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Induction of Pigmentation and Anthocyanin Biosynthesis in Harvested Strawberry Fruit by Methyl Jasmonate

메틸자스몬산에 의한 수확후 딸기 과실의 착색 및 안토시아닌 생합성 유도

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UNDER THE DIRECTION OF DR. EUN JIN LEE SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL OF SEOUL NATIONAL UNIVERSITY

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ABSTRACT

Strawberry (*Fragaria* × *ananassa*) is an important fruit worldwide. The fruit has high nutritional value such as anthocyanins and ascorbic acid. However, strawberry fruit is delicate and highly perishable to mechanical injury, desiccation, and physiological disorders after harvest. Methyl jasmonate (MeJA), a naturally occurring plant endogenous hormone, plays an important role in plant development, fruit ripening, and defense mechanism.

Furthermore, postharvest application of MeJA has been reported to effectively enhance antioxidant capacity in berry fruits including strawberry and suppress postharvest diseases caused by necrotrophic pathogens. In this study, the effects of postharvest MeJA treatment on anthocyanin biosynthesis and postharvest decay in 'Seolhyang' strawberry fruit were investigated. Peduncles of strawberry fruits were immersed in 100 μM MeJA solution and stored at 10 ± 0.5ºC and 90 ± 5% relative humidity for 3 weeks. Fruits firmness and respiration rate were not significantly affected by MeJA. Red coloration by MeJA was significantly increased to a greater degree compared to that in control. Total anthocyanins contents were higher in MeJA treatment (27.5 ± mg·100g^{-1} fresh weight) than in control (13.4 ± mg·100g^{-1} fresh weight) at 2 weeks in storage. Changes in phenylalanin ammonia-lyase (PAL), chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H), dihydroflavonol 4-reductase (DFR), anthocyanidin synthase (ANS), and flavonoid 3-*O*-glucosyltransferase (UFGT) mRNA levels involved in anthocyanin biosynthesis were measured by quantitative reverse transcription polymerase chain reaction (RT-qPCR). PAL and CHS expression levels were higher than CHI, F3H, DFR, ANS, and UFGT expression levels in control fruits at 3 and 6 days in storage. CHI, F3H, DFR, ANS, and UFGT expression levels in MeJA treatment were higher than those in control fruit at 6 and 8 days in storage. On the other hand, the expression level of genes involved in anthocyanin biosynthesis were higher in control fruits than in

MeJA treated fruits after 10 days in storage. These results suggest that postharvest MeJA treatment on ‘Seolhyang’ strawberry fruits increased the red coloration by up-regulating mRNA expression of these genes during early storage. The MeJA treatment significantly reduced fruit decay. At the end of storage, decay rate in MeJA treatment was only 21%, whereas 67% of fruit decay was shown in control fruit. Exogenous MeJA treatment also increased endogenous MeJA contents in the strawberry fruits. These results indicated that MeJA can effectively improve red coloration by increasing anthocyanin biosynthesis and reduce decay on ‘Seolhyang’ strawberry fruits. Thus it will help to elucidate the ripening mechanism of non-climacteric strawberry fruit, and it will be beneficial in distributing the high quality strawberry through developing the decay inhibition technique by MeJA.

Keywords: antioxidant capacity, coloration, defense, *Fragaria* × *ananassa*, ripening

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LIST OF ABBREVIATIONS

ABA	Abscisic acid
ANS	Anthocyanidin synthase
AOC	Allene oxide cyclase
AOS	Allene oxide synthase
CHI	Chalcone isomerase
CHS	Chalcone synthase
CK	Cytokinin
DFR	Dihydroflavonol 4-reductase
ET	Ethylene
F3H	Flavanone 3-hydroxylase
GA	Gibberellin
GC	Gas chromatograph
HPLC	High performance liquid chromatography
JA	Jasmonic acid
JAR1	Jasmonic acid amino acid synthetase
JMT	Jasmonic acid carboxy methyl transferase
LC-MS	Liquid chromatography-mass spectrometer
LOX	13-Lipoxygenase
MeJA	Methyl jasmonate

OPDA	12-Oxo-phytodienolic acid
OPR3	*Cis*-12-oxo-phytodienoic acid reductase
PAL	Phenylalanine ammonia-lyase
RT-qPCR	Quantitative reverse transcription polymerase chain reaction
SA	Salicylic acid
TCD	Thermal conductivity detector
UFGT	Flavonoid 3-*O*-glucosyltransferase
UV	Ultraviolet ray

INTRODUCTION

Strawberry (*Fragaria* × *ananassa* Duch.) fruit is one of the most widely consumed berries, and it has high nutritional value such as anthocyanin and ascorbic acid (Capocasa et al., 2008). 'Seolhyang' strawberry, a breed between 'Red pearl' and 'Akihime', has been known as one of the major cultivars in Korea (Kim et al., 2014). However, strawberry fruits are highly perishable and susceptible, with short shelf life due to their soft texture and high sensitivity to fungal diseases (Landi et al., 2014; Vicente et al., 2002). Because of these characteristics, when harvesting full-ripen strawberry, shelf life of fruits is short due to high decay rate. On the other hand, when harvesting at immature stage, the marketability decreases due to bitter taste, as well as uneven coloration.

Natural compounds have been applied to prolong the postharvest shelf life and to provide safety for consumers (Landi et al., 2014). Several natural volatile compounds have been reported to possess antimicrobial activity. Methyl jasmonate (MeJA), a naturally occurring plant endogenous hormone, plays an important role in development, fruit ripening, and defense mechanism (Concha et al., 2013; Moreno et al., 2010; Wang et al., 2008). Application of MeJA has been reported to increase ethylene production, red coloration, and anthocyanin contents in apple fruit (Rudell et al., 2002). MeJA

treatment in strawberry fruit stimulated red coloration through faster chlorophyll degradation and anthocyanin accumulation (Concha et al., 2013; Perez et al., 1997).

Jasmonic acid (JA) biosynthesis is initiated by the oxygenation of α-linolenic acid in the chloroplast. JA biosynthesis pathway involves at least six enzymes including lipoxygenase (LOX), allene oxide synthase (AOS), allene oxide cyclase (AOC), 12-oxophytodienoate reductase 3 (OPR3), JA carboxyl methyltransferase (JMT), and jasmonate amino acid synthetase (JAR1) (Fonseca et al., 2009; Turner et al., 2002). In peach fruit, MeJA increased the expression of LOX, AOS, and OPR3 genes, which result in increase of JA concentration (Ziosi et al., 2008). These results indicated positive feedback of JA on their own biosynthesis pathway during fruit ripening. Additionally, MeJA treatment stimulated anthocyanin biosynthesis in several fruits such as apple and strawberry fruits (Moreno et al., 2010; Rudell et al., 2002; Wang et al., 2008; Wang et al., 2009).

Chemical treatments have been applied to prevent postharvest diseases. However, chemical treatments were minimized for food and environment safety. For these reasons, many physical methods to prolong postharvest shelf life are well documented. The exposure to temperature at 45ºC for 3 h in air could delay fruit ripening and reduce faunal attack in strawberries (Civello et al., 1997; Garcia et al., 1995; Vicente et al., 2002). Furthermore, natural compounds such as MeJA, chitosan, and mixture of calcium and organic acids

have been applied to prevent insect attack and prolong postharvest shelf life, through the reduction of decay and with increase of ecological security, and safety for consumers (Landi et al., 2014).

