Evaluation of gene expression levels in grapevine cultivars after Erysphe necator andmethyl jasmonate treatments

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Abstract: Abstract: Powdery mildew (PM - *Eryshpe necator*) is one of the most important diseases threatening the world of viticulture. This study determined that the gene expression levels of Myc2, WRKY1, and DHN1a in Boğazkere (*V. vinifera* L. PM susceptible), Cabernet Sauvignon (*V. vinifera* L. PM susceptible), Kishmish Vatkana (*V. vinifera* L. PM tolerant), and Regent (*V. vinifera* L. PM tolerant) cultivars after PM and PM-Methyl jasmonate (MeJa) treatments by real-time polymerase chain reaction (RT-PCR). The susceptible and tolerant cultivars that were compared in the study were inoculated with PM which was taken from the natural environment and was sprayed with 50 **µM MeJa. Leaf samples of cultivars were collected at 0, 6, 12, 24, 48, 72, 96, and 120** h postinoculation (hpi). RT-PCR analyses of PM and PM-MeJa treatments were performed on WRKY1, DHN1a, and Myc2 genes. Actin1 was used as the reference gene. The analyses concluded that the susceptible and tolerant cultivars utilized different mechanisms and that TF genes were induced in the presence of PM and MeJa. It was determined that PM showed different levels of expression in 3 genes and 4 cultivars. MeJa treatment had a stimulating effect on the response to *E. necator* infection.

Key words: *Vitis vinifera* L., *Erysiphe necator*, MeJa, gene expression, WRKY1, DHN1a, Myc2

1. Introduction

Powdery mildew (*Erysiphe necator* Schwein (syn. *Unciluna necator* (Schw.)) (Burr.)) is the leading factor that threatens viticulture and causes significant yield and quality losses. All the varieties of *Vitis vinifera* L. species are considered susceptible to PM (Husfeld, 1962).

Jasmonic acid (JA) and Methyl jasmonate (MeJa) are the key regulators of jasmonate response (Staswick, 2008). Jasmonates regulate the environment-specific developmental processes and plant adaptation by controlling responses to biotic and abiotic stimuli (Fonseca et al., 2009). Methyl jasmonate can use the plant›s self-defense system as a signal, and it can be spread by physical contact or airborne to produce a defensive reaction in undamaged plants. MeJa can be stimulated to produce different defense chemicals such as phytoalexins, nicotine, or proteinase inhibitors in the plant. MeJa activates the proteinase inhibitor genes through receptor-mediated signal transmission pathway (Vidhyasekaran, 2016).

Elisitor (such as SA, MeJa, ABA, and Eth) or pathogen-activated TF receptors play an important role in the control of defense gene expression and plant resistance responses (Marchive et al., 2007). TFs regulate the expression of

defense genes in plant immune signal systems induced by PAMPs (pathogen-associated molecular patterns) (Century et al., 2008; Moreau et al., 2012). PAMPs and PIMPs (pathogen-induced molecular pattern) / HAMPs (host-associated molecular pattern) enable the expression of various TF genes involved in plant defense responses (Denoux et al., 2008; Higashi et al., 2008; Chujo et al., 2013; McLellan et al., 2013). Many TFs have been shown to induce the priming and 'systemic acquired resistance' (SAR) of defense responses in plants (Chavan and Kamble, 2013; Nakayama et al., 2013).

In cells containing low levels of bioactive jasmonate, JAZ (Jasmonate-Zim Domain) proteins suppress the activity of positive TFs (e.g., MYC2 and MYC3) involved in the expression of early response genes. JA treatment and environmental stress conditions rapidly trigger the expression of JAZ genes. It creates a negative feedback loop that fills JAZ protein pool and suppresses the response to JA (Zhang et al., 2012).

Myc2 gene is the basic regulator of JA signal pathway. By antagonistically regulating two different paths of JA signal pathway, Myc2 coordinates JA-mediated defense responses that determine resistance to pests and pathogens. Another

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important function of Myc2 is that it regulates the crosstalk between signal pathways of JA and other phytohormones such as ABA, SA, GA (gibberellic acid), and IAA (indole acetic acid), and the interactions of JA signal with light and phytochrome signal with circadian rhythm (Montiel et al., 2011). Myc2 gene has been found to be a positive regulator against defense to insect, response to wounds, flavonoid metabolism, and oxidative stress tolerance during JA signaling. In contrast, Myc2 regulates pathogen defense and secondary metabolism expression negatively during JA signal. In other words, Myc2 acts as both a transcriptional activator and a transcriptional repressor in different aspects of JA signal (Kazan and Manners, 2013). Myc2 activation results in the expression of other TFs, such as MYBs and WRKYs, which are important in stress defense.

Most of the WRKY gene family members are associated with pathogen infection and are, therefore, important factors for plant immunity (Wang et al., 2014). Interactions between WRKY and WRKY-related elicitors may be involved in signaling, transcription, chromatin remodeling, and other cellular processes (Chi et al., 2013). TFs formed by WRKY genes activate expression of defense genes in SA- or JA-dependent signal pathway. It has been found that the resistance of grapevine to PM increases with the activation of JA signal pathway. SA and JA are antagonists for the activation of many genes and have different effects on defense mechanisms. JA accumulates with pathogen attack or injury, and creates a different pathway of defense responses such as accumulation of secondary metabolites (alkaloids, phenolic compounds, and terpenes) and pathogenesis-related proteins (Marchive et al., 2013; Guo et al., 2014). The expression and function of WRKY TF have been induced by MeJa in many studies (Mao et al., 2007; Peng et al., 2012; Le Hénanff et al., 2013; Sun et al., 2013; Jiang et al., 2014; Li et al., 2014).

