Inhibition of Ethylene Binding and Biosynthesis Maintains Fruit Quality of 'Formosa' Plums during Postharvest Storage

Yong-Tae Kim[†], Suong Tuyet Thi Ha[†], Ikjo Chun, and Byung-Chun In[*]

Division of Horticulture and Medicinal Plant, Andong National University, Andong 36792, Korea

*Corresponding author: bcin@anu.ac.kr

[†]These authors contributed equally to the work.

Abstract

This study was conducted to develop an ethylene inhibition method using aminoethoxyvinylglycine (AVG) and 1-methylcyclopropene (1-MCP) to improve postharvest quality and extend the shelf life of plums (*Prunus subg. Prunus* 'Formosa'). Plums were sprayed preharvest with 150 mg·L^{-1} AVG and postharvest with 1 µL·L^{-1} 1-MCP for 24 h. The results revealed that the combined treatment with AVG and 1-MCP (AVG+1-MCP) effectively suppressed skin color change, flesh firmness reduction, decay, and weight loss of plum fruit during postharvest storage. The acidity ratio was also significantly maintained during storage in AVG+1-MCP treated groups. The transcript levels of ethylene biosynthesis genes (*PsACS3*, *PsACS4*, and *PsACO1*) showed the same pattern as the amount of ethylene produced in plums. AVG+1-MCP treatment significantly inhibited transcript levels of *PsACS3*, *PsACS4*, and *PsACO1* and the reduction in expression of signaling genes (*PsETR1*, *PsERS1*, and *PsCTR1*), resulting in a longer shelf life compared to the untreated control plums. Inhibition of ethylene biosynthesis and binding effectively suppressed senescence and ripening of plum fruit that show a climacteric rise of ethylene synthesis and respiration.

Additional key words: ethylene inhibitor, gene expression, *Prunus subg. Prunus*, ripening, shelf life

Introduction

The plum (*Prunus subg. Prunus* 'Formosa') is a climacteric fruit with a shortened shelf life after harvest due to the changes in physicochemical properties related to the ripening process, which are all stimulated by ethylene production (Singh and Khan, 2010). Furthermore, continuous respiration of plums after harvest also facilitates postharvest softening of plum fruit and decreases the commercial quality in a very short storage period (Lelièvre et al., 1997; Singh and Khan, 2010).

Senescence and ripening progression in plants accelerated by ethylene can be delayed by using inhibitors of ethylene biosynthesis and action. Aminoethoxyvinylglycine (AVG), an ethylene biosynthesis inhibitor, suppresses ethylene synthesis through inhibition of the activity of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) enzyme, a key enzyme in the ethylene biosynthetic pathway (Capitani et al., 2002). AVG inactivates the structure of the ACS enzyme and blocks the

conversion of S-adenosyl-L-methionine (SAM) to ACC (Capitani et al., 2002; Capitani et al., 2005). It has been shown that AVG treatment effectively decreases ethylene production and the postharvest disorders in many climacteric fruits, such as tomatoes (Saltveit, 2005), plums (Ozturk et al., 2015), apples (Yuan and Carbaugh, 2007), pears (Xie et al., 2015), and apricots (Muñoz-Robredo et al., 2012). The ethylene binding inhibitor 1-methylcyclopropene (1-MCP) has a strong chemical affinity to ethylene receptors, thereby preventing ethylene binding to the receptors and repressing the ethylene signaling pathway (Kamiyoshihara et al., 2012). The application of 1-MCP significantly prolongs the postharvest storage period and quality of many horticultural crops, such as broccoli, cucumbers, carnations, roses, petunias, delphiniums (Sisler et al., 1986; Serek et al., 1995; Cameron and Reid, 2001; Ha et al., 2020; Yoo et al., 2020), bananas, apples, avocados, pears, persimmons, and peaches (Watkins, 2006; Kim et al., 2018; Win et al., 2018).

Although 1-MCP treatment significantly retards fruit ripening and extends the postharvest life of plums and peaches (Khan and Singh, 2007; Hayama et al., 2008; Luo et al., 2009), it is not generally used in the postharvest industry of climacteric fruits due to its lower effectiveness during postharvest distribution of the fruit (Mathooko et al., 2001; Cin et al., 2006; Hayama et al., 2008). To maintain the high quality of plums during distribution, effective and practical techniques that can protect fruit against ethylene damage need to be developed. In this study, we investigated the effectiveness of suppressing ethylene action using preharvest AVG and postharvest 1-MCP treatments to maintain the fruit quality attributes of 'Formosa' plum fruit in a postharvest distribution system. Furthermore, we analyzed the expression patterns of ethylene biosynthesis and signaling genes in plums after AVG and 1-MCP treatments to elucidate the relationship between ethylene responses and postharvest quality of the fruit.

Materials and Methods

Preharvest Treatment and Harvest

Plum (*Prunus subg. Prunus* 'Formosa') fruit were harvested at a conventional plum orchard in Uiseong, Gyeongsangbuk-do, Republic of Korea. Ten days before harvest, the fruit were sprayed with 150 mg·L^{-1} AVG to inhibit ethylene biosynthesis. AVG powder (ReTain^{TM}, AVG 15%, ValentBioSciences, Libertyville, IL, USA) was dissolved in distilled water to prepare a 150 mg·L^{-1} solution. Untreated (control) fruit were sprayed with distilled water. Plum fruit were harvested at commercial maturity stage (9 N firmness and 12% acidity ratio) and packed in cardboard boxes and transported within an hour to a postharvest physiology laboratory at the Division of Horticulture and Medicinal Plant, Andong National University in Andong, Republic of Korea. At the laboratory, disease-free fruit of a uniform size and quality were randomly selected for postharvest treatments.

