TÜBİTAK

Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2021) 45: 273-284
© TÜBİTAK
doi:10.3906/tar-2009-109

Evaluation of powdery mildew resistance of a diverse set of grape cultivars and testing the association between powdery mildew resistance and PR gene expression

Sinem DEMİREL AŞÇI[1], Serpil TANGOLAR[2], Kemal KAZAN[3], Canan YÜKSEL ÖZMEN[1], Mert ÖKTEM[4], Umut KİBAR[5], Muhammad MUJTABA[1], Semih TANGOLAR[2], Ali ERGÜL[1,*]

[1]Ankara University, Biotechnology Institute, Ankara, Turkey
[2]Çukurova University, Faculty of Agriculture, Department of Horticulture, Adana, Turkey
[3]Commonwealth Scientific and Industrial Research Organization (CSIRO) Agriculture and Food, Queensland, Australia
[4]Mersin University, Faculty of Science, Department of Biotechnology, Mersin, Turkey
[5]Republic of Turkey, Ministry of Agriculture and Forestry, Agriculture and Rural Development Support Institution, Ankara, Turkey

Received: 01.10.2020 • **Accepted/Published Online:** 10.02.2021 • **Final Version:** 23.06.2021

Abstract: Powdery mildew caused by the fungal pathogen *Erysiphe necator* is a global disease that reduces yield and quality in grapes (*Vitis vinifera* L.). Most grape cultivars are susceptible to this pathogen and thus the selection of powdery mildew resistant cultivars is a major objective of grape breeding programs. In this study, we evaluated powdery mildew resistance of a diverse set of 28 commonly cultivated local and international *V. vinifera* cultivars after artificial inoculations with *Erysiphe necator*. Of these, 7 cultivars were found to be resistant while 10 and 11, were found to be moderately resistant and susceptible to powdery mildew, respectively. We also analyzed pathogen-responsive expression profiles of two defense genes, *VvPR1* and *VvGLP3* encoding a pathogenesis-related protein 1 homolog and a Germin-Like protein, respectively, in the same cultivars by qRT-PCR to determine whether defense gene expression is correlated with powdery mildew resistance. There was no significant correlation between powdery mildew ratings and induction patterns of these two defense genes. However, a significant correlation was found between *VvPR1* and *VvGLP3* expression only in cultivars that showed increased powdery mildew resistance. In addition, the grape cultivars we analyzed differed by berry ripening times, were shown to influence disease resistance. However, no significant correlation was found between powdery mildew ratings and the ripening times. Although potential mechanisms of powdery mildew resistance or susceptibility remain unclear, powdery mildew resistant grape cultivars identified here will be useful for growers and breeders interested in using these lines as parents in grape breeding programs.

Key words: *Erysiphe necator*, *Vitis vinifera* L., *VvPR1*, *VvGLP3*

1. Introduction

The grape (*Vitis vinifera* L.) is one of the most important fruit species cultivated all over the world. Powdery mildew caused by the fungal pathogen *Erysiphe necator*, is one of the most important diseases of grapes (Dry et al., 2010; Xu et al. 2010; Dufour et al. 2011; Gaforio et al., 2011). This pathogen infects the leaves and the berries of susceptible grape cultivars causing significant losses in yield and quality as infected berries would not be suitable for fresh consumption or winemaking (Wang et al., 1995; Xu et al., 2010; Gadoury et al., 2012a, 2012b; Xu et al., 2016). Fungicides are currently commonly used to protect grapes from powdery mildew. However, fungicide applications are often costly and can lead to harmful environmental and health effects. In addition, extensive fungicide use is known to lead to the emergence of fungicide-resistant pathogen strains (Erickson and Wilcox, 1997; Savocchia et al., 2004; Barker et al., 2005; Xu et al., 2010; Gaforio et al., 2011; Kabaktepe et al., 2015). Therefore, genetic resistance to powdery mildew in grapes is an important breeding objective.

Although many members of the *Vitaceae* family including *Vitis*, *Ampelopsis*, *Cissus*, and *Parthenocissus* have been reported to be susceptible to powdery mildew (Boubals, 1961), within the *Vitis* genus, North American *Vitis* species such as *V. riparia*, *V. aestivalis*, and *V. rupestris* are known to be less susceptible to powdery mildew than *V. vinifera* (Cadle-Davidson et al., 2011; Fung et al., 2008). For instance, in a study conducted on 24 grape species, *V. cordifolia*, *V. lanata*, *V. rupestris* and *V. vulpina* showed high susceptibility, while *V. candicans, V. munsoniana, V. piasezkii* and, *V. rotundifolia* were reported to be powdery mildew resistant (Staudt, 1997). Another resistant grape species is *Muscadinia rotundifolia* (Olmo, 1971), a vine

* Correspondence: ergul@ankara.edu.tr

This work is licensed under a Creative Commons Attribution 4.0 International License.

species native to the USA. In later studies, it was found that this resistance was due to the *Run1* (for resistance to *Uncinula necator* 1) locus, which is linked to the family of Interleukin-1 receptor (TIR), nucleotide binding site (NBS), C-terminal leucine-rich repeat (LRR) resistance gene candidates (Dry et al., 2010).

Although, most *V. vinifera* species that are mostly commercially-grown grape varieties of highest quality are susceptible to powdery mildew (Donald et al., 2002; Cadle-Davidson et al., 2011), a complete natural resistance or immunity to powdery mildew has also been identified in few cultivars (e.g., 'Kishmishvatkana' and 'Karadzhandal') (Godfrey et al., 2007; Khiavi et al., 2012; Riaz et al., 2013). Many studies (Eibach, 1994; Özer et. al., 2012; Khiavi and Davoodi, 2016) have investigated powdery mildew disease resistance of different grape hybrids and cultivars, and penetration resistance in *V. vinifera* has been found to be associated with actin cytoskeleton polymerization and vesicle trafficking (Feechan et. al, 2011).

Plants respond to pathogenic assaults by producing a broad spectrum of pathogenesis-related (PR) proteins (Van Loon et al., 1994; Van Loon and Van Strien, 1999; Van Loon et al., 2006; Li et al., 2011). The expression of genes encoding PR proteins increases significantly during infection (Godfrey et al., 2007; Roubelakis-Angelakis, 2009; León-Galván et al., 2011). The first family of these proteins (PR1 or pathogenesis-related protein 1) was identified in the 1980s (Antoniw et al., 1980). Since then, a number of PR proteins have been identified in many mono- and di-cotyledons plant species (Liu and Xue 2006; Mitsuhara et al., 2008; Li et al., 2011). These proteins are classified into 17 classes by Van Loonet et al. (2006). Genes encoding PR1 proteins are typically induced in response to pathogen attack in many plant species including grapes (Ficke et al., 2003).

In the first study of PR1 protein in grapes, changes in PR1 protein composition with powdery mildew in grape leaves were analyzed by SDS-PAGE and three PR1-like proteins (*g*PR-1a, *g*PR-1b and *g*PR-1c) were significantly induced after fungal infection (Repka, 2000). However, until recently the exact mode of antimicrobial action of the PR1 protein in plants has been enigmatic. A recent study showed that the tobacco PR1 protein inhibits fungal growth by binding and inhibiting fungal sterols, the lipid molecules that are essential components of the cell walls of many pathogenic fungi including smuts and powdery mildews (Gamir et al., 2017; Kazan and Gardiner, 2017). *PR1* expression is regulated by NPR1 (nonexpresser of pathogenesis-related genes 1), a master regulator of salicylic acid (SA) signaling pathway in plants (Kazan, 2018). Although NPR1 is required for the expression of *PR* genes, its expression is not responsive to pathogen infection in many plant species including grapes (Le Henanff et al., 2009; Le Henanff et al., 2011).

Another class of PR proteins identified in grapevine and other plant species such as barley is germins and germin-like proteins (GLPs) (El-Sharkawy et al., 2010; Emanuelli et al., 2010). GLPs are a large family of proteins with more than 25 members. Phylogenetically, GLPs are divided into 5 subgroups (true GER, gymnosperm GLPs, GLP subfamily-1, GLP subfamily-2, and the GLP subfamily-3) (Carter and Thornburg, 2000).