DHNs respond to PM infection by showing a high degree of expression. In particular, drought, cold, heat, embryogenesis, ABA, SA, and MeJa treatments induced DHN1 gene expression in vitis species. DHN1 transcripts reached maximum induction after MeJa treatment (Yang et al., 2012). DHN1 affects SAR by inducing some defense response molecules such as SA and MeJa (Pieterse et al., 2009).

The present study aimed to determine and compare gene expression levels in WRKY1, DHN1a, and Myc2 genes responding to PM infection and the effect of MeJa treatment on resistance to PM diseases with real-time polymerase chain reaction (RT-PCR). Gene expression levels in PM-infected and MeJa-treated genotypes were compared in four grapevine cultivars susceptible and tolerant at different levels to the disease.

2. Materials and methods

2.1. Plant material and growth conditions

The study was conducted by establishing an experimental setup at Department of Horticulture, Faculty of Agriculture, Ankara University between 2014 and 2018. Boğazkere (*Vitis vinifera* L. PM susceptible), Cabernet Sauvignon (*Vitis vinifera* L. PM susceptible), Kishmish Vatkana (*Vitis vinifera* L. PM tolerant), and Regent (*Vitis vinifera* L. PM tolerant) cultivars have been used as plant material.

Cuttings were prepared to have 2 buds before planting. Cuttings and planting media (Perlite + Peat) were treated with 15% NaOCl for 15 min before planting. Then, the cuttings were rinsed with distilled water 3 times for 1 min each. Next, they were rooted in a climate chamber. After rooting, they were left to develop in a controlled growth chamber where temperature and humidity were adjusted. The climate room was set as temperature: 25 °C; humidity: 75%; light intensity: 3 lux; and lighting time: 16 h day/8 h night. The cultivars were irrigated with Hoagland medium at certain intervals (Hoagland and Arnon 1950).

2.2. *Erysiphe necator* inoculation and Methyl Jasmonate treatments

The cultivars which were used in the present study developed for approximately 45 days until they contained at least 10 leaves and reached 15–30 cm of shoot length. Leaf samples were taken from each cultivar before the treatments for control. *E. necator* was collected for inoculation from the infected C. Sauvignon leaves from the vineyard. Inoculation was performed with the conidial suspension at the rate of 2×10^5 conidia/mL by spraying the upper surface of the leaves (Atak et al., 2017). After all the experimental pots were infected with PM, 50 µM of MeJa was applied to half of the pots by spraying the leaves (Repka et al., 2004). After the treatments, leaf samples were collected in 3 biological and 2 technical repetitions, provided that they were from different pots for each cultivar, to be used in at 6, 12, 24, 48, 72, 96, and 120 hpi analyses. The samples were stored in a deep freezer at –80 °C. After the sampling, the conditions of the growth chamber were changed in order to provide high temperature and high humidity, which are suitable growth conditions for PM. The conditions were adjusted to have a temperature of 25–30 °C, humidity of 80%–90%, light intensity of 3 lux, and illumination duration of 12 h day/12 h night.

2.3. RNA isolation

Total RNA isolations from the control group and the treated grapevine leaves were performed according to 'Ribospin Plant Total RNA Purification Kit' (Cat no: 307–150) protocol. After the isolation, RNAs were stored in a

deep freezer at –80 °C. The quality and concentration of the isolated RNA were measured using a Nanodrop ND-Spectrometer 1000 (Thermo–Fisher Scientific, USA).

2.4. cDNA synthesis
cDNA synthesis from RNA samples was performed according to 'First-strand cDNA synthesis kit' (Gene All, Hyperscript First strand synthesis kit, Cat no: 601–005) protocol. cDNAs were stored in a deep freezer at –80 °C. After cDNA synthesis, cDNA quality and concentration were measured with a Nanodrop ND-Spectrometer 1000.

2.5. Primer design and real-time PCR reaction
The primers which were used in RT-PCR were designed with specific oligonucleotides of conserved regions using WRKY1, DHN1a, Myc2, and Actin1 gene sequences from NCBI database. Actin1 was selected as the reference (housekeeping) gene and used for normalization. The primers were designed using PerlPrimer v.1.1.21 and the OligoAnalyzer Tool software. DNA sequences and accession numbers of the primers are given in Table.

RT-PCR reaction was performed using SYBR Green Master mix (GeneAll SybrGreen Master Mix Cat No: 801-521) in an 'Applied Biosystems StepOnePlus Real-time PCR' device. The peak profiles of RT-PCR reaction of leaf samples taken at different times for 2 different treatments (PM inoculation and PM-MeJa inoculation) of all 4 genes (WRKY1, Myc2, DHN1a, Actin1) were used in the study. Ct values of each sample were determined from these profiles. The obtained Ct values and standard curve graphs of 4 genes were determined quantitatively.

