n*-Propyl dihydrojasmonates influence ethylene signal transduction in infected apple fruit by *Botrytis cinerea

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The effects of the jasmonic acid derivative *n*-propyl dihydrojasmonate (PDJ) on ethylene signal transduction and endogenous jasmonic acid (JA) in apples infected with *Botrytis cinerea* (gray mold) were investigated. Apples were dipped into 400 μ M PDJ solution and then inoculated with *B. cinerea*. The fruit were stored at 25°C and 95% relative humidity for 16 days after PDJ treatment. The inoculation without PDJ application (PDJ⁻ Ino⁺) showed larger *B. cinerea* lesion diameters compared to the PDJ application with inoculation (PDJ⁺ Ino⁺) and the untreated controls. In contrast, the PDJ⁺ Ino⁺ group showed a higher ethylene production rate, higher 1-aminocyclo-propane-1-carboxylic acid (ACC) concentration, and greater expressions of ethylene-related genes [*MdACS1* (ACC synthase), *MdACO1* (ACC oxidase), *MdETR1* (Ethylene receptor 1), *MdERS1* (Ethylene response sensor 1), and *MdCTR1* (Constitutive triple response 1)], and endogenous JA, *MdAOS1* (Allene oxide synthase 1) gene. However, the abscisic acid concentrations were decreased in the PDJ⁺ Ino⁺ group. The results suggest that PDJ application induces ethylene production through ethylene-related genes and endogenous JA, resulting in *B. cinerea* infection inhibition.

Key Words: abscisic acid, gray mold disease, jasmonic acid, PDJ, volatile compounds.

Introduction

A synthetic analog of jasmonic acid (JA), *n*-propyl dihydrojasmonate (PDJ), has been widely used in Japan for the promotion of apple (*Malus domestica* Borkh.) coloration. PDJ application induced enzymatic antioxidant systems, resulting in reduced chilling injury symptoms in banana peel (*Musa* spp.) (Kondo et al., 2005; Pongprasert et al., 2006), and ethylene production was increased in pears treated with PDJ (Kondo et al., 2007). Nimitkeatkai et al. (2011) and Wang et al. (2015b) reported that PDJ treatment effectively inhibited growth of *Collectotrichum gloeosporioides* in apricots (*Prunus mume* Sieb. et Zucc.) and *Glomerella*

cingulate in grape berries (*Vitis labrusca* $\times$ *Vitis vinifera*).

JA and ethylene may be associated either synergistically or antagonistically in the regulation of various stress-related responses such as pathogen infection and wounding (Grennan, 2008; Khan et al., 2014). Ethylene was observed to effectively retard *Erwinia carotovora* and *Botrytis cinerea* by the up-regulation of pathogenesis-related (PR) genes in *Arabidopsis thaliana*, but promoted disease development in tomatoes (*Solanum lycopersicum*) and soybeans (*Glycine max* (L.) Merr.) (Kunkel and Brooks, 2002). *ETHYLENE RESPONSE FACTOR1* (*ERF1*) is a functional transcription factor which is a key gene regulated by ethylene or JA or both, and it is involved in pathogen resistance through the activation of the expression of PR genes (Lorenzo et al., 2003).

Shin et al. (2014) reported that ethylene and JA treatment up-regulated the expressions of *MdERF* and the PR protein encoding gene (β -chitinase) in apple roots infected with *Pythium ultimum*. JA and ethylene appli-

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cation synergistically induced JA-dependent defense genes in *A. thaliana* (Norman-Setterblad et al., 2000; Penninckx et al., 1998), and osmotin and *PR1b* in tobacco (*Nicotiana tabacum*) (Xu et al., 1994). PDJ application in apricot fruit infected with *C. gloeosporioides* increased the ethylene production, the expression level of the *PmACO1* gene, and aroma volatile emissions (Nimitkeatkai et al., 2011).