In this study, MeJA, known as plant endogenous hormone involved in plant ripening and defense mechanism, was applied to ‘Seolhyang’ strawberry fruit. This study investigated the role of postharvest MeJA treatment to extend shelf life by inhibiting fruit decay, as well as to enhance the red coloration by inducing anthocyanin biosynthesis during ripening of non-climacteric strawberry fruit.

LITERATURE REVIEW

1. ‘Seolhyang’ strawberry

‘Seolhyang’ is a new strawberry (*Fragaria* × *ananassa* Duch.) cultivar, which was breed by Nonsan Strawberry Experiment Station of Chungnam Agricultural Research and Extension Services in 2005. This cultivar was originated from a cross between ‘Red pearl’ and ‘Akihime’ and shows excellent characteristics including vigorous growth, high yield and fruit quality, and high disease resistance. It was named ‘Nonsan No. 3’ as line after examining the productivity in forcing culture. After regional adaptability test, the cultivar name, ‘Seolhyang’, was named on this cultivar. The flowering and harvesting dates of ‘Seolhyang’ are slightly slower than those of ‘Akihime’. The fruit shape is conic, and fruit color is red. The average fruit weight of ‘Seolhyang’ is about 14.7 g and the marketable yield is relatively 50% higher than that of ‘Redpearl’ because of low percentage of abnormal fruit bearing. It is resistant against powdery mildew at the season of harvest but sensitive to anthracnose and aphids (Table 1). ‘Seolhyang’ strawberry has a sweet taste because of low acidity and juicy even though the total soluble solid contents is not high as 10-11 ^{o}Brix (RDA, 2008). ‘Seolhyang’ strawberry is one of the major cultivars in Korea, and cultivation area of ‘seolhyang’ strawberry is on

a growing trend.

Table 1. Disease and insect pest resistance of 'Seolhyang' strawberry.

Cultivar	Powdery mildew	Anthracnose	Gray mold rot	Wilt disease	Aphid	Leaf mite
Seolhyang	+	+++	++	+	++	++
Akihime	++++	++++	++	+	++	++

+, low; ++, middle; +++, high; ++++, highest frequency.

2. Role of plant hormone to induce fruit ripening and inhibit decay

Plant hormones are a group of naturally occurring compounds which influence physiological processes at low concentrations. Auxin, gibberellin (GA), and cytokinin (CK) involved in plant growth and development by stimulating cell division and elongation, and delaying leaf senescence. On the other hand, ethylene (ET) promotes leaf and fruit abscission and fruit ripening. Abscisic acid (ABA) and JA inhibit many plant processes such as growth and seed germination. Especially JA promote senescence, abscission, fruit ripening, and pigment formation (Davis, 2010; Fig. 1A).

Plant hormones also play an important role in plant defense mechanism (Fig. 1B). Defense hormones such as salicylic acid (SA), JA, and ET play a main role in the plant-pathogen interactions. Herbivore damage increases JA and ET signaling and activates resistance against necrotrophic pathogens (Bostock, 1999; Reymond et al., 2000). In contrast, infection by biotrophic and hemibiotrophic pathogens increases SA signaling and triggers resistance against these pathogens (Gaffney et al., 1993; Ryals et al., 1994). SA, JA, and ET induce the expression of resistance proteins such as PDF 1, 2 and pathogenesis-related protein. Ultimately, the hypersensitive response occurs in the infected plant cell. Auxin, ABA, GA, CK, and brassinosteroid known as

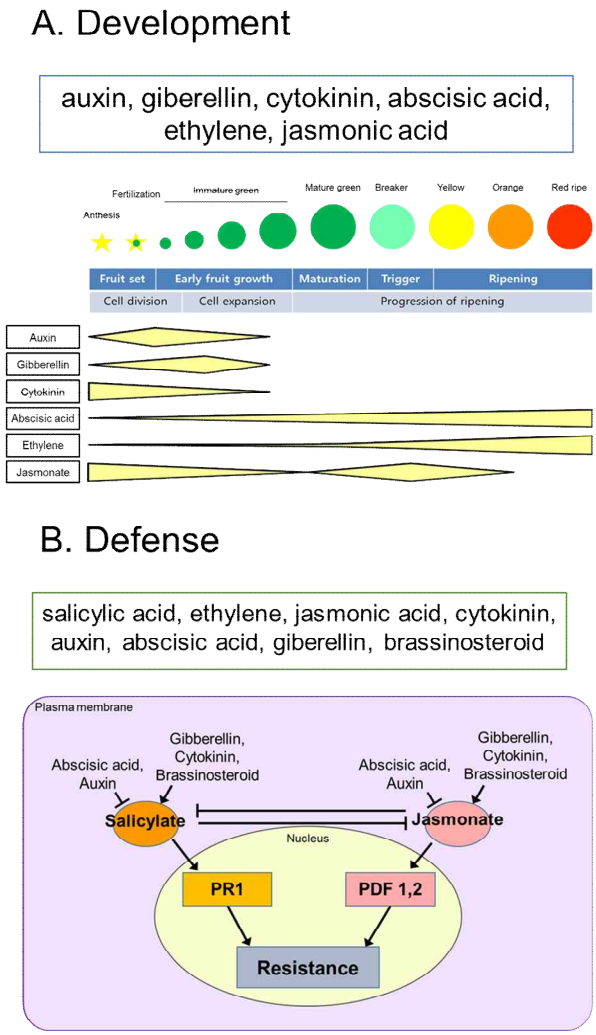

Fig. 1. The roles of the plant hormones in plant development (A) and defense mechanism (B).

plant growth hormones also modulate the plant-pathogen interactions. Auxin and ABA signaling trigger the suppression of SA signaling (Asselbergh et al., 2007; Robert-Seilaniantz et al., 2007; Ton and Mauch-Mani, 2004). GA elevates SA signaling and attenuates JA signaling (Achard et al., 2003; Navarro et al., 2006). CK and brassinosteroid also enhance the SA signaling (Choi et al., 2010; Divi et al., 2010). Levels of resistance in plants are affected by systemic signals mediated by plant hormone (Robert-Seilaniantz et al., 2011).

3. Jasmonate

JA and MeJA, naturally occurring plant endogenous hormones (Fig. 2), play important roles in pollen viability, root growth, fruit ripening, and resistance to insect and pathogen attack (Concha et al., 2013; Moreno et al., 2010; Wang et al., 2008). Endogenous concentration of JA increases during fruit ripening, and exogenous JA treatment stimulates ET production and coloration (Srivastava and Handa, 2005). Especially, JA regulates diverse aspects of plant defense. Endogenous JA levels increase rapidly in response to abiotic/biotic stresses. Exogenous JA treated plant result in re-programming of defense-related genes that are activated by wounding and herbivore attack (Davis, 2010).

As shown in Fig. 3, octadecanoid pathway for JA biosynthesis is initiated by LOX in the chloroplast. The 13-hydroperoxylinoleic acid is converted to an unstable 12, 13-epoxylinoleic acid by the action of AOS. AOC transforms the AOS reaction product to 12-oxo-phytodienolic acid (OPDA). The cyclopentenone ring of OPDA is reduced by OPR3. Three cycles of β-oxidation remove six carbons from the carboxyl side chain, competing the biosynthesis of JA. JA is converted to jasmonolyl-L-isoleucine and MeJA by the action of JAR1 and JMT, respectively (Cheong and Choi, 2003).

