wake of previous functional studies in Arabidopsis (Zeilmaker et al., 2015) and tomato (de Toledo Thomazella et al., 2016), an attempt to characterize the role *VvDMR6.1* in the resistance process against DM was carried out. Multiple *dmr6_dlo* Arabidopsis mutants already showed enhanced resistance to DM, increased levels of SA and lower accumulation of its inactive forms 2,3-DHBA and 2,5-DHBA, and upregulation of non-edited *AtDMR6* and *AtDLO* genes in response to pathogen inoculation (Van Damme et al., 2008; K. Zhang et al., 2013; Zeilmaker et al., 2015; Y. J. Zhang et al., 2017).

No previous functional investigation of these genes was done in tree species.

Transcriptomic, metabolic and phenotypic data collected drew a complex framework which does not exclude the involvement of other genes besides *VvDMR6.1* to modulate recessively inherited resistance to *P. viticola* in grapevine. The results obtained in this study suggest the need of further investigation, in particular by expanding the functional analysis to both *VvDMR6* genes, by studying mutants in which *VvDMR6.1* and *VvDMR6.2* are knocked-out, and by gaining additional knowledge on the *VvDLOs* compensative roles. However, according to literature, this could bring to enhanced resistance but also to undesired pleiotropic effects such as early senescence, as shown in Zeilmaker et al. (2015) and de Toledo Thomazella et al.(2016).

REFERENCES

Adrian, M., & Jeandet, P. (2012). Effects of resveratrol on the ultrastructure of Botrytis cinerea conidia and biological significance in plant/pathogen interactions. *Fitoterapia*, *83*(8), 1345–1350. https://doi.org/10.1016/j.fitote.2012.04.004

Alonso-Villaverde, V., Voinesco, F., Viret, O., Spring, J.-L., & Gindro, K. (2011). The effectiveness of stilbenes in resistant Vitaceae: ultrastructural and biochemical events during Plasmopara viticola infection process. *Plant Physiology and Biochemistry : PPB*, *49*(3), 265–274. https://doi.org/10.1016/j.plaphy.2010.12.010

Bézier, A., Lambert, B., & Baillieul, F. (2002). Study of defense-related gene expression in grapevine leaves and berries infected with Botrytis cinerea. *European Journal of Plant Pathology*, *108*(2), 111–120. https://doi.org/10.1023/A:1015061108045

Charrier, A., Vergne, E., Dousset, N., Richer, A., Petiteau, A., & Chevreau, E. (2019). Efficient targeted mutagenesis in apple and first time edition of pear using the CRISPR-Cas9 system. *Frontiers in Plant Science*, *10*(February), 1–12. https://doi.org/10.3389/fpls.2019.00040

Chitarrini, G., Soini, E., Riccadonna, S., Franceschi, P., Zulini, L., Masuero, D., … Vrhovsek, U. (2017). Identification of biomarkers for defense response to Plasmopara viticola in a resistant grape variety. *Frontiers in Plant Science*, *8*(September), 1–11. https://doi.org/10.3389/fpls.2017.01524

Collins, T. J. (2007). ImageJ for microscopy. *BioTechniques*, *43*(1S), S25–S30. https://doi.org/10.2144/000112517

de Toledo Thomazella, D. P., Brail, Q., Dahlbeck, D., & Staskawicz, B. J. (2016). CRISPR-Cas9 mediated mutagenesis of a DMR6 ortholog in tomato confers broad-spectrum disease resistance. *BioRxiv*, (July), 064824. https://doi.org/10.1101/064824

Díez-Navajas, A. M., Greif, C., Poutaraud, A., & Merdinoglu, D. (2007). Two simplified fluorescent staining techniques to observe infection structures of the oomycete Plasmopara viticola in grapevine leaf tissues. *Micron*, *38*(6), 680–683. https://doi.org/10.1016/j.micron.2006.09.009

Ding, P., & Ding, Y. (2020). Stories of Salicylic Acid: A Plant Defense Hormone. *Trends in Plant Science*, *25*(6), 549–565. https://doi.org/10.1016/j.tplants.2020.01.004

Falcone Ferreyra, M. L., Emiliani, J., Rodriguez, E. J., Campos-Bermudez, V. A., Grotewold, E., & Casati, P. (2015). The Identification of Maize and Arabidopsis Type I FLAVONE SYNTHASEs Links Flavones with Hormones and Biotic Interactions. *Plant Physiology*, *169*(2), 1090–1107. https://doi.org/10.1104/pp.15.00515

Fang, L., Hou, Y., Wang, L., Xin, H., Wang, N., & Li, S. (2014). Myb14, a direct activator of

STS, is associated with resveratrol content variation in berry skin in two grape cultivars. *Plant Cell Reports*, *33*(10), 1629–1640. https://doi.org/10.1007/s00299-014-1642-3

Fasoli, M., Dal Santo, S., Zenoni, S., Tornielli, G. B., Farina, L., Zamboni, A., … Pezzotti, M. (2012). The grapevine expression atlas reveals a deep transcriptome shift driving the entire plant into a maturation program. *Plant Cell*, *24*(9), 3489–3505. https://doi.org/10.1105/tpc.112.100230

Fröbel, S., & Zyprian, E. (2019). Colonization of Different Grapevine Tissues by Plasmopara viticola—A Histological Study. *Frontiers in Plant Science*, *10*(July), 1–13. https://doi.org/10.3389/fpls.2019.00951

Gabaston, J., Cantos-Villar, E., Biais, B., Waffo-Teguo, P., Renouf, E., Corio-Costet, M. F., ... & Mérillon, J. M. (2017). Stilbenes from *Vitis* vinifera L. waste: a sustainable tool for controlling Plasmopara viticola. *Ournal of Agricultural and Food Chemistry*, *65*(13), 2711–2718.