B. cinerea is one of the major postharvest diseases in apples, peaches (*Prunus persica* Batsch.), strawberries (*Fragaria × ananassa* Duch.), grapes (*Vitis vinifera*), and pears (*Pyrus* spp.). *B. cinerea* infection occurs in the maturation stage and appears at the ripening stage (Williamson et al., 2007). Applications of salicylic acid (SA) and JA have been reported to induce priming defense in plants (Conrath, 2011; Wang et al., 2015a). JA application was effective in enhancing disease resistance and inhibiting disease incidence in the postharvest shelf life of loquats (*Eriobotrya japonica*), peaches, grapes, and Chinese bayberries (*Myrica rubra* Sieb. et Zucc. ‘Wumei’) (Cao et al., 2008; Jin et al., 2009; Wang et al., 2014, 2015a). Pichersky et al. (2006) observed that volatile compounds functioned against herbivores and pathogens. Absciscic acid (ABA) responded to abiotic stress, i.e., drought, salt, and cold. ABA also responded to plant pathogens that are thought to be involved in stress signaling (Cao et al., 2011; Mauch-Mani and Mauch, 2005).

However, the interactions between JA, ethylene, and the changes in volatile compounds in apples infected with a pathogen are not well understood. In this study, the effects of PDJ application on ethylene signal transduction, endogenous JA, ABA, volatile compounds, and lesion diameter in apples infected with *B. cinerea* (gray mold) were examined.

Materials and Methods

Fruit material and pathogens

Nine randomly selected 12-year-old ‘Fuji’ apple trees (*Malus domestica* Borkh.) grafted onto Malling 26 (M.26 EMLA) rootstocks in an open field at Chiba University, located at 36°N latitude, 139°E longitude and 747 m altitude were trained as central leaders. Two hundred and twenty-five fruit (25 fruit per tree) were sampled at the climacteric stage (180 days after full bloom). The fruit weight was 260–300 g. The fruit were rinsed in tap water, and dipped into 150 mg·L⁻¹ sodium hypochlorite (NaClO) for 3 min. They were then rinsed with distilled water and air-dried at 25°C.

The causative agent for gray mold disease in apples, *B. cinerea* (MAFF Registration No. 712208), was used in this experiment. The pathogen was cultured in potato dextrose agar (PDA) at 25°C for 10 days.

PDJ application and inoculation

The fruit were randomly divided into three groups of 75 fruit (three replications of 25 fruit per tree) per treat-

ment. The first and second groups were dipped into 0.1% surfactant approach BI (50% polyoxyethylene hexitan fatty acid ester; Kao, Osaka, Japan) for 5 min. In the third group, the fruit were dipped into a 400 µM PDJ solution containing 0.1% surfactant approach BI for 5 min; these fruit are referred to hereafter as the PDJ-treated group. The concentration of PDJ was decided based on previous studies which showed the enlargement of an inoculated pathogen was suppressed in Japanese apricots and grapes (Nimitkeatkai et al., 2011; Wang et al., 2015b). After treatment, the fruit were air-dried.

For inoculation, all fruit were wounded (5 mm dia. × 3 mm deep) with a sterilized knife and inoculated with a mycelial block (6 mm dia.). The *B. cinerea* block was transferred from PDA and placed on the wounded surface of the second group (PDJ⁻ Ino⁺) and PDJ-treated groups (PDJ⁺ Ino⁺). The first group was inoculated without fungal mycelial and is referred to hereafter as the untreated control group. All fruit were stored at 25°C with 95% relative humidity. The peels of 15 apples (three replications of five apples) were sampled every 4 days following treatment (DAT), and frozen immediately in liquid nitrogen. They were then stored at -80°C until the analysis of ACC, JA, ABA, and aromatic compounds and their related gene expression levels.

Determination of lesion diameter

Each lesion diameter (15 fruit per treatment) was measured using Vernier calipers daily after inoculation, and the diameters were calculated from two equidistant wound areas in each fruit.