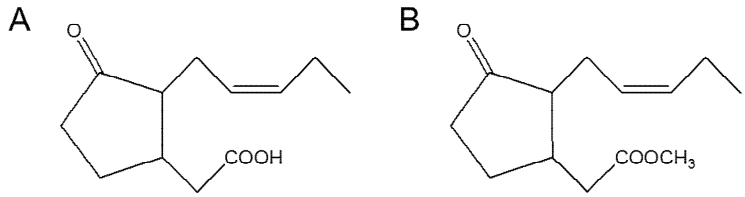

Fig. 2. Chemical structures of jasmonic acid (A) and MeJA (B).

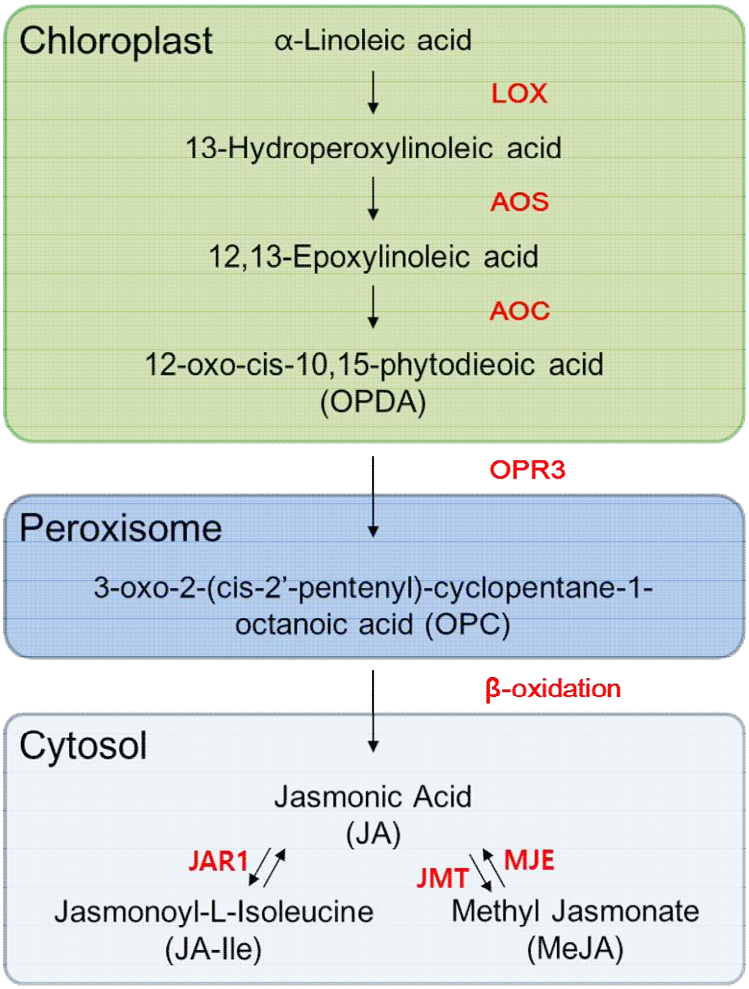

Fig. 3. Basic biosynthesis pathway of MeJA (from Cheong and Choi, 2003).

It has been reported that MeJA treatment could suppress postharvest diseases and enhance antioxidant capacity in various berry fruits (Wang et al., 2009). MeJA may have possibility commercial applications for fruit shelf life by reducing decay and enhancing antioxidant activity because it is already classified as a Generally Recognized As Safe substance by the U.S. Food and Drug Administration (Wang et al., 2009). Application of MeJA has been reported to increase ET production, red coloration, and anthocyanin contents in apple fruit (Rudell et al., 2002). MeJA treatment in strawberry fruit stimulated red coloration through faster chlorophyll degradation and anthocyanin accumulation (Concha et al., 2013; Perez et al., 1997).

MATERIALS AND METHODS

1. Plant material

Strawberry fruits (*Fragaria* × *ananassa* Duch.) were harvested from a commercial field in Nonsan, Korea. Immature, green strawberry fruits were harvested and immediately transported to the laboratory. Fruits of uniform size were selected for the in vitro ripening experiment (Fig. 4A).

2. MeJA treatment

Fruit peduncles were trimmed and immersed in 50 mL tubes containing incubation solution. The solution consisted of distilled H_2O containing 88 mM sucrose and 1 mM hydroxyl quinoline hemisulfate with two different MeJA (Sigma Aldrich, St. Louis, MO, USA) concentrations: 0 (control), and 100 μM (Fig. 4B). The fruits in solution were incubated in a storage room at 10 ± 0.5ºC and 90 ± 5% relative humidity for 3 weeks. Fruits were sampled at 0, 1, 3, 5, 7, 9, and 13 days in storage.

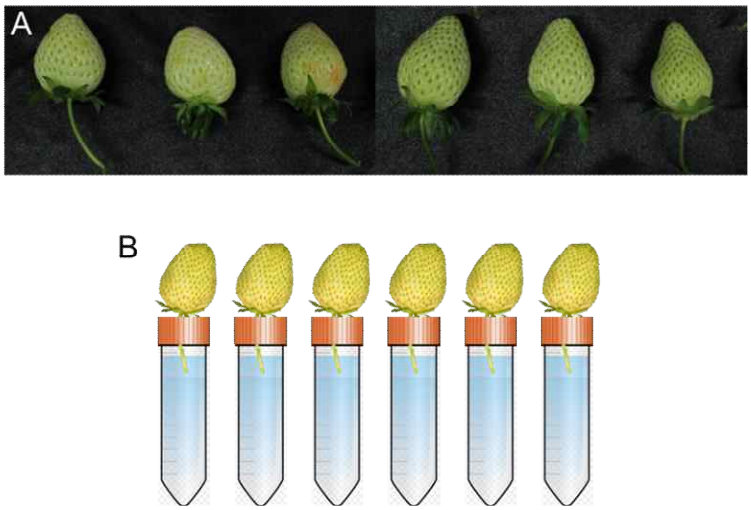

Fig. 4. Immature, green strawberry fruits (A) and MeJA treatment (B).

3. Fruit quality assessments

At each sampling time point, fruits from each treatment were observed for fungal presence, firmness, skin color, and respiration rate.

3. 1. Fungal presence

Fruit decay by pathogen (gray mold) was calculated as the percentage of total strawberry fruits.

3. 2. Firmness

Firmness was measured using a texture analyzer (model CT3, Brookfield, MA, USA) with a 5 mm flat probe. Each fruit was compressed 5 mm at a rate of 1 $mm \cdot s^{-1}$. Fifteen fruits were measured at each sampling time and the results were expressed in Newtons (N).

3. 3. Skin color

Fruit skin color was recorded by photograph (Canon power shot SX 510, Seoul, Korea).

3. 4. Respiration rate

Strawberry fruits were put into a 10 L air-tight polycarbonate jar. After 2 h incubation in a chamber at 10ºC, 1 mL of gas samples were taken. The gas samples were injected into a gas chromatography (GC; YL6400, Younglin Co., Anyang, Korea) equipped with a thermal conductivity detector (TCD), and CO_2 gas concentration (%) was measured. One% of CO_2 standard (Supelco, Bellefonte, PA, USA) was used. The conditions of GC were shown in Table 2.