Gamm, M., Héloir, M. C., Kelloniemi, J., Poinssot, B., Wendehenne, D., & Adrian, M. (2011). Identification of reference genes suitable for qRT-PCR in grapevine and application for the study of the expression of genes involved in pterostilbene synthesis. *Molecular Genetics and Genomics*, *285*(4), 273–285. https://doi.org/10.1007/s00438-011-0607-2

Gauthier, A., Trouvelot, S., Kelloniemi, J., Frettinger, P., Wendehenne, D., Daire, X., … Poinssot, B. (2014). The sulfated laminarin triggers a stress transcriptome before priming the SA- And ROS-dependent defenses during Grapevine's induced resistance against Plasmopara viticola. *PLoS ONE*, *9*(2). https://doi.org/10.1371/journal.pone.0088145

Giacomelli, L., Zeilmaker, T., Malnoy, M., Rouppe van der Voort, J., & Moser, C. (2018). Generation of mildew-resistant grapevine clones via genome editing. *ISHS Acta Horticulturae 1248: XII International Conference on Grapevine Breeding and Genetics*. https://doi.org/10.17660/ActaHortic.2019.1248.28

Guerreiro, A., Figueiredo, J., Sousa Silva, M., & Figueiredo, A. (2016). Linking jasmonic acid to grapevine resistance against the biotrophic oomycete Plasmopara viticola. *Frontiers in Plant Science*, *7*(APR2016), 1–7. https://doi.org/10.3389/fpls.2016.00565

Haile, Z. M., Pilati, S., Sonego, P., Malacarne, G., Vrhovsek, U., Engelen, K., … Moser, C. (2017). Molecular analysis of the early interaction between the grapevine flower and Botrytis cinerea reveals that prompt activation of specific host pathways leads to fungus quiescence. *Plant Cell and Environment*, *40*(8), 1409–1428. https://doi.org/10.1111/pce.12937

Hellemans, J., Mortier, G., De Paepe, A., Speleman, F., & Vandesompele, J. (2007). qBase relative quantification framework and software for management and automated analysis of

real-time quantitative PCR data. *Genome Biology*, *8*(2). https://doi.org/10.1186/gb-2007-8-2-r19

Höll, J., Vannozzi, A., Czemmel, S., Donofrio, C., Walker, A. R., Rausch, T., … Bogsa, J. (2013). The R2R3-MYB transcription factors MYB14 and MYB15 regulate stilbene biosynthesis in *Vitis* vinifera. *Plant Cell*, *25*(10), 4135–4149. https://doi.org/10.1105/tpc.113.117127

Jiang, W., Zhou, H., Bi, H., Fromm, M., Yang, B., & Weeks, D. P. (2013). Demonstration of CRISPR/Cas9/sgRNA-mediated targeted gene modification in Arabidopsis, tobacco, sorghum and rice. *Nucleic Acids Research*, *41*(20), 1–12. https://doi.org/10.1093/nar/gkt780

Klessig, D. F., Choi, H. W., & Dempsey, D. A. (2018). Systemic Acquired Resistance and Salicylic Acid: Past, Present, and Future. *Molecular Plant-Microbe Interactions*, *31*(9), MPMI-03-18-0067. https://doi.org/10.1094/MPMI-03-18-0067-CR

Langcake, P., & Pryce, J. (1976). The production of resveratrol by *Vitis* vinifera and other members of the Vitaceae as a response to infection or injury. *Physiological Plant Pathology*, *9*(1), 77–86. https://doi.org/https://doi.org/10.1016/0048-4059(76)90077-1

Le Henanff, G., Heitz, T., Mestre, P., Mutterer, J., Walter, B., & Chong, J. (2009). Characterization of *vitis* vinifera NPR1 homologs involved in the regulation of pathogenesis-related gene expression. *BMC Plant Biology*, *9*, 1–14. https://doi.org/10.1186/1471-2229-9-54

Li, M. Y., Jiao, Y. T., Wang, Y. T., Zhang, N., Wang, B. B., Liu, R. Q., … Liu, G. T. (2020). CRISPR/Cas9-mediated VvPR4b editing decreases downy mildew resistance in grapevine (*Vitis* vinifera L.). *Horticulture Research*, *7*(1). https://doi.org/10.1038/s41438-020-00371-4

Malacarne, Giulia, Vrhovsek, U., Zulini, L., Cestaro, A., Stefanini, M., Mattivi, F., … Moser, C. (2011). Resistance to Plasmopara viticola in a grapevine segregating population is associated with stilbenoid accumulation and with specific host transcriptional responses. *BMC Plant Biology*, *11*, 1–13. https://doi.org/10.1186/1471-2229-11-114

