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Inhibition of *Botrytis cinerea* and accumulation of stilbene compounds by light-emitting diodes of grapevine leaves and differential expression of defense-related genes

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Abstract Grey mold (*Botrytis cinerea*) is one of the most common diseases to attack grapes, causing serious damage during grape production. In the present study, the effects of light-emitting diodes (LED) on the suppression of fungal growth, defense related gene expression and accumulation of stilbenic compounds were investigated. Irradiation with blue and red light inhibited lesion development relative to fluorescent light in detached leaves. Treatment of detached leaves with LED light, especially blue and red, resulted in accumulation of stilbenic compounds and differential expression of genes involved in defense response. Among five stilbenic compounds, concentrations of *trans*- and *cis*-piceid were higher than those of *trans*- and *cis*-resveratrol, as well as piceatannol in both 'Campbell Early' and 'Kyoho' leaves treated with blue and red light. The gene expression of beta-1,3 glucanase (*Glu*), osmotin (*OSM*), pathogen-related protein 4a (*PR*4a), protease inhibitor-like protein (*PILP*), thaumatin-like protein (*TLP*), glutathione-*S*-transferase (*GST*), phenylalanine ammonia-lyase (*PAL*), chalcone synthase (*CHS*), and stilbene synthase (*STS*) were highly upregulated under blue and red LED light. The results reported here will facilitate development of alternative methods to enhance the accumulation of resveratrol compound and protect grapevine from fungal pathogen infections.

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Abbreviations

AOC	Allen oxide cyclase
APX	Ascorbate peroxidase
CAT	Catalase
CHS	Chalcone synthase
CLP	Chitinase-like protein
Glu	Beta-1,3 glucanase
GPX	Glutathione peroxidase
GST	Glutathione-*S*-transferase
LED	Light emitting diodes
LOX	Lipoxigenase
OSM	Osmotin
PAL	Phenylalanine ammonia-lyase
PILP	Protease inhibitor-like protein
PR4a	Pathogen-related protein 4a
ROMT	Resveratrol *O*-methyltransferase
STS	Stilbene synthase
TLP	Thaumatin-like protein

Introduction

Light plays a key role throughout the entire lifecycle of plants. Spectral quality can have significant effects on plant photosynthesis, growth, development, and secondary metabolites (Kondo et al. 2014; Lee et al. 2013; Muneer et al. 2014; Tennessen et al. 1994). Light quality has been considered a particularly important factor in

management of plant disease associated with plant-pathogen interactions (Colhoun 1973; Nilsen and Hodges 1980). Light influences plant diseases via direct and indirect mechanisms, including suppression of fungal growth (Islam et al. 1998; Rahman et al. 2003; Suthaparan et al. 2010a, 2012) and induction of plant resistance activities of the host plant (Imada et al. 2014; Kim et al. 2013; Kobayashi et al. 2013).

Red light induced resistance in some plants was previously reported against fungal pathogens such as *Botrytis cinerea* (Islam et al. 1999), *Alternaria tenuissima* (Rahman et al. 2003), *Phytophthora capsici* (Islam et al. 2004), and *Alternaria alternate* (Tabira et al. 1989). Interactions between light sensors and fungal pathogens have been identified (Chen et al. 2010; Idnurm et al. 2010). Additionally, light was recently reported to modify morphogenesis and pathogenicity in fungal pathogens (Canessa et al. 2013; Idnurm and Crosson 2009). Briggs et al. (2001) reported that red and blue light were absorbed by different photoreceptors, indicating that they had different effects on plant development and biosynthesis of cell components in higher plants. Overall, these studies suggest that red or blue light might be a useful source and alternative method to chemical sprays for controlling diseases in plants.

In addition to induction of resistance in light treated plants, secondary metabolites also accumulate. Among the secondary metabolites produced by lights in plants, polyphenolic compounds and antifungal substances such as resveratrol, antioxidants, and glycoprotein have the ability to suppress the development of fungal pathogens (Kim et al. 2013; Islam et al. 1999, 2002). Stilbenes exhibit antimicrobial activities as a phytoalexin group in grapevines produced naturally in leaves and berries, playing important roles against biotic or abiotic stresses during defense response (Chong et al. 2009; Dixon and Paiva 1995; Jeandet et al. 2002). The best studied stilbene is resveratrol (3,5,4′-trihydroxy-*trans*-stilbene), which is well-known to take part in both constitutive and inducible defense mechanisms in plants (Schmidlin et al. 2008) and to have pharmacological properties useful for maintenance of human health (Ndiaye et al. 2011; Stef et al. 2006).

The grey mold (*Botrytis cinerea*) is a very successful necrotroph that causes significant economic losses in at least 200 plants species (Jarvis 1977). The grey mold results in serious economic damage due to decreased yield of grapes owing to breaking buds during harvest, when the wet conditions are favorable to the spread of this fungus Korea (Jang et al. 1995). As there have been increasing public concerns regarding the toxicity and adverse environmental effects of use of synthetic fungicides to control pests, environmentally friendly strategies are required for plant disease management. Therefore, in the present study, the effects of LED lights on the accumulation of stilbenic compounds and suppression of *B. cinerea* growth were investigated in both 'Campbell Early' and 'Kyoho' grapevines as an alternative to chemical control. In addition, the expression of genes related to defense was analyzed to screen for the induction of defense responses in LED-treated grapevine leaves.

