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Effect of 1-MCP on Persimmon Fruit Quality and Expression of Ethylene Response Genes During Ripening

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Abstract

This study was conducted to investigate the effects of 1-MCP on the quality and ethylene response gene expression in astringent persimmon 'Bansi' during ripening. Ethylene production was reduced from 0.59 $\mu L \cdot kg^{-1} \cdot hr^{-1}$ immediately after harvest to 0.14 $\mu L \cdot kg^{-1} \cdot hr^{-1}$ and 0.04 $\mu L \cdot kg^{-1} \cdot hr^{-1}$ within five days in the control and 1-MCP treated fruit, respectively. Firmness was 13.8N immediately after harvest and declined rapidly to 7.4N within 1 day for control fruit, on the other hand 1-MCP fruit softened slightly to 11.1N up to the 7 day. Treatment with 1-MCP did not influence soluble solids concentrations. Soluble tannin declined significantly from 399.5 $mg \cdot 100g^{-1}$ to 248.5 $mg \cdot 100g^{-1}$ in control fruit but tannin level for 1-MCP treated fruit was 357.8 $mg \cdot 100g^{-1}$ one day after harvest and did not change significantly through the ripening period. Expression of all the ethylene response genes during ripening was lower in 1-MCP treated fruit than in the control fruit. These results indicate that the inhibition of expression of ethylene receptor genes by 1-MCP treatment resulted in extended shelf life of astringent persimmons. The ethylene response genes mainly associated with this 1-MCP effect appear to be DKERF1, DKERF3, and DKERF8.

The genus of *Diospyos* consists of about 400 species and is distributed in Africa, Asia, and America. Of these a few species can be cultivated in the temperature region, and the best known one is the persimmon (*Diospyros kaki* Thunb.). Persimmon is mainly grown in East Asia, including China, Japan and Korea. In Korea, persimmon ranks the fourth in fruit production following apple, pear and citrus; thus, it is an important fruit crop. Persimmon fruit contains mainly glucose and fructose, beta-carotene and high levels of functional materials, such as vitamin C, gallic acid and catechin (Hiroshi and Akira, 2007). Persimmon cultivars can be classified into two groups based on dissimilarity in flesh coloration as affected by seed formation during pollination (Miller, 1984). The first one is pollination-constant (PC), and the other is pollination-variant (PV). The fruit flesh in PC persimmon cultivars does not change color by seed formation while the fruit flesh in PV persimmon cultivars has dark coloration. In addition, PC and PV persimmon cultivars have astringent and non-astringent types depending on fruit loss (non-astringent type) or no fruit loss (astringent type) of astringency at maturation. Based on these two classification methods, persimmon cultivars are classified into four types: pollination-constant non-astringent (PCNA), pollination-variant non-astringent (PVNA), pollination-constant astringent (PCA), and pollination-variant astringent (PVA) (Xue-ren et al, 2012). The main native and cultivated types of East Asia are astringent (Xue-ren et al, 2012; Yamada et al, 1994). Astringent persimmon is one of the most important fruit due to its high eco-

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nomic value in major producing countries such as Korea, Japan and China (Pang et al., 2007; Qinggang et al., 2013).

Various aspects of ripening and astringency removal of persimmon have been studied, including ethylene treatment (Lim et al., 2015), CO_2 treatment (Arnal and Rio, 2003; Salvador et al., 2007), ethanol treatment (Ortiz et al., 2005), and high temperature treatment, etc. Kato (1987) reported that ethylene effectively removed astringency. However, after astringency removal and ripening, the fruits became softer and sensitive to damage resulting in shorter shelf life (Guinevere, 2005; Akira, et al., 2011). Therefore, Korea's export is limited regardless of production quality and intensity of management. 1-Methylcyclopropene (1-MCP) is a material that blocks the effects of ethylene by binding to the ethylene receptor in plants and is used to study the mechanisms of the ripening process (Sisler et al.,1995 ; Zisheng, 2007).

1-MCP delays ripening of climacteric fruits and has been used on various fruits including persimmon (Luo, 2007), banana (Pathak et al., 2003), and tomato (Opiyo and Ying, 2005; Wang et al., 2010). The effect of 1-MCP has been reported recently on pear fruit as it delays softening and reduced respiration and ethylene production (Villalobos-Acuna et al., 2011; Liu et al., 2013; Ioannis et al., 2013; Hanxu et al., 2016). These results suggest that 1-MCP treatment may extend the shelf life of *'Bansi'* astringency persimmon. Effects of 1-MCP, however, may vary depending on the genetic ability of cultivars to coordinate physiological, biochemical and molecular responses. Thus, it is important to test the efficacy of 1-MCP for extending persimmon shelf life. In this study, we investigated the effect of 1-MCP treatment on the shelf life of 'Bansi' persimmon by observing physiological and molecular changes of fruits.

