Original article

UV-C irradiation before or after inoculation with *Colletotrichum gloeosporioides* affected phytohormones and antioxidants in apple fruit (*Malus domestica* Borkh.)

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Summary

Decay due to fungi is a major problem in the storage of apples. The effect of 3 kJ m⁻² ultraviolet (UV)-C radiation applied both before and after inoculation with *Colletotrichum gloeosporioides* was examined. Both UV-C treatments significantly decreased the diameter of *C. gloeosporioides* lesions and maintained fruit firmness. The total phenolic concentration, total flavonoid concentration, and 2,2-diphenyl-1-picrylhydrazyl antioxidant activity were increased by the UV-C irradiation, but malondialdehyde (MDA) concentrations were significantly suppressed. UV-C irradiation before inoculation (UV-BI) increased endogenous jasmonic acid (JA) concentrations and *allene oxide synthase 1* (*MdAOS1*), but decreased ethylene production and 1-aminocyclo-propane-1-carboxylic acid (ACC) accumulation and down-regulated their related genes [*ACC synthase 1* (*MdACS1*), *ACC synthase 3* (*MdACS3*), *ACC oxidase 1* (*MdACO1*), *ethylene receptor 1* (*MdETR1*), *ethylene response sensor 1* (*MdERS1*), and *constitutive triple response 1* (*MdCTR1*)] compared to UV-C after inoculation (UV-AI). In addition, UV-BI up-regulated the expression of the pathogenesis-related (PR) genes *MdPR1a*, *MdPR2*, and *MdPR4*. These results suggest that UV-BI could inhibit the development of decay caused by *C. gloeosporioides* compared to UV-AI due to the induction of endogenous JA accumulation and the up-regulation of PR genes. The crosstalk between JA and ethylene may also play a role in the defense mechanism against pathogen infection in apple fruit.

Keywords

pathogenesis-related genes, jasmonic acid, ethylene, decay, UV-C irradiation

Significance of this study

What is already known on this subject?

- UV-C application inhibited pathogen development in several fresh fruit and vegetables.

What are the new findings?

- UV-C radiation before *Colletotrichum gloeosporioides* infection decreased the *C. gloeosporioides* lesion diameter through the upregulation of pathogenesis-related genes.

What is the expected impact on horticulture?

- UV-C radiation before infection with *C. gloeosporioides* can inhibit decay during the storage of apples.

tutive triple response 1 (*MdCTR1*) were induced in apple fruit (*Malus domestica*) infected by *Botrytis cinerea* (Suktawee et al., 2019). JA is regarded as an important plant signal molecule in the defense mechanism against necrotrophic phytopathogens (Ali et al., 2018). JA application can enhance disease resistance against *Fusarium oxysporum* in *Solanum quitoense* plants (Avila et al., 2019) and can up-regulate the expression level of pathogenesis-related (PR) genes in apple leaves (Bai et al., 2013).

PR proteins are classified in various families such as antifungal proteins (PR1), β -1,3-glucanases (PR2), and chitinases (PR4), and they have properties related to plant defense against biotrophic and necrotrophic pathogens (Sels et al., 2008). For example, enhanced activity of β -1,3-glucanase and chitinase enzymes was found as part of the defense against *Alternaria alternata* in tomato fruit (*Solanum lycopersicum*) (Cota et al., 2007). PR genes have been reported in various pathosystems such as apple – *Venturia inaequalis*, potato – *Phytophthora infestans*, and tobacco – *Phytophthora nicotianae* (Jain and Khurana, 2018). Furthermore, the expression of PR genes has been shown to be correlated with the activation of JA and ethylene, with up-regulation of their biosynthetic-related genes (Ali et al., 2018).