Materials and methods

Plant materials and light treatment

Cuttings from 'Campbell Early' and 'Kyoho' grapevines were grown in an experimental greenhouse of Yeungnam University, Gyeongsan, Korea at 25 °C/18 °C (day/night) and 65 % relative humidity. Leaves from the shoot apex of grapevines with 8–10 true leaves were harvested and kept in separate black chambers (24×25×60 cm) equipped with three different LEDs. An LED system (B07, Parus Co., Cheonan, Korea) with purple (380 nm), blue (440 nm), and red (660 nm) was used for light treatment. Detached leaves were irradiated with white fluorescent light (FL), as well as purple (380 nm), blue (440 nm), and red (660 nm) LEDs at an intensity of 80 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) for 48 h at 25±2 °C.

Inoculation procedure

B. cinerea was grown in Petri dishes at 25 °C on potato dextrose agar (potato starch 4 g, dextrose 20 g, and agar 15 g l^{-1}, PDA; Difco, Sparks, MD, USA) 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 per ml after centrifugation at 3000×*g* for 5 min to remove debris. Leaves treated with light for 48 h were placed lower face up and injured by creating tiny wounds without punching out tissue using a pencil tip. The injured leaves were inoculated with 20 μl of spore suspension 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 incubated in the dark at 25±2 °C for 4 days, at which time the diameter of the lesions was measured. Leaf samples were harvested at 0, 12, 24, and 48 h after light irradiation, then immediately frozen in liquid nitrogen for RNA extraction from control and light treated leaves before and 24 h after *B. cinerea* inoculation. Twelve leaves were used for each assay, and the experiment was conducted two times.

Quantification and HPLC assay of stilbene compounds

About 1 g of leaves treated with light for 48 h from both grape cultivars was extracted with 4 mL of 80 % methanol for 3 min in the dark. After centrifugation at 25,000×*g* for 20 min, the supernatant was filtered using a syringe (Norm-ject, HSW, Germany) and 0.45 μm syringe filter (PTFE filter media, Whatman, USA), after which it was stored at −20 °C until high-performance liquid chromatography (HPLC) analysis. HPLC analyses (Table 1) were carried out using an HPLC-mass spectrometer (LC-MS) (model 2695 HPLC, model 3100 MS, Waters, USA) as previously described (Choi 2011). Five leaves were used per assay and each treatment was performed in triplicate.

Extraction of RNA and real-time PCR

Total RNA from grapevine leaves at each time point was extracted by a slightly modified version of the previously described (Ahn et al. 2015) pine tree method (Chang et al. 1993). RNA quality and quantity were measured using a Nano Drop spectrophotometer (ND-1000, Technologies Inc., Wilmington, DE, USA). The reverse transcription reactions were performed with 1 μg of total RNA using a GoScriptTM Reverse Transcription System (Promega, Madison, USA) and subsequently used as a template for PCR. Transcript levels of selected genes were performed by quantitative PCR on a C1000TM Thermal Cycler (CFX96TM Real-Time System, BioRad, Foster City, CA, USA) using SYBR Premix Ex (TaKaRa Bio Inc., Osaka, Japan). Amplification was conducted by subjecting the samples to one cycle at 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 30 s. Transcript levels were calculated using the standard-curve method and normalized against the grapevine beta-actin gene (AB372563) as an internal control, after which melting curves of the amplified products were recorded. Untreated leaves (at time zero) were tested as the reference sample. For each gene, the reference sample was defined as the 1× expression level, and the results were expressed as the fold increase in mRNA over the reference sample. All reactions were performed in triplicate to ensure consistency of the results. The expression of genes was determined using the gene-specific primers listed in Table 2. Gene specific primer pairs were designed using the Primer3 (http://frodo.wi.mit.edu/primer3) software and employed for real-time PCR amplification.

Table 1 HPLC conditions for detection and quantification of stilbene compounds (Choi 2011)

Item	Condition For STS compounds
Column	XTerra MS C18 (3.5 μ, 150×2.1 mm, Waters, USA)
Column temperature	40 °C
Eluent	A: 0.1 % formic acid, B: acetonitrile
Flow rate	0.2 ml min^{-1}
Wavelength	*cis*: 285 nm, *trans*: 307 nm
Injection volume	5 μl

Results

Inhibitory effects of three light sources on *Botrytis cinerea* infection

The disease lesions of two grapevines were evaluated 5 days after *B. cinerea* inoculation. Necrotic areas appeared in response to droplets of *B. cinerea* on detached leaves in both grapevines under different light irradiation. The lesion development was significantly suppressed by both red (660 nm) and blue (440 nm) light treatment, while it was slightly suppressed by purple (380 nm) and FL light (Fig. 1). Even though there were similar developed lesion areas in both grapevines treated with the same light sources, different fungal incidence appeared between the two (Fig. 2). In 'Kyoho', the hypha formation on leaves treated

Table 2 NCBI gene accession numbers and sequences of gene primers used for quantitative real-time PCR analysis