Malnoy, M., Viola, R., Jung, M., Koo, O., Kim, S., Kim, J., … Kanchiswamy, C. N. (2016). *DNA-Free Genetically Edited Grapevine and Apple Protoplast Using CRISPR / Cas9 Ribonucleoproteins*. *7*(December), 1–9. https://doi.org/10.3389/fpls.2016.01904

Marguerit, E., Boury, C., Manicki, A., Donnart, M., Butterlin, G., Némorin, A., … Decroocq, S. (2009). Genetic dissection of sex determinism, inflorescence morphology and downy mildew resistance in grapevine. *Theoretical and Applied Genetics*, *118*(7), 1261–1278. https://doi.org/10.1007/s00122-009-0979-4

Merz, P. R., Moser, T., Höll, J., Kortekamp, A., Buchholz, G., Zyprian, E., & Bogs, J. (2015). The transcription factor VvWRKY33 is involved in the regulation of grapevine (*Vitis* vinifera)

defense against the oomycete pathogen Plasmopara viticola. *Physiologia Plantarum*, *153*(3), 365–380. https://doi.org/10.1111/ppl.12251

Milli, A., Cecconi, D., Bortesi, L., Persi, A., Rinalducci, S., Zamboni, A., … Polverari, A. (2012). Proteomic analysis of the compatible interaction between *Vitis* vinifera and Plasmopara viticola. *Journal of Proteomics*, *75*(4), 1284–1302. https://doi.org/10.1016/j.jprot.2011.11.006

Moniruzzaman, M., Zhong, Y., Yan, H., Yuanda, L., Jiang, B., & Zhong, G. (2020). Exploration of Susceptible Genes with Clustered Regularly Interspaced Short Palindromic Repeats–Tissue-Specific Knockout (CRISPR-TSKO) to Enhance Host Resistance. *Critical Reviews in Plant Sciences*, *39*(5), 387–417. https://doi.org/10.1080/07352689.2020.1810970

Nakajima, I., Ban, Y., Azuma, A., Onoue, N., Moriguchi, T., Yamamoto, T., … Endo, M. (2017). *CRISPR / Cas9-mediated targeted mutagenesis in grape*. 1–16.

OEPP/EPPO. (2001). Guidelines for the efficacy evaluation of fungicides. *Bulletin*, *31*, 313–317.

OIV (International Organisation of Vine and Wine). (2009). *OIV descriptor list for grape varieties and Vitis species*. Paris.

Pessina, S., Lenzi, L., Perazzolli, M., Campa, M., Dalla Costa, L., Urso, S., … Malnoy, M. (2016). Knockdown of MLO genes reduces susceptibility to powdery mildew in grapevine. *Horticulture Research*, *3*(November 2015). https://doi.org/10.1038/hortres.2016.16

Pezet, R., Gindro, K., Viret, O., & Richter, H. (2004). Effects of resveratrol, viniferins and pterostilbene on Plasmopara viticola zoospore mobility and disease development. *Vitis - Journal of Grapevine Research*, *43*(3), 145–148.

Pieterse, C. M. J., Leon-Reyes, A., Van Der Ent, S., & Van Wees, S. C. M. (2009). Networking by small-molecule hormones in plant immunity. *Nature Chemical Biology*, *5*(5), 308–316. https://doi.org/10.1038/nchembio.164

Pimentel, D. (2005). Environmental and economic costs of the application of pesticides primarily in the United States. *Environment, Development and Sustainability*, *7*(2), 229–252. https://doi.org/10.1007/s10668-005-7314-2

Pinello, L., Canver, M. C., Hoban, M. D., Orkin, S. H., Kohn, D. B., Bauer, D. E., & Yuan, G.-C. (2016). Analyzing CRISPR genome-editing experiments with CRISPResso. *Nature Biotechnology*, *34*(7), 695–697. https://doi.org/10.1038/nbt.3583

Polesani, M., Bortesi, L., Ferrarini, A., Zamboni, A., Fasoli, M., Zadra, C., … Polverari, A. (2010). General and species-specific transcriptional responses to downy mildew infection in a susceptible (*Vitis* vinifera) and a resistant (V. Riparia) grapevine species. *BMC Genomics*, *11*(1). https://doi.org/10.1186/1471-2164-11-117

Porterfield, R., & Meru, G. (2017). Candidate Susceptibility Genes for Powdery and Downy Mildew in Watermelon and Squash. *Journal of Phylogenetics & Evolutionary Biology*, *05*(02). https://doi.org/10.4172/2329-9002.1000186

Rasband, W. (1997). *ImageJ*.