4. Anthocyanin analysis

4. 1. Sample extraction

Fruit (3 g fresh weight) was ground with liquid nitrogen in 50 mL of methanol/HCl (99:1, v/v) and stirred for 1 h. Methanol extracts (10 mL) were evaporated by nitrogen gas, and dissolved in 5 mL water containing 3% formic acid. The extracted samples were purified by solid phase extraction using Sep-Pak silica cartridge (Waters, Milford, MA, USA). Cartridge inserted in 5 mL syringe and conditioned with 10 mL methanol. Extracted sample by passing 5 mL through cartridge, washing with 2 mL water and 2 mL 2.5% aqueous formic acid. Anthocyanin was eluted with 3 mL methanol containing 3% formic acid. Methanol extracts were evaporated by nitrogen gas and dissolved in 500 μL methanol containing 3% formic acid. The

Table 2. GC conditions for respiration rate analysis.

Parameter		Condition
GC system		Younglin 6400
Detector		Thermal conductivity detector
Column		Para Pak Q (packed column)
Mobile phase		Helium
Temperature (°C)	Oven	50
	Injector	250
	TCD Detector	200

extracts were quantitatively analyzed by high performance liquid chromatography (HPLC).

4. 2. HPLC and liquid chromatography-mass spectrometer (LC-MS) analysis

Purified extracts (500 μL) were analyzed using YL9100 HPLC system (Younglin, Anyang, Korea) with UV detector set at 520 nm and Symmetry C18 column (4.6 × 250 mm, USA). A gradient mobile phase of aqueous 5% formic acid (A) and acetonitrile (B) separated the compounds by decreasing B from 95 to 20% in 40 min. The conditions of HPLC were shown in Table 3. The individual anthocyanins were identified using LC-MS. The conditions of LC-MS were shown in Table 4.

5. Gene expression analysis

5. 1. RNA isolation

Total RNA was isolated from 3 g of fruit prepared for each treatment using a modified cetyl trimethylammoniun bromide method (Gasic et al., 2004). Three biological replicates were used for each treatment. cDNA synthesis was performed using amfiRivert platinum cDNA synthesis master mix kit (GenDEPOT, Katy, TX, USA) for quantitative reverse transcription

Table 3. HPLC conditions for anthocyanin analysis.

Parameter	Condition
HPLC system	YL9100 HPLC system
Detector	UV detector
Column	Symmetry C18
Mobile phase	ACN, aqueous 5% formic acid
Flow rate	1.0 $mL \cdot min^{-1}$
Colum temperature	Room temperature
Injection volume	10 μL
Absorbance	520 nm

Table 4. LC-MS conditions for anthocyanin analysis.

Parameter	Condition
HPLC system	Dionex u3000
Detector	UV detector
Column	Kinetex F5
Mobile phase	ACN, aqueous 0.1% formic acid
Flow rate	0.3 $mL \cdot min^{-1}$
Injection volume	2 μL
Absorbance	520 nm

polymerase chain reaction (RT-qPCR, Bio-Rad, Seoul, Korea) according to the manufacturer's instructions.

5. 2. Gene analysis

The transcriptional profile of 13 genes related to different pathways during postharvest storage was analyzed by RT-qPCR. In each treatment, the expressions of the genes involved in anthocyanin biosynthesis (phenylalanin ammonia-lyase (PAL), chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H), dihydroflavonol 4-reductase (DFR), anthocyanidin synthase (ANS), and flavonoid 3-*O*-glucosyltransferase (UFGT)) and MeJA biosynthesis (LOX, AOS, AOC, OPR3, JMT, and JAR1) pathways were analyzed. 18S was used as the reference housekeeping gene. Specific primer sequences for PAL, CHS, CHI, F3H, DFR, ANS, and UFGT were obtained from previous reports (Concha et al., 2013). Specific primers for the LOX, AOS, AOC, OPR3, JMT, and JAR1 genes were designed from full length cDNA sequences (Table 5).

5. 3. RT-qPCR analysis

The amplification reactions were performed using the CMQE 500 (Cosmogenetech, Seoul, Korea) according to the manufacturer's instructions in a CFX connect Real-Time system. The PCR conditions were as follows: 40 cycles of 94ºC for 30 s, 95ºC for 10 s, 60ºC for 20 s, and 72ºC for 20 s;

Table 5. Primer sequences (5'→3') used for RT-qPCR analysis of the 13 genes involved in anthocyanin and MeJA biosynthesis.

Gene	Forward primer sequence	Reverse primer sequence	Product size (bp)
PAL	GATTTGAGGCATTTGGAGGA	CTTGCCTTAGCCTTTGCATC	217
CHS	GCCGAGGAGTTGACAGAGTC	TTTCAATGGCTTTCGCTTCT	190
CHI	TGATGATTGGCATCTCCAAA	TGCCTTGTTTTCTGCTTCCT	233
F3H	TTGTCCATAGCGACATTCCA	AGTTGCTCCTTTGCATGCTT	176
DFR	ACCCTGCAATCAAAGGAACC	TAAATGCTGCTTCCTCCGTG	234
ANS	AGTGCGTACCCAACTCCATC	TGTGCTGGATATGCTCGAAG	240
UFGT	GTGGTCACTTCGGGACAACT	AGTTTCTGATCGCCGAAGAA	219
LOX	TCTGCATTTTAGGCCACCAG	GATGAGAGTGCTCCACACGG	232
AOS	CCCCGAGTTTCACTCCAGCT	AAGAGAACCCATTTCCGGGAC	197
AOC	CCCCAAGACCCACAAAAGTT	CTTTCTTTTCCGGCTTGCTT	216
OPR3	GCTATGGACTCCGACCCAGT	TGCTTCATCTTGATCACTGCC	168
JMT	CCGGTCTTGATCATCCTTCG	ACCGGGGACAGCAGAAATC	185
JAR1	TCTCCGGTTCTCACTGGAA	TGAACTGTAAGGCCTTCCCG	183

melting curve from 65 to 95°C for 10 min. Each reaction was performed in triplicate. The relative expression levels correspond to the mean of three biological replicates were normalized against the mean calculated for the expression level of the housekeeping gene. Control fruits at 0 day were used as the calibrator sample and assigned as a nominal value of 1. The expression level was calculated according to the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001) and expressed in arbitrary units.

6. Statistical analysis

The data were statistically evaluated using SPSS statistical software. Analysis of variance (ANOVA) was used, and comparison of means was done by the Duncan's multiple range test at $P = 0.05$.

RESULTS

1. Changes of quality and physiological parameters of strawberry fruit by MeJA treatment

A representative image showing the visual changes observed in fruits at 0, 3, 9, and 15 days under different MeJA treatment was shown in Fig. 5. Change in fruit color during postharvest storage included a decrease in green color with an increase in red color. MeJA treatment significantly affected the acquisition of red color at 9 days compared to the control.

Fruit firmness decreased during storage in all treatments (Fig. 6). Firmness (12.1 ± 5.8 N) significantly increased in MeJA treatment compared with the control (7.7 ± 2.4N) at 1 day. However, MeJA treated fruit firmness decreased after 1 day. Firmness was slightly lower in MeJA treatment (5.7 ± 1.2 N) than in the control (8.3 ± 3.4 N) at 6 days. There were no difference between the treatment and control fruit at 8 days.