1-MCP Treatment

Plum fruit were fumigated in the treatment chambers with 1 μL·L^{-1} 1-MCP (Ecoplants Co., Ltd., Gyeonggi-do, Republic of Korea) for 24 h at $20 \pm 1°C$ for 1-MCP treatment. Untreated control fruit were incubated in similar chambers with normal air. The 1-MCP (provided by Ecoplants Co., Ltd., Gyeonggi-do, Republic of Korea) was applied via FreshLong^{TM} sticks in the treatment chambers at $20 \pm 1°C$. To prepare 1 μL·L^{-1} 1-MCP gas, the stick was broken twice and directly put into the chambers. After the treatment, the fruit were packed in cardboard boxes and stored in the chambers in the dark at $20 \pm 1°C$ and $60 \pm 2\%$ relative humidity for postharvest quality evaluation.

Assessment of Fruit Quality Attributes

Flesh firmness, fresh weight loss, soluble solid content (SSC), titratable acidity ratio, skin color, decay, and ethylene production of fruit were measured during the postharvest period. After removing the skin of the fruit, the flesh firmness of each fruit was measured using a fruit texture analyzer fitted with ø 5-mm cylindrical probe (TA-XT2 Texture Analyzer, SMS, UK). For SSC and acidity ratio measurements, the fruit tissues were ground into a juice using a mortar and pestle. Subsequently, 1 g of fruit juice was poured into a beaker containing 50 mg distilled water and diluted by gentle stirring. The SSC and acidity ratio of the fruit were measured using a Pocket Brix-Acidity Meter (Plum) (Atago Co., LTD, Tokyo, Japan). The quality of plum fruit was also assessed by determining the extent of decay with three replicates per treatment. Plums were assessed for decay symptoms at 6 and 10 d after storage when the fruits showed a visible decay symptom on the peel. The fruit decay was assessed on a relative scale of 0 to 3 according to the percentage of rotten area on the fruit skin, as follows: 0: 0%, 1: 1 − 30%, 2: 31 − 50%, and 3: 51 − 100%. The peel color of fruit was measured over three replicates using a chromameter (CR-10, Minolta Corp., Osaka, Japan). Ethylene production was measured using gas chromatography (GC, Hewlett-Packard 5890, Palo Alto, CA, USA) equipped with a flame ionization detector (FID) and alumina column. The temperatures for the injector, column, and detector were 110°C, 50°C, and 250°C, respectively. Plum fruit were incubated in a 1 L jar at 20 ± 1°C for 1 h, and 1 mL of air was taken from the headspace of the jar and injected into the GC for ethylene analysis.

cDNA Synthesis and Quantitative Real-Time PCR (qRT-PCR)

Six flesh portions were detached from each plum fruit on day 6 of the storage period. The fruit flesh (200 mg) was mashed with liquid nitrogen using a pestle and mortar for total RNA extraction using the Ribospin Plant kit (Gene All, Gen All Biotechnology Co., LTD, Seoul, Republic of Korea). The first strand of cDNA was synthesized from sterile water, total RNA, and oligo $(dT)_{15}$ primers by heating to 70°C for 5 min. After placing on ice for 1 min, the reagents were supplemented and mixed gently as the follows: 5× RT buffer (4 µL), dNTP (2 µL), DTT (2 µL), and AMV RT enzyme (0.5 µL) (INTRON Biotechnology, Inc., Gyeonggi-do, Republic of Korea). The reverse transcription was conducted in a Bio-Rad PTC-100 Programmable Thermal Controller (MJ Research, Inc., Hercules, CA, USA) at 42°C (1 h) and heated to 70°C (5 min) to complete the reaction.

The expression levels of five ethylene biosynthesis (*PsACS1*, *PsACS3*, *PsACS4*, *PsACS5*, and *PsACO1*) and signaling

Table 1. The primer sequences used for gene expression analysis in plum fruit

Gene (accession number)	Forward primer	Reverse primer
PsACS1 (EU034649.1)	5'-ACCGCGTTCACGTTGTTT-3'	5'-TGCCACAACCATGTCGTC-3'
PsACS3 (EU034650.1)	5'-GTGCAACTGCAGCCAATG-3'	5'-GGTTGGCACAAGCAAAGC-3'
PsACS4 (EU034653.1)	5'-GCGTCGATGCTCTTGGAT-3'	5'-AAAACCCCGTGCCTCTTC-3'
PsACS5 (EU034654.1)	5'-GATCTTGGATGGCGAACG-3'	5'-AGCCTCCAAGGCTGCTCT-3'
PsACO1 (KM030036.1)	5'-TGTCATCTACCCTGCACCAA-3'	5'-TTGAAGCCATGAAAGCAGTG-3'
PsETR1 (EF585294.1)	5'-CAACCGCGCTTCAAAAAT-3'	5'-CCTGGCATGTGCTTTCCT-3'
PsERS1 (EF585295.1)	5'-CCAAATGGCACCTCAACC-3'	5'-GGTCTGTGCGCAATGAGA-3'
PsCTR1 (EF585298.1)	5'-AAGCCCCCAAACTTGTCC-3'	5'-CCATTGCGCCAGGTTTAT-3'
PsACT (EF585293.1)	5'-CTGGACCTTGCTGGTCGT-3'	5'-ATTTCCCGCTCAGCAGTG-3'

genes (*PsETR1*, *PsERS1*, and *PsCTR1*) in plums were detected using the Bio-Rad CFX Connect Real-Time System (Life Science, Hercules, CA, USA). Primer sequences used for qRT-PCR are shown in Table 1. The reaction mixtures contained 5 μL cDNA as a template, 10 μL iQ™ SYBR® Green Supermix (Bio-Rad, Life Science, Hercules, CA, USA), 1 μL forward and reverse primers (10 μM), and 4 μL water. The qRT-PCR reaction was performed using the following thermocycling pattern: 95°C for 60 s for pre-denaturation (one cycle), followed by 40 cycles of 95°C for 10 s (denaturation), 65°C for 10 s (annealing), and 72°C for 40 s (extension).