Seven members of the grapevine germin-like multigene family were cloned from *V. vinifera* (Godfrey et al., 2007). Similarly to *VvPR1*, the expression of the *VvGLP3* encoding a GLP is strongly and specifically induced in grape epidermal cells at the site of infection by *E. necator* (Godfrey et al., 2007; Roubelakis-Angelakis, 2009), providing a useful marker to follow the activation of defense responses. The *Vv*GLP3 protein was found to have superoxide dismutase (SOD) activity (Godfrey et al., 2007). Another study examining the physiochemical, functional, and structural properties of VvGLP3, revealed that this protein has a probable role in defense against powdery mildew (Ahmad et al., 2019). However, the exact mode of action of this protein in the defense against powdery mildew is unclear.

Interestingly, both *VvPR1* and *VvGLP3* expression were associated with powdery mildew susceptibility and resistance, respectively, in a grape cultivar (Ficke et al., 2003). A stronger *VvPR1* induction was found in powdery mildew susceptible berries of the grape cultivar Chardonnay. In contrast, the expression of *VvGLP3* was induced only in the resistant berries (Ficke et al., 2003).

In *Vitis* spp., *E. necator* can infect all green tissues (leaves, grains, shoots, and buds) and some grape species and varieties have been described as having tissue-specific resistance (Eibach, 1994; Cadle-Davidson et al., 2011). Nevertheless, to the best of our knowledge, there has not been any report systematically examining whether there is a direct correlation between the induction of these defense genes and powdery mildew resistance in the leaves of grape cultivars that differ by powdery mildew resistance. These two genes were also found to be rapidly induced in grape flowers in response to infection by *Botrytis cinerea*, another important fungal pathogen of grapes, suggesting that these genes can be used as markers to study defense responses in grapes (Haile et al., 2017).

Genetic resistance to powdery mildew and other fungal diseases can also be determined by disease resistance (R) genes. Goyal et al. (2019) have identified a number of NBS-LRR genes that may provide resistance to powdery mildews in grapes. In addition, improving genetic resistance to powdery mildew can also be achieved by removing factors that condition genetic susceptibility. For instance, *MLO* (*Mildew Locus O*)-like genes found in grapes condition powdery mildew susceptibility (Feechan

et al., 2008; Pessina et al., 2016). However, to the best of our knowledge, the genetic association between powdery mildew susceptibility and the MLO gene family has not been analyzed in a diverse set of grape cultivars that differ by powdery mildew resistance.

Finally, plant development can play a significant role in plant disease resistance. For instance, as indicated above, mature grape berries are known to be less susceptible to powdery mildew infection (Viala, 1893; Delp, 1954; Chellemi and Marois, 1992; Gee et al., 2008; Bioletti, 2016). In addition, leaf age is correlated with increased powdery mildew resistance in grapes with older leaves being more resistant to this pathogen (Sall and Teviotdale, 1981; Doster and Schnathorst, 1985a, 1985b; Calonnec et al., 2018). This phenomenon is known as ontogenic or age-related resistance (Ficke et al., 2003; Qiu et al., 2015). However, to the best of our knowledge, the association between berry ripening time/maturity and powdery mildew resistance in grapes has not been investigated.

In the current study, we evaluated powdery mildew resistance of 28 grape cultivars after artificial inoculations with *E. necator* to assess the genetic variation available for disease resistance within the cultivated grape germplasm. These grape cultivars also differed by bud bursting and berry ripening times, the developmental traits that might influence disease resistance. In addition, we measured the expression profiles of *VvPR1* and *VvGLP3* after powdery mildew inoculations in the leaves of the same cultivars. Our results from these experiments indicate that although grape cultivars could be clearly distinguishable based on their powdery mildew ratings, no clear correlation between powdery mildew resistance ratings and the expression profiles of the 2 defense gene examined here or bud bursting and berry ripening times could be found. Possible reasons for these findings are discussed.

2. Materials and methods

2.1. Plant material

The leaves harvested from the cuttings of 28 commonly cultivated native and international cultivars that were obtained from the collection vineyard of the Department of Horticulture, Çukurova University, Adana, Turkey, were used in this study (2007). The cuttings of the tested cultivars were grown, and powdery mildew inoculations were carried-out in a climate chamber of the Plant Protection Research Institute, Adana, Turkey.

2.2. Powdery mildew inoculations

2.2.1. Preparation of powdery mildew inoculum

The seeds of the powdery mildew susceptible cultivar Alphonse Lavallee´ were stratified in moist sand for 3 months at 4 °C and then germinated in growth chambers (24 ± 1 °C). Optimum temperature for conidial germination is 24–25 °C (Doster and Schnathorst, 1985a; Fessler and Kassemeyer, 1995; Willocquet et al., 1996) and fungal germination is completed within 30 h at this temperature (Delp 1954). At the two-leaf stage, the plantlets were transferred to 10 x 2 cm pots. The pots were then kept in a growth chamber at 25 °C, 16: 8-h light: dark conditions. To collect spores, the cuttings (one-year-old) infected with powdery mildew were collected from vineyards and placed on Alphonse Lavallee´ plantlets until the pathogen sporulated on the leaves.

2.2.2. Inoculation procedure

The inoculation of plants using the powdery mildew conidia harvested from the leaves of Alphonse Lavalleé performed using the following method described by Le Henanff et al. (2011). In this inoculation experiment, 7–14 days old conidia were used. Briefly, 50 mL of sterile water suspension (0.05% Tween 20 (v/v)) containing about 10^5 conidia per mL was prepared and applied for 30 min by spraying onto upper surface of plants (Yamamoto et al., 2000; Ramming et al., 2011) using a hand atomizer until suspension droplets were seen on the leaves which were subsequently dried for approximately 20 min.

2.3. Evaluation of powdery mildew resistance in grapes

To evaluate powdery mildew tolerance of 28 grape cultivars, 18 cuttings (30–35 cm) per cultivar were planted in perlite-filled 8 L plastic pots (3 pots for each variety and 6 cuttings in each pot). Pots were kept in a growth chamber at 25 °C with relative humidity of 80% and 16:8-h light: dark photoperiod. Initial infection signs started within seven days after inoculations. Disease symptom development was then assessed four weeks after inoculations according to the procedure described by Gadoury et al. (2003) and Gadoury et al. (2012a).

Briefly, disease ratings were determined by examining young 5–6 leaves (average) from the top for each cutting (or plant). Disease severity was calculated according to a 0 to 3 scale where, 0: no spot on the leaf, 1: 1–2 spots on the leaf, 2: 3–10 spots on the leaf, and 3: more than 10 spots on the leaf (Delen et al., 1987; T.C. Tarım ve Köyişleri Bakanlığı Tarımsal Araştırmalar Genel Müdürlüğü, 1996; Albayrak et al., 2018).

A control experiment without any spores was conducted in parallel. The leaves from inoculated plants were frozen in liquid nitrogen and then stored in at –80 °C for RNA isolation.

2.4. Bud bursting and berry ripening time evaluations

The bud bursting and berry ripening times of grape cultivars were evaluated in Adana, Turkey with coordinates of 36.9914° N, and 35.3308° E. Ripening times were evaluated using the following criteria previously reported by Tangolar et al. (1996) ; very early (ripening date before 30 June and ripening class 1), early (ripening date 1–15

July and ripening class 2), medium early (ripening date 16–31 July, ripening class 3), medium (ripening date 1–15 August, ripening class 4), medium late (ripening date 16–31 August, ripening class 5) and late (ripening date after 1 September 1, ripening class 6). Bud bursting times were evaluated using the following criteria; early (1) (bursting time in the second or third week of March); medium (2) (bursting time in the last of week of March or first week of April); late (3) (bursting time in the second week of April).

