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Induction of Defense-related Responses and Suppression of Grey Mold in Grapevines Treated with Defense Response Signaling Mulecules

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Abstract

The induction of defense responses in grapevines to fungal pathogens was studied on leaves of the cultivars 'Campbell Early' and 'Kyoho' which were treated with ethephon, hydrogen peroxide (H_2O_2), methyl jasmonate (MeJA), and salicylic acid (SA). Applying the chemicals resulted in an increase in expression of several defense-related genes and accumulation of stilbene compounds and gamma-aminobutyric acid (GABA). The expression of plant defense-related genes such as catalase (*Cat*), chalcone synthesis (*CHS*), chitinase-like protein (*CLP*), flavonol synthase gene (*FLS*), glutathione-*S*-transferase (*GST*), stilbene synthase (*STSY*), and thaumatin-like protein (*TLP*), as well as signal transduction-related genes including lipoxygenase (*LOX*), was variously induced by the treatments in both cultivars. Additionally, cell wall modification-related genes such as polygalacturonase-inhibiting protein (*PGIP*) and proline rich protein (*PRP2*) were slightly upregulated by treatment with signaling molecules in both 'Campbell Early' and 'Kyoho'. Stilbenoid glucosides, including *trans*- and *cis*-piceid, accumulated to high concentrations in grapevines that were subjected to chemical treatment. The concentration of GABA in leaves increased following pretreatment with all of the signaling molecules tested. Additionally, pretreatment with signaling molecules resulted in reduced lesion size after inoculation of leaves with *Botrytis cinerea*. These results indicate that plant signaling molecules can be used to elicit a resistance responses against infection by pathogens in grapevine and might offer a tool to manage certain diseases.

Grapes (*Vitis vinifera*) are one of the most economically important fruit crops worldwide (Pearson and Goheen, 1998). However, cultivated grapevines are subject to a number of bacterial, fungal, and viral diseases (Wang et al., 2011). Grey mold caused by the necrotrophic pathogen *Botrytis cinerea* is one of the most common diseases that afflict grapes, resulting in serious economic damage during grape production from bud break to harvest in protected cultivation in Korea (Jang et al., 1995). In grapevines, botrytis rot has been associated with infections of mature grapes following late season rains or prolonged periods of high relative humidity (McClellan and Hewitt, 1973). However, owing to the difficulty in controlling this fungus by fungicides due to the appearance of fungicide resistant strains (Kim et al., 1993; Kim and Kwon, 1993), the development of eco-friendly management systems including the use of induced resistance is being considered (Belhadj et al., 2008; Jeandet et al., 2002).

Plant defense responses are regulated through a complex network of signaling pathways that involve endogenous plant signaling molecules including salicylic acid (SA), jasmonic acid (JA), ethylene (ET), and hydrogen peroxide (H_2O_2) (Hung et al., 2005; Kunkel and Brooks, 2002). These signaling molecules are important regulators known to induce disease resistance against many plant pathogens (Chong et al., 2008; Koehl et al., 2007; Malamy et al., 1990; Repka, 2006; Walters et al., 2002). Moreover, exogenous application of SA, JA and ET induces the production of antimicrobial compounds such as phytoalexins and pathogenesis-related

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(PR) proteins (Belhadj et al., 2006; Lamb and Dixon, 1997; Larronde et al., 2003; Pieterse and van Loon, 1999).

Phytoalexins are defined as low molecular weight, anti-microbial secondary metabolites that are both synthesized and accumulated by plants in response to fungal infection or abiotic stresses such as UV irradiation and heavy metal ions (Adrian et al., 1996; Bailey, 1982; Paxton, 1981). Stilbene compounds, resveratrol (trans-3,5,4'-trihydroxystilbene) (Jeandet et al., 1995; Langcake and Pryce, 1977) and its oligomers (Waterhouse and Lamuela-Raventos, 1994), viniferins and *trans*-pterostilbene (3,5 dimethoxy-4'-hydroxystilbene) are produced as phytoalexins in grapevines as a response to infection with various pathogens including *Botrytis cinerea* (Adrian et al., 1997), *Plasmopara viticola* (Pezet et al., 2004), and *Erysiphe necator* (Schnee et al., 2008).

Gamma-aminobutyric acid (GABA) is a non-protein amino acid that is present in a number of plant species (Steward et al., 1949) and is synthesised from glutamate by glutamate decarboxylase, whose activity is highly regulated by calmodulin (Baum et al., 1996). The role of GABA in plants has remained unclear over the past 60 years following its discovery (Park et al., 2010), but GABA is known to play a role in pH regulation (Bown and Shelp 1997), nitrogen storage (Selman and Cooper, 1978), plant development (Baum et al., 1996), and plant defense responses (Bown et al., 2006).

Exogenous application of signaling molecules to plants is assumed to increase the defense response and accumulation of stilbene compounds and GABA have been shown to occur in response to environmental stress (Shelp et al., 1999; Wang et al., 2010) and pathogen attack (Schnee et al., 2008; Solomon and Oliver, 2001, 2002).

This study was conducted to evaluate the inhibition of disease development and to screen expression of defense-related genes and accumulation of various stilbenes and GABA as a resistance response in grapevine leaves that have been treated with different signaling molecules.

Materials and Methods

Application of signaling molecules to grapevine leaves. Shoots with 15-20 leaves from rooted cuttings of 'Campbell Early' and 'Kyoho' grapevines, which are both susceptible to gray mold, were grown in a greenhouse at 25°C and a 16 h photoperiod and sprayed to run-off with either ethephon (500 and 1000 ppm), or hydrogen peroxide (H_2O_2; 5 and 10 mM), or methyl jasmonate (MeJA; 0.1 and 0.5 mM), or salicylic acid (SA; 0.5 and 1 mM) dissolved in distilled water. Leaves were then harvested at 0, 1, 6, 24, 48, and 72 h after treatment, immediately frozen in liquid nitrogen, and stored at -80°C for future evaluation.

RNA isolation and semi-quantitative RT-PCR analysis. At each harvest time, total RNAs were extracted from grapevine leaves using a modified pine tree method (Chang et al., 1993). The differential expressions of genes were confirmed by semi-quantitative RT-PCR using 15 gene specific primer pairs (Table 1). First-strand cDNA was synthesized from the total RNA (500 ng) using a PrimeScriptTM 1st strand cDNA synthesis kit (Takara Bio Inc., Japan) and subsequently used as the template for PCR. Actin gene primers were used as an internal control. The PCR reaction was as follows: initial denaturation at 94°C for 5 min, followed by 30 cycles at 94°C for 45 sec, 55°C for 45 sec, and 72°C for 1 min and then final extension at 72°C for 7 min. The PCR products were identified by 1% (W/V) agarose gel electrophoresis with 0.5X TBE running buffer. After taking the gel picture using a benchtop variable transilluminator (UVP, CA, USA), the images were analyzed and the amount of expression was assessed using a public domain image analysis system (NIH ImageJ, NIH Image, Bethesda, USA).

Stilbene quantification and analysis of stilbene using HPLC. A 1 g sample of fresh grapevine leaves treated with each signal-

Table 1. Gene accession numbers and sequences of gene primers used for RT-PCR analysis.