2.6. Normalization and statistical analysis
Transcript profiles of the plants which were treated with PM and PM-MeJa were compared with the control profiles and the reference gene. Statistical evaluation of the obtained data was made with Livak and Schmittgen's $2^{-\Delta\Delta CT}$ formula and one-way ANOVA (Livak and Schmittgen, 2001). Analysis of variance was used to test the hypothesis whether the difference between the means of two or more groups was significant. If the means of more than two groups are to be compared, F-test, also known as the analysis of variance (ANOVA), was applied. When the researchers need to compare a control group with more than one experimental group, it is recommended to use the Dunnet test (Dunnet, 1955). In this way, the results of differentiating gene expression in stress treatments were normalized by considering the reference gene Actin1 and control profiles. The mean, standard deviation, standard error, and statistical significance of these data were calculated with the statistical program (SPSS 18). Thus, mRNA expression levels of 4 genes were determined in the plants treated with PM and PM-MeJa. The small Sig value indicates that the change in gene expression level depending on time in PM and PM-MeJa treatments is statistically significant at $p < 0.05$ level and there is a significant difference between the means of the groups being compared. As the analysis of variance cannot report which one of the compared groups is differed, the post hoc test was performed to find it out in case of a significant difference.

3. Results
3.1. Normalization and statistical analysis
In the study, the differences in Myc2, WRKY1, and DHN1a gene expressions in the selected cultivars were determined under stress conditions after 0, 6, 12, 24, 48, 72, 96, and 120 hpi PM and PM-MeJa treatments.

The responses of the control and the treated cultivars by RT-PCR reaction were determined using the primers given in Table.

The graphs of the gene expressions obtained as a result of PM and PM-MeJa treatments of the cultivars, and comparison between the treatmens and the cultivars for each gene are given in Figures 1 and 2, respectively.

Table. DNA sequences, amplicon lengths, and accession numbers of the primers.

Primer	DNA sequences	Amplicon length	Accession number
Reference gene			
Vv-Actin1	F: TCACCACTACTGCTGAACGG R: AGAGGACTTCTGGACAACGG	185 bp	AY680701.1
Target genes			
Vv-DHN1a	F: TCTGTAAGTTGCCATGCCA R: TCCCTTTCTTCCTTCTCCCA	130 bp	JN689936.1
Vv-Myc2	F: CTTAGCCAGTTCCAGCCT R: GTTCAGTTTCTCACGCCTC	254 bp	EF636725.2
Vv-WRKY1	F: AGATGACTGAAGAGGAGCCC R: GTGCTGTCCGTACAAGAAGAG	341 bp	GQ884198.1

Figure 1. Comparison of Myc2 (A,D,G,J), WRKY1 (B,E,H,K) and DHN1a (C,F,I,L) gene expression levels in Boğazkere (A,B,C), C. Sauvignon (D,E,F), K. Vatkana (G,H,I), and Regent (J,K,L) cultivars to PM and PM+MeJa treatments analyzed using Actin1 gene.

The mean, standard error, and standard deviation of gene expression data were calculated after normalization. The value in Sig (Significant) column of ANOVA table is seen as 0.00. Additionally, post hoc test data of PM- and PM-MeJa–treated samples were evaluated (data not shown).