Name	Accession no.	Primer sequence
Chitinase-like protein (*CLP*)	XM002269123.1	5′-CCGATTTCTTCCAGACCTACCA-3′ 5′-CAAATCCGTGAGGCTGGTAAAC-3′
Beta-1,3 glucanase (*Glu*)	DQ267748.1	5′-GGGGTTATTTGGATCCCATCAT-3′ 5′-CAGAAGCGGCGACTTATTGTCT-3′
Osmotin (*OSM*)	Y10992.1	5′-ACTGCAACTTCGATGCGTCA-3′ 5′-TGCGAATTCGGCTAAGGTGT-3′
Pathogen-related protein 4a (*PR4a*)	AF061329.1	5′-GCTGCCCAGAGCGCTAGTAA-3′ 5′-TCCCAAGTGGAGCAGTAGGC-3′
Protease inhibitor-like protein (*PILP*)	XM002284418.3	5′-CTGGTGGGAGTTCAGGGAGA-3′ 5′-CCAAACACGGACCCTAGTGC-3′
Thaumatin-like protein (*TLP*)	XM002282928.2	5′-TTCGCACTTAACCAATTCAGCA-3′ 5′-TGCACCCATTGGAAGTAGGATT-3′
Ascorbate peroxidase (*APX*)	NM001281059.1	5′-GGTCCGTTTGGGACAATGAA-3′ 5′-CGGAAATTGCTCCTTGATCG-3′
Catalase (*CAT*)	AF236127.1	5′-GTCCGTCAAGTGCCTTCAAT-3′ 5′-CGAAAAACCCTTTGGCACTA-3′
Glutathione peroxidase (*GPX*)	XM003631370.1	5′-GAGCACAGGAACCTGGGAGTAA-3′ 5′-AGCACTATCGCCATTCACATCA-3′
Glutathione-*S*-transferase (*GST*)	AY156048	5′-TATAATGTGTGGGCAGCAAACG-3′ 5′-CCAATGTCCAGAAAACCCAAAG-3′
Lipoxigenase (*LOX*)	NM001281094.1	5′-AACCTTGCGAGGTAATGGTCAA-3′ 5′-TACCACCAAGTACCGGTCGAGT-3′
Allen oxide cyclase (*AOC*)	DQ406694.1	5′-CTACACGGGAGACCTGGAGAAG-3′ 5′-CTCCCTTCTTCCCTGGAACATT-3′
Phenylalanine ammonia-lyase (*PAL*)	X75967.1	5′-TGAACAATGGCGAAAGTGAGAA-3′ 5′-TCTCTTGCGCTCTCAACCTCTT-3′
Chalcone synthase (*CHS*)	EF192464.1	5′-AGTTCAAGCGCATGTGTGAAAA-3′ 5′-CTTCAACCACCACCATGTCTTG-3′
Stilbene synthase (*STS*)	X76892.1	5′-GGTGCCATTGCAGGAAACTTAC-3′ 5′-CAAGTGGGTCAAAAGCCTGAGT-3′
Resveratrol *O*-methyltransferase (*ROMT*)	NM001281115	5′-CTAGGCATCCCAGACATCATCC-3′ 5′-ATGAACAAGAATGCGCATGAGA-3′
Beta-actin	AB372563	5′-ACGAGAAATCGTGAGGGATG-3′ 5′-ATTCTGCCTTTGCAATCCAC-3′

with lights had a larger amount of mycelia and dark necrotic lesions than ‘Campbell Early’ grapevine leaves.

Analysis of stilbene concentrations in response to light treatment of two grapevine leaves

Five stilbenic compounds (*trans*-, and *cis*-resveratrol, piceatannol, *trans*- and *cis*-piceid) were detected in both ‘Campbell Early’ and ‘Kyoho’ grape leaves exposed to four different light sources (Fig. 3). The total amount of stilbenic compounds was higher in the leaves of both grapevines treated with blue and red light than in FL, purple, and untreated leaves. The content of total stilbenic compounds ranged from 37.0 (FL) to 84.8 (red) μg g^{-1} in grapevine leaves in ‘Campbell Early’ (Fig. 3a and b). The *trans*-resveratrol and *cis*-piceid accumulation were significantly higher in red and blue light-treated leaves, with concentrations of 18.2 and 55.7 μg g^{-1} FW, respectively.

Among stilbenic compounds tested in ‘Campbell Early’, *trans*-resveratrol was present at 5.4-, 4.0-, 5.4-, and 2.8-fold higher levels in red light-treated leaves than under 0 h, FL, purple, and blue light, respectively. In blue light-treated leaves, the *cis*-

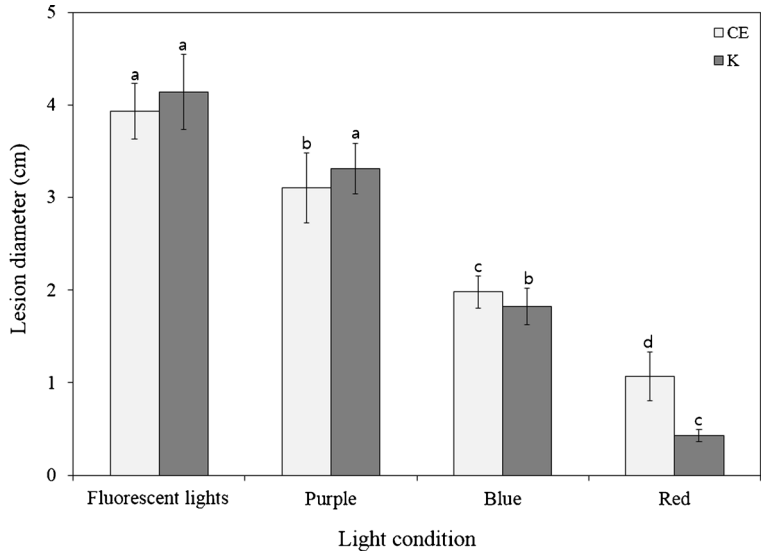

Fig. 1 Lesion diameters of 'Campbell Early' and 'Kyoho' grapevine leaves treated with fluorescent lights (FL) and purple, blue, and red LEDs 4 days after pathogen inoculation with *B. cinerea*. Mean separation within treatments was determined by Duncan's multiple range test, *letters* indicate significance at the p value<0.05. Vertical bars indicate the SE (n=5)

piceid concentration was present at levels 2.4-, 2.0-, 1.7-, and 1.6-fold higher than under 0 h, FL, purple, and red light, respectively.