2.5. RNA isolations and real-time PCR analysis

Three biological replications were performed for RNA isolation and real-time quantitative PCR (RT-qPCR) experiments. Briefly, severely infected five to 6 leaves from 6 cuttings as each biological replication were collected 4 weeks after inoculations and frozen in liquid nitrogen. For RNA isolations, the samples were homogenized in porcelain mortars following the protocol described by Gambino et al. (2008). The concentrations of isolated RNA samples were measured quantitatively using a Nanodrop ND 1000 spectrophotometer. Nanodrop results were validated by running RNA samples on 1.2% agarose gel electrophoresis.

RT-qPCR reactions were performed in a Light Cycler 480 (Roche) for 2 genes *V. vinifera pathogenesis-related protein 1* (*VvPR1*) (AY298726.1) and *V. vinifera germin-like protein 3 (VvGLP3)* (AY298727) with 3 biological and 3 technical replicates and gene expression values were normalized to the expression of *V. vinifera Actin 1 (VvACT1)* (AF369524) housekeeping gene in the same samples. The primers for these genes are given in Table. The primers we used were already validated for grapes by Ficke et al. (2003). Our melting curve analyses also revealed the presence of a single amplification product, suggesting that these primers were specific. cDNA was synthesized using a First Strand cDNA Synthesis Kit (Roche, Cat no: 04897030001). The cDNA was quantified using a Nanodrop ND-1000 spectrophotometer. The purity of cDNAs was checked and the concentrations were adjusted to (approximately 2000 ng/µL). Amplification reactions were then performed on a Roche Light Cycler-LC 480 PCR using the SYBR Green I (Roche) Master Kit using the appropriate reaction mix and PCR program.

2.6. Data analysis

For each gene, Ct (cycle threshold) values of infected and control samples were obtained after RT-qPCR analysis. Expression levels of *VvPR1* and *VvGLP3* genes were normalized with the expression level of the housekeeping gene (*VvACT1*). The gene expressions were calculated according to the $2^{\Delta CT}$ method (Pfaffl, 2001). Correlations between powdery mildew resistance and defense gene expression and powdery mildew resistance and the maturity time were tested using the SPSS software package.

3. Results

3.1. Powdery mildew and ripening time evaluations

In this study, we first determined powdery mildew ratings of a diverse set of locally and internationally important grape cultivars that can be potentially used as parents in grape breeding programs to improve powdery mildew resistance. To the best of our knowledge, disease ratings of these cultivars have not been comparatively examined previously in a single study. Disease resistance was evaluated using a scale of 0–3 (see materials and methods). In these experiments, most, if not all, cultivars have developed disease symptoms. We ranked these grape cultivars from more resistant to less resistance in an attempt to group these cultivars into somewhat distinct classes (as shown in Figure 1A). For instance, the cultivars, Reçel Üzümü, Bone Seedless, Muscat of Hamburg, Italia, Kyoho, Horoz karası, and Boğazkere had either no or 1 spot and thus were considered highly resistant to powdery mildew (as shown in Figure 1A). Another group of cultivars, including Yalova Çekirdeksizi, Sugraone, Trakya İlkeren, Cardinal, Early Cardinal, Black Perl, Royal, Victoria, and Öküzgözü, showed only few spots (disease rating 1) after inoculations and thus were considered moderately resistant (as shown in Figure 1A). A third group of cultivars, including Ergin Çekirdeksizi, Flame Seedless, Tekirdağ Çekirdeksizi, Güz Üzümü, Crimson Seedless, King's Ruby, Perlette, Atasarısı, Razakı, Alphonse Lavalle and Karasakız, showed more lessions (3–10) (disease ratings 2 and 3) and were considered susceptible to powdery mildew (see Supplementary Figure for examples). Together, these experiments showed that the grape cultivars used in this study display a quantitative response to powdery mildew infection (as shown in Figure 1A) and facilitated the identification of highly resistant and highly susceptible cultivars.

3.2. *VvPR1* and *VvGLP3* expression analysis in diverse grape cultivars

We next tested whether there is a correlation between powdery mildew and the resistance expression of the two well-known defense-associated genes in grapes. To test this possibility, we have determined pathogen-responsive expression of *VvPR1* and *VvGL3* by RT-qPCR in the leaves of the same grape cultivars.

Different grape cultivars used in this study responded differently to powdery mildew inoculations as far as the inducibility of the *VvPR1* is considered. At least three groups of cultivars could be observable among the 28 grape cultivars used in this study. The highest fold-induction (more than 50-fold) values for *VvPR1* were observed in Reçel Üzümü, Güz Üzümü, Black Perl, and Italia (as shown in Figure 2A). Relatively lower (1–8 fold) induction values were observed for a subset of grape cultivars that included Tekirdağ Çekirdeksizi, Yalova Çekirdeksizi, King's Ruby,

Table. Forward and reverse primer sequences used for *VvPR1*, *VvGLP3*, and *VvACT1*.

Gene	Forward primer sequences (5'-3')	Reverse primer sequences (5'-3')
Vitis vinifera Pathogenesis related Protein 1 gene (VvPR1)	cgtccttattaaacatcatggaact	caacacaccatattgttctgacc
Vitis vinifera Germin like Protein 3 gene (VvGLP3)	ccctcgtgctactgagat	gactttgctgatgaggcg
Vitis vinifera Actin 1 gene (VvACT1) (housekeeping gene)	gggcgtacaactggtattgtg	cataaattggcacagtgtgactc

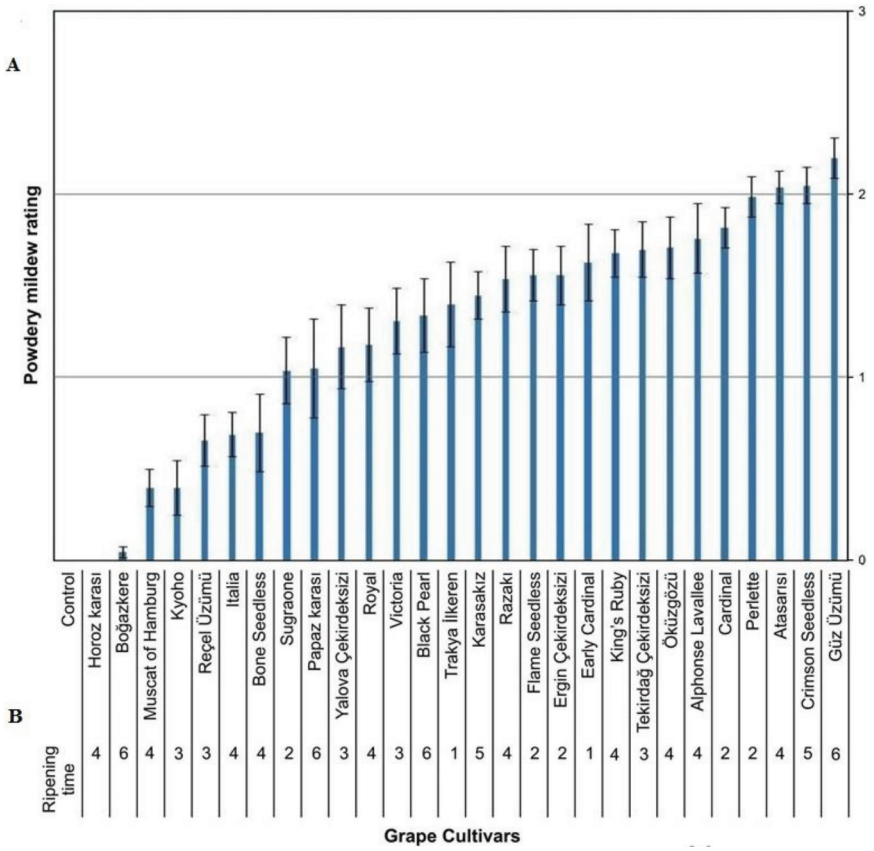


Figure 1. Evaluation of powdery mildew resistance in a diverse set of 28 grape cultivars after artificial inoculations with *Erysiphe necator*. (A) Average disease rating for each cultivar. Please see 'Materials and methods' for a detailed description of disease ratings, (B) Berry ripening times in the cultivars used in the study (Very early: 1, Early: 2, Medium early: 3, Medium: 4, Medium late: 5, and Late: 6).