Statistical Analyses

All measurements were conducted on three biological replicates for each treatment and three fruits per replicate. Data are shown as mean ± standard error (SE). Data were subjected to two-way analysis of variance at $p = 0.05$ using SPSS 20.0 (IBM, Somers, NY, USA), and Duncan's multiple range test was used to compare the differences among the treatments.

Results

Fruit Quality Attributes

Changes in postharvest quality attributes of fruit during storage period were investigated to assess the effectiveness of the AVG and 1-MCP applications. After 6 d of postharvest storage, untreated control fruit exhibited a dark red color of the skin through an increase in the a* value. Color changes were effectively delayed by the combination treatment of AVG and 1-MCP at 6 d after storage, compared with the result from other treatments (Figs. 1 and 2A).

Flesh firmness of untreated control plums rapidly decreased with progression in storage duration (Fig. 2B). The flesh firmness values were higher in AVG and single 1-MCP treated plums, compared with untreated control plums (Fig. 2B). However, the combination treatment of AVG and 1-MCP was the most effective treatment in delaying fruit softening and maintaining flesh firmness during postharvest storage period (Fig. 2B). Weight loss increased in untreated control plums as the postharvest storage period increased (Fig. 2C). In contrast, weight loss was lowest in the combination treatment of AVG and 1-MCP, 1-MCP, and AVG, compared with untreated control fruit (Fig. 2C).
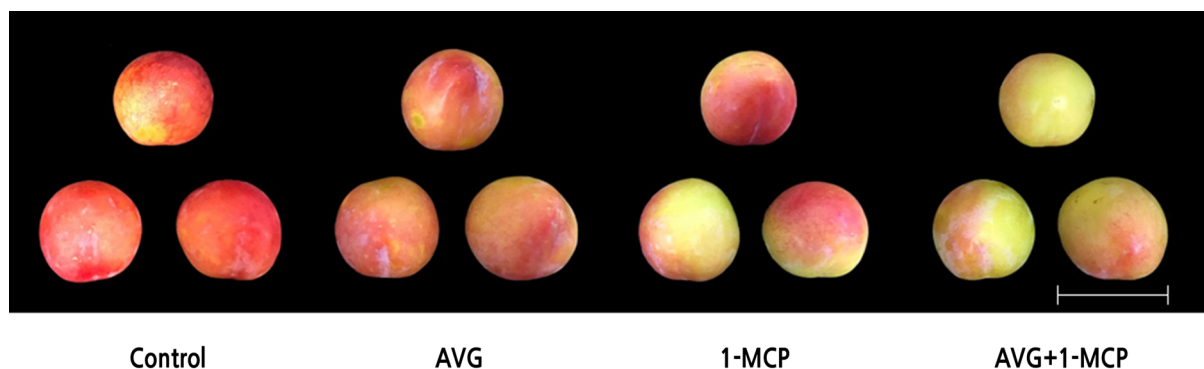

Control   **AVG**   **1-MCP**   **AVG+1-MCP**

Fig. 1. Effect of AVG and 1-MCP treatments on the appearance of plum fruit after 6 d of storage in the dark at 20 ± 1°C and 60 ± 2% relative humidity. Plum fruit were sprayed preharvest with 150 mg·L⁻¹ AVG and postharvest with 1 μL·L⁻¹ 1-MCP for 24 h. Control, untreated fruit; AVG, 1-MCP, and AVG+1-MCP, fruit were treated with AVG (aminoethoxyvinylglycine), 1-MCP (1-methylcyclopropene), and combination of AVG and 1-MCP. Scale bar = 6 cm.

The SSC of plum fruit juice was significantly decreased by the combination treatment of AVG and 1-MCP (Fig. 2D). The highest change in acidity ratios was observed in control fruit during postharvest storage (Fig. 2E). Both preharvest AVG treatment and postharvest 1-MCP treatment also reduced SSC acidity ratios in the fruit juice of plums (Fig. 2F).