3.2. Gene expression levels of cultivars

3.2.1. Boğazkere Myc2 gene expression levels
Myc2 gave the strongest gene expression at 6 hpi PM treatment in Boğazkere. At 6 hpi, an increase of approximately 5-fold was observed compared to the control. It was evaluated as the first moment when the plant encountered stress. No significant difference was observed compared to the control at 12–96 hpi range. Twenty-four, 72, and 96 hpi were found to be statistically nonsignificant. At 120 hpi, Myc2 gene expression showed the greatest increase (more than 6-fold). In Boğazkere's response to PM-MeJa treatment, Myc2 gene showed the strongest expression at 6 hpi and 12 hpi (6 hpi: 12-fold; 12 hpi: 14-fold). Similarly, at other times, there was a continuous increase compared to the control. The
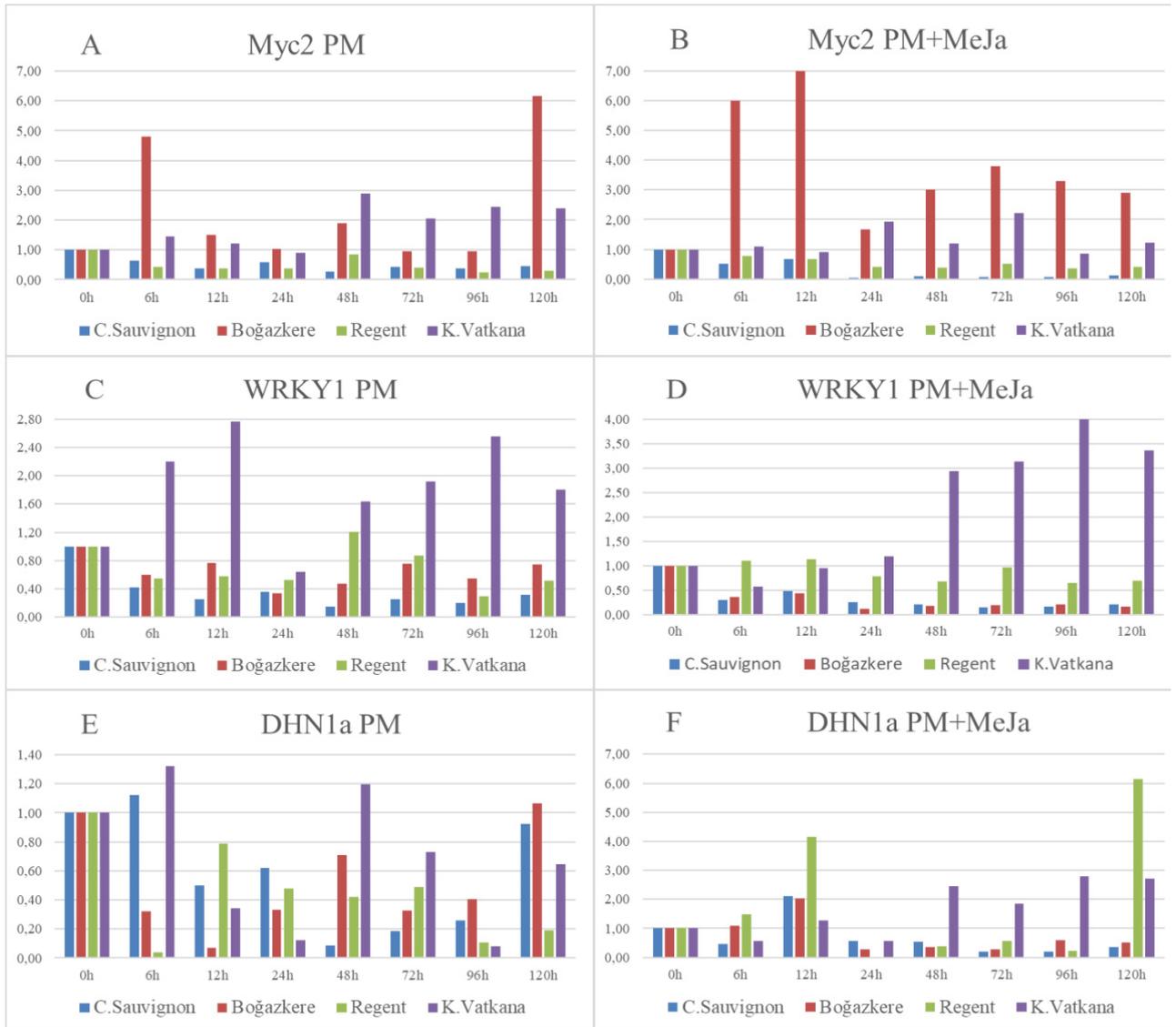
Figure 2. Comparison of Myc2 (A,B), WRKY1 (C,D), and DHN1a (E,F) gene expression levels in 4 cultivars to PM and PM+MeJa treatments analyzed using Actin1 gene.

detection of significant increases relative to the control at all treatment durations clearly indicates that MeJa affects Myc2 gene region. When PM and PM-MeJa treatments on Boğazkere were compared, it became clearer how effective MeJa was in plant defense in Myc2 gene expression. The comparison of graphs showed that MeJa treatment produced significant increases in Myc2 gene expression. The expressions were level at 120 hpi in both treatments, indicating that the effect of MeJa disappeared. In all other time periods, more gene expression was observed in MeJa treatment compared to *E. necator* treatment. These results suggest that MeJa with Myc2 may play a role in plant disease resistance (Figure 1A).

3.2.2. Boğazkere WRKY1 gene expression levels
Boğazkere demonstrated a decreased WRKY1 gene expression in response to PM treatment compared to the control. It was concluded that PM suppressed WRKY1 gene expression. In Boğazkere's response to PM-MeJa treatment, a decreased expression was observed in WRKY1 gene expression compared to the control as well. PM-MeJa treatment greatly suppressed WRKY1 gene expression. When PM and PM-MeJa treatments on Boğazkere were compared, it was observed that WRKY1 gene expression was a negative regulator and MeJa treatment reduced WRKY1 gene expression further (Figure 1B).

3.2.3. Boğazkere DHN1a gene expression levels

DHN1a gene expression was suppressed in Boğazkere's response to PM treatment. There was only a slight increase at 120 hpi compared to the control, which was found to be statistically nonsignificant. In Boğazkere's response to PM-MeJa treatment, DHN1a gene expression was first increased and then decreased compared to the control. MeJa caused a slight increase in DHN1a gene expression at 6 hpi, but at other times, decreased expressions were determined compared to the control. When evaluated statistically, this slight increase at 6 hpi was found to be nonsignificant. When PM and PM-MeJa treatments were compared, MeJa treatment increased DHN1a gene expression up to 12 hpi and gave the strongest defense responses (6 hpi: 3.5-fold; 12 hpi: 29-fold). MeJa effect disappeared at 24 hpi and upwards (Figure 1C).

3.2.4. Cabernet Sauvignon Myc2 gene expression levels

In terms of PM treatment on C. Sauvignon, Myc2 gene expression was upregulated relative to the control. Myc2 gene expression was observed to decrease in PM-MeJa treatment on C. Sauvignon. Almost no expression was found in the time periods except for 6 hpi and 12 hpi. Comparing PM and PM-MeJa treatments for C. Sauvignon, the effect of MeJa was increased 2-fold only at 12 hpi while MeJa had a negative effect on Myc2 gene at the rest of the other times (Figure 1D).