Name (Accession no.)	Primer sequences
Cell wall protein (XM002277798.1)	5'-GCCCTCCTTGTCATCTTCAA-3'
	5'-GTAAATGCAGGGGCAAGGTA-3'
Chalcone isomerase (*CHI*) (XM002282072.2)	5'-TCCCATCTCTCCTTCAACCA-3'
	5'-TATCCCCGAAGATGTCTCCA-3'
Chalcone synthase (*CHS*) (EF192464.1)	5'-TCCAACTACCACAAGCCTCA-3'
	5'-GAGCAGACAACAAGGACACG-3'
Chitinase-like protein (*CLP*) (XM002269123.1)	5'-GAAGCCATTGGTGAAGGTGT-3'
	5'-GGGTGGCGTTCTGTTCTATG-3'
Cold induced protein (*CI*) (XM002283501.2)	5'-CCAAGTGTTTGGGAGTCCAT-3'
	5'-GCAGAACCCCTCTTCTTTGA-3'
Cytochrome p450 (*CYP*) (CAB85635.1)	5'-AAGCAACGGTTACAGCTAAG-3'
	5'-GCCATATCTGTCTTCCATGT-3'
Flavonol synthase (*FLS*) (XM002285805.1)	5'-AGCGGTACTCAGCAAAGGTT-3'
	5'-TGAGAAGGTTGAGTGGGTTG-3'
Glutathione-*S*-transferase (*GST*) (AY156048)	5'-GGCGATCAAAGTCCATGGTAG-3'
	5'-GCTTCTCCAATCCCTTAACCC-3'
Lipoxygenase (*LOX*) (XM002285538.2)	5'-TAACCTTAAGAGGAGATGGAACTG-3'
	5'-TATCCTCTTGAATAACCTGAGGAG-3'
Meiosis 5 (*Mei5*) (XM002283847.2)	5'-AGGCTTCACCATTCATCACC-3'
	5'-TCCCTTCCCGGTATAGTTCC-3'
Polygalacturonase-inhibiting protein (*PGIP*) AF305093.1)	5'-CTCTCCTCCTCTCTCCTCCT-3'
	5'-CGGTGAGGTTAGAGAGCTTG-3'
Proline rich protein 2 (*PRP2*) (XR078193.2)	5'- TTGCTAGTGGTGTTGCTTGG-3'
	5'-ACACCATATCCATGGTAGCC-3'
Sirtuin (AK227432)	5'-CTTCAGRMAACWCCRAARGAYAARAARGC-3'
	5'-AWRRTCWRTWCKKAYAWATGGAGGAAT-3'
Stilbene synthase (*STSY*) (X76892.1)	5'-TTAACATACGCCAAGAGATTATCA-3'
	5'-CCTGCAGAATTAGGAATAAATGTT-3'
Thaumatin-like protein (*TLP*) (XM002282928.2)	5'- GTCAACCAATGCACCTAC-3'
	5'- GGTGGATCATCCTGTGGA-3'
β-actin (AB372563)	5'-ACGAGAAATCGTGAGGGATG-3'
	5'-ATTCTGCCTTTGCAATCCAC-3'

ing chemical from both cultivars and at each time point was extracted with 4 ml methanol (80%) for 3 min in the dark and the extracts centrifuged for 20 min (25,000 g). The supernatant was transferred into a new tube for HPLC analysis and stored at -20°C. Stilbene compounds were analyzed by HPLC-mass spectrometry (model 2695 HPLC, model 3100 MS, Waters, USA), according to the method described by Choi (2011). Three leaves were used per treatment and each treatment was performed in triplicate.

γ-Aminobutyric acid (GABA) quantification and analysis of GABA using HPLC. A 0.5 g sample of grapevine leaves from both cultivars at each harvest time was air dried prior to extraction in 10 mL of 3% trichloroacetic acid (TCA) at room temperature for 1 h (samples were vortexed (Genie 2, Scientific Industries, Inc., USA)). The samples were subsequently centrifuged (15,000 g) for 30 min, after which the supernatants were col-

lected, filtered through 0.45 μm membranes, and analyzed by HPLC (2690, Waters Co, USA) according to the method described by Lee and Lee (2004). The HPLC analysis conditions were as described in Table 2. Three leaves were used per treatment and each treatment was performed in triplicate.

Evaluation of grey mold severity on plants treated with signaling molecules. Leaves were sprayed with various signaling molecules 24 h before inoculation with *B. cinerea*. *Botrytis cinerea* was grown in petri-dishes at 25°C on potato dextrose agar (PDA, potato starch 4 g, dextrose 20 g, and agar 15 g·L^{-1}) under 12/12 h light/dark conditions. Spores of *B. cinerea* were collected from the plates and suspended in 0.24% potato dextrose broth at a concentration of 10^6 spores·mL^{-1} (after centrifugation (3,000 g) for 5 min to remove debris). A mycelial block (5 mm in diameter) was excised from the pathogenic isolate of *B. cinerea* grown on PDA, and used for inoculation. The spore suspensions and mycelial blocks were inoculated to compare the development of symptoms caused by *B. cinerea*. The upper fourth or fifth leaves from the shoot apex were detached, and the underside injured by slightly scratching the epidermis with a pencil tip. The injured leaves were inoculated either with 20 μL of spore suspension or with a mycelial block on both the wounded and non-wounded areas. Leaves inoculated with the pathogen were placed on two layers of moist paper towel in a closed box, and kept in the dark at 25°C for 4 d, when the diameter of the lesions was measured. Five leaves were used for each treatment, and the experiment was repeated twice.

Results

Expression profiles of defense-related genes following treatment with plant signaling molecules. In both 'Campbell Early' and 'Kyoho', the expressions of the plant defense-related genes catalase (*Cat*), chitinase-like protein (*CLP*), glutathione *S*-transferase (*GST*), stilbene synthase (*STSY*), thaumatin-like protein (*TLP*), and signal transduction-related genes such as lipoxygenase (*LOX*), were strongly induced by treatment with the signaling molecules (Fig. 1). The flavonol synthase gene (*FLS*) and cell wall modification-related genes such as polygalacturonase-inhibiting protein (*PGIP*) and proline rich protein (*PRP2*) were slightly upregulated in response to these treatments.

JA-dependent response genes such as *CLP*, *LOX* and *TLP* were strongly induced in response to all signaling molecules in both grapevine cultivars.

Analysis of stilbene and GABA accumulation. In both grapevine cultivars, *trans*- and *cis*-piceid, which are stilbenoid glucosides showing low biological activity compared to

Table 2. HPLC conditions for detection and quantification of stilbene compounds and γ-aminobutyric acid (GABA) (Choi, 2011; Lee and Lee, 2004).