Material and Methods

Plant material and 1-MCP treatment. Astringent persimmon fruit (*Diospyros kaki* Thunb. 'Bansi'), an astringent persimmon cultivar (PCA), were harvested from Gyeongsangnam-do, Miryang, Korea on 14 Oct. 2014. The fruit were transported to Kangwon national University horticulture laboratory within 24 h of harvest. A total of 135 persimmon fruits were treated with 1-MCP on the same day after harvest. 1-MCP was generated from commercial powder (EthylBloc, Bio Technologies for Horticulture, IL, USA). The treatment was applied at 1.0 $\mu L \cdot L^{-1}$ in a sealed 62.0 L container for 12 h at 20℃. Six containers were used for this study, and each container contained 45 fruits. The 1-MCP concentration chosen as optimal from preliminary experiments was from 0.1 $\mu L \cdot L^{-1}$ to 100 $\mu L \cdot L^{-1}$(Zisheng, 2007). Control fruit were treated similarly but without 1-MCP. After 1-MCP treatment, all fruit were ripened with ethylene, at 100 $\mu L.L^{-1}$ in a sealed 62L container at 20℃ (Akaura, 2010), generated from an ethylene producing tablet. *Measurement of ethylene production*. Persimmon fruit samples were placed in air tight 4.0 L volume containers for three hours and ethylene concentration was analyzed using GC2010 Shimadzu (Shimadzu Corporation, Japan) equipped with BP 20 Wax column (30 m x 0.25 mm x 0.25 μm, SGE analytical science, Australia) and a flame ionization detector (FID). The rate of ethylene production was expressed as $\mu L \cdot kg^{-1} \cdot hr^{-1}$.

*Measurement of fruit quality***.** Effect of 1-MCP application on fruit quality was evaluated by measuring fruit firmness, total soluble solids concentration, fruit skin color and soluble tannin concentration. Fruit firmness was measured in an equatorial end area using a Rheo meter (Sun Scientific Co. Ltd., Japan), fitted with a 3mm diameter head (Agusti et al., 2004). Fifteen fruits were measured for each treatment and firmness was expressed in newton (N). Total soluble solids concentration was measured on each fruit by expressing juice from each side of the fruit onto a digital refractometer (Model-Atago, USA). Fruit skin color was evaluated on the most colored parts of 15 fruit from

Table 1. Real-time PCR primers of ethylene signaling related genes.

Gene	Primary PCR (5'-3')	Secondary PCR (5'-3')
DkCTR1	GGCTTGTAACCCCACCAATA	CCATTGAAGCCCAGAGAAAC
DkEIL1	GCCTACCCTGGTCAAGTGAA	GAGACCAGCATGGGACAAGT
DkERF1	GCTGCTGTCGGAGAGTGAT	TCTCGGGCCTTACAAAGAAG
DkERF2	AAGCCCGACTTGAACGAATA	AAGGTCACAATCCCTTTGGA
DkERF3	AAGAGGCGGTGACAAACAAG	TCACCACATTCCATCATCCA
DkERF5	GGCCGTAGACAGGTTCTTGA	AAAAAGGGAAACTCCTCAACG
DkERF7	GACGACGGAGATGGAGACAT	ATCAACATCAGAGGCGAAGG
DkERF8	ATCTGGAAGGGGGACAATTC	AGAGTAGCGCGGCAAAATTA

each treatment using a Minolta Colorimeter (Model CR-400, Japan) and calibrated with a white and black standard tile. Result were expressed in Hunter 'L' and 'a' values. Soluble tannin concentration was measured according to the method of Folin-Dennis method described by Taira (1995). 5.0 g of the sample were placed directly into a solution of 25 mL of 80% methanol. 1 mL of this sample solution and 6 mL of distilled water were mixed. Then, 0.25 mL of 2N Folin-Ciacalteau reagent was added and vortexed. After 3min, 1mL of saturated Na_2CO_3 plus and 1.5 mL of distilled water was added. After incubation for 1 h at 25℃, the solution was measured using a spectrometer by reading absorbance at 725 nm. The results were expressed as mg/100g F-W.

Gene expression analysis. Transcript accumulation of DkCTR1, DkEIL1, DkERF1, DkERF2, DkERF3, DkERF5, DkERF7 and DkERF8 was evaluated via quantitative real-time RCR(RT-PCR). Total RNA was isolated from frozen fruit samples with the Robospin Plant TM Kit (GeneAll, Korea) according to the manufacturer's instructions, and treated with RNA-free DNAase I to remove genomic DNA. The quality and concentration of the extracted RNA were measured using a Nano-drop and then cDNA was synthesized with oligo $d(T)_{18}$ primer and SuperScript® III Reverse Transcriptase (Life Technologies, USA) from 5 μg of total RNA. Subsequently, the cDNA was utilized to conduct real time PCR using gene-specific primers. Specific primers were as reported in Table 1 and adapted from an earlier study (Xueren et al, 2012). 1μl of cDNA template was amplified using the Platinum SYBR Green qPCR supermix-UDG (Invitrogen, the Netherlands) in a 20μl qPCR reaction according to the manufacturer's protocol. The samples were amplified with PCR as follows: 3min 50°C, 3min 95°C, 45 cycles of 10 sec at 95°C followed by 30 sec at 60°C. Melting curve analyses were performed on the PCR products. DtActin was used as the reference gene to calculate relative expression levels, using the ΔΔCt method (Livak and Schmittgen, 2001). Three RT-PCR runs were performed per each treatment.