Total resveratrol accumulated to a concentration of 63.9 μg•g^{-1} in response to red light in the leaves of 'Kyoho' grapevine (Fig. 3c and d). In contrast to 'Campbell Early', the maximum content of *trans*-resveratrol was shown in 'Kyoho' grapevine leaves in response to purple light-treatment (Fig. 3c). The *cis*-resveratrol was not detected in 'Kyoho' grapevine leaves treated with any light. The glycosylated stilbene, piceid, accumulated at high levels in response to light treatments (Fig. 3d). Particularly, red light enhanced the accumulation of *trans*-piceid, with high levels of 27.0 μg g^{-1} being found in grapevine leaves. In red light-treated leaves, the *trans*-piceid was present at levels 3.9 to 4.5-fold higher than in leaves treated with other lights. Among the five stilbenic compounds,

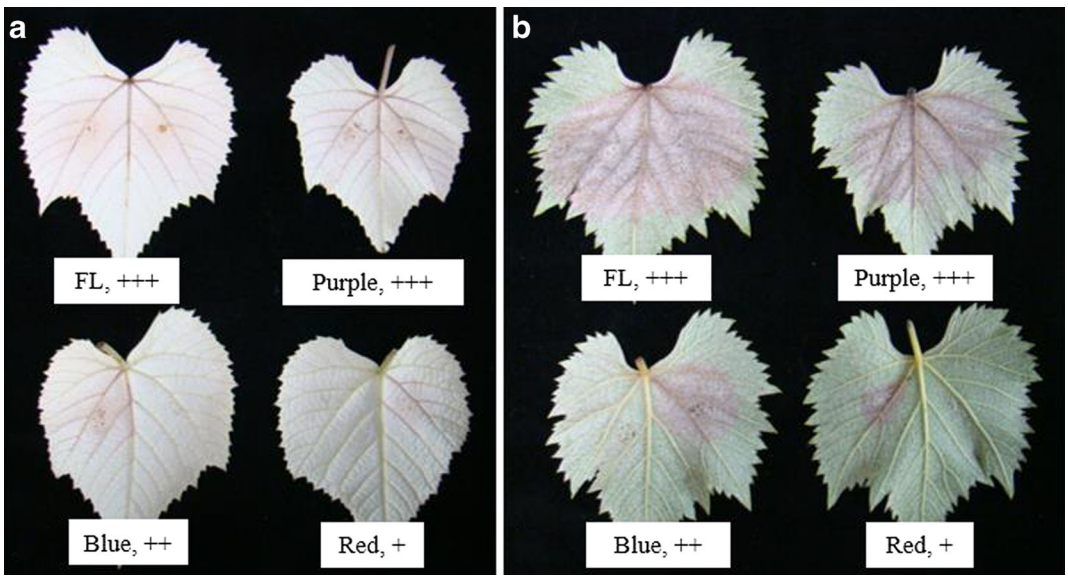

Fig. 2 Effects of fluorescent lights (FL) and purple, blue, and red LEDs on infection of 'Campbell Early' (**a**) and 'Kyoho' (**b**) grapevine leaves 4 days after *B. cinerea* inoculation. Disease severity were determined as follows: +++, necrotic area>3 cm from wounded spot; ++, necrotic area of 2–3 cm over wounded spot; +, necrotic area of 1–2 cm over wounded spot

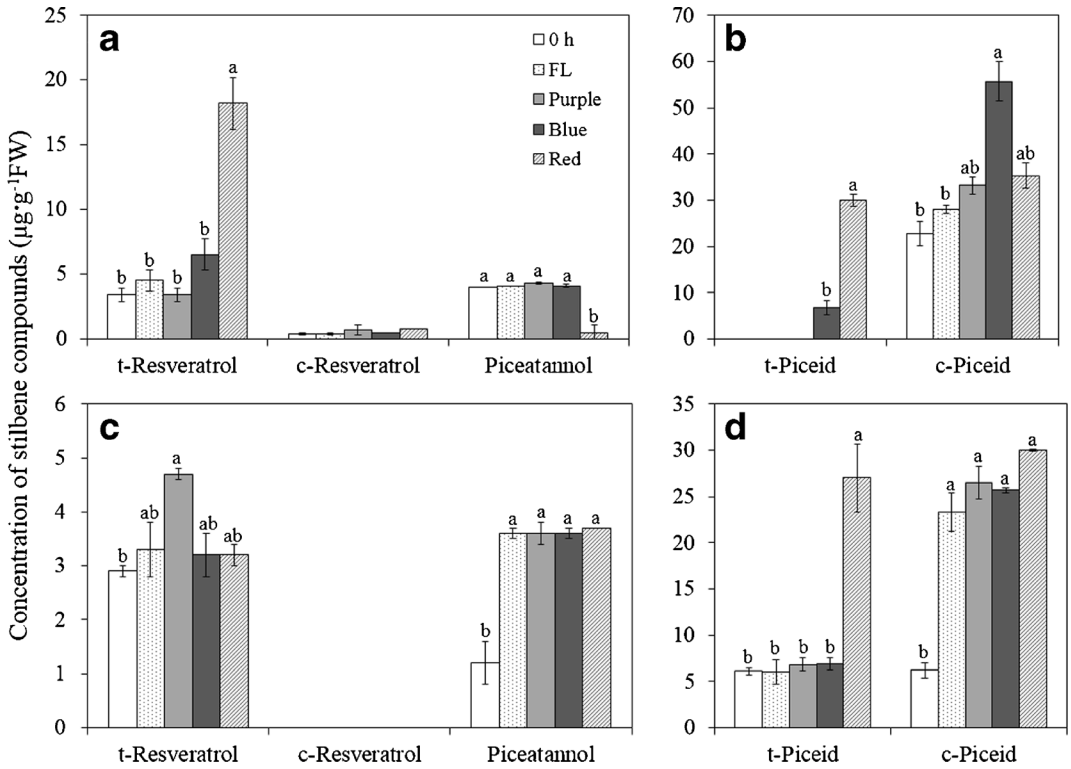

Fig. 3 Effect of fluorescent lights (FL) and purple, blue, and red LEDs on levels of stilbenic compounds in 'Campbell Early' (**a** and **b**) and 'Kyoho' (**c** and **d**) grapevine leaves at 24 h after treatment. Mean separation within treatments was determined by Duncan's multiple range test, *letters* indicate significance at the p value<0.05. Vertical bars indicate the SE (n=3)

trans- and *cis*-piceid were present at higher levels than *trans*- resveratrol, *cis*-resveratrol, and piceatannol in both 'Campbell Early' and 'Kyoho' leaves.