Item	Condition	
	For STS compounds	For GABA
Column	XTerra MS C18	Waters AccQ-tag column
	150 x 2.1mm	150 x 3.9 mm
Column temperature	40°C	37°C
Eluent	A: 0.1% formic acid	A: acetate-phosphate buffer (AccQ-tag eluent A)
	B: acetonitrile	B: 60% ACN solution
Flow rate	0.2 mL / min.	1.0 mL / min.
Wavelength	*Cis*: 285 nm, *trans*: 307 nm	Ex: 250 nm, Em: 395 nm
Injection volume	5 μL	5 μL

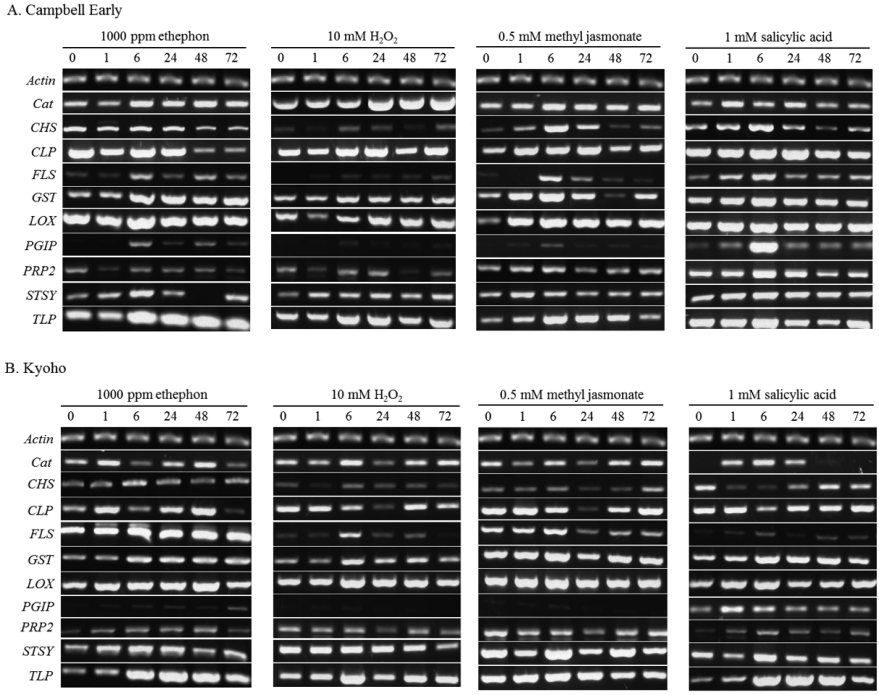

Fig. 1. RT-PCR analysis for evaluation of the expression of ten defense- and stress-related genes in grapevine leaves of cultivars 'Campbell Early' and 'Kyoho' treated with different signaling molecules. Total RNA was extracted at the indicated time points (hours). Equal volume usage of total RNA was identified by comparing the expression levels of the actin gene. *Cat*; catalase1, *CHS*; chalcone synthase, *CLP*; chitinase-like protein, *FLS*; flavonol synthase, *GST*; glutathione-*S*-transferase, *LOX*; lipoxygenase, *PRP*2; proline rich protein 2, *PGIP*; polygalacturonase-inhibiting protein, *STSY*; stilbene synthase, *TLP*; thaumatine-like protein.

resveratrol and piceatannol accumulation in response to application of the signaling molecules (Tables 3 and 4). Among these, *trans*-piceid concentration was higher compared to *cis*-piceid in 'Campbell Early' grapevine leaves. However, in 'Kyoho', while resveratrol concentration was low, the concentration of piceatannol, a hydroxylated derivative of resveratrol, was high. Although, the concentrations of both *trans* and *cis*-resveratrol and piceatannol were low, they declined rapidly after a peak at between 6 and 24 h, whereas *trans*-piceid reached a peak 48-72 h after treatment.

The concentration of GABA in leaves treated with each signaling molecule increased compared to untreated leaves (Fig. 2), with a two-fold increase occurring in ethephon-treated leaves at 48 h after treatment. With other signaling molecules the concentration of GABA was typically higher 48 h after treatment compared to that at 24 h.

Inhibition of grey mold disease by Botrytis cinerea. Grapevine leaves pretreated with the signaling molecules were assessed for the development of grey mold symptoms 4 d after inoculation with *B. cinerea*. Treatment with the signaling molecules reduced the development of lesions when leaves were inoculated with either mycelia blocks or spore suspensions of *B. cinerea* on both 'Campbell Early' and 'Kyoho' grapevine leaves (Fig. 3). The development of lesions in response to inoculation with the pathogen was higher in

Table 3. Concentrations of stilbene compounds in 'Campbell Early' grapevine leaves treated with different signaling molecules.

Signaling molecule	Sample time (h)	µg·0.1 g^{-1} fresh wt.					
		trans-Resveratrol	*cis*-Resveratrol	*trans*-Piceatannol	*trans*-Piceid	*cis*-Piceid	Total
ET[z]	0	1.5 ± 0.1cd[w]	0.2 ± 0.0b	1.2 ± 0.3b	54.2 ±0.8d	13.6 ± 1.0c	70.7 ± 1.4e
1000 ppm	1	1.8 ± 0.3c	0.1 ± 0.1b	0.9 ± 0.2b	48.2 ± 4.2e	45.3 ± 2.9d	96.4 ± 7.1d
	6	10.7 ± 1.1a	2.0 ± 1.5a	1.5 ± 0.1b	87.5 ± 2.3c	64.6 ± 1.0a	166.2 ± 2.8a
	24	0.8 ± 0.2d	0.2 ± 0.1b	2.2 ± 0.5a	83.1 ± 2.6c	7.4 ± 1.9d	93.6 ± 4.8d
	48	2.8 ± 0.4b	0.3 ± 0.1b	1.4 ± 0.4b	123.8 ± 2.3a	7.4 ± 2.3d	135.6 ± 4.2b
	72	0.7 ± 0.3d	0.2 ± 0.1b	1.1 ± 0.6b	96.0 ± 2.2b	9.6 ± 2.2d	107.7 ± 1.5c
H_2O_2	0	1.5 ± 0.1ab	0.2 ± 0.0b	1.2 ± 0.3b	54.2 ±0.8e	13.6 ± 1.0c	70.7 ± 1.4f
1 mM	1	0.6 ± 0.2b	0.2 ± 0.1b	3.0 ± 1.4a	183.7 ± 3.6a	4.7 ± 0.5d	192.0 ± 1.4a
	6	1.3 ± 0.6b	1.6 ± 0.6a	1.6 ± 0.2b	53.9 ± 2.3e	39.3 ± 2.0a	97.8 ± 3.0d
	24	1.5 ± 0.6ab	1.9 ± 0.5a	0.9 ± 0.4b	83.2 ± 2.6d	3.5 ± 1.5d	90.9 ± 0.7e
	48	2.4 ± 1.2a	0.3 ± 0.1b	1.2 ± 0.5b	111.5 ± 2.8c	11.4 ± 1.0c	126.8 ± 2.5c
	72	0.9 ± 0.4b	0.2 ± 0.1b	0.8 ±0.4b	122.1 ± 2.5b	20.4 ± 3.5b	144.4 ± 1.7b
MeJA[y]	0	1.5 ± 0.1cd	0.2 ± 0.0b	1.2 ± 0.3a	54.2 ±0.8e	13.6 ± 1.0d	70.7 ± 1.4e
0.5 mM	1	5.5 ± 1.8a	2.3 ± 1.3a	1.7 ± 0.2a	33.2 ± 3.0f	39.5 ± 0.7b	82.2 ± 1.6d
	6	3.6 ± 0.9b	2.9 ± 0.4a	1.8 ± 0.2a	96.0 ± 2.8d	60.7 ± 1.1a	165.1 ± 1.5b
	24	1.9 ± 0.6bcd	3.0 ± 2.2a	1.8 ± 1.3a	199.5 ± 2.8a	10.3 ± 2.3e	216.5 ± 6.5a
	48	3.2 ± 1.0bc	4.2 ± 0.5a	0.9 ± 0.6a	189.0 ± 4.2b	19.3 ± 1.9c	216.6 ± 3.4a
	72	1.0 ± 0.7d	0.4 ± 0.1b	1.3 ± 0.4a	104.3 ± 1.5c	6.5 ± 0.8f	113.4 ± 2.6c
SA[x]	0	1.5 ± 0.1a	0.2 ± 0.0d	1.2 ± 0.3ab	54.2 ±0.8d	13.6 ± 1.0bc	70.7 ± 1.4e
1 mM	1	<0.1 ±0.0b	1.2 ± 0.5cd	<0.1 ±0.0c	45.7 ± 1.5e	17.2 ± 2.2b	64.1 ± 4.1e
	6	1.5 ± 0.2a	2.1 ± 0.7bc	1.5 ± 0.5a	85.1 ± 3.2c	31.7 ± 2.5a	121.8 ± 5.6b
	24	1.1 ± 0.6a	3.6 ± 0.6a	0.9 ± 0.6ab	98.6 ± 5.7b	8.1 ± 2.1d	112.2 ± 8.3c
	48	0.9 ± 0.3a	0.2 ± 0.1d	0.8 ± 0.2ab	138.2 ± 3.0a	9.3 ± 1.2d	149.4 ± 3.7a
	72	1.0 ± 0.5a	3.3 ± 1.3ab	0.7 ± 0.4b	84.9 ± 1.4c	10.1 ± 3.1cd	100.1 ± 4.1d