3.2.5. Cabernet Sauvignon WRKY1 gene expression levels

WRKY1 gene expression decreased in PM treatment on C. Sauvignon compared to the control. Similarly, the expression decreased with PM-MeJa treatment. When PM and PM-MeJa treatments were compared, it is seen that the effect of MeJa increased at 12 hpi (2-fold) and 48 hpi (1.5-fold). The decreases observed at other times were small (Figure 1E).

3.2.6. Cabernet Sauvignon DHN1a gene expression levels

In the response of C. Sauvignon to PM treatment, DHN1a gene expression was only slightly increased at 6 hpi compared to the control. In case of PM-MeJa treatment, DHN1a gene expression was decreased in all time periods except for 12 hpi. An approximate 2-fold increase was seen at 12 hpi. The effect of MeJa varied in PM and PM-MeJa treatments for C. Sauvignon. It decreased at 6 hpi and 120 hpi but increased at 12 hpi and 48 hpi. MeJa was not effective at 24, 72, and 96 hpi (Figure 1F).

3.2.7. Kishmish Vatkana Myc2 gene expression levels

The effects of PM treatment on Myc2 gene expression in K. Vatkana showed that the defense-related mechanisms emerged at 48 hpi, and Myc2 gene expression was positively regulated (48 hpi: 3-fold; 72 hpi: 2-fold; 96 hpi: 2.5-fold; 120 hpi: 2.5-fold). The expressions at 12 and 24 hpi were found to be nonsignificant. As a response to PM-MeJa treatment,

Myc2 gene expression in K. Vatkana increased at 24 hpi (~2-fold) and 72 hpi (~2.5-fold). The responses at 6, 12, 48, 96, and 120 hpi were found to be nonsignificant. A comparison of PM and PM-MeJa treatments show that MeJa treatment decreased at 6, 12, 48, 96, and 120 hpi but increased at 24 hpi and 72 hpi (Figure 1G).

3.2.8. Kishmish Vatkana WRKY1 gene expression levels

WRKY1 gene expression showed an increased expression profile in K. Vatkana as a response to PM treatment. It decreased only at 24 hpi. In K. Vatkana's response to PM-MeJa treatment, WRKY1 gene expression decreased at 6 hpi and 12 hpi while it increased at other time periods. Statistically, at 12 hpi and 24 hpi were found to be nonsignificant. When PM and PM-MeJa treatments were compared, the effect of MeJa showed a decreased expression at 6 hpi and 12 hpi while it increased at other times. These results show that the effect of MeJa appeared at 24 hpi and played a role in its defense by increasing WRKY1 gene expression (Figure 1H).

3.2.9. Kishmish Vatkana DHN1a gene expression levels

DHN1a gene expression demonstrated an irregular expression in K. Vatkana's response to PM treatment. While there was a slight increase in 6 hpi and 48 hpi compared to the control, it decreased at other times. Statistically, the response at 48 hpi was found to be nonsignificant. DHN1a gene expression, as a response to PM-MeJa treatment, decreased at 6 hpi and 24 hpi compared to the control but increased at other times. Statistically, at 12 hpi was found to be nonsignificant. Comparing PM and PM-MeJa treatments, the effect of MeJa decreased only at 6 hpi, while it increased at other times. These results suggest that MeJa treatment in K. Vatkana effectively stimulated the defense responses of DHN1a gene at 12 hpi (Figure 1I).

3.2.10. Regent Myc2 gene expression levels

It was observed that Myc2 gene expression in Regent was suppressed in its response to PM treatment and had similar expression levels except for 48 hpi. Myc2 gene expression was decreased in PM-MeJa treatment compared to the control. When PM and PM-MeJa treatments were compared, it was observed that the effect of MeJa increased 2-fold at 6 hpi and 12 hpi (Figure 1J).

3.2.11. Regent WRKY1 gene expression levels

In Regent's response to PM treatment, WRKY1 gene expression showed a decreased expression compared to the control except for 48 hpi. The responses at 48 hpi and 72 hpi were statistically nonsignificant. As a response to PM-MeJa treatment, an increase was observed in WRKY1 gene expression in Regent at 6 hpi and 12 hpi, although it was not as high as the control. When PM and PM-MeJa treatments were compared, it was seen that WRKY1 gene expression increased with MeJa treatment (Figure 1K).

3.2.12. Regent DHN1a gene expression levels

In Regent's response to PM treatment, DHN1a gene expression exhibited a decreased expression compared to the control. In particular, there was no DHN1a gene expression at 6 hpi. The gene expression increased again at 12 hpi but continued to decrease over subsequent time periods. After PM-MeJa treatment, DHN1a gene expression increased at 6 hpi (1.5-fold) and 12 hpi (4-fold) compared to the control. In the following time periods, the expressions decreased compared to the control. These results suggested that the effect of MeJa appeared at 6 hpi and 12 hpi in Regent and was effective in plant defense. At 120 hpi, it was determined that DHN1a gene expression increased 6-fold compared to the control. When PM and PM-MeJa treatments were compared, MeJa was found to significantly increase DHN1a gene expression. These increases were the greatest at 6 hpi (37-fold), 12 hpi (5-fold), and 120 hpi (32-fold) (Figure 1L).