Ethylene production and 1-aminocyclopropane-1-carboxylic acid (ACC) concentration

The ethylene production and ACC concentration were analyzed by gas chromatography with a flame ionization detector (GC-2014; Shimadzu, Kyoto, Japan) according to Kondo et al. (1991) and Lizada and Yang (1979), respectively. For ethylene analysis, the apples were placed in a plastic box with a lid and incubated at 25°C for 1 hr. Headspace gas (1 mL) was extracted and injected into a gas chromatography system. ACC concentration was analyzed in the peel [1 g fresh weight (FW), three replications].

Jasmonic acid concentration

One gram (FW) of peel (three replications) was used for JA analysis. JA with ibuprofen as an internal standard was analyzed according to Kondo et al. (2005) with gas chromatography/mass spectroscopy-selective ion monitoring (GC-MS-SIM) (QP 5000; Shimadzu, 25 m × 0.25 mm I.D. column). The column temperature gradient was 60°C for 2 min, 60–270°C at 10°C·min⁻¹, and 270°C for 35 min with a 50.2 cm·s⁻¹ linear helium flow and 70 eV electron potential. The ions were measured as *m/z* 390, 264, and 209. The concentration of

JA was determined from the ratio of peak areas for m/z 209 (jasmonic acid)/264 (ibuprofen).

Abscisic acid concentration

The peel (1 g FW, three replications) was homogenized in 20 mL cold 80% (v/v) methanol including 0.5 polyvinylpyrrolidone with 0.2 μg ABA- d_6 as an internal standard and analyzed with GC-MS-SIM (QP 5000; Shimadzu, 25 m $\times$ 0.25 mm I.D. column) (Setha and Kondo, 2009). The column temperature gradient was 60°C for 2 min, 60–270°C at 10°C·min⁻¹, and 270°C for 35 min with a 50.2 cm·s⁻¹ linear helium flow. The ions were measured as m/z 190, 194, 260, and 264. The concentrations of ABA were determined from the ratio of peak areas for m/z 190 (d_0)/194 (d_6)

RNA extraction, cDNA synthesis, and quantitative real-time RT-PCR analysis

Total RNA in the peel (0.3 g FW, three replications) was extracted using a column-based extraction method (Henderson and Hammond, 2013) with a cetyltrimethylammonium bromide buffer. RNA was converted to cDNA using ReverTra Ace[®] qPCR RT Master Mix (FSQ-201; Toyobo, Osaka, Japan) following the manufacturer's instructions. Table 1 shows the specific primer of each gene for the quantitative real-time polymerase chain reaction (qRT-PCR) analysis. The transcription levels were estimated by qRT-PCR (StepOnePlus[™]; Life Technologies, Tokyo, Japan) with KAPA SYBR[®] FAST qPCR Master Mix (Kapa

Biosystems, Woburn, MA, USA) according to the instruction manual. The expression level of each gene was calculated as the coefficient of variation and normalized to the transcript level of the average of *HISTONE H3* (*HISH3*), *UBIQUITIN* (*UBQ*), and *SAND* genes.

Volatile compound analysis

Volatile compounds were measured according to Wang et al. (2015b) with a slight modification. One gram (FW) of peel (three replications) with cyclohexanol as an internal standard was incubated at 40°C in a 5-mL vial with a cover lid. Headspace gas was absorbed by solid-phase microextraction (SPME) fibers and then injected into a gas chromatography system with a flame ionization detector (GC-4000; GL Sciences, Tokyo; DB-Wax, 60 m $\times$ 0.25 mm I.D. capillary column; Agilent, Santa Clara, CA, USA). The oven, detector, and injection temperatures were 50°, 250°, and 230°C, respectively. The compounds were identified by commercial reference compounds with a GC-MS QP2010 standard gas chromatography mass spectrometer (Shimadzu). The concentrations of individual compounds were determined using the peak of the internal standard as a reference value and calculated based on the standard curves of pure compounds.