Respiration rate (33.6 ± 8.1 mL CO_2 $kg^{-1}h^{-1}$) of the control fruits was higher than that (22.6 ± 2.6 mL CO_2 $kg^{-1}h^{-1}$) of MeJA treated fruits at 1 day. For 10 days, the respiration rate maintained at 21.6 ± 2.5 mL CO_2 $kg^{-1}h^{-1}$ in both treatments. At 13 days, the respiration rate slightly increased in the

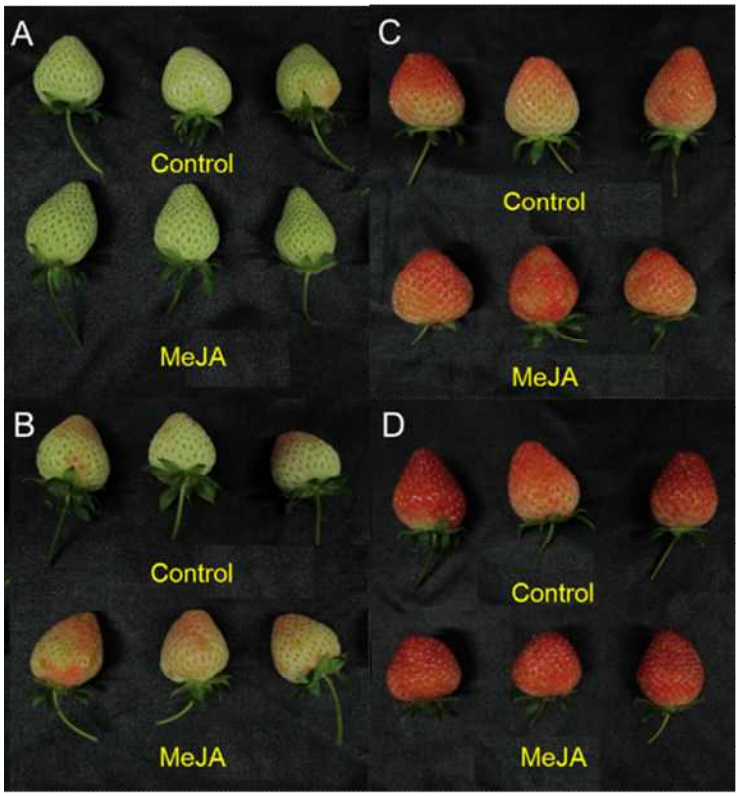

Fig. 5. Changes in pigmentation of strawberry fruits by MeJA during storage at 0°C. A, 0 day; B, 3 days; C, 9 days; D, 15 days in storage.

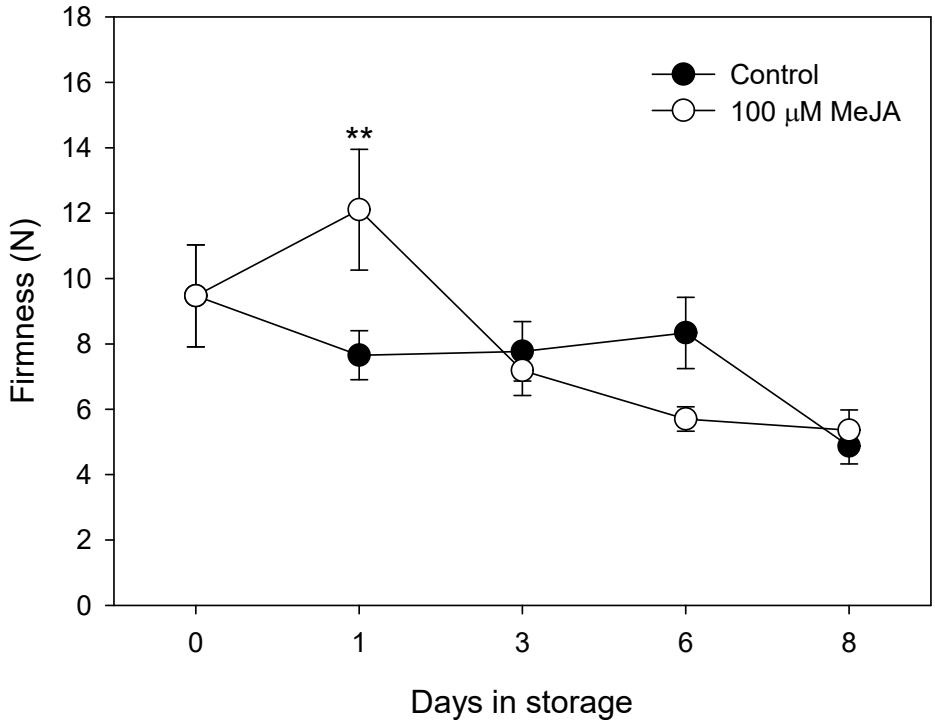

Fig. 6. Change in firmness of strawberry fruit by MeJA during storage at 0°C. The means are significant at $P = 0.01$ (**).

control and MeJA treated fruits at 34.7 ± 6.2 and 28.8 ± 4.0 ml CO_2 $kg^{-1}h^{-1}$, respectively. During storage, no sharp changes in respiration rate was observed with no significant differences between treatment and the control fruit (Fig. 7).

The effect of MeJA treatment on decay of fruit was shown in Fig. 8. Decayed fruits appeared at 7 days storage in MeJA treatment. MeJA treatment significantly inhibited fruit decay after 10 days storage. At the end of storage, decay rate in MeJA treatment was only 21%, whereas 67% of fruit decay was shown in the control fruit.

These results indicate that MeJA can effectively improve red coloration and inhibit decay in 'Seolhyang' strawberry fruit after harvest.

2. Quantitative analysis of anthocyanin and biosynthesis gene expression in 'Seolhyang' strawberry fruit by MeJA treatment

The HPLC chromatograms of 'Seolhyang' strawberry extracts were shown in Fig. 9. Cyanidin-3-glucoside, pelargonidin-3-glucoside, and petunidin-3-glucoside, the major anthocyanin in strawberry fruits, were confirmed by LC-MS. LC-MS results of the cyaniding-3-glucoside, pelargonidin-3-glucoside, and petunidin-3-glucoside showed a molecular ion