Raskin, I. (1992). Role of Salicylic Acid in Plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, *43*(1), 439–463. https://doi.org/10.1146/annurev.pp.43.060192.002255

Reid, K. E., Olsson, N., Schlosser, J., Peng, F., & Lund, S. T. (2006). An optimized grapevine RNA isolation procedure and statistical determination of reference genes for real-time RT-PCR during berry development. *BMC Plant Biology*, *6*, 1–11. https://doi.org/10.1186/1471-2229-6-27

Ren, C., Guo, Y., Kong, J., Lecourieux, F., Dai, Z., Li, S., & Liang, Z. (2020). Knockout of VvCCD8 gene in grapevine affects shoot branching. *BMC Plant Biology*, *20*(1), 1–8. https://doi.org/10.1186/s12870-020-2263-3

Ren, C., Liu, X., Zhang, Z., Wang, Y., Duan, W., & Li, S. (2016). CRISPR / Cas9-mediated efficient targeted mutagenesis in Chardonnay (*Vitis* vinifera L .). *Nature Publishing Group*, 1–9. https://doi.org/10.1038/srep32289

Ruijter, J. M., Ramakers, C., Hoogaars, W. M. H., Karlen, Y., Bakker, O., van den hoff, M. J. B., & Moorman, A. F. M. (2009). Amplification efficiency: Linking baseline and bias in the analysis of quantitative PCR data. *Nucleic Acids Research*, *37*(6). https://doi.org/10.1093/nar/gkp045

Schenke, D., & Cai, D. (2020). Applications of CRISPR/Cas to Improve Crop Disease Resistance: Beyond Inactivation of Susceptibility Factors. *IScience*, *23*(9), 101478. https://doi.org/10.1016/j.isci.2020.101478

Schouten, H. J., Krauskopf, J., Visser, R. G. F., & Bai, Y. (2014). Identification of candidate genes required for susceptibility to powdery or downy mildew in cucumber. *Euphytica*, *200*(3), 475–486. https://doi.org/10.1007/s10681-014-1216-z

Sun, K., van Tuinen, A., van Kan, J. A. L., Wolters, A. M. A., Jacobsen, E., Visser, R. G. F., & Bai, Y. (2017). Silencing of DND1 in potato and tomato impedes conidial germination, attachment and hyphal growth of Botrytis cinerea. *BMC Plant Biology*, *17*(1), 235. https://doi.org/10.1186/s12870-017-1184-2

Team, R. C. (2013). *A language and environment for statistical computing*. Retrieved from http://www.r-project.org/

van Butselaar, T., & Van den Ackerveken, G. (2020). Salicylic Acid Steers the Growth–Immunity Tradeoff. *Trends in Plant Science*, *25*(6), 566–576.

https://doi.org/10.1016/j.tplants.2020.02.002

Van Damme, M., Andel, A., Huibers, R. P., Panstruga, R., Weisbeek, P. J., & Van Den Ackerveken, G. (2005). Identification of Arabidopsis loci required for susceptibility to the downy mildew pathogen Hyaloperonospora parasitica. *Molecular Plant-Microbe Interactions*, *18*(6), 583–592. https://doi.org/10.1094/MPMI-18-0583

Van Damme, M., Huibers, R. P., Elberse, J., & Van Den Ackerveken, G. (2008). Arabidopsis DMR6 encodes a putative 2OG-Fe(II) oxygenase that is defense-associated but required for susceptibility to downy mildew. *Plant Journal*, *54*(5), 785–793. https://doi.org/10.1111/j.1365-313X.2008.03427.x

van Schie, C. C. N., & Takken, F. L. W. (2014). Susceptibility Genes 101: How to Be a Good Host. *Annual Review of Phytopathology*, *52*(1), 551–581. https://doi.org/10.1146/annurev-phyto-102313-045854

Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., & Speleman, F. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology*, *3*(7), 1–12. https://doi.org/10.1186/gb-2002-3-7-research0034

Vannozzi, A., Dry, I. B., Fasoli, M., Zenoni, S., & Lucchin, M. (2012). Genome-wide analysis of the grapevine stilbene synthase multigenic family: genomic organization and expression profiles upon biotic and abiotic stresses. *BMC Plant Biology*, *12*, 130. https://doi.org/10.1186/1471-2229-12-130

Vannozzi, A., Wong, D. C. J., Höll, J., Hmmam, I., Matus, J. T., Bogs, J., … Lucchin, M. (2018). Combinatorial Regulation of Stilbene Synthase Genes by WRKY and MYB Transcription Factors in Grapevine (*Vitis* vinifera L.). *Plant and Cell Physiology*, *59*(5), 1043–1059. https://doi.org/10.1093/pcp/pcy045

Vezzulli, S., Vecchione, A., Stefanini, M., & Zulini, L. (2018). Downy mildew resistance evaluation in 28 grapevine hybrids promising for breeding programs in Trentino region (Italy). *European Journal of Plant Pathology*, *150*(2), 485–495. https://doi.org/10.1007/s10658-017-1298-2

Vlot, A. C., Dempsey, D. A., & Klessig, D. F. (2009). Salicylic Acid, a Multifaceted Hormone to Combat Disease. *Annual Review of Phytopathology*, *47*(1), 177–206. https://doi.org/10.1146/annurev.phyto.050908.135202

Wan, D. Y., Guo, Y., Cheng, Y., Hu, Y., Xiao, S., Wang, Y., & Wen, Y. Q. (2020). CRISPR/Cas9-mediated mutagenesis of VvMLO3 results in enhanced resistance to powdery mildew in grapevine (*Vitis* vinifera). *Horticulture Research*, *7*(1).