Defense-related gene expression in grapevine leaves during the infection process following LED treatment

To understand the profile of gene expression involved in light induced resistance to *B. cinerea*, real-time PCR was conducted. Genes tested in this study were clustered into three groups based on their roles in defense responses in plants. Group 1 includes the defense-related genes chitinase-like protein (*CLP*), beta-1,3 glucanase (*Glu*), osmotin (*OSM*), pathogen-related protein 4a (*PR*4a), protease inhibitor-like protein (*PILP*), and thaumatin-like protein (*TLP*). Group 2 includes antioxidant enzymes, such as ascorbate peroxidase (*APX*), catalase (*CAT*), glutathione peroxidase (*GPX*), glutathione-*S*-transferase (*GST*), lipoxygenase (*LOX*), and allen oxide cyclase (*AOC)*. Group 3 consists of stilbene biosynthesis related genes, including phenylalanine ammonia-lyase (*PAL*), chalcone synthase (*CHS*), stilbene synthase (*STS*), and resveratrol *O*-methyltransferase (*ROMT*). All genes were differentially expressed in response to LED irradiation in both 'Campbell Early' and 'Kyoho' grapevines. Increased gene expression of *CLP*, *Glu*, *OSM*, *PR*4a, *PILP*, and *TLP* was observed in leaves exposed to FL, purple, blue, and red light in both 'Campbell Early' and 'Kyoho' grapevine (Fig. 4). In red light-treated leaves, there was a significant increase in the expression of *Glu*, *OSM*, *PR*4a, *PILP*, and *TLP* genes at 48 h after treatment in both grape cultivars. Specifically, the maximum expression levels were observed at 48 h after infection for the *OSM* and *TLP* genes in 'Kyoho' leaves. Consequently, various defense-related genes were highly up-regulated by irradiation with blue and red light in grapevine leaves.

As shown in Fig. 5, the expression of *APX*, *CAT*, *GPX*, *GST*, *LOX*, and *AOC* was induced differentially in the grapevine leaves. In 'Campbell Early', the expression of *APX* was down-regulated by irradiation with all lights except FL (Fig. 5a). The expression of *LOX* increased at 12 h after blue light treatment, declined at 24 h and then increased again at 48 h. In red light-