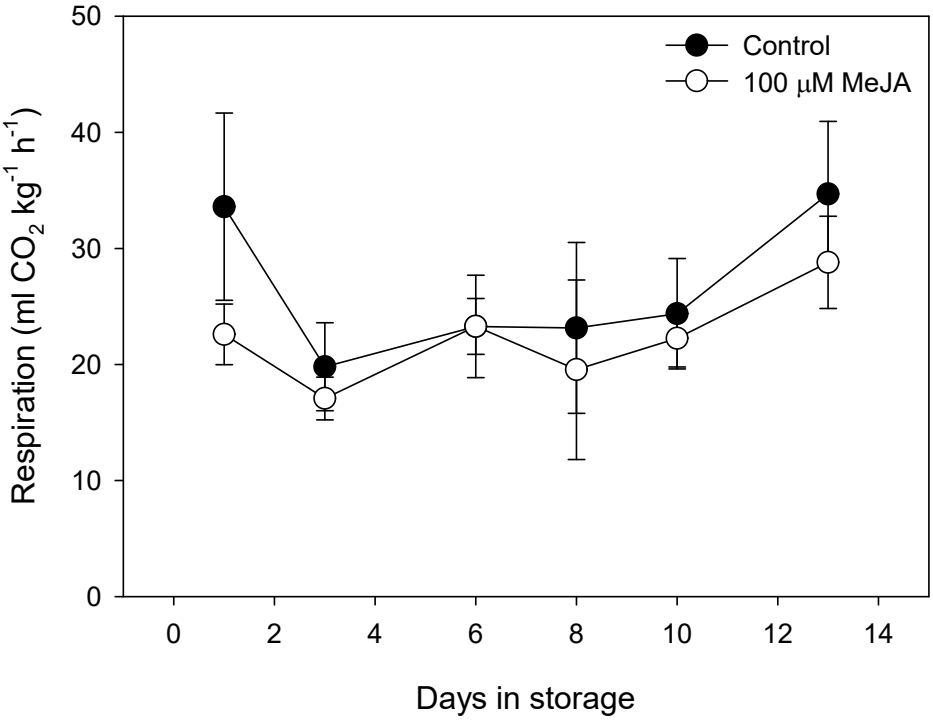

Fig. 7. Change in respiration rate of strawberry fruits by MeJA during storage at 0°C.

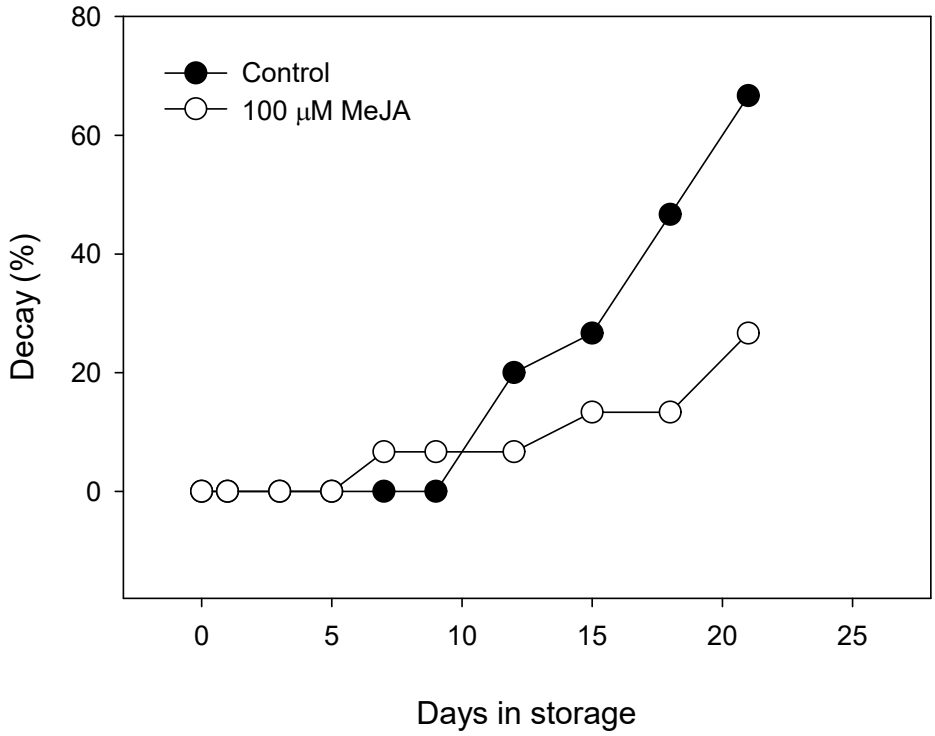

Fig. 8. Decay inhibition of strawberry fruit by MeJA during storage at 0ºC.

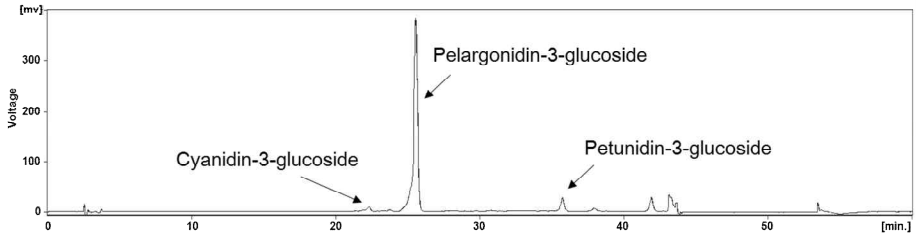

Fig. 9. HPLC chromatogram of anthocyanin in ‘Seolhyang’ strawberry fruit.

peak $[M-H]^{-1}$ at *m/z* 448.1057, 442.1125, and 478.1158, respectively. The total anthocyanin contents

expressed as pelargonidin-3-glucoside were determined during storage. The total anthocyanin content in two treatment fruit gradually during storage. The levels of total anthocyanin were 2.1, 2.0, 2.3, and 1.8 times higher in MeJA treatment fruit than in control fruit on 5, 7, 9, and 13 days in storage, respectively (Fig. 10).

Changes in PAL, CHS, CHI, F3H, DFR, ANS, and UFGT mRNA levels involved by RT-qPCR (Fig. 11). PAL and CHS expression levels in MeJA treatment were higher than those in the control at 3 and 6 days in storage. CHI, F3H, DFR, ANS, and UFGT expression levels in MeJA treatment were higher than those in control fruit at 6 and 8 days in storage. On the other hand, the expression levels of PAL, CHS, CHI, F3H, DFR, ANS, and UFGT genes were higher in the control than those in MeJA treatment after 10 days in storage. Increased gene expression level by MeJA treatment at 3, 6, and 8 days correlated with the higher anthocyanin content.

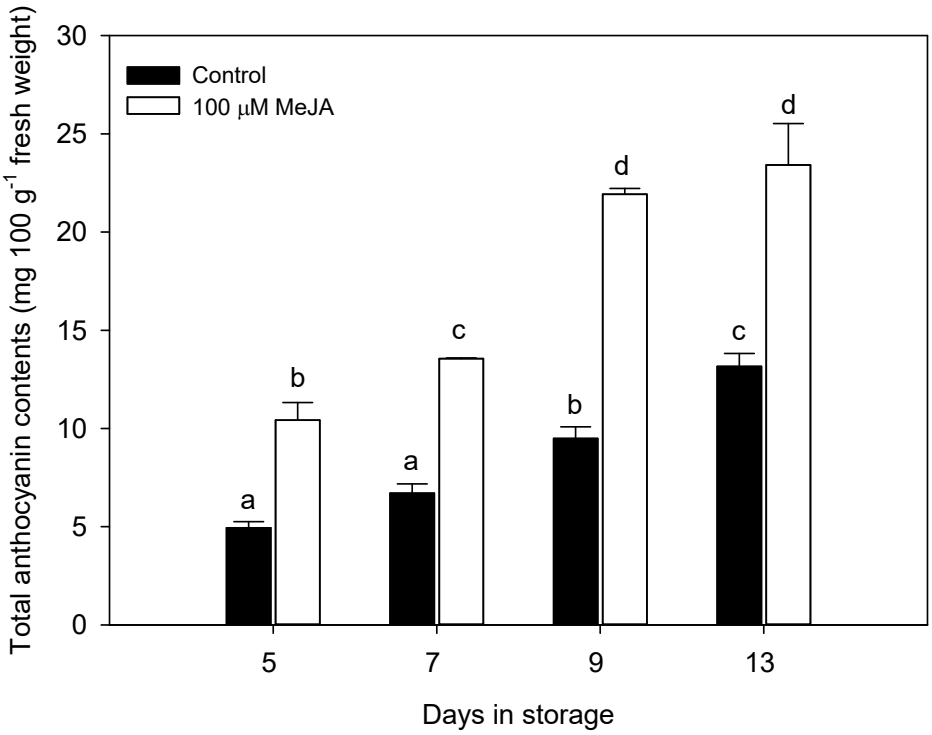

Fig. 10. Total anthocyanin contents of strawberry fruit by MeJA during storage at 0ºC. Values followed by the same letter are not significantly different based on Duncan's multiple range test at P=0.05.