https://doi.org/10.1038/s41438-020-0339-8

Wang, X., Tu, M., Wang, D., Liu, J., Li, Y., Li, Z., … Wang, X. (2018). CRISPR/Cas9-mediated efficient targeted mutagenesis in grape in the first generation. *Plant Biotechnology Journal*, *16*(4), 844–855. https://doi.org/10.1111/pbi.12832

Wang, Y., Cheng, X., Shan, Q., Zhang, Y., Liu, J., Gao, C., & Jin-Long, Q. (2014). Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nature Biotechnology*, (32), 947–951. https://doi.org/https://doi.org/10.1038/nbt.2969

Winkler, A. M., Ridgway, G. R., Webster, M. A., Smith, S. M., & Nichols, T. E. (2014). Permutation inference for the general linear model. *NeuroImage*, *92*, 381–397. https://doi.org/10.1016/j.neuroimage.2014.01.060

Zeilmaker, T., Ludwig, N. R., Elberse, J., Seidl, M. F., Berke, L., Doorn, A. Van, … Ackerveken, G. Van Den. (2015). *DOWNY MILDEW RESISTANT 6 and DMR6-LIKE OXYGENASE 1 are partially redundant but distinct suppressors of immunity in Arabidopsis*. 210–222. https://doi.org/10.1111/tpj.12719

Zhang, K., Halitschke, R., Yin, C., Liu, C.-J., & Gan, S.-S. (2013). Salicylic acid 3-hydroxylase regulates *Arabidopsis* leaf longevity by mediating salicylic acid catabolism. *Proceedings of the National Academy of Sciences*, *110*(36), 14807–14812. https://doi.org/10.1073/pnas.1302702110

Zhang, W., Mirlohi, S., Li, X., & He, Y. (2018). Identification of functional single-nucleotide polymorphisms affecting leaf hair number in Brassica rapa. *Plant Physiology*, *177*(2), 490–503. https://doi.org/10.1104/pp.18.00025

Zhang, Y. J., Zhao, L., Zhao, J. Z., Li, Y. J., Wang, J. Bin, Guo, R., … Zhanga, K. W. (2017). S5H/DMR6 encodes a salicylic acid 5-hydroxylase that fine-tunes salicylic acid homeostasis. *Plant Physiology*, *175*(3), 1082–1093. https://doi.org/10.1104/pp.17.00695

CONCLUSIONS AND FUTURE PERSPECTIVES

The aim of this research work was to provide new insights on the role and function of *S* genes to DM and PM in grapevine.

The genetic diversity survey on *VvDMR6.1, VvDMR6.2, VvDLO1*, *VvDLO2* and *VvMLO7* provided a list of valuable natural mutations able to disrupt protein function. Particular interest was generated in some specific mutations since their occurrence can be possibly linked to resistance phenotypic data. Interestingly, a disrupting mutation shared between two haplotypes in *VvDMR6.2* was significantly recurrent in DM resistant individuals. These findings can be exploited as new resources in resistance-aimed breeding programs and as molecular tools to integrate *R* loci-driven resistance.

Regarding the functional study of *VvDMR6.1, VvDMR6.2, VvDLO1* and *VvDLO2,* high variability in the results was found among the different edited lines that were investigated. Interestingly, our *dmr6.1* plants did not show any pleiotropic effect, which was instead observed in previous studies on herbaceous plant species. This study suggested that *VvDMR6.1* is not able to modulate susceptibility by itself, but its coordinated interaction with *VvDMR6.2* or *VvDLO* genes may be needed in order to observe stable phenotypic effects.

According to conclusions drawn from the different research approaches, it would be desirable that further studies focus on double or triple knock-out mutants of the investigated genes in order to observe how their additive loss of function is able to modulate resistance to *P. viticola*.

PUBLICATIONS

I) Pirrello, C., Mizzotti, C., Tomazetti, T. C., Colombo, M., Bettinelli, P., Prodorutti, D., … Vezzulli, S. (2019). Emergent Ascomycetes in Viticulture: An Interdisciplinary Overview. Frontiers in Plant Science, 10(November), 1–30. https://doi.org/10.3389/fpls.2019.01394

Front. Plant Sci., 22 November 2019

Emergent Ascomycetes in Viticulture: An Interdisciplinary Overview

Carlotta Pirrello[1,2†], Chiara Mizzotti[3†], Tiago C. Tomazetti[4†], Monica Colombo[1], Paola Bettinelli[1], Daniele Prodorutti[5], Elisa Peressotti[1], Luca Zulini[1], Marco Stefanini[1], Gino Angeli[5], Simona Masiero[3], Leocir J. Welter[6], Ludger Hausmann[7] and Silvia Vezzulli[1]

[1]Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Italy
[2]Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Udine, Italy
[3]Department of Biosciences, University of Milan, Milan, Italy
[4]Center of Agricultural Sciences, Federal University of Santa Catarina, Rodovia Admar Gonzaga, Florianópolis, Brazil
[5]Technology Transfer Centre, Fondazione Edmund Mach, San Michele all'Adige, Italy
[6]Department of Natural and Social Sciences, Federal University of Santa Catarina, Campus of Curitibanos, Rodovia Ulysses Gaboardi, Curitibanos, Brazil
[7]Julius Kühn Institute (JKI), Institute for Grapevine Breeding Geilweilerhof, Siebeldingen, Germany
[†] First authors