Statistical analysis

The data are presented as the mean values of three replications $\pm$ the standard error (SE), subjected to anal-

Table 1. Specific primers for real-time PCR.

Gene		Forward/reverse primer (5 prime-3 prime)	References/Accession no.
<i>MdACS1</i>	(F)	ACAGCCTCTCTAAGGATCTTGGTCT	U89156
	(R)	TTTGGTTCTCGGCTATGTAGTTCTT	
<i>MdACS3</i>	(F)	TGAGTCAAGCAACCCATCTG	U73816
	(R)	AATTTGCCATTGCCTTTCTG	
<i>MdACO1</i>	(F)	GTTCTACAACCCAGGCAACG	M81794
	(R)	TCTCAGAGCTCAGGCAGTTG	
<i>MdETR1</i>	(F)	GGGGCGACTCATCTTATCAA	AF032448
	(R)	CACCACCGCAGTTAAACCT	
<i>MdERS1</i>	(F)	CAGATGAGCTGCTGGTGAAA	AY083169
	(R)	TATGAGCTCCAAGGGAATGG	
<i>MdCTR1</i>	(F)	ACTTCTTGGATCCAGTGCCG	DQ847146
	(R)	GACAGACAGCCATTACCCA	
<i>MdAOS1</i>	(F)	AAGAAGGATATCTTCACCGAAC	XM_008379148.2
	(R)	CGACTCGACTTGAGGAGGTAG	
<i>MdAOS2</i>	(F)	GGGAGAAGCTGTTGAAGCAC	TC59991
	(R)	TCCAGCACACTGTTTGTTC	
<i>MdHISH3</i>	(F)	GTCAAGAAGCCCCACAGATAC	Mimida et al. (2015) AY347801
	(R)	CTGGAAACGCAGATCAGTCTTG	
<i>MdUBQ</i>	(F)	CTCCGTGGTGGTTTTTAAGT	Mimida et al. (2015) U74358
	(R)	GGAGGCAGAAACAGTACCAT	
<i>MdSAND</i>	(F)	CCCAGGACTTTGAGCTTTATGC	Mimida et al. (2015) MDP0000088431
	(R)	TATCACCATGAAAAGGGGCTTG	

ysis of variance (ANOVA) procedures, and separated by the Tukey-Kramer test at $P \leq 0.05$ using the SAS statistical analysis package ver. 8.2 (SAS, Cary, NC, USA).

Results

Lesion diameter

The lesion diameters of the apples infected by *B. cinerea* are shown in Figure 1. The PDJ⁺ Ino⁺ treatment significantly decreased the lesion diameter from 3 to 16 DAT compared to the PDJ⁻ Ino⁺ treatment.

Ethylene production, ACC concentration, and the expression levels of ethylene-related genes

The ethylene production in the PDJ⁺ Ino⁺ group was significantly increased at 8 and 12 DAT compared to the untreated control and PDJ⁻ Ino⁺ groups (Fig. 2A). The ACC concentrations were highest in the PDJ⁺ Ino⁺ group at 8–16 DAT compared to the PDJ⁻ Ino⁺ group. The ACC concentration in the untreated control was lowest at 16 DAT.

In general, the expression levels of *MdACS1* and *MdACO1* genes showed a pattern similar to that of the

ACC concentrations (Fig. 2C, E). That is, the PDJ⁺ Ino⁺ group showed significantly higher expression levels compared to the untreated control and PDJ⁻ Ino⁺ groups. The *MdACS3* expression levels in the PDJ⁺ Ino⁺ group were increased at 4 and 8 DAT compared to the

Fig. 1. Lesion diameters in apples infected with *B. cinerea*, and PDJ treatment. Different letters indicate significant differences ($P \leq 0.05$) by Tukey-Kramer test at each date. Data show the mean $\pm$ SE of three replications.