Ultraviolet (UV)-C irradiation is considered to be a low-cost non-chemical treatment against postharvest diseases (Sripong et al., 2019). It has been demonstrated that UV-C radiation at a dose of 5 kJ m⁻² inhibited *Monilinia fruticola* growth in pears (*Pyrus bretschneideri* Rehd.) through the induction of defense-related enzyme activity (Li et al., 2010). Sheng et al. (2018) reported that UV-C up-regulated phenolic and flavonoid biosynthetic-related genes such as *phenylalanine ammonia-lyase* (*PAL*), *chalcone synthase*, and *flavanone 3-hydroxylase* in grapes (*Vitis vinifera*). Moreover, the application of UV-C enhanced the signaling and biosynthesis

Introduction

The development of decay after harvest caused by *Colletotrichum gloeosporioides* contributes to major postharvest losses in apples. Wounding or infection with a pathogen affected the signaling molecules of plant hormones such as jasmonic acid (JA) and ethylene (Khan et al., 2014). As a defensive response against pathogen attack, systemic resistance (ISR) through the JA was induced (Poveda, 2020). For example, endogenous JA and the ethylene production and ethylene-related genes; 1-aminocyclo-propane-1-carboxylic acid (ACC) *ACC synthase 1* (*MdACS1*), *ACC oxidase 1* (*MdACO1*), and the ethylene signal transduction genes; *ethylene receptor 1* (*MdETR1*), *ethylene response sensor 1* (*MdERS1*), and *consti-*

of JA in *Arabidopsis thaliana* (Xu et al., 2016). In addition, Gangopadhyay et al. (2016) reported that UV-C priming radiation reduced the root rot disease caused by *Macrophomina phaseolina* in Indian Coleus (*Coleus forskohlii*). Therefore, UV-C irradiation may induce antioxidants, higher JA concentrations, and PR genes, and can result in the inhibition of postharvest disease. However, there are few reports on the effect of applying UV-C irradiation before pathogen infection on postharvest disease. This study investigated the effects of UV-C irradiation on *C. gloeosporioides* infection, PR genes, ethylene, and JA.

Materials and methods

Plant material

'Tsugaru' apple fruit were harvested at a pre-climacteric stage (See Figure 3) (5 months after full bloom) from the experimental field at Chiba University located at 36°N latitude, 139°E longitude, and 747 m altitude. After being harvested, the apples were thoroughly cleaned in tap water and disinfected with 0.002 M of sodium hypochlorite (NaOCl) for 1 min. The fruit were then rinsed with distilled water and dried at 25°C overnight.

Pathogen inoculation and UV-C treatment

A *C. gloeosporioides* (Registration No. MAFF 239927) spore suspension at a concentration of 6×10^5 spores mL⁻¹ was cultured in potato dextrose agar at room temperature for one week. The inoculation was performed according to Suktawee et al. (2019). A wound (5 mm diameter × 3 mm deep) was made on each apple fruit. The *C. gloeosporioides* mycelia agar block (6 mm diameter) was put on each wound.

Fruit were divided into three groups with 60 fruit each. The UV-C treatments were performed as follows. For UV-C before inoculation (UV-BI), UV-C irradiation was administered before the inoculation of fruit with *C. gloeosporioides*. The UV-C irradiation dose of 3 kJ m⁻² was provided by six UV lamps with a peak at 253 nm (GL-20W, Toshiba, Japan) from a distance of 25 cm. For UV-C after inoculation (UV-AI), UV-C irradiation was administered after the inoculation of fruit with *C. gloeosporioides*. An inoculated fruit that was not exposed to UV-C light was the untreated control. Following the UV-C treatment, the treated apples were kept at 25°C with 95% relative humidity for 16 days. The peels from 15 apples in each treatment were sampled at 0, 4, 12, and 16 days after treatment (DAT), the samples were immediately frozen in liquid nitrogen and stored at -80°C for analysis.

Determination of lesion diameter and measurement of fruit firmness

The lesion diameter (15 fruits per treatment) was measured using calipers every day after the inoculation. Fifteen apples per treatment were analyzed with a texture analyzer with a cylindrical probe (2 mm diameter) at a speed of 100 mm min⁻¹ (Rheo Meter CR-100, Sun Scientific, Japan). The measurement was performed after peeling.