3.3. Comparison of gene expression levels of cultivars

3.3.1. Comparison of Myc2 gene expression levels by cultivar

When Myc2 gene expression was compared in 4 cultivars after PM treatment, there was a 5-fold increase in 6 hpi, especially in Boğazkere, compared to other cultivars. Boğazkere and K. Vatkana showed more expression at 12 hpi and 24 hpi, respectively, than the other 2 cultivars. However, K. Vatkana demonstrated more expression at 48, 72, and 96 hpi in Myc2 gene than Boğazkere. At 120 hpi, Boğazkere exhibited 2.5-fold more expression than K. Vatkana. Myc2 gene expressions of C. Sauvignon and Regent were close to each other in all time periods (Figure 2A).

Myc2 gene expression was compared in 4 cultivars after PM-MeJa treatment, and it was observed that Boğazkere showed more gene expression than other cultivars (except at 24 hpi). It was observed that the gene expression difference between the cultivars increased from 2-fold to 94.5-fold. After Boğazkere, K. Vatkana was the cultivar with the most expression, but it was not as remarkable as Boğazkere. Regent and C. Sauvignon showed decreasing expression following K. Vatkana. Gene expression in C. Sauvignon was less than other cultivars. Between the two tolerant cultivars, K. Vatkana showed higher gene expression than Regent (Figure 2B).

3.3.2. Comparison of WRKY1 gene expression levels by cultivar

When WRKY1 gene expression was compared in 4 cultivars after PM treatment, it was seen that K. Vatkana showed a much higher gene expression (between 1.5- and 13-fold) than all other cultivars. This expression occurred at most at 12 hpi. Other cultivars showed similar expressions (Figure 2C).

WRKY1 gene expression was compared in 4 cultivars after PM-MeJa treatment, and the conclusion is that K. Vatkana showed a higher gene expression than other cultivars. This difference was evident at 48 hpi and higher. C. Sauvignon and Boğazkere showed similar expressions, and the cultivar with the most expression was Regent following K. Vatkana (Figure 2D).

3.3.3. Comparison of DHN1a gene expression levels of cultivars

When DHN1a gene expression was compared in 4 cultivars after PM treatment, a variable distribution was observed. The highest expressions were recorded at 6, 48, and 72 hpi for K. Vatkana; at 12 hpi for Regent; at 24 hpi for C. Sauvignon; and at 96 hpi and 120 hpi for Boğazkere (Figure 2E).

When DHN1a gene expression was compared in 4 cultivars after PM-MeJa treatment, Regent was determined to show significantly increased expression at 12 hpi and 120 hpi compared to other cultivars. K. Vatkana exhibited an increased expression profile at 48 hpi and higher. DHN1a gene was expressed the most at 12 hpi in all cultivars compared to the control (Figure 2F).

4. Discussion

Disease resistance in plants depends on many reactions from the cytological level to the molecular level, including the morphological characteristics of the epidermal cell, signal transmission, and the number, diversity, and severity of genes related to disease resistance mechanism (Zhu et al., 2012). Therefore, although it is natural that there are differences in the expressions of TF genes in susceptible and tolerant plants, examining and evaluating other mechanisms and pathways collectively will contribute to understanding the differences in gene expressions.

In the present study, Myc2 gene expression was seen to differ in susceptible and tolerant cultivars. Susceptible Boğazkere was the cultivar that demonstrated the most expression in both PM and PM-MeJa treatments. Tolerant K. Vatkana stood out as the second most expressed cultivar after Boğazkere. These results may be interpreted as that Myc2 was positively expressed on PM in Boğazkere and K. Vatkana and reflects basic plant-triggered immunity by participating in basal defense. In this comparison, although susceptible Boğazkere and tolerant K. Vatkana cultivars followed each other, the upregulation in Boğazkere only at 6 hpi and in K. Vatkana after at 48 hpi indicates that defense mechanisms are activated in different time periods. This dissimilarity also indicates itself in operating genes in the same pathways. A similar result was observed by Zhang et al. (2019) who reported *E. necator* treatment in the tolerant V. *quinquangularis* Shang-24 which demonstrated the highest expression of VqJAZ4 gene at 72 hpi, followed by 96 hpi and 120 hpi as a higher increase

compared to the control. These transcriptional responses showed that VqJAZ4 can affect the regulation of responses to PM. These results, which are parallel to the expressions of Myc2 and JAZ4 genes and are related to each other in the pathways in which PM resistance genes are regulated, suggested that 2 genes participate in PM response. In the present study, susceptible C. Sauvignon and tolerant Regent exhibited decreased expressions. These results showed that different mechanisms may be involved in the suppression of this gene in K. Vatkana and Regent. The cultivar with the least expression in Myc2 gene was found to be C. Sauvignon. However, it was determined as a remarkable result because Boğazkere, as a susceptible cultivar, showed maximum expression of a gene such as Myc2, which has important roles in plant defense. Therefore, it is important to study the defense mechanisms of Boğazkere against pathogens in detail. In the present research, other cultivars except Boğazkere provided results in parallel with the expectations in terms of Myc2 gene expression.