Perlette, Cardinal, Early Cardinal, Kyoho, Royal, Victoria, and Boğazkere (as shown in Figure 2A). In the remaining 11 cultivars, either no or repressed *VvPR1* expression was observed (as shown in Figure 2A). Overall, these analyses indicate that powdery mildew mediated induction of *VvPR1* varies from one cultivar to another.

We then examined the induction profiles of *VvGLP3* in the same grape cultivars. The highest induction of the *VvGLP3* gene was recorded for Güz Üzümü followed by Reçel Üzümü and Victoria. We observed relatively low or modest induction of *VvGLP3* in another subset of grape cultivars that included Tekirdağ Çekirdeksizi, Yalova Çekirdeksizi, King's Ruby, Ata Sarısı, Trakya İlkeren, Cardinal, Italia, Black Perl, and Royal. No or little induction or even repression was observed for *VvGLP3* in the remaining 16 cultivars (see Figure 2B).

3.3. Correlations between powdery mildew ratings and defense gene expression

As indicated above, PR1 was found to bind ergosterols, lipid molecules found in fungal membranes and such binding most likely inhibits fungal growth (Porep et al., 2014; Gamir et al., 2017; Kazan and Gardiner, 2017). Most

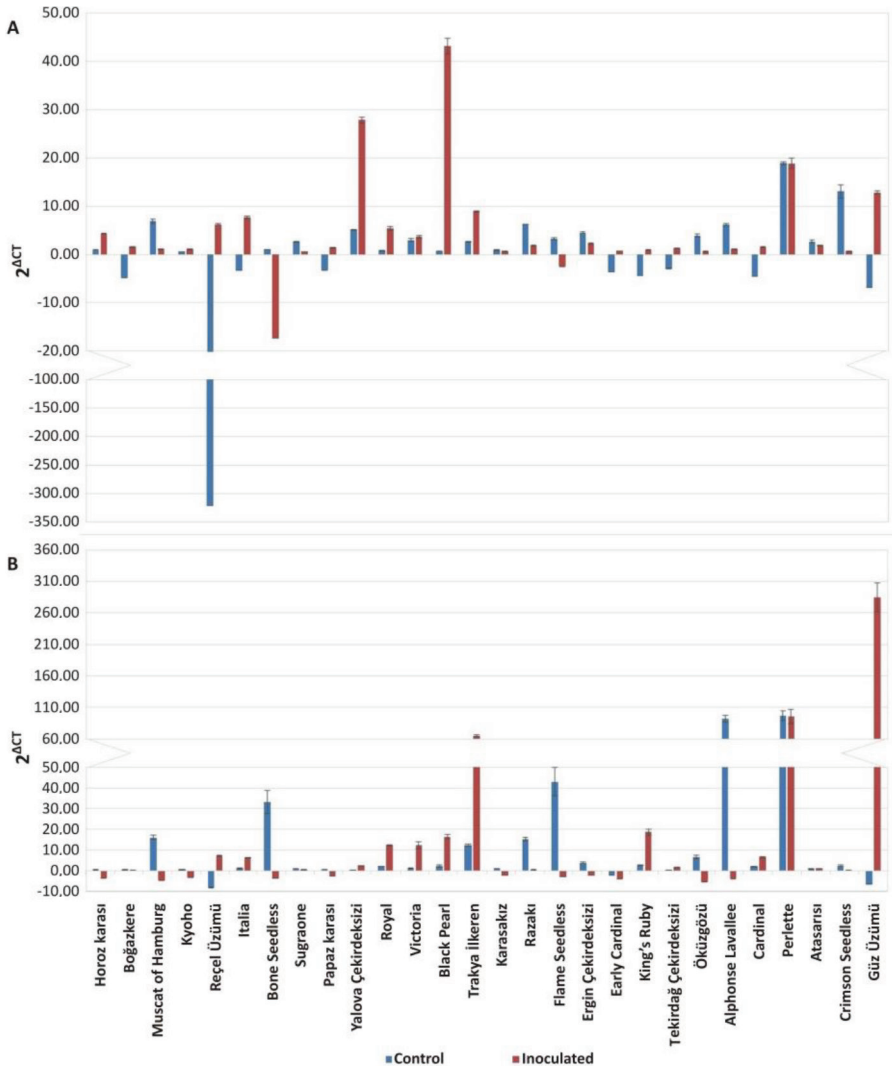


Figure 2. RT-qPCR analysis of powdery mildew responsive expression of *VvPR1*. (A) and *VvGLP3*, (B) expression in the same grape cultivars. Average expression values from 3 biological replicates with standard errors are shown.

members of the Pezizomycotina subphylum that includes powdery mildews (*Erysiphales*) are known to contain ergosterol in their membranes (Weete et al., 2010). We therefore wanted to test if there is a correlation between powdery mildew disease ratings and *VvPR1* expression. The disease evaluation experiments presented above showed that the cultivars Reçel Üzümü, Bone Seedless, Muscat of Hamburg, Kyoho, Italia, Horoz karası, and Boğazkere cultivars were highly resistant to powdery mildew (as shown in Figure 1A). Of these cultivars, Reçel Üzümü, and Italia showed highly induced *VvGLP3* expression, suggesting a positive correlation between disease resistance and *VvGLP3* expression. However, when all cultivars used in this study are considered, our correlation analyses did not reveal any significant association between defense gene induction profiles and disease resistance cultivars, suggesting that these two defense genes are most likely not responsible for disease phenotypes observed in the resistant cultivars. In fact, in some resistant cultivars *VvPR1* expression is suppressed while in some susceptible cultivars, it is strongly induced (as shown in Figure 2A).

Our gene expression analyses presented above showed that *VvPR1* was highly responsive to powdery mildew inoculation in 16 cultivars while *VvGLP3* was induced in 12 of the 28 grape cultivars inoculated with *E. necator*. Furthermore, the expression of *VvPR1* was induced in 12 cultivars that also showed an increased expression of *VvGLP3*. This suggested that there might be a positive correlation between powdery mildew responsive expressions of these two defense genes. When fold-induction values of *VvPR1* and *VvGLP3* from all cultivars are used in correlation analyses, no such correlation could

be observed. However, when only 6 resistant cultivars (Horoz Karası, Boğazkere, Muscat of Hamburg, Kyoho, Italia and Bone Seedless) were included in correlation analyses, a significant association between *VvPR1* and *VvGL3* could be observed (as shown in Figure 3). This finding suggests that expression of these genes might be regulated by the same signaling pathways in the resistant cultivars.

3.4. Correlation between ripening time and disease resistance

We have noted that the grape cultivars evaluated for powdery mildew resistance in this study differ by their ripening times, which might affect ontogenic resistance against powdery mildew (Ficke et al., 2003). Indeed, under our experimental conditions, 2 cultivars showed very early maturity, 6 cultivars early maturity, 4 cultivars intermediate early, 8 cultivars intermediate maturity, 3 cultivars intermediate late maturity and 4 cultivars with late maturity (as shown in Figure 1B). The bud bursting times were also correlated with the berry ripening times in these cultivars (Supplementary Table).

To determine whether ripening time affects defense gene expression and powdery mildew resistance in grapes, we have also tested potential correlations between berry ripening times and defense gene expression and powdery mildew resistance. However, no significant associations between these traits could be found (data not shown).