Evaluation of total phenolic and total flavonoid concentrations and 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant activity

Three grams of fresh samples (3 replications of 15 fruits per treatment) were extracted with 20 mL of 80% methanol and centrifuged at 15,000 *g* for 10 min at 4°C. The total phenolic concentrations of the peels were evaluated using Folin-Ciocalteu's method according to Sheng et al. (2018).

The phenolic concentrations were monitored at 765 nm by spectrophotometer (U-2910, Hitachi, Japan). The total flavonoid concentration was evaluated according to Sheng et al. (2018) with modifications. The peel extract (250 µL) was added to 1 mL of deionized water and 75 µL of 5% sodium nitrite (NaNO₂). Then, 75 µL of 10% aluminum chloride (AlCl₃) was added to the mixture. After 5 min, 1 mL of 4% sodium hydroxide (NaOH) was added. This reaction mixture was measured at 510 nm. The DPPH antioxidant activity was determined according to Phonyiam et al. (2016) with slight modifications. A 500 µL volume of the sample extract was mixed with 1.5 mL of 0.1 mM DPPH-methanolic solution. The decrease of absorbance was monitored at 517 nm after incubation in the dark for 30 min.

Measurement of malondialdehyde (MDA) concentration

Lipid peroxidation was examined based on the production of MDA according to Phonyiam et al. (2016) with modifications. One gram of fresh sample (3 replications of 15 fruits per treatment) was homogenized with 7 mL of 10% trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000 *g* for 10 min at 4°C. Thereafter, the supernatant of 500 µL was mixed with 1.5 mL of 0.5% thiobarbituric acid in 10% TCA and thereafter incubated in 85°C for 30 min. The cooled mixture was measured at 532 nm using a spectrophotometer. The non-specific turbidity of MDA was determined at the absorbance of 600 nm.

Measurement of JA concentration

The endogenous JA concentration was determined according to Segarra et al. (2006). Fresh samples, that were 0.5 grams (3 replications of 15 fruits per treatment) were homogenized with 5 mL of MeOH-H₂O-CH₃COOH (90:9:1, v/v/v) and 100 µL of 10⁻⁴ M of ibuprofen, which was used as the internal standard. The homogenate was separated after centrifugation at 15,000 *g* for 10 min at 4°C. The supernatant was dried at 37°C and filtered with 1 mL of methanol using absolute high-performance liquid chromatography with an Omnipore 0.45-µm filter (Millipore, Bedford, U.S.A.). Analysis and quantification of extract containing JA was carried out using a liquid chromatograph mass spectrometer (LCMS-2010EV; Shimadzu, Kyoto, Japan). A 5 µL injected volume was monitored through an RP-18 octadecylsilyl column (5 µm, 2 mm I.D. × 150 mm; Kanto Chemical, Tokyo, Japan). The JA concentration was calculated from the ratio of the peak area for *m/z* 206/161 (ibuprofen).

Determination of ethylene and ACC concentration

Ethylene production was examined according to Suktawee et al. (2019). Apple fruits (3 replications of 15 fruits per treatment) were sealed in a plastic jar of 1.7 L and incubated at 25°C for one hour. A 1 mL gas sample was analyzed by gas chromatography (GC-2014; Shimadzu, Kyoto, Japan). ACC concentrations were evaluated based on Suktawee et al. (2019) with slight modifications. One gram of peel (3 replications of 15 fruits per treatment) was extracted with 10 mL of 0.1 M HCl. The reaction mixture, which contained 2 mL of crude extract, 0.2 mL of 0.1 M HgCl₂, and 0.2 mL of 5% NaOCl, was rapidly mixed and monitored by gas chromatography.

Measurement of total RNA extraction and quantitative reverse-transcription polymerase chain reaction (qRT-PCR) analysis

The total RNA was extracted from the apple peel. A 500 mg sample of peel (3 replications of 15 fruits per treat-

TABLE 1. Primer sequences used for quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) analyses.