[z] Ethephon; [y]Methyl jasmonate; [x]Salicylic acid.
[w] Means±SE (n=3). Mean separation within columns and within each signaling molecule treatment by the Duncan's multiple range test, P<0.05.

wounded leaves than in non-wounded leaves. Among the treatments, there was a trend for MeJA to most effectively suppress lesion development.

Discussion

Exogenous application of ethylene, methyl jasmonate, hydrogen peroxide, and salicylic acid induced the expression of PR and *GST* genes and induced acquired immunity in grapevine plants, as has been previously reported in other different hosts (Chen et al., 1996; Enyedi et al., 1992; Malamy et al., 1990; Marrs, 1996; Pieterse and van Loon, 1999; Wagner et al. 2002; Zhou and Goldsbrough, 1993).

Ethylene has been reported to play a role in disease resistance by inducing the formation of phenolic compounds, phytoalexins, lignin, ethylene-induced enzymes such as β-1, 3-glucanase, chitinase, phenylalanineammonia-lyase (*PAL*), peroxidase (Abeles et al., 1992; Archer and Hislop, 1975), and zeta-*GST* (Meyer et al., 1991). Jasmonates have been shown to induce genes encoding pathogenesis-related (PR) proteins in to-

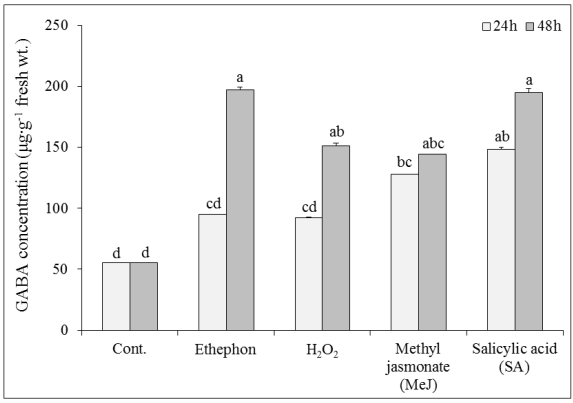

Fig. 2. Concentrations of γ-aminobutyric acid (GABA) in 'Kyoho' grapevine leaves treated with signaling molecules. Mean separation within treatments by Duncan's multiple range test, P<0.05. Vertical bars indicate the SEs (n=3).

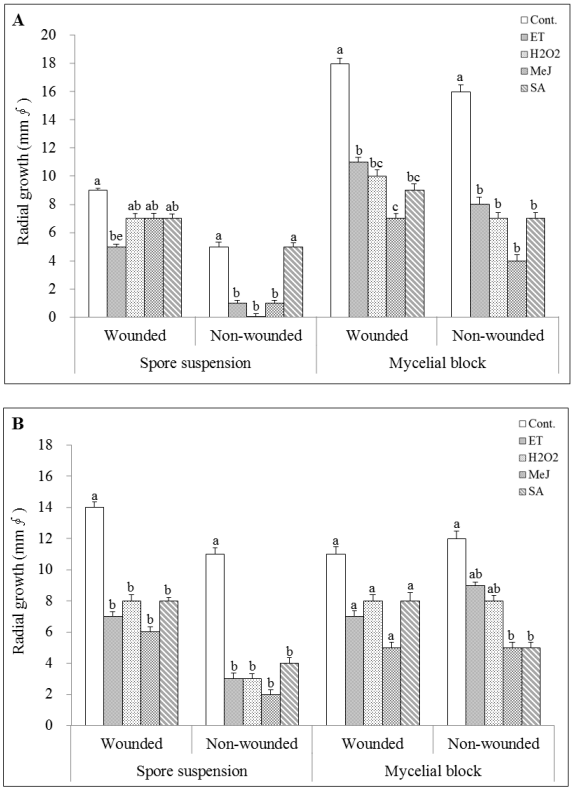

Fig. 3. The size of lesions on 'Campbell Early' (A) and 'Kyoho' (B) grapevine leaves inoculated with *B. cinerea* three days after pathogen inoculation. Mean separation within treatments by Duncan's multiple range test, P<0.05. Vertical bars indicate the SEs (n=5).

bacco (Fukuda and Shinshi, 1994) and tomato (Enkerli et al., 1993). Our results showed that the expression of defense related genes such as *CLP* and *GST* were activated in both ethylene and MeJA treated leaves.

The mRNA expression levels of several defense-related genes including *TLP*, *CHS*, and *LOX*, were higher in grapevine leaves that had been inoculated with *Rhizobium vitis* and treated with SA (Choi et al., 2008). Grapevine chitinase genes are known to be inducible under stress conditions or in response to exposure to ethylene, jasmonic acid, and salicylic acid (Graham and Sticklen, 1994; Kombrink and Somssich, 1995). According to previous reports (Aziz et al., 2003; Busam et al., 1997; Jacobs et al., 1999) the induction of the chitinase gene was dependent on the stress applied, the organ studied, and the grapevine cultivar. However, in this study, *CLP* was slightly upregulated in response to all treatments in both of the grapevine cultivars.