Abstract

The reduction of pesticide usage is a current imperative and the implementation of sustainable viticulture is an urgent necessity. A potential solution, which is being increasingly adopted, is offered by the use of grapevine cultivars resistant to its main pathogenic threats. This, however, has contributed to changes in defense strategies resulting in the occurrence of secondary diseases, which were previously controlled. Concomitantly, the ongoing climate crisis is contributing to destabilizing the increasingly dynamic viticultural context. In this review, we explore the available knowledge on three Ascomycetes which are considered emergent and causal agents of powdery mildew, black rot and anthracnose. We also aim to provide a survey on methods for phenotyping disease symptoms in fields, greenhouse and lab conditions, and for disease control underlying the insurgence of pathogen resistance to fungicide. Thus, we discuss fungal genetic variability, highlighting the usage and development of molecular markers and barcoding, coupled with genome sequencing. Moreover, we extensively report on the current knowledge available on grapevine-ascomycete interactions, as well as the mechanisms developed by the host to counteract the attack. Indeed, to better understand these resistance mechanisms, it is relevant to identify pathogen effectors which are involved in the infection process and how grapevine resistance genes function and impact the downstream cascade. Dealing

with such a wealth of information on both pathogens and the host, the horizon is now represented by multidisciplinary approaches, combining traditional and innovative methods of cultivation. This will support the translation from theory to practice, in an attempt to understand biology very deeply and manage the spread of these Ascomycetes.

II) Pirrello, C., Zeilmaker, T., Bianco, L., Giacomelli, L., Moser, C., & Vezzulli, S. (2020). Mining downy mildew susceptibility genes: a diversity study in grapevine. https://doi.org/10.1101/2020.01.15.898700

bioRxiv, January 15, 2020.

Mining downy mildew susceptibility genes: a diversity study in grapevine

Carlotta Pirrello[1,2], Tieme Zeilmaker[3], Luca Bianco[1], Lisa Giacomelli[1,3], Claudio Moser[1], Silvia Vezzulli[1]

[1]Research and Innovation Centre, Fondazione Edmund Mach, via E. Mach 1, 38010 San Michele all'Adige (TN), Italy
[2]Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, via delle Scienze 206, 33100 Udine, Italy
[3]SciENZA Biotechnologies B.V., Sciencepark 904, 1098 XH Amsterdam, The Netherlands

Abstract

Several pathogens continuously threaten viticulture worldwide. Until now, the investigation on resistance loci has been the main trend to understand the interaction between grapevine and mildew causal agents. Dominantly inherited gene-based resistance has shown to be race-specific in some cases, to confer partial immunity and to be potentially overcome within a few years since its introgression. Recently, on the footprint of research conducted on Arabidopsis, the putative hortologues of genes associated with downy mildew susceptibility in this species, have been discovered also in the grapevine genome. In this work, we deep-resequenced four putative susceptibility genes in 190 highly genetically diverse grapevine genotypes to discover new sources of broad-spectrum recessively inherited resistance. The scouted genes are VvDMR6-1, VvDMR6-2, VvDLO1, VvDLO2 and predicted to be involved in susceptibility to downy mildew. From all identified mutations, 56% were Single Nucleotide Polymorphisms (SNPs) in heterozygosity, while the remaining 44% were homozygous. Regarding the identified mutations with putative impact on gene function, we observed ~4% genotypes mutated in VvDMR6-1 and ~8% mutated in VvDMR6-2, only a handful of genotypes that were mutated in both genes. ~2% and ~7% genotypes showed mutations in VvDLO1 and VvDLO2 respectively, and again a few genotypes resulted mutated in both genes. In particular, 80% of impacting mutations were heterozygous while 20% were homozygous. The current results will inform grapevine genetics and corroborate genomic-assisted breeding programs for resistance to biotic stresses.

III) Pirrello, C., Zeilmaker, T., Bianco, L., Giacomelli, L., Moser, C., & Vezzulli, S. (2021). Mining Grapevine Downy Mildew Susceptibility Genes: A Resource for Genomics-Based Breeding and Tailored Gene Editing. https://doi.org/10.3390/biom11020181

Biomolecules, 28 January 2021

Special Issue *Molecular-Genetic Bases of Plant Breeding*

Mining Grapevine Downy Mildew Susceptibility Genes: A Resource for Genomics-Based Breeding and Tailored Gene Editing

Carlotta Pirrello[1,2], Tieme Zeilmaker[3], Luca Bianco[1], Lisa Giacomelli[1,3], Claudio Moser[1], Silvia Vezzulli[1]

[1]Research and Innovation Centre, Fondazione Edmund Mach, via E. Mach 1, 38010 San Michele all'Adige (TN), Italy
[2]Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, via delle Scienze 206, 33100 Udine, Italy
[3]SciENZA Biotechnologies B.V., Sciencepark 904, 1098 XH Amsterdam, The Netherlands