Gene name	Primer sequences (5'–3')	References/Accession No.
<i>MdACS1</i>	(F) TCACCTCAATATATCTCCTGGA (R) CCTCAGGGACGTTGTAATAC	U89156
<i>MdACO1</i>	(F) GTTCTACAACCCAGGCAACG (R) TCTCAGAGCTCAGGCAGTTG	NM001294118
<i>MdETR1</i>	(F) GGGGCGACTCATCTTATCAA (R) CACCACCGCAGTTAAACCT	AF032448
<i>MdERS1</i>	(F) CAGATGAGCTGCTGGTGAAA (R) TATGAGCTCCAAGGGAATGG	AY083169
<i>MdCTR1</i>	(F) ACTTCTTGGATCCAGTGCCG (R) GACAGACAGCCATTACCCA	DQ847149
<i>MdAOS1</i>	(F) GGGAGAAGCTGTTGAAGCAC (R) TCCAGCACACTGTTTGTTC	XM008366758
<i>MdPR1a</i>	(F) GCTCAGCCGTAATACAATCCTCTC (R) TACCCCACTACTGCACCTCACT	DQ318212
<i>MdPR2</i>	(F) CTTCAGAGTCACCATCTTCAACA (R) GGTGCACCAGCTTTTTCAA	AY548364
<i>MdPR4</i>	(F) ATACCACCTCTACAATCCACA (R) GTCCAAGTCCAATCCTCC	XM008372483
<i>MdSAND</i>	(F) CCCAGGACTTTGAGCTTTATGC (R) TATCACCATGAAAAGGGGCTTG	Mimida et al. (2015)
<i>MdUBQ</i>	(F) CTCCGTGGTGGTTTTAAGT (R) GGAGGCAGAAACAGTACCAT	U74358
<i>MdHISH3</i>	(F) GTCAAGAAGCCCCACAGATAC (R) CTGGAACGCAGATCAGTCTTG	AY347801

FIGURE 1. Effect of UV-C irradiation on lesion diameters (A), fruit firmness (B), total phenols (C), total flavonoids (D), DPPH antioxidant capacity (E), and malondialdehyde (F) in the untreated control, UV-BI, and UV-AI treatments. The different letters indicate significant differences at $P \leq 0.05$ as determined by an LSD test each day with the mean $\pm$ SE of three replications of 15 fruits per treatment; ns: no significant difference.

ment) was extracted by the modified cetyl trimethylammonium bromide method (Henderson and Hammond, 2013). The purified RNA quality and integrity were determined using a UV spectrophotometer (SmartSpec Plus, Bio Rad, U.S.A.) in the ratio of A260/A280 and by agarose gel electrophoresis, respectively. The first strand cDNA was synthesized by the reversion of RNA using Revertra Ace qPCR RT Master Mix (Toyobo, Osaka, Japan), and a final concentration of 500 ng $50 \mu\text{L}^{-1}$ was used as the reaction mix for qRT-PCR analysis. The gene specific primers were used for qRT-PCR examination (Table 1). The analysis was performed using qRT-PCR (StepOnePlus™; Applied Biosystems, Foster City, CA, U.S.A.) with Kapa Sybr Fast qPCR Master Mix ABI Prism (Kapa Biosystems, Wilmington, MA, U.S.A.). The expression of each gene was examined using the quantitation comparative cycle threshold ($2^{-\Delta\Delta C_t}$) to normalize to the C_t values of *MdSAND*, *MdUBQ*, and *MdHISH3*.

Statistical analysis

All results are shown as the mean $\pm$ standard error (SE). The statistical analysis of variance was performed using the least significant difference (LSD) test at $P \leq 0.05$ (SPSS 16; IBM Corp., Armonk, NY, U.S.A.).