STSY genes, which have been reported to be involved in the regulation of fungal growth and development in grapevines (Hammerschmidt, 1999), were induced by all of the chemical treatments. *PGIP*, which is regulated by pathogen attack, fungal elicitors, and salicylic acid (Maleck et al., 2000; Rushton et al., 1996; Schenk et al., 2000), was also slightly upregulated in response to all of the treatments that were studied. In 'Campbell Early' grapevines, the SA concentrations increased sharply within 1 h of inoculation with downy mildew and anthracnose (Roh et al., 2005). Consistently, defense- and stress-related genes regulated by SA

Table 4. Concentrations of stilbene compounds in 'Kyoho' grapevine leaves treated with different signaling molecules.

Signaling molecule	Sample time (h)	µg·0.1 g^{-1} fresh wt.					
		trans-Resveratrol	*cis*-Resveratrol	*trans*-Piceatannol	*trans*-Piceid	*cis*-Piceid	Total
ET[z]	0	0.4 ±0.2d[w]	0.3 ±0.2ab	4.0 ±2.0e	39.5 ±3.3e	26.7 ±1.3b	70.9 ± 2.6d
1000 ppm	1	0.9 ±0.3cd	0.4 ±0.2ab	3.9 ±0.2e	43.7 ±1.3e	29.3 ±3.8ab	78.2 ± 5.3d
	6	3.1 ±1.2a	0.4 ±0.1ab	7.8 ±0.5d	79.1 ±1.2d	31.1 ±1.9a	121.5 ± 1.3c
	24	2.7 ±0.5ab	0.5 ±0.2a	27.8 ±0.4a	193.3 ±3.0c	16.2 ±3.1c	240.5 ± 6.2b
	48	0.6 ±0.1d	0.1 ±0.1b	20.3 ±2.3c	200.3 ±6.9b	14.8 ±1.4cd	236.1 ± 10.7b
	72	1.9 ±0.4bc	0.5 ±0.2a	24.8 ±1.4b	234.6 ±2.8a	10.8 ±1.4d	272.6 ± 3.1a
H_2O_2	0	0.4 ±0.2b	0.3 ±0.2b	4.0 ±1.0c	39.5 ±3.2e	26.7 ±2.2a	70.9 ± 0.1e
1 mM	1	0.5 ±0.3b	0.4 ±0.1b	9.4 ±2.9b	108.8 ±2.4b	11.0 ±2.2cd	130.1 ± 7.8b
	6	1.3 ±0.4a	0.4 ±0.3ab	12.5 ±2.5b	105.5 ±3.2b	14.9 ±1.9b	134.6 ± 7.7b
	24	0.9 ±0.2b	0.3 ±0.1b	8.1 ±2.2b	62.4 ±2.5d	12.6 ±1.9bc	84.3 ± 6.8d
	48	0.8 ±0.4b	0.3 ±0.2b	10.4 ±1.2b	97.2 ±2.0c	8.5 ±1.8de	117.2 ± 4.5c
	72	0.7 ±0.5b	0.7 ±0.1a	20.5 ±2.7a	180.6 ±2.6a	7.8 ±0.4e	210.3 ± 4.6a
MeJ[y]	0	0.4 ±0.2b	0.3 ±0.3ab	4.0 ±3.0d	39.5 ±2.8e	26.7 ±1.5a	70.9 ± 1.3e
0.5 mM	1	0.5 ±0.2b	0.5 ±0.1ab	4.2 ±0.5d	40.5 ±1.7e	21.0 ±2.1b	66.7 ± 4.4e
	6	4.2 ±1.3a	0.6 ±0.3a	19.5 ±2.5b	137.6 ±0.7b	26.9 ±1.1a	188.8 ± 5.2b
	24	0.4 ±0.2b	0.2 ±0.1b	11.7 ±0.5c	133 ±2.2c	8.0 ±0.7d	153.3 ± 3.6c
	48	<0.1 ±0.0b	0.4 ±0.1ab	13.5 ±1.8c	103.3 ±2.3d	12.3 ±1.6c	129.5 ± 5.7d
	72	0.7 ±0.7b	0.3 ±0.2ab	24.9 ±1.3a	205 ±0.3a	12.1 ±1.4c	243.0 ± 0.4a
SA[x]	0	0.4 ±0.1b	0.3 ±0.1d	4.0 ±2.2d	39.5 ±1.2f	26.7 ±2.4c	70.9 ± 1.0f
1mM	1	0.4 ±0.2b	0.5 ±0.2cd	5.5 ±0.5cd	64.2 ±0.4e	34.1 ±1.8b	104.7 ± 1.9d
	6	5.2 ±2.7a	4.0 ±0.2a	7.1 ±1.4c	81.9 ±2.1d	58.6 ±1.6a	156.8 ± 4.8b
	24	1.0 ±0.8b	0.3 ±0.2d	24.1 ±1.5a	181.4 ±2.4a	5.9 ±0.4d	212.7 ± 2.2a
	48	<0.1 ±0.0b	1.3 ±0.2b	1.7 ±0.2e	91.9 ±2.4c	<0.1 ±0.0e	94.9 ± 2.4e
	72	0.3 ±0.1b	0.8 ±0.2c	9.8 ±0.4b	104.5 ±3.3b	26.0 ±1.3c	141.4 ± 4.7c

[z] Ethephon; [y]Methyl jasmonate; [x]Salicylic acid.
[w] Means±SE (n=3). Mean separation within columns and within each signaling molecule treatment by the Duncan's multiple range test, P<0.05.

were up-regulated in both 'Campbell Early' and 'Kyoho' grapevines in the present study, providing further evidence of their role in induced resistance responses.

Treatment with SA, chitosan, MeJA, and elicitor released from *B. cinerea* has been shown to highly stimulate accumulation of PR proteins and key enzymes of the phenylpropanoid pathway (Renault et al., 1996; Repka, 2001). In grapevine cells, oligogalacturonide (OGA) induced the expression of nine defense-related genes, and treatment of grapevine leaves with OGA also reduced infection by *B. cinerea* development (Aziz et al., 2004). Oligoglucuronans as elicitors have also been demonstrated to induce defense reactions dependent on degree of polymerization and the nature of substituents in grapevine. Acetylated oligoglucuronans with different degrees of polymerization induced transient production of H_2O_2, induced expression of some defence-related genes (*PAL*, *STS*, *Chit4c* and *PGIP*), and reduced the infection of grapevine leaves by *B. cinerea* (Caillot et al., 2012). Belhadj et al. (2008) also reported that ethephon treatment triggered the protection of grapevine against powdery mildew.

In many previous reports, treatment with elicitors has been reported to induce defense-related responses and to protect grapevines against pathogen infection. However, in our research, differential expression patterns of specific defense-related genes were shown in response to particular signal molecules including the activation of *CAT*, *CLP*, *GST*, *LOX*, *PGIP*, *STSY*, and *TLP*. These responses were common in the two grapevine cultivars.