Fruit decay was also determined during the storage period to assess the effectiveness of AVG and 1-MCP treatments during the storage period. All treatments significantly reduced fruit decay, compared with the untreated control plums on days 6 and 10 of the storage period (Fig. 3). Notably, the combination treatment of AVG and 1-MCP did not result in any fruit decay on day 6 and furthermore showed the lowest fruit decay (< 5%) on day 10 (Fig. 3). In contrast, untreated control plums exhibited the highest decay (9% and 27%) on days 6 and 10 of storage, respectively (Fig. 3). Overall, these results revealed that the combination treatment of AVG and 1-MCP effectively decreased the deterioration of plum fruit, thereby prolonging fruit storability of plums.
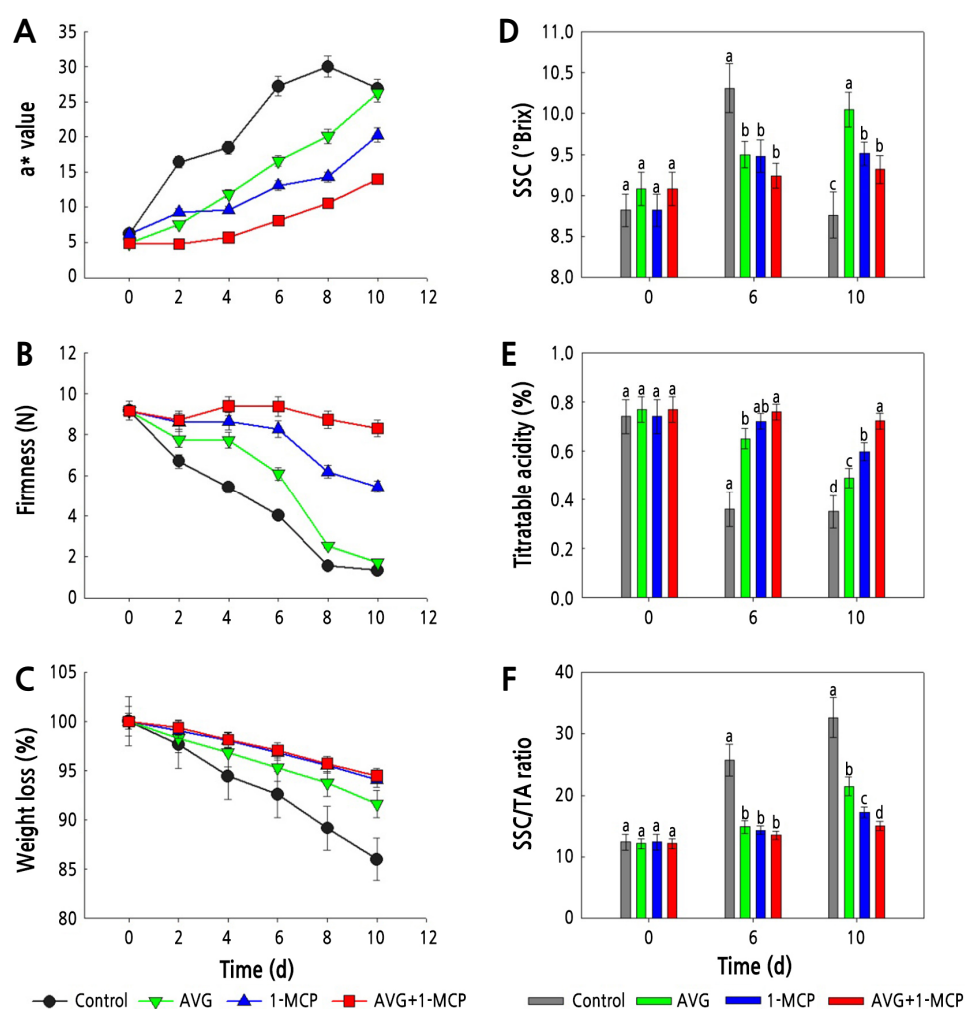

Fig. 2. Effect of AVG and 1-MCP treatments on skin color change (A), flesh firmness (B), weight loss (C), SSC (D), titratable acidity ratio (E), and SSC/TA ratio (F) of plum fruits during storage in the dark at $20 \pm 1°C$ and $60 \pm 2\%$ relative humidity. Plum fruit were sprayed preharvest with 150 mg·L^{-1} AVG and postharvest with 1 μL·L^{-1} 1-MCP for 24 h. Control, untreated fruit; AVG, 1-MCP, and AVG+1-MCP, fruit were treated with AVG (aminoethoxyvinylglycine), 1-MCP (1-methylcyclopropene), and combination of AVG and 1-MCP. Error bars are SE of 3 replications. The values followed by different letters in D, E, and F are significantly different ($p < 0.05$) based on Duncan's multiple range test (n = 3).
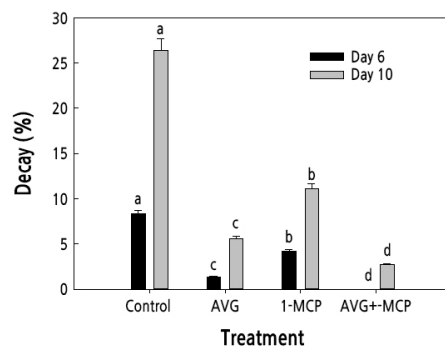
Fig. 3. Effect of AVG and 1-MCP treatments on fruit decay on days 6 and 10 of storage in the dark at $20 \pm 1\,°C$ and $60 \pm 2\%$ relative humidity. Plum fruit were sprayed preharvest with 150 mg·L^{-1} AVG and postharvest with 1 µL·L^{-1} 1-MCP for 24 h. The fruit decay was assessed on a relative scale of 0 to 3 according to the percentage of rotten area on the fruit skin, as follows: 0: 0%, 1: 1-30%, 2: 31-50%, and 3: 51-100%. Control, untreated fruit; AVG, 1-MCP, and AVG+1-MCP, fruit were treated with AVG (aminoethoxyvinylglycine), 1-MCP (1-methylcyclopropene), and combination of AVG and 1-MCP. Values followed by different letters are significantly different ($p < 0.05$) based on Duncan's multiple range test (n = 3).
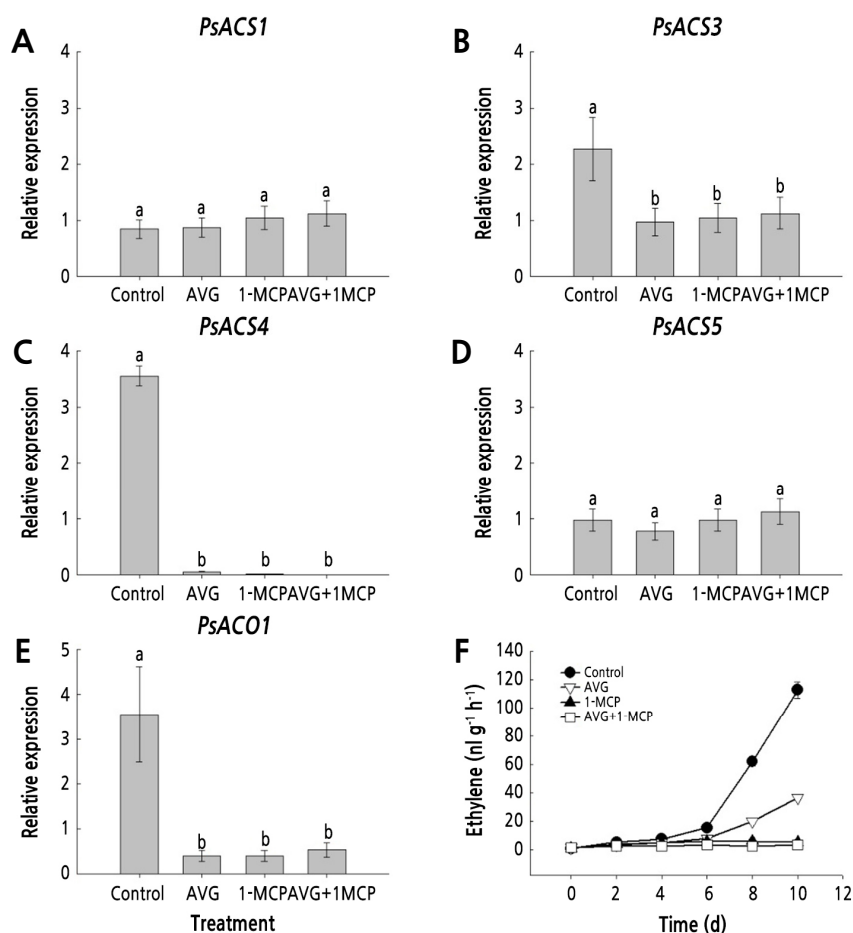
Fig. 4. Effect of AVG and 1-MCP treatments on the transcript levels of *PsACS1* (A), *PsACS3* (B), *PsACS4* (C), *PsACS5* (D), *PsACO1* (E), and ethylene production (F) in plum fruit on day 6 of the storage in the dark at $20 \pm 1\,°C$ and $60 \pm 2\%$ relative humidity. Plum fruit were sprayed preharvest with 150 mg·L^{-1} AVG and postharvest with 1 µL·L^{-1} 1-MCP for 24 h. Control, untreated fruit; AVG, 1-MCP, and AVG+1-MCP, fruit were treated with AVG (aminoethoxyvinylglycine), 1-MCP (1-methylcyclopropene), and combination of AVG and 1-MCP. Values followed by different letters are significantly different ($p < 0.05$) based on Duncan's multiple range test (n = 3).