Results

Lesion diameter and fruit firmness

UV-C irradiation significantly reduced the lesion diameter compared to the untreated control throughout the storage period with a profound effect at 16 DAT (UV-BI was 4.51 ± 0.15 cm, UV-AI was 4.49 ± 0.11 cm, and the untreated

control was 5.04 ± 0.08 cm) (Figure 1A). UV-C irradiation significantly delayed the decrease of firmness in apple fruit at 12 and 16 DAT compared with the untreated control (Figure 1B). The soluble solid content and titratable acidity did not show any significant difference among the treatments (data not presented).

Total phenolic and total flavonoid concentrations, DPPH antioxidant activity, and MDA concentration

The total phenolic concentration was generally increased by UV-BI throughout the storage period compared with the other treatments (Figure 1C). UV-BI significantly enhanced the total flavonoid concentration at 4 and 16 DAT compared with the other groups (Figure 1D). In the UV-AI, the total flavonoid concentration was most increased at 12 DAT and was decreased at 16 DAT. UV-BI irradiation significantly increased DPPH activity compared to the untreated control (Figure 1E). The DPPH activity in the UV-AI group at 12 DAT was higher than that in the untreated control, but it decreased at 16 DAT. The MDA concentration was significantly decreased by both UV-BI and UV-AI irradiation at 4 DAT. There were no significant differences at 16 DAT among the treatments.

JA concentration and expression of allene oxide synthase 1 (*MdAOS1*)

The UV-BI significantly increased the JA concentration at 4, 12, and 16 DAT compared with the UV-AI and untreated control groups (Figure 2A). UV-AI also showed higher JA concentrations than the untreated control. The expression of *MdAOS1* in both the UV-BI and UV-AI groups was significantly up-regulated compared to the untreated control at 4, 12, and 16 DAT (Figure 2B).

Ethylene production, ACC concentration, and ethylene-related genes

In general, ethylene production in the untreated control was higher than that with UV-C irradiation during storage (Figure 3A). Ethylene production in the untreated control was highest at 16 DAT. The ACC concentration in the untreated control was highest at 12 DAT (Figure 3B). The UV-BI and UV-AI generally down-regulated the expression levels of *MdACS1* compared with that of the untreated control (Figure 3C). The *MdACO1* expression levels in UV-BI and UV-AI decreased significantly at 4 and 16 DAT (Figure 3D). The expression levels of *MdETR1*, *MdERS1*, and *MdCTR1* under both the UV-BI and UV-AI treatments were significantly lower than those in the untreated control (Figure 3E–G).

PR genes

The expression level of *MdPR1a* was significantly increased by UV-BI and UV-AI irradiation compared to the untreated control at 4 and 12 DAT (Figure 4A). In general, *MdPR2* in each treatment gradually decreased during storage (Figure 4B). The expression of *MdPR2* was up-regulated by UV-BI at 4 and 12 DAT compared to that of the untreated control group. The expression of *MdPR4* was significantly up-regulated by the UV-AI treatment (Figure 4C).

Discussion

UV-C irradiation limited the proliferation of foodborne pathogens including *Escherichia coli*, *Listeria innocua* or *Salmonella enterica* in apples (Graça et al., 2013) and inhibited decay caused by *B. cinerea* in strawberries (*Fragaria ananassa*) (Jin et al., 2017). In our study, both UV-BI and UV-AI at a dose of 3 kJ m^{-2} decreased the lesion diameter

FIGURE 2. Effect of UV-C irradiation on jasmonic acid (A) and the expression of *MdAOS1* (B) in the untreated control, UV-BI, and UV-AI treatments. The different letters indicate significant differences at $P \leq 0.05$ as determined by an LSD test each day with the mean $\pm$ SE of three replications of 15 fruits per treatment; ns: no significant difference.