Stilbene compounds exhibit a wide range of biological activities including antioxidant and radical scavenging properties. Their effectiveness is related to the degree of hydroxylation. For example, the hydroxylated derivative of resveratrol, piceatannol, has more effective antioxidant activity than resveratrol (Lorenz et al., 2003). A significant proportion of total stilbenes was reported to be accumulated as glucosides in stilbene-producing plant species (Chong et al., 2009). In our study, the total concentration of stilbene-related compounds increased and the development of grey mold was reduced in both cultivars when treated with either ET, H_2O_2, MeJA, or SA. Accumulation of stilbene phytoalexin by chemical treatments was considered to inhibit grey mold development through enhanced disease resistance to *B. cinerea* in grapevines, as evidenced by the concentration of resveratrol being positively correlated with resistance (Adrian et al., 1997; Bavaresco et al., 1997; Langcake, 1981; Stein and Blaich, 1985). Jeandet et al. (2002) reported that phytoalexin gene expression in various transgenic plants, such as *Arabidopsis thaliana*, *Nicotiana tabacum*, *Hordeum vulgare*, *Actinidia deliciosa*, and *Malus domestica* as well as *Vitis vinifera*, was related to antifungal activity. These previously reported results are in agreement with the results of the present study where the concentration of stilbene metabolites increased subsequent to application of the resistance induction signaling molecules. In this study, five types of stilbene derivatives were shown to be accumulated differentially in the time courses tested in grapevine leaves in response to the application of signaling molecules.

It has been reported that *trans*/*cis*-resveratrol, piceatannol, and *trans*/*cis*-piceid concentrations increased in the initial stages after UV irradiation and that *trans*/*cis*-resveratrol and piceatannol concentrations decreased gradually, while *trans*/*cis*-piceid concentrations were maintained consistently for 7 to 9 days following treatment (Choi, 2012; Schnee et al. 2008; Wang et al., 2010). The results from our study showed similar patterns.

GABA increased in the treated grapevine leaves, which has been reported previously in plants exposed to various environmental stresses (Kinnesley and Turano, 2000) leading to the suggestion that GABA plays a role in protecting yeast against oxidative stress (Coleman et al., 2001), tomato leaves from virus attack (Cooper and Selman, 1974), resistance to the root-knot nematode and tobacco budworm larvae in tobacco plants (MacGregor et al., 2003; McLean et al., 2003), and in the pathogen-induced oxidative burst in asparagus cells (Janzen et al., 2001).

Although there have been few reports that have shown that accumulation of GABA is correlated with induction of resistance to pathogens in plants, in this study, the concentration of GABA in leaves treated with signaling molecules increased, particularly in SA-treatments. This increase in GABA, in response to exogenous application of signaling molecules, is likely due to the induction of a similar disease resistance mechanism.

Exogenous application of various chemicals is considered to induce defense responses to inhibit disease development in many plants (Ryals et al., 1996; Xu et al., 1994). This study showed that pretreatment with the signaling molecules ethephon, H_2O_2, MeJA and SA induced the expression of pathogen- and stress-related genes including *STSY*, as well as the accumulation of stilbene compounds and GABA, which have been reported to inhibit plant pathogens (Schnee et al., 2008; Solomon and Oliver, 2002). The

results of the present study suggest that treatment with signaling molecules might be used as an alternative to conventional fungicides by inducing resistance responses to diseases, including grey mold of grapevines. Apart from reducing the risk of pathogen resistance to fungicides, the use of induced resistance is likely to have lower environmental impact. However, these laboratory-based responses on leaves require validation on berries and on field-grown grapevines.

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Literature Cited

Abeles, G.B., P.W. Morgan, and M.E. Saltveit. 1992. Regulation of ethylene production by internal, environmental, and stress factors. p.117-119. In: Ethylene in plant biology (2nd ed.). Academic Press, San Diego.

Adrian, M., P. Jeandet, J. Veneau, L.A. Weston, and R. Bessis. 1997. Biological activity of resveratrol, a stilbenic compound from grapevines, against *Botrytis cinerea*, the causal agent for gray mold. J. Chem. Ecol. 23:1689-702.

Adrian, M., P. Jeandet, R. Bessis, and J.M. Joubert. 1996. Induction of phytoalexin (resveratrol) synthesis in grapevine leaves treated with aluminum chloride ($AlCl_3$). J. Agric. Food Chem. 44:1979-1981.

Archer, S.A. and E.C. Hislop. 1975. Ethylene in host–pathogen relationships. Ann. Appl. Biol. 81:121-126.

Aziz, A., A. Heyraud, and B. Lambert. 2004. Oligogalacturonide signal transduction, induction of defense-related responses and protection of grapevine against *Botrytis cinerea*. Planta 218:767-774.

Aziz, A., B. Poinssot, X. Daire, M. Adrian, A. Bezier, B. Lambert, J.M. Joubert, and A. Pugin. 2003. Laminarin elicits defense responses in grapevine and induces protection against *Botrytis cinerea* and *Plasmopara Viticola*. Mol. Plant Microbe Interact. 16:1118-1128.

Bailey, J.A. 1982. Mechanisms of phytoalexin accumulation. p. 289-318. In Phytoalexins; Bailey, J.A., Mansfield, J.W. (eds.). Blackie, Glasgow.

Baum, G., S. Lev-Yadun, Y. Fridmann, T. Arazi, H. Katsnelson, M. Zik, and H. Fromm. 1996. Calmodulin binding to glutamate decarboxylase is required for regulation of glutamate and GABA metabolism and normal development in plants. EMBO J. 15:2988-2996.

Bavaresco, L., D. Petegolli, E. Cantu, M. Fregni, G. Chiusa, and M. Trevisan. 1997. Elicitation and accumulation of stilbene phytoalexins in grapevine berries infected by *Botrytis cinerea*. Vitis 36:77-83.

Belhadj, A., C. Saigne, N. Telef, S. Cluzet, J. Bouscaut, M.F. Corio-Costet, and J.M. Mérillon. 2006. Methyl jasmonate induces defense responses in grapevine and triggers protection against *Erysiphe necator*. J. Agric. Food Chem. 54:9119-9125.

Belhadj, A., N. Telef, S. Cluzet, J. Bouscaut, M.F. Corio-Costet, and J.M. Merillon. 2008. Ethephon elicits protection against *Erysiphe necator* in grapevine. J. Agric. Food Chem. 56:5781-5787.

Bown, A.W. and B.J. Shelp. 1997. The metabolism and functions of γ-aminobutyric acid. Plant Physiol. 115:1-5.

Bown, A.W., K.B. Macgregor, and B.J. Shelp. 2006. Gamma-aminobutyrate: defense against invertebrate pests? Trends Plant Sci. 11:424-427.

Busam, G., H.H. Kassemeyer, and U. Matern. 1997. Differential expression of chitinases in *Vitis Vinifera* L. responding to systemic acquired resistance activators or fungal challenge. Plant Physiol. 115:1029-1038.

Caillot, S., S. Rat, M.L. Tavernier, P. Michaud, J. Kovensky, A. Wadouachi, C. Clément, F. Baillieul, and E. Petit. 2012. Native and sulfated oligoglucuronans as elicitors of defence-related responses inducing protection against *Botrytis cinerea* of *Vitis vinifera*. Carbohyd. Polym. 87:1728-1736.