Abstract

Several pathogens continuously threaten viticulture worldwide. Until now, the investigation on resistance loci has been the main trend to understand the interaction between grapevine and the mildew causal agents. Dominantly inherited gene-based resistance has shown to be race-specific in some cases, to confer partial immunity, and to be potentially overcome within a few years since its introgression. Recently, on the footprint of research conducted in Arabidopsis, putative genes associated with downy mildew susceptibility have been discovered also in the grapevine genome. In this work, we deep-sequenced four putative susceptibility genes—namely *VvDMR6.1, VvDMR6.2, VvDLO1, VvDLO2*—in 190 genetically diverse grapevine genotypes to discover new sources of broad-spectrum and recessively inherited resistance. Identified Single Nucleotide Polymorphisms were screened in a bottleneck analysis from the genetic sequence to their impact on protein structure. Fifty-five genotypes showed at least one impacting mutation in one or more of the scouted genes. Haplotypes were inferred for each gene and two of them at the *VvDMR6.2* gene were found significantly more represented in downy mildew resistant genotypes. The current results provide a resource for grapevine and plant genetics and could corroborate genomic-assisted breeding programs as well as tailored gene editing approaches for resistance to biotic stresses.

GENERAL APPENDICES

Appendix I

Poster presentation at: XII International Conference on Grapevine Breeding and Genetics, Bordeaux 15-20/07/2018

This work won the prize as "best poster presentation made by a young scientist during the conference".

Scouting downy and powdery mildew susceptibility genes: a diversity study in *Vitis* spp.

Carlotta Pirrello[1,2], Tieme Zeilmaker[3], Lisa Giacomelli[1,3], Luca Bianco[1], Claudio Moser[1], Silvia Vezzulli[1]

[1]Research and Innovation Centre, Fondazione Edmund Mach, via E. Mach 1, 38010 San Michele all'Adige (TN), Italy

[2]Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, via delle Scienze 206, 33100 Udine, Italy

[3]SciENZA Biotechnologies B.V., Sciencepark 904, 1098 XH Amsterdam, The Netherlands

Abstract

World viticulture is continually threatened by both known and emerging pathogens. Until now, the investigation of resistance loci/genes has been the main trend to understand the interaction between grapevine (*Vitis* spp.) and mildew causal agents. Dominantly inherited gene-based resistance has shown to be race-specific in some cases, not to confer total immunity and to be potentially overcome within a few years. Recently, on the footprint of research conducted on Arabidopsis and barley, susceptibility genes associated to downy (DM) and powdery (PM) mildew resistance have been discovered also in the grapevine genome.

In the present work, in order to find new sources of broad-spectrum recessively inherited resistance against pathogens five susceptibility genes were re-sequenced in 96 grapevine accessions including wild, vinifera and hybrid individuals. The scouted genes were *VvDMR6-1, VvDMR6-2, VvDLO1, VvDLO2* involved in susceptibility to DM and *VvMLO7* associated with susceptibility to PM. These genes were mapped on the reference genome and analyzed to identify polymorphisms (SNPs) and haplotypes using dedicated software to study the mutation impact. Prior haplotype function confirmation, the final results will corroborate genomic-assisted breeding programs for resistance to biotic stresses.

Key words: *Vitis spp., downy mildew, DMR, DLO, SNP*

Appendix II

Oral presentation at: LXIII SIGA Annual Conference "Science and innovation for sustainable agriculture intensification: the contribution of plant genetics and breeding", Naples 12-15/09/2019

Exploring genetic variability of susceptibility genes in grapevine: a recent frontier to dissect disease resistance

Carlotta Pirrello[1,2], Tieme Zeilmaker[3], Lisa Giacomelli[1,3], Luca Bianco[1], Andrea Mattevi[4], Claudio Moser[1], Silvia Vezzulli[1]

[1]Research and Innovation Centre, Fondazione Edmund Mach, via E. Mach 1, 38010 San Michele all'Adige (TN), Italy

[2]Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, via delle Scienze 206, 33100 Udine, Italy

[3]SciENZA Biotechnologies B.V., Sciencepark 904, 1098 XH Amsterdam, The Netherlands

[4]Department of Biology and Biotechnology, University of Pavia, via Ferrata 1, 27100 Pavia, Italy

Abstract

Grapevine is one the most important and studied tree crops worldwide. Obtaining resistance to pathogens as *Plasmopara viticola* (the causal agent of downy mildew, DM) has always been a main goal in Europe since spreading of these pathogens at the end of the 19th century. In the last decades, due to the need to reduce environmentally impacting fungicides, breeders focused on crossing Eurasian *Vitis vinifera* with wild American and Asian species to obtain resistant individuals. Unfortunately, dominantly inherited gene-based resistance has shown to be race-specific in some cases, to confer partial immunity and to be potentially overcome within a few years from the introduction of the resistance trait. Recently, the identification of susceptibility genes in herbaceous and tree crops, as factors required by the pathogen to infect the host-tissue, has opened up a chance for their exploitation as an alternative to breed for resistant plants. On the footprint of the research conducted on Arabidopsis, genes associated with DM susceptibility have been discovered also in the grapevine genome.