4. Discussion

In this study, we determined powdery mildew disease ratings of a diverse set of grape cultivars after artificial inoculations with *E. necator*. Most *V. vinifera* cultivars are known to be susceptible to this pathogen and the variability observed for powdery mildew resistance in the grape cultivars we tested is very promising. This information will be useful in breeding programs for selecting disease resistant parental lines and for grape growers when deciding which cultivars to grow especially in regions where powdery mildew is a problem. Similar germplasm evaluation studies have also been conducted by others (Eibach, 1994; Staudt, 1997; Cadle-Davidson et al., 2011; Gaforio et al., 2011; Özer et al., 2012; Khiavi et al., 2012). For instance, Gaforio et al. (2011) have evaluated 159 local and international grape accessions under natural inoculation conditions and identified 35 super susceptible cultivars while the remaining accessions were either resistant or highly resistant. In addition, these authors have tested the associations between disease resistance and 14 ampelographic characters and identified a negative correlation between the degree of powdery mildew resistance and the goffering of leaf blades as well as the degree of berry resistance to powdery mildew and bunch density (Gaforio et al., 2011). In another study, among the grape cultivars tested, 11.1% were found resistant against powdery mildew, while 33.4% and 55% of the tested cultivars were classified as tolerant and susceptible, respectively (Khiavi et al., 2012).

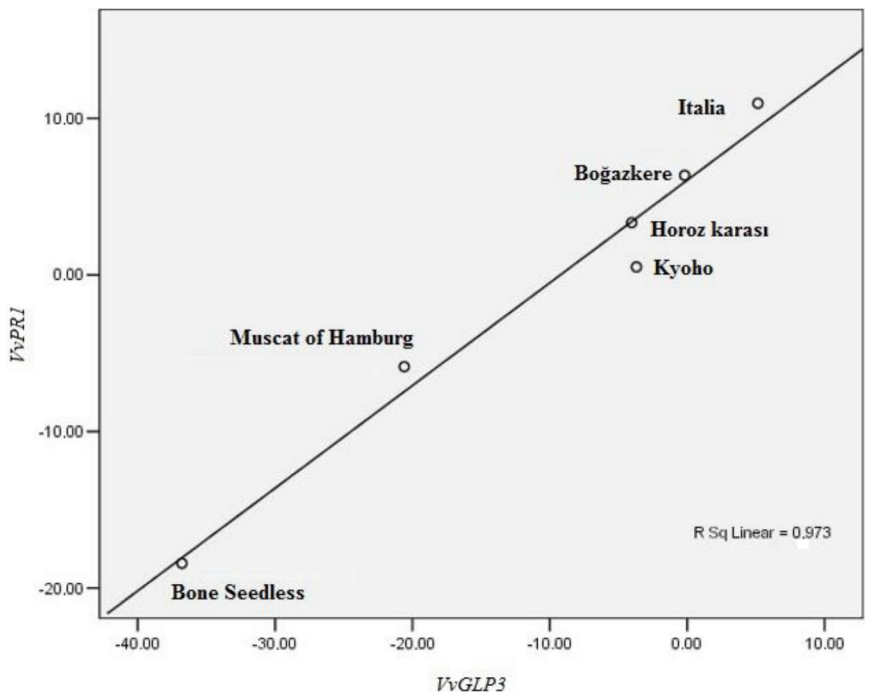


Figure 3. Powdery mildew responsive expression of *VvPR1* and *VvGLP3* shows a positive correlation ($p < 0.05$) in a subset of disease resistant grape cultivars.

The powdery mildew scoring results from this study show broad agreements with those from previous studies. For instance, the variety Perlette used in this study has previously been identified as moderately susceptible (Pospíšilová, 1978) and very susceptible (Doster and Schnathorst, 1985b). Similarly, 'Lett Perlett' and 'Flame Seedless' showed high levels of powdery mildew susceptibility (Khiavi and Davoodi, 2016) in the evaluation of infected leaves after natural and artificial inoculations. Previously, the Muscat De Yaloven variety (Eibach, 1994) showed relatively low levels of fruit and leaf infection. Similarly, in this study 'Muscat of Hamburg' was also found to be a powdery mildew tolerant cultivar rating, while 'Cardinal' was found to be a susceptible cultivar in both this and previous studies (Pospíšilová, 1978).

Grape varieties from the Black Sea region and especially those belonging to *V. labrusca* (Georgian, Spanish Grape, Native Isabella, White Fragrant, Solid Black, and Glossy Grape) have been reported to be highly tolerant to powdery mildew, but *V. vinifera* cultivar "Italia", has been reported to be less tolerant than *V. labrusca* varieties. Similarly, in this study, 'Italia' variety has been observed to be a tolerant variety. In another study 'Italia x Favli -149', 'Italia x 28/259-166', 'Italia x Coral-174' leaves of these hybrid varieties were found to exhibit stable resistance to powdery mildew (Özer et al., 2012).

To understand what makes one genotypes resistant or susceptible, we also examined the association between powdery mildew resistance and defense gene expression. If a positive correlation was found between these two variables, then selecting resistant cultivars based on their defense gene expression profiles could be exploited as a useful selection tool. Indeed, high levels of *PR1* expression was found in a subset of grape genotypes showing resistance to the downy mildew pathogen *Plasmopara viticola* (Liu et al., 2016). However, our results presented in the paper show that, although *VvPR1* and *VvGLP3* are certainly useful markers to study plant defense responses in grapes, no correlation could be found between pathogen-induced expressions of these genes and the powdery mildew resistance in grapes. There was no correlation either between basal levels of these genes measured without pathogen inoculation (data not shown) and powdery mildew resistance (data not shown). A lack of correlation between powdery mildew ratings and *PR*1 expression could be due to the fact that despite most other members of the powdery mildews which contain ergosterols in their membranes (Weete et al., 2010), the grape pathogen *E. necator* contains other types of lipids (Debieu et al., 1995) that might not be sensitive to inhibition by the PR1 protein. It should also be noted that the comparative analysis of the transcriptomes of the powdery mildew resistant (*V. aestivalis*) and susceptible (*V. vinifera*) grape species revealed that the expression of *PR1* was much less in *V. aestivalis* than in *V. vinifera* (Fung et al., 2008). This suggests that *PR1* expression might not be well correlated with powdery mildew resistance after all.

As indicated above, MLO-like genes in grapes and in many other plant species act as powdery mildew susceptibility genes (Pessina et al., 2016). Recent analyses in grapes suggested that two particular members of the MLO gene family, *VvMLO7* and *VvMLO6*, were found to be the major powdery mildew susceptibility genes in the grape cultivar Brachetto (Pessina et al., 2016). Therefore, the possibility exists that the differences we observed for powdery mildew resistance among the grape cultivars used in this study would due to the presence or one or more *VvMLO* like genes in these genotypes. Future studies should provide new insights into this possibility.

Ontogenic or age-related resistance could be another reason for the differences observed among the grape genotypes for powdery mildew resistance in this study. Indeed, as shown in Figure 1B, ripening times varied among the genotypes and this could account for differences we observed for powdery mildew resistance. The timing of bud burst also correlated strongly with the time of ripening in these cultivars (Supplementary Table). Therefore, it is conceivable that in early bud bursting cultivars, the sink to source mobilization of nutrients (e.g. sugars) would start earlier than late bud bursting cultivars to meet the nutrient demand of developing berries. Thus, one would assume that even if pathogen inoculations are conducted on developmentally comparable leaves of each genotype, it is possible that the leaves of early bud bursting and maturing genotypes are physiologically older than those from late bud bursting and maturing genotypes. The biotrophic powdery mildew pathogen is known to infect physiologically younger leaves better. Indeed, a positive correlation was found between sporulation and infection efficiency of the powdery mildew pathogen and sugar content in grape leaves (Calonnec et al., 2018). In addition, Calonnec et al. (2018) proposed that ontogenic resistance in grapes could be associated with differential expression of plant defenses at different leaf ages. However, we did not find any association between ripening time and powdery mildew resistance or pathogen-induced defense gene expression in these diverse grape cultivars, suggesting that ripening time is not a factor affecting powdery mildew resistance and/or susceptibility in grapes. This was surprising although it is possible that bud bursting and ripening time-associated physiological changes, which can potentially affect powdery mildew resistance might not be sufficiently different by the time these cultivars were inoculated. Also, a single and a relatively late time point

was used in our study when examining the expression of two *PR* genes. Therefore, a time course analysis of additional *PR* genes using a greater set of grape cultivars could be useful to identify potential correlations between powdery mildew ratings and defense gene expression profiles in the future.