Ethylene Synthesis and Gene Expression

The expression level of the ethylene biosynthesis genes (*PsACS1*, *PsACS3*, *PsACS4*, *PsACS5*, and *PsACO1*) and ethylene production were characterized in plum fruit during the storage period (Fig. 4A-F). The ethylene production level of 'Formosa' plums was very low in the early stage (2 − 4 d after storage) and was similar among the treatments (Fig. 4F). However, untreated control plums showed an increase in ethylene production after 6 d of storage and exhibited a climacteric ethylene production peak with ripening at day 10 (Fig. 4F). Both postharvest 1-MCP treatment and the combination treatment of AVG and 1-MCP completely suppressed ethylene production for 10 d of the storage period (Fig. 4F). The single AVG treatment also decreased the ethylene production of plums, compared with untreated control fruit.
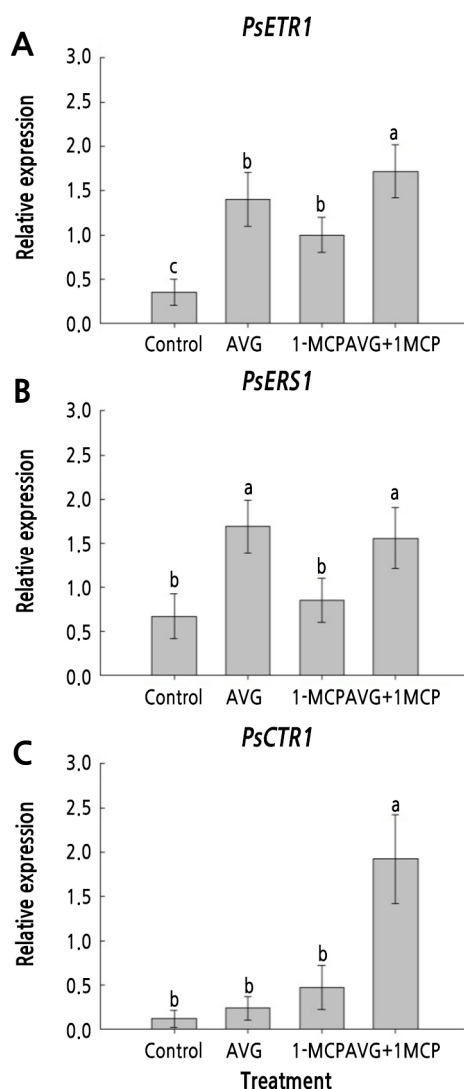

Fig. 5. Effect of AVG and 1-MCP treatments on the transcript levels of ethylene signaling genes *PsETR1* (A), *PsERS1* (B), and *PsCTR1* (C) in plum fruits on day 6 of storage in the dark at 20 ± 1°C and 60 ± 2% relative humidity. Plum fruit were sprayed preharvest with 150 mg·L^{-1} AVG and postharvest with 1 μL·L^{-1} 1-MCP for 24 h. Control, untreated fruit; AVG, 1-MCP, and AVG+1-MCP, fruit were treated with AVG (aminoethoxyvinylglycine), 1-MCP (1-methylcyclopropene), and combination of AVG and 1-MCP. Values followed by different letters are significantly different (*p* < 0.05) based on Duncan's multiple range test (n = 3).

However, its effectiveness was relatively lower than that of the postharvest 1-MCP treatment and the combination treatment of AVG and 1-MCP (Fig. 4F).

The transcript levels of the ethylene biosynthesis genes (*PsACS3*, *PsACS4*, and *PsACO1*) in untreated control plums also markedly increased on day 6 (Fig. 4B, 4C, and 4E). Treatments with AVG, 1-MCP, and AVG+1-MCP suppressed the transcript levels of *PsACS3*, *PsACS4*, and *PsACO1* in plum fruit (Fig. 4B, 4C, and 4E). The transcript levels of *PsACS1* and *PsACS5* were unaffected by the treatments (Fig. 4A and 4D), indicating that *PsACS1* and *PsACS5* may be related to fruit growth and maturation rather than fruit ripening and softening.