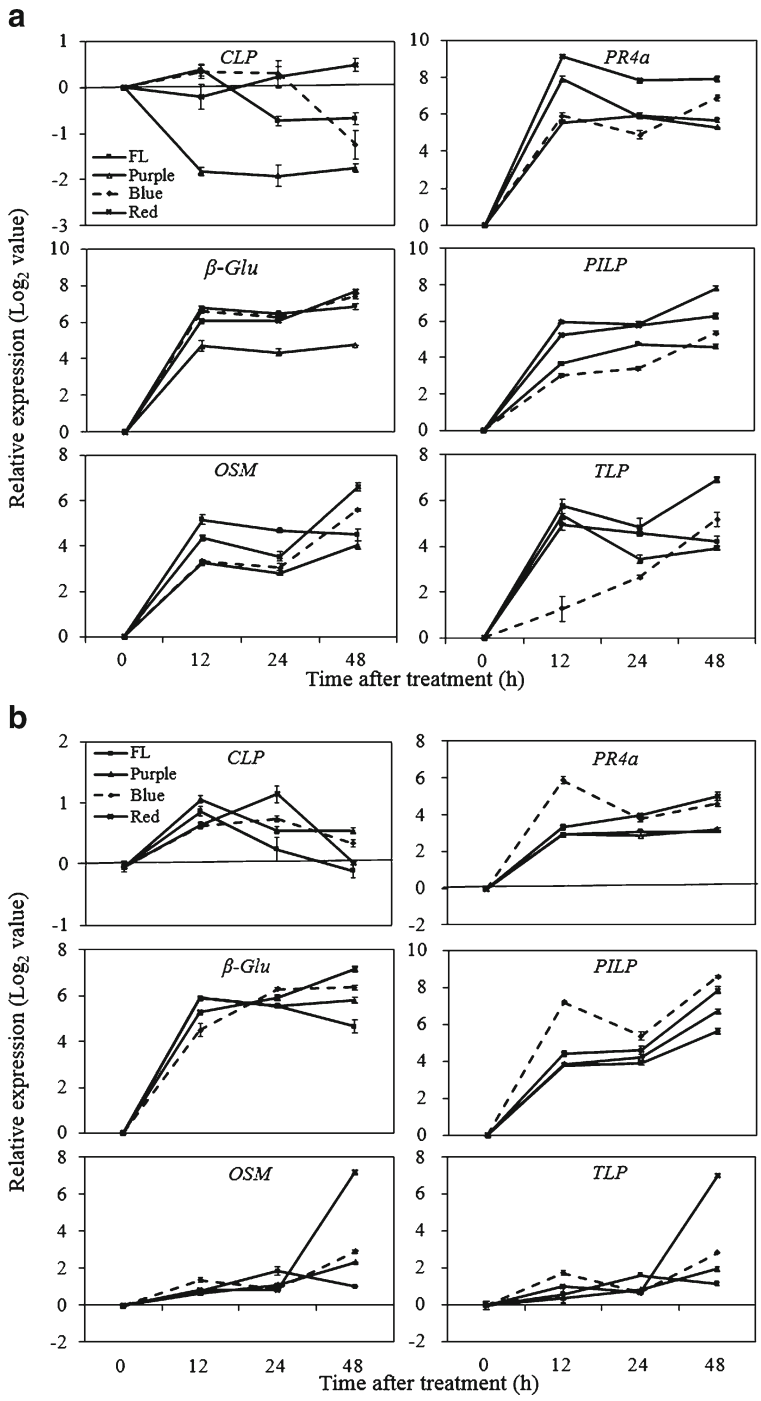

Fig. 4 Expression of defense-related genes by quantitative real-time PCR analysis in 'Campbell Early' (**a**) and 'Kyoho' (**b**) leaves exposed to fluorescent lights (FL) and purple, blue, and red LEDs. Transcript levels were calculated using the standard curve method from triplicate data with the grapevine actin gene as the internal control and untreated leaves (at time zero) as the reference sample. Results represent the mean fold increase in mRNA level over that of the untreated leaves, which was referred to as the 1× expression level. Results are the means of triplicate data from three experiments. *Bars* indicate the standard deviation

irradiated grapevine leaves, the expression of *GPX*, *GST*, and *LOX* genes was rapidly up-regulated, peaking at 12 h, after which it decreased. The maximum *APX* gene expression levels were obtained at 24 h after FL treatment in both cultivars (Fig. 5a and b). Expression of the *GPX* gene was strongly upregulated at 12 h after blue light treatment, with levels 10.2 to 30.5-fold higher being observed relative to other light treatments in 'Kyoho' grapevine leaves (Fig. 5b). *GST* gene expression was up-regulated in response to LED treatment,

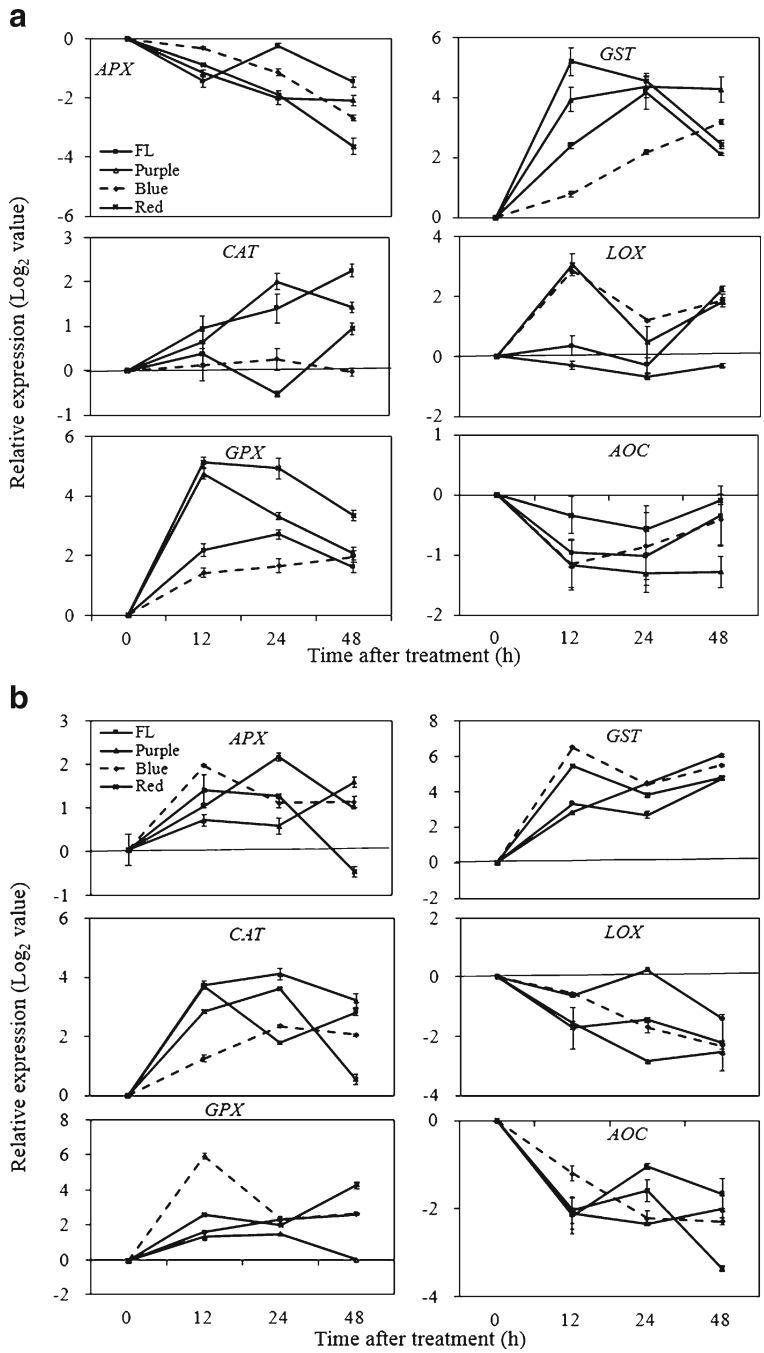

Fig. 5 Expression of antioxidant activity related genes by quantitative real-time PCR analysis of 'Campbell Early' (**a**) and 'Kyoho' (**b**) leaves exposed to fluorescent lights (FL) and purple, blue, and red LEDs. Transcript levels were calculated using the standard curve method from triplicate data with the grapevine actin gene as the internal control and untreated leaves (at time zero) as the reference sample. Results represent the mean fold increase in mRNA level over that of the untreated leaves, which was referred to as the 1× expression level. Results are the means of triplicate data from three experiments. *Bars* indicate the standard deviation

peaking at 12 and 48 h after blue and red light, and purple light treatment, respectively. *LOX* and *AOC* gene expression peaked at 24 h, then declined in FL and red light-treated leaves of 'Kyoho' grapevine.