Fig. 2. Changes in the ethylene production (A), ACC concentration (B), and expression levels of ethylene-related genes (C–H) in the untreated control, PDJ⁻ Ino⁺, and PDJ⁺ Ino⁺ groups. Different letters indicate significant differences ($P \leq 0.05$) by Tukey-Kramer test at each date. Data show the mean $\pm$ SE of three replications.

PDJ⁻ Ino⁺ group (Fig. 2D). In contrast, the expression level of the *MdACS3* gene in the PDJ⁻ Ino⁺ group was decreased at 4 DAT. However, expression levels of the *MdACS3* gene did not significantly differ between the PDJ⁺ Ino⁺ group and the PDJ⁻ Ino⁺ group at 12 and 16 DAT. During storage, the expression levels of the *MdETR1*, *MdERS1* and *MdCTR1* genes were generally higher in the PDJ⁺ Ino⁺ group compared to the other groups (Fig. 2F, G).

The JA concentration and the expression levels of *MdAOS2* gene

The JA concentration in the PDJ⁺ Ino⁺ group was increased at 4–16 DAT compared to the other groups (Fig. 3A). The expression levels of *MdAOS1* were up-regulated in the PDJ⁺ Ino⁺ group from 4 days after treatment and were significantly higher than the untreated control and PDJ⁻ Ino⁺ groups until 12 DAT, then decreased sharply at 16 DAT (Fig. 3B). The expression levels of *MdAOS2* in the PDJ⁺ Ino⁺ and PDJ⁻ Ino⁺

Fig. 3. Changes in the jasmonic acid (JA) concentration (A) and expression level of the *MdAOS1* (B) and *MdAOS2* (C) genes in the untreated control, PDJ⁻ Ino⁺, and PDJ⁺ Ino⁺ groups. Different letters indicate significant differences ($P \leq 0.05$) by Tukey-Kramer test at each date. Data show the mean $\pm$ SE of three replications.

groups were significantly higher than the untreated control group at 12 DAT, and were more strongly up-regulated in the PDJ⁺ Ino⁺ than those in the PDJ⁻ Ino⁺ and the untreated control groups at 16 DAT (Fig. 3C).

Absciscic acid (ABA) concentration

ABA concentrations rapidly decreased at 4 DAT (Fig. 4). At 8 DAT, the PDJ⁺ Ino⁺ and PDJ⁻ Ino⁺ groups showed higher ABA concentrations compared to the untreated control group. However, the ABA concentrations were not significantly different between each treatment at 4 and 16 DAT.

Volatile compound analysis

Thirty-two types of aromatic volatile compounds (four alcohols, three aldehydes, 22 esters, and three terpenes) were detected in apple skins (data not shown). The alcohol concentrations at 12 DAT and the aldehyde and α -farnesene concentrations at 12 and 16 DAT in the untreated controls were higher than those in both inoculated groups (Fig. 5A, B, D). In general, the alcohol, ester, aldehyde, and α -farnesene concentrations in the PDJ⁺ Ino⁺ group were the lowest compared to the other treatments.

Discussion

The PDJ application at 400 μ M significantly reduced the lesion diameters of *B. cinerea* in apples. Similar results have been reported; PDJ application decreased the lesion diameter of *C. gloeosporioides* in apricots and *G. cingulate* in 'Kyoho' grape berries (Nimitkeatkai et al., 2011; Wang et al., 2015b). Other research showed that preharvest methyl jasmonate application reduced growth infection of *B. cinerea* in Chilean strawberries [*F. chiloensis* (L.) Mill.] through upregulation of the β -1,3-glucanase gene (*FcBGs*) and polygalacturonase-inhibiting protein (*FcPGIP*) gene (Saavedra et al., 2017). JA is associated with defense mechanisms against *Alternaria alternata*, *Pseudomonas syringae*,

Fig. 4. Changes in the absciscic acid (ABA) concentrations in the untreated control, PDJ⁻ Ino⁺, and PDJ⁺ Ino⁺ groups. Different letters indicate significant differences ($P \leq 0.05$) by Tukey-Kramer test at each date. Data show the mean $\pm$ SE of three replications.