Chang, S., J. Puryear, and J. Cairney. 1993. A simple and efficient method for isolating RNA from pine trees. Plant Mol. Biol. 11:113-116.

Chen, W., G. Chao, and K.B. Singh. 1996. The promoter of a H_2O_2-inducible *Arabidopsis* glutathione *S*-transferase gene contains closely linked OBF- and OBP1-binding sites. Plant J. 10:955-966.

Choi, S.J. 2011. The identification of stilbene compounds and the change of their contents in UV-irradiated grapevine leaves. Kor. J. Hort. Sci. Technol. 29:374-381.

Choi, S.J. 2012. The Influence of UV irradiation on stilbene contents and gray mold incidence in grapevine leaves. Kor. J. Hort. Sci. Technol. 30:493-500.

Choi, Y.J., H.K. Yun, K.S. Park, J.H. Roh, S.T. Jeong, H.J. Lee, and H.I. Jang. 2008. Screening genes expressed by *Rhizobium vitis* inoculation and salicylic acid treatment in grapevines using genefishing. J. Japan Soc. Hort. Sci. 77:137-142.

Chong, J., A. Poutaraud, and P. Hugueney. 2009. Metabolism and roles of stilbenes in plants. Plant Sci. 177:143-155.

Chong, J., G. Le Henanff, C. Bertsch, and B. Walter. 2008. Identification, expression analysis and characterization of defense and signaling genes in *Vitis vinifera*. Plant Physiol. Biochem. 46:469-481.

Coleman, S.T., T.K. Fang, S.A. Rovinsky, F.J. Turano, and W.S. Moye-Rowley. 2001. Expression of a glutamate decarboxylase homologue is required for normal oxidative stress tolerance in *Saccharomyces cerevisiae*. J. Biol. Chem. 276:244-250.

Cooper, P. and I.W. Selman. 1974. An analysis of the effects of tobacco mosaic virus on growth and changes in the free amino compounds in young tomato plants. Ann. Bot. 38:625-638.

Enkerli, J., U. Gisi, and E. Moesinger. 1993. Systemic acquired resistance to *Phytophthora infestans* in tomato and the role of pathogenesis related proteins. Physiol. Mol. Plant Pathol. 43:161-171.

Enyedi, A.J., N. Yalphani, P. Silverman, and I. Raskin. 1992. Location, conjugation, and function of salicylic acid in tobacco during the hypersensitive reaction to tobacco mosaic virus. Proc. Natl. Acad. Sci. USA 89:2480-2484.

Fukuda, Y. and H. Shinshi. 1994. Characterization of a novel *cis*-acting element that is responsive to a fungal elicitor in the promoter of a tobacco class I chitinase gene. Plant Mol. Biol. 24:485-493.

Graham, L.S. and M.B. Sticklen. 1994. Plant chitinases. Can. J. Bot. 72:1057-1083.

Hammerschmidt, R. 1999. Phytoalexins: what have we learned after 60 years? Annu. Rev. Phytopathol. 37:285-306.

Hung, S.H., C.W. Yu, and C.H. Lin. 2005. Hydrogen peroxide functions as a stress signal in plants. Bot. Bull. Acad. Sin. 46:1-10.

Jacobs, A.K., I.B. Dry, and S.P. Robinson. 1999. Induction of different pathogenesis-related cDNAs in grapevine infected with powdery mildew and treated with ethephon. Plant Pathol. 48:325-336.

Jang, H.I, S.B. Lee, K.H. Kim, Y.M. Choi, and K.Y. Kim. 1995. Studies on ecology of grape grey mold by *Botrytis cinerea*. Agricultural Science Report 37:307-313.

Janzen, D.J., L.J. Allen, K.B. MacGregor, and A.W. Bown. 2001. Cytosolic acidification and gamma-aminobutyric acid synthesis during the oxidative burst in isolated *Asparagus sprengeri* mesophyll cells. Can. J. Bot. 79:438-443.

Jeandet, P., A.C. Douillet-Breuil, R. Bessis, S. Debord, M. Sbaghi, and M. Adrian. 2002. Phytoalexins from the Vitaceae: biosynthesis, phytoalexin gene expression in transgenic plants, antifungal activity, and metabolism. J. Agric. Food Chem. 50: 2731-2741.

Jeandet, P., R. Bessis, M. Sbaghi, and P. Meunier. 1995. Production of the phytoalexin resveratrol by grapes as a response to *Botrytis* attack under natural conditions. J. Phytopathol. 143:135-139.

Kim, B.S., K.J. Choi, and K.Y. Cho. 1993. Reaction to several benzimidazole and dicarboximide resistant *Botrytis cinerea*. Kor. J. Plant Pathol. 9:98-103.

Kim, C.H. and S.I. Kwon. 1993. Parasitic fitness of procymidone-resistant isolates of *Botrytis cinerea* on strawberry. Kor. J. Plant Pathol. 9:26-30.

Kinnersley, A.M. and F.J. Turano. 2000. Gamma aminobutyric acid (GABA) and plant responses to stress. Critical Reviews in Plant Sciences 19:479-509.

Koehl, J., A. Djulic, V. Kirner, T.T. Nguyen, and I. Heiser. 2007. Ethylene is required for elicitin-induced oxidative burst but not for cell death induction in tobacco cell suspension cultures. J. Plant Physiol. 164:1555-1563.

Kombrink, E. and I.E. Somssich. 1995. Defense responses of plants to pathogens. Adv. Bot. Res. 21:1-34.

Kunkel, B.N. and D.M. Brooks. 2002. Cross talk between signaling pathways in pathogen defense. Curr. Opin. Plant Biol. 5:325-331.

Lamb, C. and R.A. Dixon. 1997. The oxidative burst in plant disease resistance. Annu. Rev. Plant Physiol. 48:251-275.

Langcake, P. 1981. Disease resistance of *Vitis* spp. and the production of the stressmetabolites resveratrol, epsilon - viniferin, alpha - viniferin and pterostilbene. Physiol. Plant Pathol. 18:213-226.

Langcake, P. and R.J. Pryce. 1977. A new class of phytoalexins from grapevines. Experientia 33:151-152.

Larronde, F., J.P. Gaudilliere, S. Krisa, A. Decendit, G. Deffieux, and J.M. Merillon. 2003. Airborne methyl jasmonate induces stilbene accumulation in leaves and berries of grapevine plants. Am. J. Enol. Viticult. 54:63-66.

Lee, M.O. and Y.S. Lee☒2004. Studies on the institution of standardization and the screening of biological activity from Korean traditional foods (I). Rep. Busan Inst. Health Environ. 14:44-62.

Lorenz, P., S. Roychowdhury, M. Engelmann, G. Wolf, and T.F.W. Horn. 2003. Oxyresveratrol and resveratrol are potent antioxidants and free radical scavengers: Effect on nitrosative and oxidative stress derived from microglial cells. Nitric Oxide 9:64-76.

MacGregor, K.B., K.B. MacGregor, B.J. Shelp, S. Peiris, and A.W. Bown. 2003. Overexpression of glutamate decarboxylase in transgenic tobacco plants deters feeding by phytophagous insect larvae. J. Chem. Ecol. 29:2177-2182.