When the effect of MeJa treatment on cultivars was evaluated, it was seen that Myc2 gene expression was positively effected in Boğazkere. Lorenzo et al. (2004) proposed that Myc2 gene positively regulates JA-susceptible genes. It has been clearly seen in the present results that the treatment of MeJa, which increased the expression of Myc2 gene, is effective in the defense of Boğazkere. On the other hand, Myc2 gene expression increased slightly with MeJa treatment in Regent. It is known that Myc2 gene acts as a negative regulator in JA pathway (Kazan and Manners, 2013). It also proves that MeJa acts as a negative regulator in plant defense in tolerant Regent. In K. Vatkana which is also tolerant, MeJa treatment generally decreased Myc2 gene expression and showed an inconsistent increase-decrease. It showed that MeJa treatment revealed a variable mechanism on Myc2 gene expression. It is thought that different TFs or pathways may play a role in the defense mechanisms in tolerant K. Vatkana. Induced resistance does not necessarily require direct activation of defense responses but may also result from the expression of a faster and stronger basal defense response upon pathogenic attack. The increased capacity to express infection-induced basal defenses is called 'priming' (Conrath, 2009) which causes faster and stronger induction of defense mechanisms after pathogenic attack (Jung et al., 2009; Conrath, 2011; Martinez-Medina et al., 2013; Po-Wen et al., 2013). The mechanisms involved here may have negatively affected MeJa activity through SA for tolerant K. Vatkana. In addition, MeJa treatment in tolerant Shang-24 with VqJAZ4 gene approximately increased 3-fold at 1 hpi and resulted in an expression close to the control at other intervals in a study by Zhang et al. (2019). These increases and decreases in gene expressions have similar expressions in Myc2 and JAZ4 which work synergistically. Unlike

Boğazkere, MeJa treatment in C. Sauvignon decreased Myc2 gene expression significantly. Since Myc2 is known to act as a negative regulator of JA pathway in a susceptible cultivar such as C. Sauvignon, it is expected that MeJa would reduce Myc2 gene expression. MeJa has been shown to be a stimulant on PM to regress the disease. It is thought that Myc2 displays a negative profile with the effect of other TFs that regulate down-stream JA response genes involved in MeJa signal pathways. It can be inferred that PM affects SA signal pathway in order to obstruct defense mechanisms and SA, which works antagonistically with JA, inhibits this mechanism. Therefore, MeJa might have negatively affected Myc2 gene expression. SA, JA, and Eth defense signal pathways do not function independently but exhibit complex cross-talk and interaction including synergism and antagonism during the defense response (Glazebrook, 2005).

As another gene evaluated in the present study, WRKY1 provided expected expressions in susceptible and tolerant cultivars. It has decreasing expressions in susceptible Boğazkere and C. Sauvignon but increasing expressions in tolerant Regent and K. Vatkana. Li et al. (2010) studied VpWRKY1 expression induced by E. necator infection in 11 grapevine genotypes (5 tolerant, 6 susceptible). Maximum induction of VpWRKY1 was found to be higher in E. necator-resistant grapevine genotypes than in susceptible ones after E. necator inoculation. These results are in parallel with the findings obtained in the present study. All E. necator-resistant grapevine genotypes had more than four-fold maximum VvWRKY1 induction. In the present study, the highest expression of VvWRKY1 in K. Vatkana occurred during early infection times. Li et al. (2010) observed the maximum induction of WRKY1 in E. necator-resistant V. pseadoreticulata Baihe-35-1 genotype at 12 hpi, similar to the results obtained in this study. VpWRKY1 expression levels peaked at 6–12 hpi and then decreased to original levels at 96–120 hpi in all genotypes. The maximum induction of VpWRKY1 was observed at 12 hpi in E. necator-resistant genotype Baihe-35-1. Considering that WRKY1 gene expression is the highest at 12 hpi in Boğazkere, it may be stated that 12 hpi is an important time period in the response to PM. Guo et al. (2014) reported that VvWRKY53 (named as VvWRKY1 by Marchive et al. 2007) significantly increased expressions 24 h after inoculation. In the present study, WRKY1 gene expressions were generally found to be at their highest level after 12 hpi PM inoculation in Boğazkere and K. Vatkana. Therefore, it is concluded that VvWRKY53 and WRKY1 genes which were studied here may play a role in the emergence of the resistance response during the early phase of the infection. It has been suggested that WRKY1 gene has an important role in inducing the basal defense response against E. necator, with little or no involvement

in the mechanism mediated by resistance genes (Guo et al., 2014). Wang et al. (2014) reported more VvWRKY expression in susceptible C. Sauvignon than resistant Norton after *E. necator* infection, contrary to the results in the present study. Calonnec et al. (2021) suggested that Artaban (VRH3082-1-42 x Regent), Prior, and C. Sauvignon overexpressed VvWRKY1 after inoculation in all leaves. Additionally, gene expression for VvWRKY2 was suppressed in these cultivars. Overexpression of VvWRKY2 in C. Sauvignon was associated positively with the disease. This result supports the hypothesis of an interaction between the detection of PAMP by WRKY1 and the protein encoded by WRKY2 and the resistance gene for Artaban. However, WRKY1 acted as a suppressor of PAMP-induced basal defense against PM in Regent in the present study.