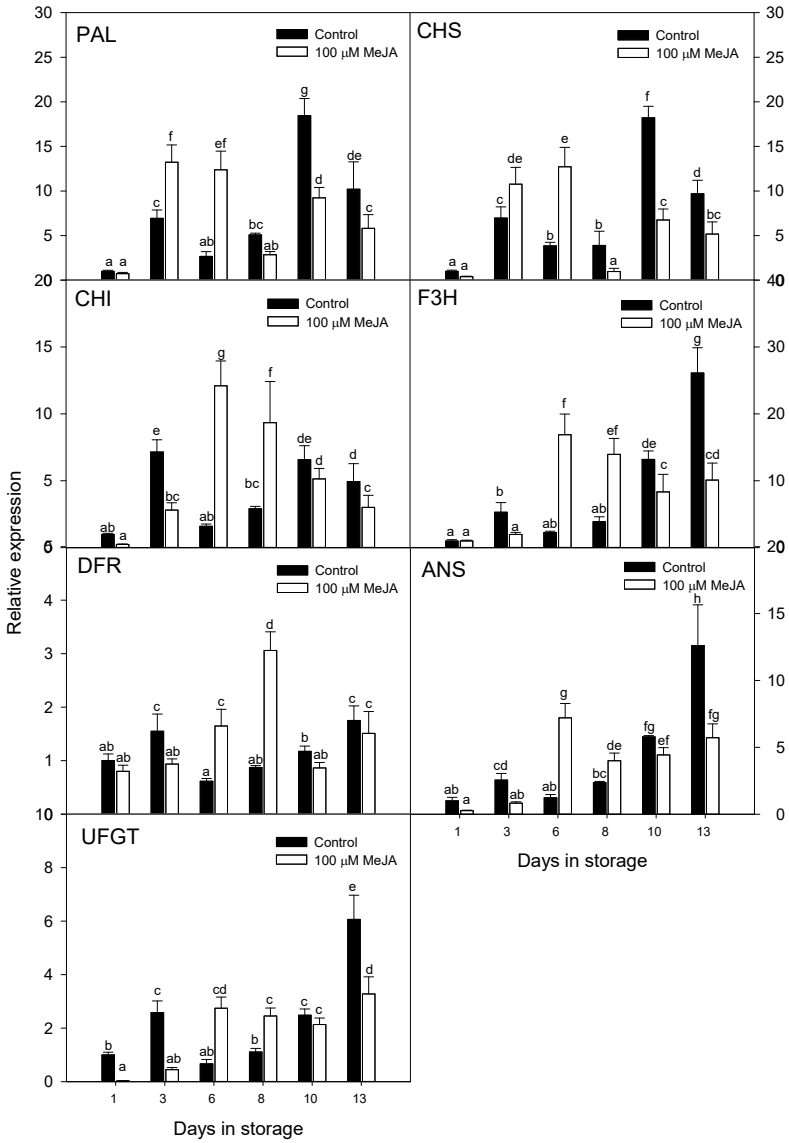

Fig. 11. RT-qPCR analysis of genes involved in anthocyanin biosynthesis in strawberry fruit by MeJA during storage at 0°C. Values followed by the same letter are not significantly different based on Duncan's multiple range test at $P = 0.05$.

3. Quantitative analysis of biosynthesis gene expression in ‘Seolhyang’ strawberry fruit by MeJA treatment

Changes in LOX, AOS, AOC, OPR3, JMT, and JAR1 mRNA levels involved in JA biosynthesis were measured by RT-qPCR (Fig. 12). LOX expression level in MeJA treatment was higher than that in the control at 1 and 8 days during storage. AOS expression level in the control was higher than that in MeJA treatment during storage except 6 days. AOC, OPR3, and JMT expression levels in MeJA treatment were higher than those in the control after 6, 8, and 3 days in storage, respectively. On the other hand, JAR1 expression level was higher than that in the control at only 3, and 6 days during storage.

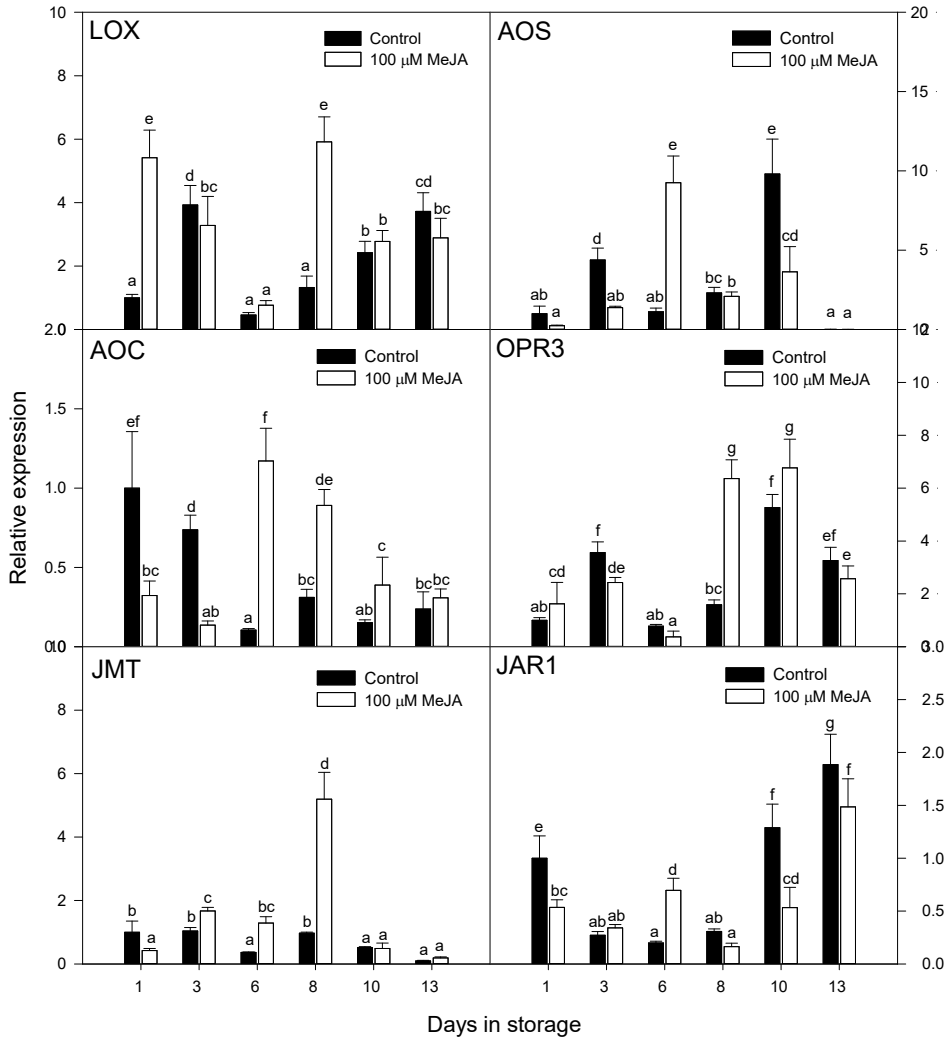

Fig. 12. RT-qPCR analysis of genes involved in MeJA biosynthesis in strawberry fruit by MeJA during storage at 0°C. Values followed by the same letter are not significantly different based on Duncan's multiple range test at $P = 0.05$.