Expression of Ethylene Receptor and Signaling Genes

The expression patterns of *PsETR1*, *PsERS1*, and *PsCTR1* were also analyzed in plum fruit after treatment with AVG and 1-MCP. The transcript levels of *PsETR1*, *PsERS1*, and *PsCTR1* were lowest in untreated control fruit on day 6 (Fig. 5). The *PsETR1* and *PsERS1* reduction was suppressed by AVG and 1-MCP treatment (Fig. 5A and 5B). The AVG+1-MCP treatment significantly increased the expression of *PsCTR1* in plum fruit on day 6, but the single AVG or 1-MCP treatment did not clearly affect the expression of *PsCTR1*, compared with the untreated control plums (Fig. 5C). Overall, the combination treatment of AVG and 1-MCP was the most effective in inhibiting the ripening-related degradation of the ethylene receptors and the signaling genes in plums.

Discussion

The ethylene production during ripening of climacteric plum fruit is altered based on harvest maturity, cultivar, and pre/postharvest treatments (Singh and Khan, 2010). During storage, the ethylene production in 'Formosa' plum fruit was different in each treatment. High ethylene production leads to fruit ripening and consequently shortens the shelf life of plums. Ethylene antagonists (AVG and 1-MCP) are used to retard the ripening process in climacteric fruits (including plums) due to their strong effectiveness in inhibiting ethylene production and respiration rates (Sisler and Serek, 1997). Although the ripening accelerated by ethylene in climacteric fruits can be suppressed by AVG or 1-MCP application, the effectiveness is stable only in the short term because of the differences in ethylene sensitivity of the genotypes and the recovery of hormone sensitivity after the treatments (Sisler and Serek, 1997). Our results indicated that the combination treatment of AVG and 1-MCP effectively reduced ethylene production in fruit during the storage period, decreased postharvest losses, and extended the shelf life of 'Formosa' plums, compared with the single treatment of AVG or 1-MCP. These results indicate that the primary cause of early ripening in plum fruit would be ethylene and the ethylene-inducible process would be effectively delayed by the simultaneous suppression of ethylene binding and biosynthesis with 1-MCP and AVG. Previous studies have also indicated that the combined application of 1-MCP and AVG would be more effective in delaying fruit ripening and softening than a single treatment of the ethylene antagonists (Hayama et al., 2008).

In this study, the expression of three ethylene biosynthesis genes (*PsACO1*, *PsACS3*, and *PsACS4*) exhibited a rapid increase with the climacteric rise of ethylene synthesis in plum fruit at a later stage (day 6). This result indicates that *PsACO1*, *PsACS3*, and *PsACS4* are presumably involved in autocatalytic ethylene synthesis (system 2 ethylene), which is responsible for senescence and ripening in plums. Our results imply that the autocatalytic ethylene synthesized by

untreated control fruit was bound to ethylene receptors and thereby decreased the expression of the receptor genes, *PsETR1* and *PsERS1*. This leads to a decrease in the downstream protein kinase, *PsCTR1*, resulting in an ethylene response in the fruit. It is well known that the accumulation of the ethylene biosynthesis genes (*ACSs*) during plant senescence may be regulated by the degradation of protein kinase, CTR1 (Jones, 2003; In et al., 2013; Xu and Zhang, 2014). In this study, the combination treatment of AVG and 1-MCP significantly inhibited an increase in the expression of *PsACO1*, *PsACS3*, and *PsACS4* and the degradation of *PsETR1*, *PsERS1*, and *PsCTR1*, and thereby strongly suppressed the progression of fruit ripening. These results are supported by previous work showing that the simultaneous application of 1-MCP and AVG in carnations suppressed the ethylene-inducible increase of *DcACSs* and degradation of *DcCTRs*, which consequently progressed senescence in the petals (In et al., 2013). In this study, although the single application of 1-MCP decreased ethylene production in plum fruit, the transcript levels of *PsETR1*, *PsERS1*, and *PsCTR1* were lower than the AVG+1-MCP treatment. It has been shown that fruit treated with 1-MCP alone can overcome ethylene inhibition caused by generating new ethylene receptors that have the ability to bind with the hormone (Sisler and Serek, 1997; Kevany et al., 2007; In et al., 2013). Thus, in plums treated with 1-MCP alone in this study, ethylene receptors synthesized anew or dissociated from ethylene presumably regained ethylene responsiveness in the short period after the treatments. This reversible binding of ethylene to the receptors had been observed in *AtETR1* and *AtETR2* and in carnation flowers (Schaller and Bleecker, 1995; O'Malley et al., 2005; In et al., 2013).