Fig. 5. Changes in volatile compounds in the untreated control, PDJ⁻ Ino⁺, and PDJ⁺ Ino⁺ groups. Different letters indicate significant differences ($P \leq 0.05$) by Tukey-Kramer test at each date. Data show the mean $\pm$ SE of three replications.

B. cinerea, and *Penicillium citrinum* through the induction of pathogenesis-related (PR) protein (Ding et al., 2002; Jung et al., 2009; Yu et al., 2009; Wang et al., 2014; Saavedra, et al., 2016).

Ethylene is a plant hormone that regulates ripening and the stress process. For instance, the increase in ethylene production through the expression of *CrACS1*, *CrASC2* and *CrACO* genes resulted in a reduction in *P. digitatum* infection in citrus fruit [*Citrus sinensis* (L.) Osbeck] (Marcos et al., 2005). Tong et al. (2017) suggested that methyl jasmonates (MeJA) promoted ethylene biosynthesis during apple ripening via enhancement of the transcription of *MdACS1* and *MdACO1*. The present experiments showed that PDJ⁺ Ino⁺ treatment up-regulated the expression of ethylene signal transduction genes (i.e., *MdETR1*, *MdERS1* and *MdCTR1*), as well as the ethylene and ACC concentrations. Ethylene binds with membrane-bound receptors such as *ETR1* and ethylene response factor (*ERF*) genes (Plett et al., 2014). Similar to our study, Lv et al. (2018) reported that methyl jasmonate application enhanced the expression of ethylene biosynthesis-related genes such as *MdACS*, *MdACO*, *MdETR*, *MdERS*, *MdCTR*, *MdEIN* (*Ethylene insensitive*), *MdEIL* (*ein3-like*) and *MdERF* during apple ripening.

JA is a phytohormone synthesized in response to biotic and abiotic stress. JA application inhibited post-harvest pathogens in fruit such as loquats, sugarbeet roots (*Beta vulgaris*), grapes, peaches, and apricots (Cao et al., 2008; Fugate et al., 2012; Nimitkeatkai et al., 2011; Wang et al., 2015a; Yao and Tian, 2005). Fugate et al. (2017) found that MeJA application up-regulated genes for PR proteins as a defense response in sugar beet roots. In our study, the JA concentrations increased in the PDJ⁺ Ino⁺ group, whilst the expression levels of *MdAOS1* also increased. These results are in agreement with our previous finding that PDJ applica-

tion induced the accumulation of endogenous JA at the ripening stage in pears (*Pyrus communis*) (Kondo et al., 2007). The *AOS* gene is a major gene in the JA biosynthetic pathway. Our results agree with the previous research of Ruiz et al. (2013) which reported that *PpAOS1* gene levels were enhanced by methyl jasmonate treatment during development. However, the coincident expressions in *MdAOS2* were not observed in our study, although previous research showed that *MdAOS2* could be involved in the JA biosynthesis pathway during maturation (Lv et al., 2015).

JA application promoted ethylene production in apples, Japanese plums (*Prunus salicina* Lindl.), and mangoes (*Mangifera indica*) (Kondo et al., 2009; Khan and Singh, 2015; Lalel et al., 2015). Kondo et al. (2009) reported that JA treatment increased the expression levels of *MdACS1* and *MdACO1*, as well as ethylene production, in apples. Previous research demonstrated that *MdACS3* functions in system I and that *MdACS1* was expressed at the fruit ripening stage for system II ethylene biosynthesis (Tan et al., 2013; Wiersma et al., 2007). Our present investigation revealed that PDJ⁺ Ino⁺ increased the expression levels of *MdACS3* gene prior to the increase in the *MdACS1* level. These results suggest that JA application induced system I ethylene biosynthesis in apples infected with *B. cinerea*.