Four susceptibility genes were re-sequenced (Illumina, 1,000X depth) in 190 grapevine accessions including 23 wild, 28 *vinifera* and 139 hybrid individuals to discover new sources of broad-spectrum recessively inherited resistance against *P. viticola*. The scouted genes were *VvDMR6-1, VvDMR6-2, VvDLO1, VvDLO2* involved in susceptibility to DM. These genes were mapped on the reference genome and analysed to identify polymorphisms and haplotypes using dedicated software to study the mutation effect. Regarding those mutations with putative impact on gene function, within the 190 accessions we observed ~14% accessions mutated in *VvDMR6-1* and ~18% mutated in *VvDMR6-2*,

only a handful of accessions that were mutated in both genes. ~21% and ~16% accessions showed mutations in *VvDLO1* and *VvDLO2* respectively, and again only a few accessions were mutated in both genes. 86% of the total impacting mutations were SNPs while 14% were substitutions. Out of the 129 accessions carrying selected mutations ~83% were hybrids while 7% and 10% were respectively wild species and vinifera varieties. When taking into account haplotype frequencies, highly shared haplotypes (in ~40% of the mutation-carrying accessions) were observed for *VvDMR6-2* and *VvDLO1*, whereas for *VvDMR6-1* and *VvDLO2* almost every accession showed a specific haplotype.

Moreover, a validation of Illumina results was carried out with a Sanger sequencing on 25 selected accessions as informative for interesting non-synonymous or synonymous substitutions in one or more of the genes under investigation. Basing on these findings, the *VvDMR6-1* and *VvDLO1* protein model based on confirmed mutations-carrying haplotypes was drafted with the aim to investigate the impact of amino acids substitution on protein folding and function.

These results will inform grapevine genetics and corroborate genomic-assisted breeding programs for resistance to biotic stresses.

Key words: *Vitis spp., downy mildew, DMR, DLO, SNP*

Appendix III

Poster presentation at: 7th International Horticulture Research Conference, online, 1-30/07/2020

Investigating DMR6 susceptibility genes role in grapevine-downy mildew interaction

Carlotta Pirrello[1,2], Lisa Giacomelli[3], Tieme Zeilmaker[3], Giulia Malacarne[1], Claudio Moser[1]

[1]Research and Innovation Centre, Fondazione Edmund Mach, via E. Mach 1, 38010 San Michele all'Adige (TN), Italy

[2]Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, via delle Scienze 206, 33100 Udine, Italy

[3]SciENZA Biotechnologies B.V., Sciencepark 904, 1098 XH Amsterdam, The Netherlands

Abstract

In viticulture, downy mildew (DM) is one of the most destructive fungal diseases. It is caused by the oomycete *Plasmopara viticola* to which *Vitis vinifera* L. is highly susceptible. To date, the control of this pathogen mainly relies on an intensive use of chemical fungicides sprayed on vineyards several times a year with negative consequences both on human health and the environment. In the last decades many cultivated varieties have been developed taking advantage of Resistance (*R*) genes from wild species in resistance-aimed breeding programs. As long as the use of resistant hybrids has become common practice, it's getting clear that the *R* gene strategy needs to be deployed with other tools able to stall the fast overcoming of resistance by pathogens. In this framework, Susceptibility (S) genes represent a valid instrument since their inactivation can lead to broad-spectrum and durable resistance. *Downy Mildew Resistance 6* (*DMR6*) susceptibility gene has been discovered and characterized in Arabidopsis thaliana and its orthologs *VvDMR6.1* and *VvDMR6.2* and *VvDMR6-like Oxygenases 1* (*DLO1*), *VvDLO2* and *VvDLO3* have been recently identified in grapevine. In the present work, *dmr6.1* edited grapevine plants were used for DM assays. Since more replicates are needed to confirm a reduction of susceptibility in edited plants, the phenotypic analysis has been coupled with a gene expression analysis on *DMR6* and *DLO* genes to evaluate the editing effect at transcriptional level. From preliminary results a certain trend of expression is visible but still further analyses need to be done.

ACKNOWLEDGEMENTS

At the end of my PhD, I would like to express my gratitude to the people who have supported me over these three years.

I must thank the Edmund Mach Foundation, the University of Udine and Dr. Giuseppe Firrao, who has always shown great effort in coordinating the research activities of PhD students with interesting training initiatives.

A special thank goes to my FEM supervisors Prof. Claudio Moser, Dr. Silvia Vezzulli and Dr. Giulia Malacarne for welcoming me with kindness and accompanying me in my personal and professional growth during these three years.

My thanks go to my supervisor at SciENZA Biotechnologies Dr. Tieme Zeilmaker and to Dr. Jeroen Rouppe van der Voort for their support during the months I spent at ENZA Zaden and for the points of reflection given.

I thank Dr. Lisa Giacomelli for her precious scientific support; Susanna Micheli and Giorgio Sordato for the constant technical help; Luca Zulini, Raffaele Filippi and Alessandra Zatelli for their assistance in greenhouse activities; Dr. Luca Bianco and Dr. Pietro Franceschi for computational and statistical support.

Many thanks to all the co-authors and collaborators who have dedicated some of their time to my PhD study. In particular, I thank Paola Bettinelli and Dr. Monica Colombo for the teamwork done, under a great coordination, in writing our review.

Finally, I would like to thank my family, my friends and all colleagues for the emotional support and the good times spent together.