Acidity ratio, SSC, color, and flesh firmness are the most important postharvest quality attributes in plum fruit (Crisosto et al., 2007; Singh and Khan, 2010). Ethylene production and respiration during ripening results in various biochemical and physical changes in plums, such as the degradation of pigment, softening of fruit tissues, and variations in organic acids and soluble carbohydrates (Valero et al., 2003; Singh and Khan, 2010). Flesh firmness in plums is negatively correlated with ethylene production during ripening (Ozturk et al., 2015). Anthocyanins are responsible for the red coloration in plum fruit during maturity and ripening, and they are regulated by ethylene production (Singh and Khan, 2010; Fang et al., 2016). Since the ethylene responses in plum fruit are strongly blocked by the combination treatment of AVG and 1-MCP, the transcript levels of softening-related genes (*endo-PG*, *exo-PG*, *pectin esterase*, and *endo-1,4-β-glucanase*), *polyphenol oxidase*, and anthocyanin-related genes (*MYB* and *bHLH*) (Khan and Singh, 2007; Fang et al., 2016; Lee et al., 2020) can be inhibited after harvest. Therefore, AVG+1-MCP strongly inhibited the color change, maintained the firmness, and significantly reduced the decay of plum fruits during the storage period. Our results are consistent with a previous study indicating that a combined application of AVG and 1-MCP was effective in delaying softening and stabilizing the acidity in peach fruits (Hayama et al., 2008). Similar to our results, inhibition of ethylene synthesis and binding by AVG and 1-MCP decreased ethylene production and the activity of softened enzymes, maintained the hardness, and decreased red peel color development in 'Qingnai' and 'Tegan Blue' plum fruit (Khan et al., 2009; Luo et al., 2009). In this study, AVG was not effective in retarding the early change in color and the loss of flesh firmness in fruit. The limitation can be overcome by the simultaneous application of AVG and 1-MCP pre- and postharvest in plum fruit.

Overall, our results indicated that AVG+1-MCP treatment effectively suppressed ethylene production and ripening, and consequently resulted in longer shelf life of plum fruit. The results also revealed that the ethylene-inducible reduction of ethylene receptor transcripts (*PsETR1* and *PsERS1*) was blocked by the AVG+1-MCP treatment, thereby the active *PsCTR1* and suppressed ethylene signaling, resulting in delayed ethylene responses in plum fruit. Our results facilitate the

improvement of postharvest treatment techniques for plums that show a climacteric rise of respiration and ethylene synthesis. Based on the current results, further research on the effectiveness of AVG and 1-MCP on postharvest quality of fruit at different storage temperatures is needed to develop a practical postharvest treatment technique for plums.

Literature Cited

Cameron AC, Reid MS (2001) 1-MCP blocks ethylene-induced petal abscission of *Pelargonium peltatum* but the effect is transient. Postharvest Biol Technol 22:169-177. doi:10.1016/S0925-5214(00)00189-7

Capitani G, McCarthy DL, Gut H, Grütter MG, Kirsch JF (2002) Apple 1-aminocyclopropane-1-carboxylate synthase in complex with the inhibitor L-aminoethoxyvinylglycine. Evidence for a ketimine intermediate. J Biol Chem 277:49735-49742. doi:10.1074/jbc.M208427200

Capitani G, Tschopp M, Eliot AC, Kirsch JF, Grütter MG (2005) Structure of ACC synthase inactivated by the mechanism-based inhibitor l-vinylglycine. FEBS Letters 579:2458-2462. doi:10.1016/j.febslet.2005.03.048

Cin VD, Rizzini FM, Botton A, Tonutti P (2006) The ethylene biosynthetic and signal transduction pathways are differently affected by 1-MCP in apple and peach fruit. Postharvest Biol Technol 42:125-133. doi:10.1016/j.postharvbio.2006.06.008

Crisosto CH, Crisosto GM, Echeverria G, Puy J (2007) Segregation of plum and pluot cultivars according to their organoleptic characteristics. Postharvest Biol Technol 44:271-276. doi:10.1016/j.postharvbio.2006.12.005

Fang Z-Z, Zhou D-R, Ye X-F, Jiang C-C, Pan S-L (2016) Identification of candidate anthocyanin-related genes by transcriptomic analysis of 'Furongli' plum (*Prunus salicina* Lindl.) during fruit ripening using RNA-Seq. Front in Plant Sci 7. doi:10.3389/fpls.2016.01338

Ha STT, Lim J-H, In B-C (2020) Simultaneous inhibition of ethylene biosynthesis and binding using AVG and 1-MCP in two rose cultivars with different sensitivities to ethylene. J Plant Growth Regul 39:553-563. doi:10.1007/s00344-019-09999-6

Hayama H, Tatsuki M, Nakamura Y (2008) Combined treatment of aminoethoxyvinylglycine (AVG) and 1-methylcyclopropene (1-MCP) reduces melting-flesh peach fruit softening. Postharvest Biol Technol 50:228-230. doi:10.1016/j.postharvbio.2008.05.003

In B-C, Binder BM, Falbel TG, Patterson SE (2013) Analysis of gene expression during the transition to climacteric phase in carnation flowers (*Dianthus caryophyllus* L.). J Exp Bot 64:4923-4937. doi:10.1093/jxb/ert281

Jones ML (2003) Ethylene biosynthetic genes are differentially regulated by ethylene and ACC in carnation styles. Plant Growth Regul 40:129-138. doi:10.1023/A:1024241006254

Kamiyoshihara Y, Tieman DM, Huber DJ, Klee HJ (2012) Ligand-induced alterations in the phosphorylation state of ethylene receptors in tomato fruit. Plant Physiol 160:488-497. doi:10.1104/pp.112.202820

Kevany BM, Tienman DM, Taylor MG, Cin VD, Klee HJ (2007) Ethylene receptor degradation controls the timing of ripening in tomato fruit. Plant J 51:458-467. doi:10.1111/j.1365-313X.2007.03170.x

Khan AS, Singh Z (2007) 1-MCP regulates ethylene biosynthesis and fruit softening during ripening of 'Tegan Blue' plum. Postharvest Biol Technol 43:298-306. doi:10.1016/j.postharvbio.2006.10.005

Khan AS, Singh Z, Swinny EE (2009) Postharvest application of 1-methylcyclopropene modulates fruit ripening, storage life and quality of 'Tegan Blue' Japanese plum kept in ambient and cold storage. Int J Food Sci tech 44:1272-1280. doi:10.1111/j.1365-2621.2009.01957.x

Kim KO, Yoo J, Lee J, Win NM, Ryu S, Han JS, Jung HY, Choung MG, Kwon YD, et al. (2018) Effects of 1-methylcyclopropene (1-MCP) and polyethylene (PE) film liner treatments on the fruit quality of cold-stored 'Gamhong' apples. Hortic Environ Biotechnol 59:51-57. doi:10.1007/s13580-018-0006-2.