of *C. gloeosporioides* in apple fruit. This is comparable to the findings of earlier studies by Charles et al. (2009) and Sripong et al. (2019), who demonstrated that UV-C could induce disease resistance through increasing plant-defense-related enzymes like PAL, peroxidase, and chitinase, which are involved in activating PR proteins such as PR2 in the man-

gosteen (*Garcinia mangostana* L.) and tomato. On the other hand, Severo et al. (2015) indicated that UV-C may delay fruit softening due to the decrease in the expression of firmness-associated genes such as *pectate lyase a (PLa)*, *PLb*, and *PLc* in the strawberry. These results are consistent with our finding that both UV-BI and UV-AI significantly delayed soften-

FIGURE 3. Effect of UV-C irradiation on ethylene production (A), ACC concentration (B), and the expressions of *MdACS1* (C), *MdACO1* (D), *MdETR1* (E), *MdERS1* (F), and *MdCTR1* (G) in the untreated control, UV-BI, and UV-AI treatments. The different letters indicate significant differences at $P \leq 0.05$ as determined by an LSD test each day with the mean $\pm$ SE of three replications of 15 fruits per treatment; ns: no significant difference.

ing in infected apple fruit. Moreover, Kesari et al. (2010) reported that a decrease in fruit firmness could promote the activity of *PR1a*.

UV-C radiation stimulated the total phenolic and flavonoid concentrations in wheat (*Triticum aestivum*) and lettuce (*Lactuca sativa*) (Ouhibi et al., 2014; Badridze et al., 2015). Our study showed that UV-BI increased the total phenolic and flavonoid concentrations compared to UV-AI and the untreated control in apples infected with *C. gloeosporioides*. These results suggest that the increases in bioactive substances such as phenols and flavonoids were immediately induced after irradiation with UV-C. UV-C irradiation may enhance the synthesis of secondary metabolites associated with the inhibition of pathogen infection. Furthermore, our study also revealed that UV-BI increased DPPH antioxidant activity. Jin et al. (2017) confirmed that UV-C treatment promoted antioxidant enzyme activities and inhibited gray mold

development in strawberry fruit. Li et al. (2019) and Xu et al. (2019) showed that phenolic, flavonoid, and overall antioxidant activities were increased via the stimulation of their key enzymes, namely PAL, cinnamate-4-hydroxylase, and 4-coumarate coenzyme A ligase, after exposure to UV-C radiation. Our results suggest that the UV-BI treatment induced disease resistance prior to infection with *C. gloeosporioides* through the enhanced accumulation of phenols and flavonoids as well as through increased DPPH antioxidant activity.

MDA is part of the secondary end-product of lipid peroxidation which results in membrane structure damage (He et al., 2019). The previous research showed that UV-C irradiation suppressed the production of MDA and lipoxygenase activity, which caused lipid membrane degradation and resulted in control of crown rot disease in bananas (*Musa AAA*) (Pongprasert et al., 2011). In our study, UV-C treatment decreased MDA concentrations. Therefore, lower MDA production may preserve the integrity of the cell membrane structure and subsequently promote resistance against infection with *C. gloeosporioides*.

It has been shown that JA promoted plant resistance against pathogens by inducing antimicrobial compounds and activating plant signaling molecules (Shigenaga and Argueso, 2016). A previous study demonstrated that UV-C treatment induced JA accumulation in strawberry fruit during storage (Xu et al., 2019). Moreover, Xu et al. (2017) reported that JA affected the antioxidant activity in plants. These results agree with our results, which showed that apple fruit exposed to UV-BI significantly promoted endogenous JA and increased total phenolic and flavonoid concentrations as well as antioxidant activity and PR gene expression. Our results suggest that the increase of JA accumulation may have increased

FIGURE 4. Effect of UV-C irradiation on the expressions of *MdPR1a* (A), *MdPR2* (B), and *MdPR4* (C) in the untreated control, UV-BI, and UV-AI treatments. The different letters indicate significant differences at $P \leq 0.05$ as determined by an LSD test each day with the mean $\pm$ SE of three replications of 15 fruits per treatment; ns: no significant difference.