Acknowledgments
A major portion of the current study was conducted as a master's thesis of the first author.

Author contributions
AE and ST designed and outlined the research. SDA and ST performed the experiment, KK, CYÖ, UK, MM, and MÖ drafted and edited the manuscript and also analyzed the data.

Data archiving statement
The data from the grape cultivars used in the study were presented in the text and in supplementary tables and figures.

References

Ahmad A, Ahmed A, Essa R, Baber S, Jamshed A et al. (2019). In-silico analysis of grapevine Germin like protein (VvGLP3) and its probable role in defense against powdery mildew disease. Life Science Journal of Pakistan 1 (2): 17-23.

Albayrak S, Karabıçak Y, Tuncer S, Alaserhat İ, Karadoğan B et al. (2018). Determination of efficiency of different spraying programs to powdery mildew (*Erysiphe necator* Schw.) in vineyard. Plant Protection Bulletin 58 (4): 199-206. doi: 10.16955/bitkorb. 430594

Antoniw J, Ritter C, Pierpoint W, Van Loon L (1980). Comparison of three pathogenesis-related proteins from plants of two cultivars of tobacco infected with TMV. Journal of General Virology 47: 79-87. doi: 10.1099/0022-1317-47-1-79

Barker CL, Donald T, Pauquet J, Ratnaparkhe M, Bouquet A et al. (2005). Genetic and physical mapping of the grapevine powdery mildew resistance gene, Run1, using a bacterial artificial chromosome library. Theoretical and Applied Genetics 111: 370-377. doi: 10.1007/s00122-005-2030-8

Bioletti FT (2016). *Oidium or powdery mildew of the vine*: Read Books Ltd.

Boubals D (1961). Etude des causes de la résistance des Vitacées a l'oidium de la vigne *Uncinula necator* (Schw. Burr.) et leurmoed de transmission hèrèditaire. Ann. L'Amél des Plantes 11: 401-500.

Cadle-Davidson L, Chicoine DR, Consolie NH (2011). Variation within and among Vitis spp. for foliar resistance to the powdery mildew pathogen Erysiphe necator. Plant Disease 95 (2): 202-211. doi: 10.1094/PDIS-02-10-0092

Calonnec A, Jolivet J, Vivin P, Schnee S (2018). Pathogenicity traits correlate with the susceptible *Vitis vinifera* leaf physiology transition in the biotroph fungus erysiphenecator: an adaptation to plant ontogenic resistance. Frontiers in Plant Science 9: 1808. doi: 10.3389/fpls.2018.01808

Carter C, Thornburg RW (2000). Tobacco nectarin I purification and characterization as a germin-like, manganese superoxide dismutase implicated in the defense of floral reproductive tissues. The Journal of Biological Chemistry 275: 36726-36733. doi: 10.1074/jbc.M006461200

Chellemi DO, Marois J (1992). Influence of leaf removal, fungicide applications, and fruit maturity on incidence and severity of grape powdery mildew. American Journal of Enology and Viticulture 43: 53-57.

Debieu D, Corio-Costet M-F, Steva H, Malosse C, Lereoux P (1995). Sterol composition of the vine powdery mildew fungus, *Uncinulanecator*: Comparison of triadimenol-sensitive and resistant strains. Phytochemistry 39: 293-300. doi: 10.1016/0031-9422(95)00015-Y

Delen N, Onoğur E, Öncü M (1987). Bağ Küllemesi (Uncinula Necator Sch. Burr.)'nin kimyasal savaşımı üzerinde araştırmalar. Doğa 11 (2): 303-309 (in Turkish)

Delp CJ (1954). Effect of temperature and humidity on the grape powdery mildew fungus. Phytopathology 44.

Donald TM, Pellerone F, Adam-Blondon AF, Bouquet A, Thomas MR et al. (2002). Identification of resistance gene analogs linked to a powdery mildew resistance locus in grapevine. Theoretical and Applied Genetics 104: 610-618. doi: 10.1007/s00122-001-0768-1

Doster MA, Schnathorst WC (1985a). Effects of leaf maturity and cultivar resistance on development of powdery mildew fungus on grapevines. Phytopathology 75: 318-321. doi: 10.1094/Phyto-75-318

Doster MA, Schnathorst WC (1985b). Comparative susceptibility of various grapevine cultivars to the powdery mildew fungus *Uncinula Necator.* American Journal of Enology and Viticulture 36: 1001-104.

Dry IB, Feechan A, Anderson C, Jermakow A, Bouquet A et al. (2010). Molecular strategies to enhance the genetic resistance of grapevines to powdery mildew. Australian Journal of Grape and Wine Research 16: 94-105. doi: 10.1111/j.1755-0238.2009.00076.x

Dufour MC, Fontaine S, Montarry J, Corio-Costet MF (2011). Assessment of fungicide resistance and pathogen diversity in Erysiphe necator using quantitative real-time PCR assays. Pest Management Science 67: 60-69. doi: 10.1002/ps.2032

Eibach R (1994). Investigations about the genetic resources of grapes with regard to resistance characteristiques to powdery mildew (*Oidium tuckeri*). Vitis 33: 143-150. doi: 10.5073/vitis.1994.33.143-150

El-Sharkawy I, Mila I, Bouzayen M, Jayasankar S (2010). Regulation of two germin-like protein genes during plum fruit development. The Journal of Experimental Botany 61: 1761-1770. doi: 10.1093/jxb/erq043

Emanuelli F, Battilana J, Costantini L, Le Cunff L, Boursiquot J-M et al. (2010). A candidate gene association study on muscat flavor in grapevine (*Vitis vinifera L.).* BMC Plant Biology 10: 241. doi: 10.1186/1471-2229-10-241

Erickson EO, Wilcox WF (1997). Distributions of sensitivities to three sterol demethylation inhibitor fungicides among populations of Uncinula necator sensitive and resistant to triadimefon. Phytopathology 87: 784-791. doi: 10.1094/PHYTO.1997.87.8.784

Feechan A, Jermakow AM, Torregrosa L, Panstruga R, Dry IB (2008). Identification of grapevine MLO gene candidates involved in susceptibility to powdery mildew. Functional Plant Biology 35: 1255-1266. doi: 10.1071/FP08173

Feechan A, Kabbara S, Dry IB (2011). Mechanisms of powdery mildew resistance in the *Vitaceae* family. Molecular Plant Pathology 12: 263-274. doi: 10.1111/j.1364-3703.2010.00668.x.

Fessler C. Kassemeyer HH (1995). The influence of temperature during the development of conidia on the germination of *Uncinula necator.* Vitis 34: 63-64. doi: 10.5073/vitis.1995.34.63-64

Ficke A, Gadoury DM, Seem RC, Dry IB (2003). Effects of ontogenic resistance upon establishment and growth of Uncinula necator on grape berries. Phytopathology 93: 556-563. doi: 10.1094/PHYTO.2003.93.5.556

Fung RWM, Gonzalo M, Fekete C, Kovacs LG, He Y et al. (2008). Powdery mildew induces defense-oriented reprogramming of the transcriptome in a susceptible but not in a resistant grapevine. Plant Physiology 146: 236-249. doi: 10.1104/pp.107.108712

Gadoury DM, Cadle-Davidson L, Wilcox WF, Dry IB, Seem RC et al. (2012a). Grapevine powdery mildew (*Erysiphe necator*): a fascinating system for the study of the biology, ecology and epidemiology of an obligate biotroph. Molecular Plant Pathology 13: 1-16. doi: 10.1111/j.1364-3703.2011.00728.x

Gadoury DM, Seem RC, Ficke A, Wilcox WF (2003). Ontogenic resistance to powdery mildew in grape berries. Phytopathology 93: 547-555. doi: 10.1094/PHYTO.2003.93.5.547

Gadoury DM, Wakefield LM, Cadle-Davidson L, Dry IB, Seem RC (2012b). Effects of prior vegetative growth, inoculum density, light, and mating on conidiation of Erysiphe necator. Phytopathology 102: 65-72. doi: 10.1094/PHYTO-03-11-0085

Gaforio L, Garcia-Munoz S, Cabello F, Munoz-Organero G (2011). Evaluation of susceptibility to powdery mildew (Erysiphe necator) in *Vitis vinifera* varieties. Vitis 50: 123-126.