The expression profile of four genes of the phenylpropanoid pathway known to be involved in stilbene biosynthesis, *PAL*, *CHS*, *ROMT*, and *STS*, were investigated (Fig. 6). Expression of the *PAL* and *STS* genes were highly upregulated to show two differential peaks at 12 and 48 h in 'Campbell Early' leaves in response to treatment with red light (Fig. 6a). In both grapevines, light treatments induced gradual up and down-regulation of the expression levels of the *ROMT* gene in leaves. Blue light irradiation induced *CHS* gene

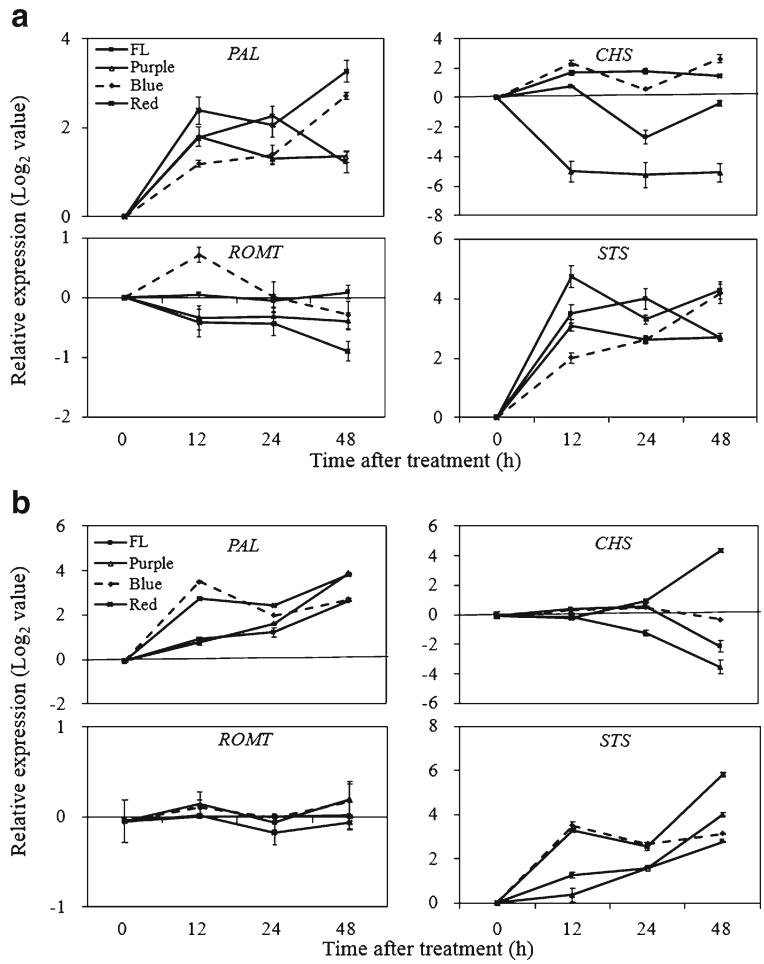

Fig. 6 Expression of flavonoid and stilbene synthetic genes by quantitative real-time PCR analysis of 'Campbell Early' (**a**) and 'Kyoho' (**b**) leaves exposed to fluorescent lights (FL) and purple, blue, and red LEDs. Transcript levels were calculated using the standard curve method from triplicate data with the grapevine actin gene as the internal control and untreated leaves (at time zero) as the reference sample. Results represent the mean fold increase in mRNA level over that of the untreated leaves, which was referred to as the 1× expression level. Results are the means of triplicate data from three experiments. *Bars* indicate the standard deviation

expression, with peaks occurring at 12 and 48 h after treatment. In 'Kyoho' grapevine, expression of *CHS* and *STS* genes was up-regulated within 12 h of red LED irradiation. Subsequently, the expression of *CHS* and *STS* genes increased progressively, reaching a peak at 48 h after treatment (Fig. 6b). *PAL* gene expression levels increased significantly in grapevine leaves treated with blue and red LED for 12 and 48 h, respectively.

Discussion

This study was conducted to evaluate the resistance in detached grapevine leaves under LED treatment. We previously reported accumulation of stilbenic compound by LED light in grape berries (Ahn et al. 2015). Although there have been several reports of induced resistance and resveratrol accumulation in response to UV irradiation (Adrian et al. 2000; Bonomelli et al. 2004; Choi 2012; Keller et al. 2003; Kobayashi et al. 2013; Wang et al. 2010), there have been no previous investigations of the induction of resistance responses against *B. cinerea* in grapevine leaves in response to different LED light sources. In this study, red light inhibited lesion development by *B. cinerea* and induced expression of defense-related genes in both 'Campbell Early' and 'Kyoho' grapevines. These findings are consistent with previous reports that red light enhanced resistance against *B. cinerea* in broad bean (Islam et al. 1998, 1999; Khanam et al. 2005), and induced resistance or suppressed lesion development against pathogens in *Arabidopsis* (Islam et al. 2008), broad bean (Rahman et al. 2003), cucumber (Rahman et al. 2010), and tomato (Schuerger and Brown 1997). Several studies have reported that exposure to blue light reduces fungal infection (Kim et al. 2013; Vakalounakis and Christias 1981). Murdoch et al. (2013) reported an

inactivation effect of blue light (405 nm) on the filamentous fungus *Aspergillus niger*. Suthaparan et al. (2010a, b) reported that development of pathogens was regulated by light in rose plants.