Malamy, J., J.P. Carr, D.F. Klessig, and I. Raskin. 1990. Salicylic acid: a likely endogenous signal in

the resistance response of tobacco viral infection. Science 250:1002-1004.

Maleck, K., A. Levine, T. Eulgem, A. Morgan, J. Schmid, K.A. Lawton, J.L. Dangl, and R.A. Dietrich. 2000. The transcriptome of *Arabidopsis thaliana* during systemic acquired resistance. Nat. Genet. 26:403–409.

Marrs, K.A. 1996. The functions and regulation of glutathione *S*-transferases in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 47:127-158.

McClellan, W.D. and W.B. Hewitt. 1973. Early botrytis rot of grape: Time of infection and latency of *Botrytis cinerea* Pers. in *Vitis vinifera* L. Phytopathol. 63:1151-1157.

McLean, M.D., D.P. Yevtushenko, A. Deschene, O.R. Van Cauwenberghe, A. Makhmoudova, J.W. Potter, A.W. Bown, and B.J. Shelp. 2003. Overexpression of glutamate decarboxylase in transgenic tobacco plants confers resistance to the northern rootknot nematode. Mol. Breed. 11:277-285.

Meyer, R.C., P.B. Goldsbrough, and W.R. Woodson. 1991. An ethylene-responsive flower senescence-related gene from carnation encodes a protein homologous to glutathione-*S*-transferases. Plant Mol. Biol. 17:277-281.

Park, D.H., R. Mirabella, P.A. Bronstein, G.M. Preston, M.A. Haring, C.K. Lim, A. Collmer, and R.C. Schuurink. 2010. Mutations in γ-aminobutyric acid (GABA) transaminase genes in plants or *Pseudomonas syringae* reduce bacterial virulence. Plant J. 64:318-330.

Paxton, J.D. 1981. Phytoalexins-a working redefinition. Phytopathol. Z. 101:106- 209.

Pearson, R.C. and A.C. Goheen. 1998. Compendium of grape disease. APS Press, St. Paul.

Pezet, R., K. Gindro, O. Viret, and J.L. Spring. 2004. Glycosylation and oxidative dimerization of resveratrol are respectively associated to sensitivity and resistance of grapevine cultivars to downy mildew. Physiol. Mol. Plant Pathol. 65:297-303.

Pieterse, C.M.J. and van Loon, L.C. 1999. Salicylic acid-independent plant defense pathways. Trends Plant Sci. 4:52-58.

Renault, A.S., A. Deloire, and J. Bierne. 1996. Pathogenesis-related proteins in grapevines induced by salicylic acid and *Botrytis cinerea*. Vitis 35:49-52.

Repka, V. 2001. Elicitor-stimulated induction of defense mechanisms and defense gene activation in grapevine cell suspension cultures. Biol. Plantarum 44:555-565.

Repka, V. 2006. Early defence responses induced by two distinct elicitors derived from a *Botrytis cinerea* in grapevine leaves and cell suspensions. Biol. Plantarum 50:94-106.

Roh, J.H., H.K. Yun, K.S. Park, Y.J. Choi, S.S. Hong, and S.H. Jeon. 2005. Salicylic acid and resveratrol content changes as affected by downy mildew and anthracnose in grapevine. J. Kor. Soc. Hort. Sci. 46:59-63.

Rushton. P.J., J.T. Torres, M. Parniske, P. Wernert, K. Hahlbrock, and I.E. Somssich. 1996. Interaction of elicitor-induced DNA binding proteins with elicitor response elements in the promoters of parsley PR1 genes. EMBO J. 15:5690-5700.

Ryals, J.A., U.H. Neuenschwander, M.G. U.H., Willits, A. M.G., Molina, A., H. Steiner, and M.D. Hunt. 1996. Systemic acquired resistance. Plant Cell 8:1809-1819.

Schenk, P.M., K. Kazan, I. Wilson, J.P. Anderson, T. Richmond, S.C. Somerville, and J.M. Manners. 2000. Coordinated plant defense responses in *Arabidopsis* revealed by microarray analysis. Proc. Natl. Acad. Sci. USA 97:11655-11660.

Schnee, S., O. Viret, and K. Gindro. 2008. Role of stilbenes in the resistance of grapevine to powdery mildew. Physiol. Mol. Plant Pathol. 72:128-133.

Selman, L.W. and P. Cooper. 1978. Changes in free amino compounds in young tomato plants in light and darkness with particular references to γ-aminobutyric acid. Ann. Bot. 42:627-636.

Shelp, B.J., A.W. Bown, and M.D. McLean. 1999. Metabolism and functions of gamma-aminobutyric acid. Trends Plant Sci. 4:446-452.

Solomon, P.S. and R.P. Oliver. 2002. Evidence that gamma-aminobutyric acid is a major nitrogen source during *Cladosporium fulvum* infection of tomato. Planta 214:414-420.

Solomon, P.S. and R.P. Oliver. 2001. The nitrogen content of the tomato leaf apoplast increases during infection by *Cladosporium fulvum*. Planta 213:241-249.

Stein, U. and R. Blaich. 1985. Studies on stilbene production and susceptibility to *Botrytis* in *Vitis* species. Vitis 24:75-87.

Steward, F.C., J.F. Tompson, and C.E. Dent. 1949. γ-amminobutyric acid: a constituent of the potato tuber? Science 110:439-440.

Wagner, U., R. Edwards, D.P. Dixon, and F. Mauch. 2002. Probing the diversity of the *Arabidopsis* glutathione *S*-transferase gene family. Plant Mol. Biol. 49:515-532.

Walters, D., T. Cowley, and A. Mitchell. 2002. Methyl jasmonate alters polyamine metabolism and induces systemic protection against powdery mildew infection in barley seedlings. J. Exp. Bot. 53:747-756.

Wang, Q., Y. Zhang, M. Gao, C. Jiao, and X. Wang. 2011. Identification and expression analysis of a pathogen responsive PR-1 gene from Chinese wild *Vitis quinquangularis*. Afri. J. Biotech. 10:17062-17069.

Wang, W., K. Tang, H.R. Yang, P-F. Wen, P. Zhang, H.L. Wang, and W.D. Huang. 2010. Distribution of resveratrol and stilbene synthase in young grape plant (*Vitis vinifera* L. cv. Cabernet Sauvignon) and the effect of UV-C on its accumulation. Plant Physiol. Biochem. 48:142-152.

Waterhouse, A.L. and R.M. Lamuela-Raventos. 1994. The occurrence of piceid, a stilbene glucoside in grape berries. Phytochem. 37:571-573.

Xu, Y., P.L.C. Chang, D. Liu, M.L. Narasimhan, G.R. Kashchandra, P.M. Hasegawa, and R.A. Bressan. 1994. Plant defense genes are synergistically induced d by ethylene and methyl jasmonate. Plant Cell 6:1077-1085.

Zhou, J.M. and P.B. Goldsbrough. 1993. An *Arabidopsis* gene with homology to glutathione-*S*-transferases is regulated by ethylene. Plant Mol. Biol. 22:517-523.

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