DISCUSSION

The findings of the present study support the role of MeJA as positive regulator during strawberry ripening: MeJA treatment enhances strawberry skin red coloration by increasing anthocyanin biosynthesis and by inhibiting fruit decay.

Induction of pigmentation and anthocyanin biosynthesis in harvested 'Seolhyang' strawberry fruit by MeJA

Color development is an important values in fruit ripening, and it is frequently used as a marketing index. Concha et al. (2013) showed that MeJA applied to *Fragaria chiloensis* fruit decreased in L*, b*, chroma, and h^O along with an increase in a*. These changes indicate a decrease in fruit brightness and an acquisition of red color. In apple, a climacteric fruit, application of MeJA increased ET biosynthesis, red color, anthocyanin, and β-carotene content (Rudell et al., 2002). In non-climacteric fruits, such as raspberry and blackberry, MeJA treatment increased the soluble solid content/titratable activity ratio and anthocyanin content (Wang and Zheng, 2005; Wang et al., 2008). In strawberry fruit, MeJA has been found to stimulate the acquisition of color through chlorophyll degradation and anthocyanin accumulation

(Concha et al., 2013, Perez et al., 1997). Strawberry color depends mainly on anthocyanin and pelargonidin 3-glucoside which is the most abundant anthocyanin in strawberry (Moreno et al., 2010; Perez et al., 1997).

In the present study, MeJA 'Seolhyang' strawberry fruit treated with exhibited a higher content of anthocyanin compared to control fruit (Fig. 10), which could be explained by a stimulatory effect of MeJA on anthocyanin biosynthesis (Fig. 11). And this higher anthocyanin content correlated with the acquisition of red color at 9 days after storage (Fig. 5). MeJA treatment up-regulated several anthocyanin biosynthesis genes (PAL, CHS, CHI, F3H, DFR, ANS, and UFGT; Fig. 11) during postharvest storage in 'Seolhyang' strawberry fruits.

PAL as a key enzyme in the first step of phenyl propanoid pathway is directly involved in the biosynthesis of anthocyanin (Dixon and Paiva, 1995). It has been reported that MeJA treatment enhanced antioxidant activity by including PAL activity in berry fruits (Ayala-Zavala et al., 2005; Chanjirakul et al., 2006, 2007; Wang and Zheng, 2005; Wang et al., 2008). The expressions of the anthocyanin biosynthesis late genes (DFR, AN, and UF3GT) were significantly induced by MeJA (Shan et al., 2009). In previous study in strawberry, MeJA application was found to increase the expression levels of UFGT along with the higher expression levels of PAL, C4H, CHI, and F3H. These changes correlated with a higher anthocyanin content (Concha et al., 2013).

The results from previous studies are consistent with the findings. In the present study significant positive relationships between total anthocyanin content and antioxidant activity have been reported in some berry fruit (Bao et al., 2005). Postharvest application of MeJA might improve the antioxidant activity of 'Seolhyang' strawberry fruit by enhancing the anthocyanin accumulation.

Control of fungal decay in harvested 'Seolhyang' strawberry fruit by MeJA

In the present study, postharvest fungal decay of 'Seolhyang' strawberry could markedly be inhibited by MeJA treatment (Fig. 8). In other studies, the effective concentration of MeJA treatment to reduce decay was 10 $\mu mol \cdot L^{-1}$ in chinesebayberry (Wang et al., 2009), loquat (Cao et al., 2008), and grapefruit (Droby et al., 1999). It has been postulated that the control of postharvest disease by MeJA is because of its direct inhibitory effect on pathogen growth and induction of natural disease resistance (Cao et al., 2008; Droby et al., 1999; Yao and Tian, 2005).

In conclusion, the findings of the present study suggest that MeJA can effectively improve red coloration by increasing anthocyanin contents and reduce fruit decay on 'Seolhyang' strawberry. Thus it will lend to explain the

ripening mechanism of non-climacteric strawberry fruit, and it will be beneficial in distributing the high quality strawberry through developing the decay inhibition technique by MeJA.

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초 록

‘설향’ 딸기는 2005년에 일본 품종인 ‘레드펄’과 ‘아키히메’를 교배하여 육종한 국내 품종 딸기이다. ‘설향’ 딸기는 국내 딸기 총 재배 면적의 70% 이상을 점유하고 있는 주요 과실이지만, 불균일한 착색으로 인한 상품성 하락, 수확 후 과육 부패로 인한 저장 수명 단축이 가장 큰 문제가 되고 있다. 따라서 본 연구에서는 식물체의 발달 단계 및 방어에 관여하는 것으로 알려진 식물 내생 호르몬 메틸자스몬산(MeJA)을 이용하여 수확 후 딸기의 부패를 억제하여 저장 수명을 연장함과 동시에 주요 색소인 안토시아닌 생합성에 관여하는 메틸자스몬산의 역할을 알아보았다. 녹숙 단계의 ‘설향’ 딸기를 100μM 메틸자스몬산 용액에 꽃자루만 침지하여 2주간 10ºC, 상대 습도 90 ± 5%에 저장하면서 부패율, 착색 정도, 안토시아닌 함량 및 안토시아닌 생합성 유전자 발현뿐만 아니라 메틸자스몬산 함량과 메틸자스몬산 생합성 유전자 발현을 조사하였다. 저장 기간 중 육안 관찰을 통해 무처리구에 비해 처리구에서 착색이 빠르게 진행되는 것을 확인하였고, 저장 13일차에 무처리구 및 처리구의 안토시아닌 함량 역시 각각 13과 27mg $100g^{-1}$ fresh weight으로 처리구에서 더 높았다. 안토시아닌

생합성 유전자 발현을 PCR로 분석한 결과, 처리 6일차와 8일차에 무처리구보다 처리구에서 안토시아닌 생합성 관련 유전자가 더 많이 발현되었다. 따라서 메틸자스몬산이 수확후 '설향' 딸기의 안토시아닌 생합성을 유도하여 착색을 촉진시키는 것을 확인하였다. 또한 저장 후 7일차부터 부패가 발생하여 13일차에 무처리구 및 처리구에서 각각 20%와 7%의 부패율을 나타냈고, 저장 15일차에는 각각 67%와 21% 부패율로 메틸자스몬산을 처리한 딸기에서 부패가 억제되었다. 향후 본 결과를 바탕으로 메틸자스몬산을 이용한 수확 후 과실인 딸기의 균일한 착색을 유도하고, 부패 억제 기술 개발하여 고품질의 딸기를 유통하는 데 활용할 계획이다.

주요어: 숙성, 착색, 항산화력, 호르몬, *Fragaria* × *ananassa*

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