Gambino G, Perrone I, Gribaudo I (2008). A rapid and effective method for RNA extraction from different tissues of grapevine and other woody plants. Phytochemical Analysis 19: 520-525. doi: 10.1002/pca.1078

Gamir J, Darwiche R, Van't Hof P, Choudhary V, Stumpe M et al. (2017). The sterol-binding activity of PATHOGENESIS-RELATED PROTEIN 1 reveals the mode of action of an antimicrobial protein. The Plant Journal 89: 502-509. doi: 10.1111/tpj.13398

Gee CT, Gadoury DM, Cadle-Davidson L (2008.) Ontogenic resistance to Uncinulanecator varies by genotype and tissue type in a diverse collection of *Vitis* spp. Plant Disease 92: 1067-1073. doi: 10.1094/PDIS-92-7-1067

Godfrey D, Able AJ, Dry IB (2007). Induction of a grapevine germin-like protein (*VvGLP3*) gene is closely linked to the site of *Erysiphe necator* infection: a possible role in defense? Molecular Plant-Microbe Interactions 20: 1112-1125. doi: 10.1094/MPMI-20-9-1112

Goyal N, Bhatia G, Sharma S, Garewal N, Upadhyay A et al. (2019). Genome-wide characterization revealed role of NBS-LRR genes during powdery mildew infection in *Vitis vinifera*. Genomics S0888-7543 (18): 30732-8. doi: 10.1016/j.ygeno.2019.02.011

Haile ZM, Pilati S, Sonego P, Malacarne G, Vrhovsek U et al. (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 & Environment 40: 1409-1428. doi: 10.1111/pce.1293

Kabaktepe Ş, Heluta V, Akata I (2015). Checklist of powdery mildews (Erysiphales) in Turkey. Biyolojik Çeşitlilik ve Koruma 8: 128-146.

Kazan K (2018). A new twist in SA signalling. Nature Plants 4 (6): 327-328. doi: 10.1038/s41477-018-0171-4

Kazan K, Gardiner DM (2017). Targeting pathogen sterols: defence and counterdefence? PLOS Pathogens 18, 13 (5): e1006297. doi: 10.1371/journal.ppat.1006297

Khiavi HK, Davoodi A (2016). Resistance evaluation of some commercial Vitis vinifera varieties to powdery mildew Erysiphe necator Schwein. in two regions of Iran. Journal of Crop Protection 5 (2): 229-237. doi: 10.18869/modares.jcp.5.2.229

Khiavi HK, Shikhlinskiy H, Ahari AB, Akrami M (2012). Evaluation of different grape varieties for resistance to powdery mildew caused by Uncinula necator. African Journal of Agricultural Research 7: 4182-4186. doi: 10.5897/AJAR12.1056

Le Henanff G, Farine S, Kieffer-Mazet F, Miclot AS, Heitz T et al. (2011). Vitis vinifera VvNPR1.1 is the functional ortholog of AtNPR1 and its overexpression in grapevine triggers constitutive activation of PR genes and enhanced resistance to powdery mildew. Planta 234 (2): 405-417. doi: 10.1007/s00425-011-1412-1

Le Henanff G, Heitz T, Mestre P, Mutterer J, Walter B et al. (2009). Characterization of Vitis vinifera NPR1 homologs involved in the regulation of pathogenesis-related gene expression. BMC Plant Biology 9: 54. doi: 10.1186/1471-2229-9-54

León-Galván F, Joaquín-Ramos AdJ, Torres-Pacheco I, De la Rosa APB, Guevara-Olvera L et al. (2011). A germin-like protein gene (CchGLP) of Capsicum chinense Jacq. Is induced during incompatible interactions and displays Mn-Superoxide dismutase activity. International Journal of Molecular Sciences 12: 7301-7313. doi: 10.3390/ijms12117301

Li ZT, Dhekney SA, Gray DJ (2011). PR-1 gene family of grapevine: a uniquely duplicated PR-1 gene from a Vitis interspecific hybrid confers high level resistance to bacterial disease in transgenic tobacco. Plant Cell Reports 30: 1-11. doi: 10.1007/s00299-010-0934-5

Liu Q, Xue Q (2006). Computational identification of novelPR-1-type genes in Oryza sativa. Journal of Genetics and Genomics 85: 193-198. doi: 10.1007/bf02935330

Liu SL, Wu J, Zhang P, Hasi G, Huang Y et al. (2016). Response of phytohormones and correlation of SAR signal pathway genes to the differentresistance levels of grapevine against *Plasmoparaviticola* infection. Plant Physiology and Biochemistry 107: 56-66. doi: 10.1016/j.plaphy.2016.05.020

Mitsuhara I, Iwai T, Seo S, Yanagawa Y, Kawahigasi H et al. (2008). Characteristic expression of twelve rice PR1 family genes in response to pathogen infection, wounding, and defense-related signal compounds (121/180). Molecular Genetics and Genomics 279: 415-427. doi: 10.1007/s00438-008-0322-9

Olmo HP (1971). Vinifera x rotundifolia hybrids as wine grapes. American Journal of Enology and Viticulture 22: 87-91.

Özer C, Solak E, Öztürk L, Özer N (2012). The development of powdery mildew-tolerant grape cultivars with standard quality characteristics by cross breeding. African Journal of. Agricultural Research 7: 1374-1380. doi: 10.5897/AJAR11.871

Pessina S, Lenzi L, Perazzolli M, Campa M, Costa Dalla L et al. (2016). Knockdown of MLO genes reduces susceptibility to powdery mildew in grapevine. Horticulture Research 3: 16016. doi: 10.1038/hortres.2016.16

Pfaffl MW (2001). A new mathematical model for relative quantification in real-time RT–PCR. Nucleic Acids Research 29 (9): e45. doi: 10.1093/nar/29.9.e45

Porep JU, Walter R, Kortekamp A, Carle R (2014). Ergosterol as an objective indicator for grape rot and fungal biomass in grapes. Food Control 37: 77-84. doi: 10.1016/j.foodcont.2013.09.012

Pospíšilová D (1978). Susceptibility of cultivars of Vitis vinifera to powdery mildew (Uncinula necator Schw. Burr.). In: Grapevine genetics and breeding. INRA, Paris, pp. 251-257.

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: 15020. doi: 10.1038/hortres.2015.20

Ramming DW, Gabler F, Smilanick J, Cadle-Davidson M, Barba P et al. (2011). A single dominant locus, ren4, confers rapid non-race-specific resistance to grapevine powdery mildew. Phytopathology. 101 (4): 502-508. doi: 10.1094/PHYTO-09-10-0237.

Repka V, Kubíková J, Fischerová I (2000). Immunodetection of PR-1-like proteins in grapevine leaves infected with Oidiumtuckeri and in elicited suspension cell cultures. Vitis 39: 123-128. doi: 10.5073/vitis.2000.39.123-127

Riaz S, Boursiquot J-M, Dangl GS, Lacombe T, Laucou V et al. (2013). Identification of mildew resistance in wild and cultivated Central Asian grape germplasm. BMC Plant Biology 13: 149. doi: 10.1186/1471-2229-13-149

Roubelakis-Angelakis KA (2009). Grapevine molecular physiology & biotechnology: Springer Science & Business Media.