FIGURE 5. Overview of the potential effect of UV-C irradiation on phytohormones and antioxidant activity in apple fruit. The solid lines indicate UV-C-mediated up-regulation or stimulation (arrow) and inhibition (blunt-end line). Dashed lines indicate some interaction or process involved in hormone crosstalk. CW = cell wall, PM = plasma membrane.

the antioxidant activity involved in the up-regulation of PR genes. The *allene oxide synthase* (AOS) is a dominant key gene for the biosynthesis of JA (Garrido-Bigotes et al., 2018), and our study found that the expression of *MdAOS1* was stimulated by UV-C irradiation. JA concentrations may have increased through the increase in *MdAOS1* expression.

Ethylene plays a role in fruit ripening and in plant defense against pathogens. For example, it has been reported that UV-C radiation suppressed ethylene biosynthesis in the tomato, banana, and mango (*Mangifera indica*) (Bu et al., 2013; Pristijono et al., 2018). Our result also showed that UV-BI decreased ethylene production as well as the ACC concentrations. Moreover, UV-BI also down-regulated the transcript levels of ethylene biosynthesis genes (*MdACS1* and *MdACO1*) and signal transduction genes (*MdETR1*, *MdERS1* and *MdCTR1*). These results may be associated with the JA accumulation. Sun et al. (2016) reported that both ethylene and JA signaling play a role in regulating necrotrophic pathogens. For example, the interaction between ethylene and JA revealed that *JASMONATE ZIM DOMAIN PROTEIN* down-regulated the transcripts of *ETHYLENE INSENSITIVE 3* (EIN3) and EIN3-like 1, resulting in the stimulation of defense proteins such as *PLANT DEFENSIN 1.2* (Yang et al., 2019). Therefore, this suggests that a reduction of ethylene biosynthesis is required during JA signaling for UV-BI to enhance the plant defense against *C. gloeosporioides*.

The accumulation of PR proteins is frequently used as a defensive marker against phytopathogens such as viruses, bacteria, and fungi (Sels et al., 2008). The induction of the *PR1* and *PR2* genes was considered to activate phytohormones such as JA and ethylene (Ali et al., 2017). For example, enhanced expression of *MpNPR1* promoted pathogen defense against apple diseases caused by *Erwinia amylovora* (Malnoy et al., 2008). Furthermore, Sarowar et al. (2005) also reported that high resistance to oomycete and bacterial pathogens in tobacco (*Nicotiana tabacum*) was stimulated by the overexpression of *CABPR1*. Besides, *PR2* family genes played a role in plant defense through the deconstruction of the cell walls of pathogenic fungi (Balasubramanian et al., 2012). Our results also showed that the expressions of *MdPR1a* and *MdPR2* were up-regulated by UV-C irradiation. Jin et al. (2017) reported that UV-C treatment prior to *B. cinerea* inoculation induced defense-related genes such as *cinnamoyl CoA reductase-1* allele, *chitinase*, *polyphenol oxidase*, and *PAL6* in strawberry fruit. *PR4* is categorized as a chitinase enzyme, which particularly functions in the plant defense response through hydrolyzing chitin-containing pathogen cell walls (Hamid et al., 2013). Our results showed that the expression level of *MdPR4* was up-regulated by UV-C application. These results agree with those of Bai et al. (2013), who demonstrated that an up-regulated expression of *MdPR4* may be related to ribonuclease activity that is associated with antifungal mechanism.

Conclusion

The UV-BI was more efficient in suppressing *C. gloeosporioides* than UV-AI through increasing the JA concentration and the expression of *MdAOS1*, decreasing ethylene production, and down-regulating *MdACS1*, *MdACO1*, *MdETR1*, *MdERS1*, and *MdCTR1* expressions, as well as up-regulating *PR* genes. In addition, the total phenolic and flavonoid concentrations and DPPH antioxidant activity were increased by UV-BI irradiation. UV-BI may induce the apple defense mechanism against *C. gloeosporioides* through the synergistic interaction of the *PR* gene and crosstalk between JA and ethylene.

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