Lee DB, Lee GJ, Kim SH, Kang IK, Choi SJ, Yun HK (2020) Changes in fruit characteristics and expression of ripening-related genes in 'Madoka' peaches treated with 1-methylcyclopropene and aminoethoxyvinylglycine. Hortic Sci Technol 38:32-43.

Lelièvre J-M, Tichit L, Dao P, Fillion L, Nam Y-W, Pech J-C, Latché A (1997) Effects of chilling on the expression of ethylene biosynthetic genes in Passe-Crassane pear (*Pyrus communis* L.) fruits. Plant Mol Biol 33:847-855. doi:10.1023/A:1005750324531

Luo Z, Xie J, Xu T, Zhang L (2009) Delay ripening of 'Qingnai' plum (*Prunus salicina* Lindl.) with 1-methylcyclopropene. Plant Sci 177:705-709. doi:10.1016/j.plantsci.2009.08.013

Mathooko FM, Tsunashima Y, Owino WZO, Kubo Y, Inaba A (2001) Regulation of genes encoding ethylene biosynthetic enzymes in peach (*Prunus persica* L.) fruit by carbon dioxide and 1-methylcyclopropen. Postharvest Biol Technol 36:93-102 doi:10.1016/S0925-5214(00)00158-7

Muñoz-Robredo P, Rubio P, Infante R, Campos-Vargas R, Manríquez D, González-Agüero M, Defilippi BG (2012) Ethylene biosynthesis in apricot: Identification of a ripening-related 1-aminocyclopropane-1-carboxylic acid synthase (ACS) gene. Postharvest Biol Technol 63:85-90. doi:10.1016/j.postharvbio.2011.09.001

O'Malley RC, Rodriguez FI, Esch JJ, Binder BM, O'Donnell P, Klee HJ, Bleeker AB (2005) Ethylene-binding activity, gene expression levels, and receptor system output for ethylene receptor family members from *Arabidopsis* and tomato. Plant J 41:651-659. doi:10.1111/j.1365-313X.2004.02331.x

Ozturk B, Kucuker E, Yıldız K, Celik SM (2015) Changes of bioactive compounds and ethylene production of Japanese plums treated with

pre-harvest aminoethoxyvinylglycine. Int J Food Prop 18:2165-2186. doi:10.1080/10942912.2014.966387

Saltveit ME (2005) Aminoethoxyvinylglycine (AVG) reduces ethylene and protein biosynthesis in excised discs of mature-green tomato pericarp tissue. Postharvest Biol Technol 35:183-190. doi:10.1016/j.postharvbio.2004.07.002

Schaller GE, Bleecker AB (1995) Ethylene-binding sites generated in yeast expressing the *Arabidopsis* ETR1 gene. Science 270:1809-1811. doi:10.1126/science.270.5243.1809

Serek M, Sisler EC, Reid MS (1995) Effects of 1-MCP on the vase life and ethylene response of cut flowers. Plant Growth Regul 16:93-97. doi:10.1007/BF00040512

Singh Z, Khan A (2010) Physiology of plum fruit ripening. Stewart Postharvest Rev 6:1-10. doi:10.2212/spr.2010.2.3

Sisler EC, Reid MS, Yang SF (1986) Effect of antagonists of ethylene action on binding of ethylene in cut carnations. Plant Growth Regul 4:213-218. doi:10.1007/BF00028164

Sisler EC, Serek M (1997) Inhibitors of ethylene responses in plants at the receptor level: Recent developments. Physiol Plant 100:577-582. doi:10.1111/j.1399-3054.1997.tb03063.x

Valero D, Martı, x, nez-Romero D, Valverde JM, Guillén F, Serrano M (2003) Quality improvement and extension of shelf life by 1-methyl-cyclopropene in plum as affected by ripening stage at harvest. Innov Food Sci Emerg Technol 4:339-348. doi:10.1016/S1466-8564(03)00038-9

Watkins CB (2006) The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. Biotechnol Adv 24:389-409. doi:10.1016/j.biotechadv.2006.01.005

Win NM, Lee JW, Yoo JG, Ryu SG, Kim KO, Kim DH, Jung HY, Choung MG, Park KI, et al. (2018) Effects of polyethylene film liner, 1-methylcyclopropene, and aminoethoxyvinylglycine treatments on fruit quality attributes of 'Tonewase' persimmon fruits during cold storage. Hortic Sci Technol 36:256-265. doi:10.12972/kjhst.20180026

Xie X, Einhorn T, Wang Y (2015) Inhibition of ethylene biosynthesis and associated gene expression by aminoethoxyvinylglycine and 1-methylcyclopropene and their consequences on eating quality and internal browning of 'Starkrimson' pears. J Am Soc Hortic Sci 140:587-596. doi:10.21273/JASHS.140.6.587

Xu J, Zhang S (2014) Regulation of ethylene biosynthesis and signaling by protein kinases and phosphatases. Mol Plant. doi:10.1093/mp/ssu059

Yoo J, Jung H, Win NM, Kwon J-G, Cho Y-J, Jung HY, Lee DH, Kang I-K (2020) Changes in fruit quality attributes, cell wall materials, and related hydrolases activities in 1-methylcyclopropene (1-MCP)-treated 'Honggeum' apples during cold storage. Hortic Sci Technol 38:870-879. doi:10.7235/HORT.20200079

Yuan R, Carbaugh DH (2007) Effects of NAA, AVG, and 1-MCP on ethylene biosynthesis, preharvest fruit drop, fruit maturity, and quality of 'Golden Supreme' and 'Golden Delicious' apples. HortScience 42:101-105. doi:10.21273/HORTSCI.42.1.101