There is very little information available regarding the mechanism of induced resistance derived from light irradiation in plants. Rahman et al. (2002) reported that photosynthetic or protein synthetic inhibitor treatment inhibited the resistance induced by red light, which suggested a possible relationship between synthetic activity and red light-induced resistance in broad bean leaves against chocolate spot disease caused by *B. cinerea*. Several photoreceptors have been revealed in fungal systems (Corrochano 2007; Herrera-Estrella and Horwitz 2007), and it has been suggested that photoexcitation of natural photosensitizer porphyrins might lead to photoinactivation of grey mold after infection (Imada et al. 2014). It has also been reported that red and blue light produced different morphogenetic and photosynthetic responses in plants (Ma et al. 2014).

Stilbenic compounds accumulation is part of the grapevine response to fungal infection (Langcake and Pryce 1976), which induces pathogen resistance. Grapes with a high resveratrol content can limit fungal attack, which would stimulate resveratrol metabolism. However, these plants lack the amount of fungal enzymes needed to degrade the stilbenic compound. Stilbene compounds are well-known for their antioxidant activities, especially their free radical scavenging properties during interaction with pathogens in plants (Chong et al. 2009; Waffo-Téguo et al. 1998). High resveratrol producers accumulate resveratrol preferentially in the glycosylated forms, *trans*- and *cis*-piceid, relative to low resveratrol producers. Moreover, piceid concentration has been found to be greater than resveratrol levels in *V. vinifera* grapes (Gatto et al. 2008; Mattivi et al. 1995; Romero-Perez et al. 1996, 2001).

Plant stilbenes can accumulate in tissues and are well known to inhibit fungal growth as phytoalexins (Jeandet et al. 2002). Accumulation of antifungal substance(s) under red-light radiation was shown to be the active response to fungal attack in a study by Islam et al. (1998). Additionally, *trans*-resveratrol content varied widely following UV irradiation depending on grape variety, degree of maturity, storage period, and treatment method in plants (Tříska and Houška (2012). Furthermore, Demkura and Ballaré (2012) reported that UVR8 plays a key role in mediating the effects of UV-B radiation on pathogenicity by controlling expression of the sinapate biosynthetic pathway. Inactivation of pathogens by exposure to UV light is a well-known method of crop protection (Demkura and Ballaré 2012; Matsuura and Ishikura 2014; Nigro et al. 1998). However, irradiation with UV light has limited applications because of its harmful effects on workers, such as injury to the skin or eye upon direct exposure (Young 2006). In this study, we found that grey mold development was inhibited and stilbenic compounds accumulated in the leaves of two grapevine cultivars in response to irradiation with different LED light sources. These findings suggest that irradiation of LEDs including red light can be used to promote accumulation of stilbenic compounds against pathogen infections as an alternative to UV treatment in grapevines.

Many studies have reported that the expression of genes related to defense and the secondary metabolite biosynthesis pathway was regulated under light (Chamnongpol et al. 1996; Ebisawa et al. 2008; Ma et al. 2014; Schmidlin et al. 2008; Zhou et al. 2013). Induction of disease resistance response to pathogens by exposing plants to several light-emitting diodes (LEDs), including blue, green, yellow, and red, has been reported in plants (Imada et al. 2014; Islam et al. 1998; Kudo et al. 2009, 2011; Suthaparan et al. 2010b; Upadhyaya 2013). LEDs are solid-state, durable, long-lived light sources that provide narrow band spectral emissions (Upadhyaya 2013).

In the present study, the expression of 16 genes involved in defense response was differentially induced by LED light treatment. Among the genes tested here, *Glu*, *OSM*, *PR*4a, *PILP*, *TLP*, *GST*, *CHS*, *PAL*, and *STS* expression was highly up-regulated in the leaves of both cultivars following blue and red light irradiation. The high expression of several defense-related genes in plants exposed to LEDs suggests that irradiation by light from LEDs at various wavelengths could prevent disease development in plants. Moreover, up-regulation of STS, which are important stilbene biosynthetic genes, was consistent with the higher total resveratrol concentration in the grapevine leaves of both cultivars treated by red light in this study. Kudo et al. (2009, 2011) reported that *AOS*1 gene expression was induced by green light radiation, resulting in inhibition of strawberry anthracnose and cucumber grey mold. The transcript levels of *PAL*, *4CL*, and *STS* were positively correlated with the concentration of stilbene compounds in grapes (Gatto et al. 2008). In the present study, our results showed that blue and red LED suppressed lesion

development and induced the expression of genes related to defense response along with accumulation of stilbenic compounds. Taken together, our results suggest that LED radiation suppresses grey mold disease by direct and indirect mechanisms. The results of the present study suggest that red light LEDs can be used to promote the accumulation of stilbenic compounds, enhance the expression of defense-related responses, and protect grapevines from pathogen attacks during cultivation.

Michaloski (1991) invented the apparatus suitable for UV-C irradiation using driving devices including an array of lamps and energy directing reflectors that provide an appropriate level of irradiance to reduce fungal diseases from grapevines in vineyards. Harvested grapes have been exposed to UV lamp to increase the content of resveratrol in berries in postharvest (Choi 2011; Gonzalez-Barrio et al. 2009). About 30 % of the grapevines are cultivated in the plastic house to protect the vine shoots and clusters against a rainfall during grape ripening season, and to promote early harvesting and increasing quality of grapes in Korea (Yun et al. 2012). Through further studies for development of efficient application systems such as irradiation time, periodic intervals, distance between vines and LED lamps, and intensity in field-grown grapevines, it is considered that LED irradiation systems proposed in this study can be applied for the protection of grapevine leaves from grey mold caused by *B. cinerea* in Korean viticulture.

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