The transcriptional response to MeJa and other elicitors is much faster than the metabolic response (Belhadj et al., 2008; Vannozzi et al., 2012). The increases in WRKY1 gene expression after MeJa treatment, especially in K. Vatkana at 48 hpi and later, support this conclusion. Considering that plant disease resistance is supported by JA pathway (Zhang et al., 2015), it has been observed that there is a large increase in PM-MeJa treatment at 48 hpi and later. The current results show that MeJa treatments increased WRKY1 gene expression significantly in K. Vatkana but a little in Regent. These results support the hypothesis that different resistance mechanisms may exist in Regent and K. Vatkana. Li et al. (2010) determined that the basal VpWRKY1 transcript level in Baihe-35-1 was not significantly induced by MeJa and reported a decreased expression compared to their control, which is similar in the current study. Similar expression profiles in tolerant Regent and Baihe-35-1 is a surprising but expected result for these two WRKY1 genes in different groups. Susceptible Boğazkere and C. Sauvignon demonstrated decreased gene expressions with MeJa treatment.

It has been suggested that WRKY1 gene may be involved in signal pathways that include SA signal-mediated hypersensitive response (HR) and a different set of defense responses may be activated by JA/Ethylene signal (Marchive et al., 2007). Marchive et al. (2007) determined that transcript accumulation reached its maximum at 4 hpi and 8 hpi and started to decrease at 24 hpi after SA treatment. Similarly, MeJa treatment reduced WRKY1 expression in Boğazkere at 24 hpi and later in the present study. The involvement of JA signal in biotrophic infection is still understood poorly. A detailed analysis of jasmonates and SA together with the metabolic flux analysis of the jasmonate pathway can confirm the modulation of jasmonate metabolism in response to PM (Pimentel et al., 2021). Additionally, exogenous treatment of MeJa to *V. vinifera* elicited tolerance to *E. necator* infection, and JA

signaling was associated with defense responses to PM in grapevine in a study by Belhadj et al. (2006). Therefore, it may be hypothesized that jasmonates may function in SA-mediated responses as cross-talk (Robert-Seilaniantz et al., 2011) and possibly in interaction with signal pathways involving ABA and IAA (Coelho et al., 2019).

DHN1a gene expression exhibited almost expected results in the susceptible and tolerant cultivars. Dehydrins protect plant cells from desiccation damage during environmental stress and participate in host resistance against various pathogens (Yang et al., 2012). In the current study, this feature of DHN1a gene was interpreted as stimulating pathways that are effective in activating the gene after PM-induced stress in leaves. It was observed that these pathways increased the expression of DHN1a gene by activating especially at 12 hpi, but this increase was still negative compared to the control. Wan et al. (2007) defined *V. yeshanensis* Yanshan-1 as susceptible to PM, whereas Yang et al. (2012) evaluated it as tolerant and stated that it showed an expression profile similar to susceptible Pinot Noir (*V. vinifera* cv.). In the present study, this gene showed early expression only at 6 hpi with a slight increase in K. Vatkana and C. Sauvignon. Boğazkere attempted to create a defense at 48 hpi, but it was negative compared with the control. Yang et al. (2012) reported that DHN1 demonstrated an increased expression in *V. yeshanensis* and *V. vinifera* L. after inoculation with *E. necator*, and the expression level of DHN1 was higher in resistant *V. yeshanensis* than susceptible *V. vinifera* L.. It has been hypothesized that tolerant cultivars activate their chemical and structural defense earlier than susceptible cultivars to prevent penetration by pathogens (Dai et al., 1995). DHN1a gene behaved differently in the presence of MeJa in tolerant cultivars. Regent increased expression up to 12 hpi but then decreased it. K. Vatkana showed an increase at 48 hpi as a late activation. PM treatment alone caused an irregular expression profile on DHN1a gene expression in K. Vatkana. An expression increase was detected with MeJa treatment at 48 hpi and later. It has been interpreted as that MeJa regulates DHN1a gene expression. The highest gene expression was detected in Regent at 120 hpi. DHN1a gene expression increased in tolerant Regent and K. Vatkana. These results show that DHN1a can be effective in plant defense mechanisms of tolerant Regent and K. Vatkana. Susceptible Boğazkere and C. Sauvignon showed increased gene expression at 12 hpi and decreased afterwards. Yang et al. (2012) reported that DHN1 transcripts from MeJa-treated leaves showed early expression with the highest stimulation approximately 4 h after treatment. In the present study, cultivars except K. Vatkana showed early expression at 12 hpi and the results were found to be compatible with each other.

Although the results of 3 gene regions and 4 cultivars studied in the study differ, the current study showed that

MeJa was an effective elicitor on *E. necator* by stimulating defense responses. Tolerant cultivars have a higher induction of programmed cell death than susceptible cultivars (Feechan et al., 2011). This suggests that susceptible and tolerant cultivars use different mechanisms and are stimulated by different TF genes in the presence of PM. A better understanding of gene expressions and the pathways of these genes can be obtained by analyzing chemical contents such as phytoalexins (stilbenes), flavonoids, and phenolic compounds, which may lead to productive knowledge for science and viticulture.

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