Previous research indicated a relationship between JA and ethylene in plant defense. JA induced the expression of the *ERF1* gene and then the expression of then *PR* gene (Lorenzo et al., 2003; Kouzai et al., 2016). Salicylic acid is a key regulator of pathogen-induced systemic acquired resistance (SAR), whereas JA and ethylene are required for induced-systemic resistance (ISR) (Ton et al., 2002; Pieterse et al., 2000; Pieterse et al., 2003). Shores et al. (2005) reported that increased levels of Lipoxygenase1 (*Lox1*), Phenylalanine ammonia-lyase1 (*Pall*), *ETR1*, and *CTR1* en-

hanced the ISR in the JA/ethylene signaling pathways in cucumber (*Cucumis sativus*) plants. Therefore, PDJ may induce ISR through JA/ethylene signaling defense response pathways. Alternatively, PDJ could directly inhibit pathogen growth.

ABA can regulate abiotic and biotic stresses in fruit and can also regulate maturation processes in non-climacteric fruit such as grapes, strawberries (*Fragaria × ananassa*) and cherries (*Prunus avium*) (Li et al., 2011; Wheeler et al., 2009; Falchi et al., 2014). ABA treatment increased the pathogen resistance of fruit (Sivakumaran et al., 2016; Lievens et al., 2017). Wang et al. (2015b) reported that PDJ application increased the endogenous ABA and JA concentrations in inoculated 'Kyoho' grape berries. In our study, the endogenous ABA concentration in the pathogen-infected fruit was increased at 8 DAT. However, the ABA concentrations generally did not show a clear tendency. Unlike the results with non-climacteric fruit such as grapes, ethylene may respond significantly against pathogen infection in apples.

Volatile compounds such as esters are synthesized from fatty acids and amino acids (El Hadi et al., 2013). In the β -oxidation pathway, the fatty acid is catabolized to alcohols and acyl co-enzyme A (acyl-CoA), then the acyl-CoA is resolved by a series of enzymes such as lipoxygenase (LOX), hydroperoxide lyase (HPL), pyruvate decarboxylase (PDC), alcohol dehydrogenase (ADH), and alcohol acyltransferase (AAT) (Defilippi et al., 2005; Wang and Luca, 2005). Ethylene was shown to play a role in the production of compounds during ripening (Johnston et al., 2009). Several compounds (e.g., hexenal isomers and 2,3-butanediol) were increased in apple plants infected with *E. amylovora* and activated by JA and ethylene (Spinelli et al., 2011). Oxylipin, which is an active lipid generated from fatty acids, is initiated by the LOX-dependent oxidation of fatty acids, and it produces green leaf volatiles (Wasternack and Kombrink, 2010). Celloni et al. (2016) reported that *LOX* genes may provide tolerance responses mediated by JA in apple plants infected with *E. amylovora*. In our study, the PDJ⁺ Ino⁺ group showed lower levels of volatile compounds; this may be related to the increase in the endogenous JA in the defense mechanism of *B. cinerea*. The increase in JA in pathogen-infected apples may not require an increase in volatile compounds against infection. Pertaining to the effect of PDJ on endogenous JA, our previous report suggested that PDJ may increase JA biosynthesis by influencing AOS in the octadecanoid pathway (Yoshikawa et al., 2007). In addition, our previous study also showed that AAT activity was increased with pathogen inoculation and decreased by PDJ treatment, indicating that AAT may be involved in the JA pathway (Nimitkeatkai et al., 2011).

Conclusion

PDJ application decreased the enlargement of *B. cinerea* lesion diameters. This result may be related to increases in endogenous JA and ethylene production, as well as increases in the expression levels of *MdAOS1*, *MdACS3*, *MdETR1*, and *MdCTR1*, in apples infected with *B. cinerea*. The apple's defense resistance system against *B. cinerea* may depend on the synergistic relationship between JA and ethylene.

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