Sall MA, Teviotdale BL (1981). Powdery mildew, in: Grape Pest Management. D. Flaherty, F. Jensen, A. Kasimatis, H. Kido and W. Moller (editors). Agricultural Sciences Publications, University of California, Berkeley, pp. 46-50

Savocchia S, Stummer B, Wicks T, Van Heeswijck R, Scott E (2004). Reduced sensitivity of Uncinula necator to sterol demethylation inhibiting fungicides in southern Australian vineyards. Australasian Plant Pathology 33: 465-473. doi: 10.1071/AP04066

Staudt G (1997). Evaluation of resistance to grapevine powdery mildew (*Uncinula necator* [Schw.] Burr., anamorph *Oidium tuckeri* Berk.) in accessions of *Vitis* species. Vitis 36: 151-154. doi: 10.5073/vitis.1997.36.151-154

Tangolar S, Ergenoğlu F, Gök S (1996). Ç.Ü. Ziraat Fakültesi Bahçe Bitkileri Bölümü Araştırma Bağı Üzüm Çeşitleri Kataloğu. Ç.Ü. Ziraat Fakültesi Yardımcı Ders Kitapları 29: 94 (in Turkish)

T.C. Tarım ve Köyişleri Bakanlığı Tarımsal Araştırmalar Genel Müdürlüğü (1996). Zirai Mücadele Standart İlaç Deneme Metodları, T.C. Tarım ve Köyişleri Bakanlığı Tarımsal Araştırmalar Genel Müdürlüğü, Ankara, 1, 447 p. (in Turkish)

Van Loon L, Pierpoint W, Boller T, Conejero V (1994). Recommendations for naming plant pathogenesis-related proteins. Plant Molecular Biology Reports 12: 245-264. doi: 10.1007/BF02668748

Van Loon L, Van Strien E (1999). The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. Physiological and Molecular Plant Pathology 55: 85-97. doi: 10.1006/pmpp.1999.0213.

Van Loon LC, Rep M, Pieterse CM (2006). Significance of inducible defense-related proteins in infected plants. Annual Review of Phytopathology 44: 135-162. doi: 10.1146/annurev.phyto.44.070505.143425

Viala P (1893). Les maladies de la vigne, 3éd. *In*.: Masson, Paris.

Wang Y, Liu Y, He P, Chen J, Lamikanra O, Lu J (1995). Evaluation of foliar resistance to Uncinula necator in Chinese wild Vitis species. Vitis 34: 159-164. doi: 10.5073/vitis.1995.34.159-164

Weete JD, Abril M, Blackwell M (2010). Phylogenetic distribution of fungal sterols. PLoS One 28, 5 (5): e10899. doi: 10.1371/journal.pone.0010899

Willocquet L, Colombet D, Rougier M, Fargues J, Clerjeau M (1996). Effects of radiation, especially ultraviolet B, on conidial germination and mycelial growth of grape powdery mildew. European Journal of Plant Pathology 102: 441-449. doi: 10.1007/BF01877138

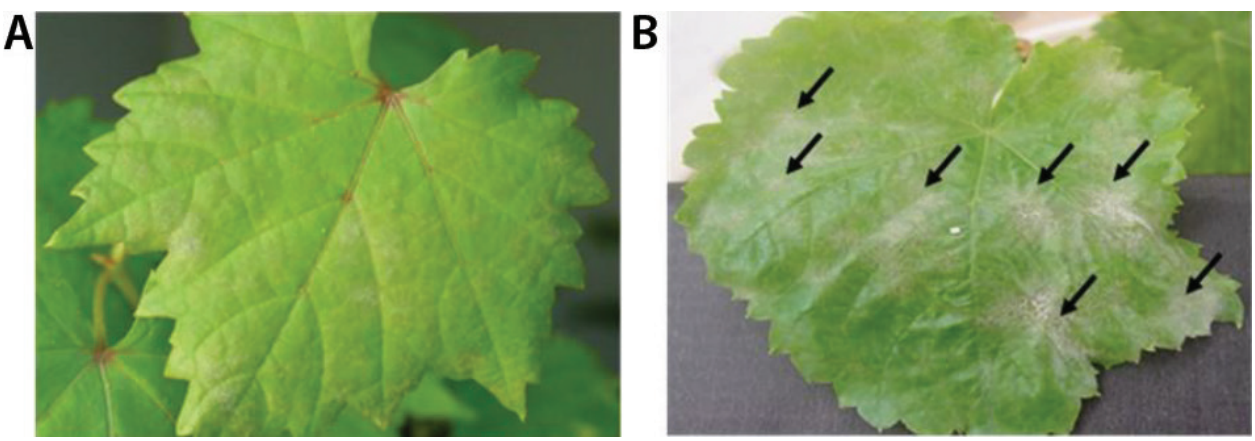
Xu W, Yu Y, Ding J, Hua Z, Wang Y (2010). Characterization of a novel stilbene synthase promoter involved in pathogen-and stress-inducible expression from Chinese wild Vitis pseudoreticulata. Planta 231: 475. doi: 10.1007/s00425-009-1062-8

Xu Y, Zhu Z, Xiao Y, Wang Y (2016). Construction of a cDNA library of Vitis pseudoreticulata native to China inoculated with Uncinula necator and the analysis of potential defence-related expressed sequence tags (ESTs). South African Journal for Enology and Viticulture 30: 65-71. doi: 10.21273/HORTSCI.41.4.993C

Yamamoto T, Iketani H, Ieki H, Nishizawa Y, Notsuka K et al. (2000). Transgenic grapevine plants expressing a rice chitinase with enhanced resistance to fungal pathogens. Plant Cell Reports 19: 639-646. doi: 10.1007/s002999900174

Supplementary Table. Powdery mildew ratings, and bud bursting and berry ripening time classes of the grape cultivars evaluated in the Adana province of Turkey in this study.

Cultivar name	Powdery mildew rating	*Bud bursting times	**Berry ripening times
Horoz karası	Resistance	Medium (2)	Medium (4)
Boğazkere	Resistance	Late (3)	Late (6)
Muscat of Hamburg	Resistance	Medium (2)	Medium (4)
Kyoho	Resistance	Early (1)	Medium early (3)
Reçel Üzümü	Resistance	Early (1)	Medium early (3)
Italia	Resistance	Medium (2)	Medium (4)
Bone Seedless	Resistance	Medium (2)	Medium (4)
Sugraone	Moderately resistant	Early (1)	Early (2)
Papaz karası	Moderately resistant	Late (3)	Late (6)
Yalova Çekirdeksizi	Moderately resistant	Early (1)	Medium early (3)
Royal	Moderately resistant	Medium (2)	Medium (4)
Victoria	Moderately resistant	Medium (2)	Medium early (3)
Black Pearl	Moderately resistant	Late (3)	Late (6)
Trakya İlkeren	Moderately resistant	Early (1)	Very early (1)
Karasakız	Moderately resistant	Medium (2)	Medium late (5)
Razakı	Moderately susceptible	Late (3)	Medium (4)
Flame Seedless	Moderately susceptible	Medium (2)	Early (2)
Ergin Çekirdeksizi	Moderately susceptible	Early (1)	Early (2)
Early Cardinal	Moderately susceptible	Early (1)	Very early (1)
King's Ruby	Moderately susceptible	Medium (2)	Medium (4)
Tekirdağ Çekirdeksizi	Moderately susceptible	Medium (2)	Medium early (3)
Öküzgözü	Moderately susceptible	Medium (2)	Medium (4)
Alphonse Lavallee´	Moderately susceptible	Medium (2)	Medium (4)
Cardinal	Moderately susceptible	Early (1)	Early (2)
Perlette	Susceptible	Medium (2)	Early (2)
Ata Sarısı	Susceptible	Medium (2)	Medium (4)
Crimson Seedless	Susceptible	Medium (2)	Medium late (5)
Güz Üzümü	Susceptible	Late (3)	Late (6)



Supplementary Figure 1. Examples of a powdery mildew resistant (A) and a susceptible (B) grape leaf following inoculations. Arrows in B show powdery mildew development.