Statistical Analysis. All results were presented as means ± standard errors and differences between treatment groups were tested for significance using t-test. Statistical analyses were performed with SPSS statistics program (Version 21, SPSS, USA).

Results and Discussion

Ethylene production immediately after harvest was 0.59 $\mu L \cdot kg^{-1} \cdot hr^{-1}$; this changed to 0.60 $\mu L \cdot kg^{-1} \cdot hr^{-1}$ for the control group and 0.36 $\mu L \cdot kg^{-1} \cdot hr^{-1}$ for 1-MCP treatment group after one day of ripening (Fig. 1). At day 5 of ripening, levels of ethylene production decreased to 0.14 $\mu L \cdot kg^{-1} \cdot hr^{-1}$ and 0.04 $\mu L \cdot kg^{-1} \cdot hr^{-1}$, for the control and 1-MCP treatment groups, respectively. Persimmon is a climac-

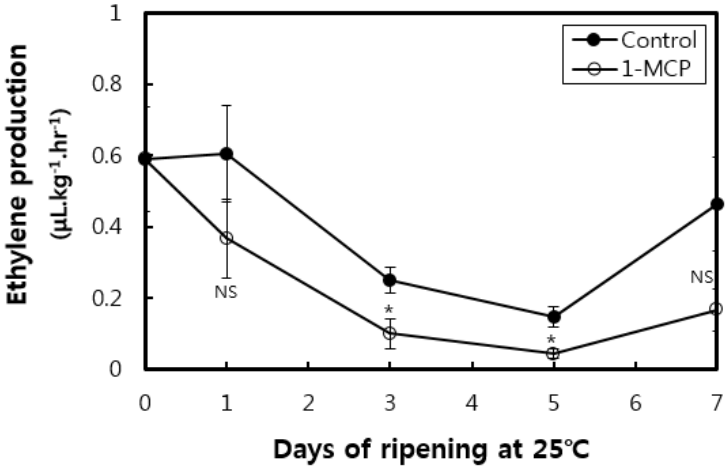

Fig 1. Effect of 1-MCP treatment on ethylene production in 'Bansi' persimmon fruit during ripening at 25°C. Vertical bars represent standard errors of the means (n=15). NS,*,** indicate nonsignificant at P>0.05, significant at P<0.05 and P<0.01 probability level, respectively.

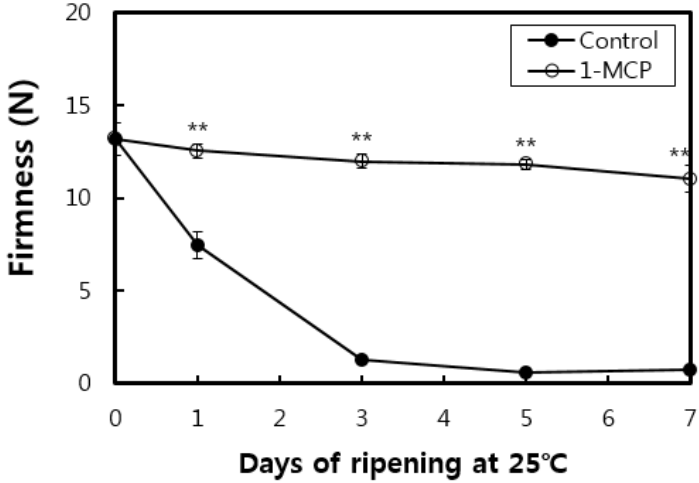

Fig 2. Effect of 1-MCP treatment on firmness in 'Bansi' persimmon fruit during ripening at 25°C. Vertical bars represent standard error of the means (n=15). NS,*,** indicate non significant at P>0.05, significant at P<0.05 and P<0.01 probability level, respectively.

teric fruit, but shows low ethylene production during ripening (Nakano et al. 2002; Pang et al. 2007). The results demonstrated that 1-MCP treatment can reduce ethylene production, similar to previous reports for persimmon fruit (Shinji et al. 2003).

Fruit firmness, a typical fruit ripening indicator, decreased after harvest, from 3.8 N to 7.4 N in the control group at day 1 after ethylene treatment, followed by a steady decrease to 0.7 N over 7 days (Fig. 2). The 1-MCP fruit, softened only a slightly, from 13.8 N to 12.5 N at day 1, with an overall decrease to 11.1 N over 7 days. This inhibition of fruit ripening and softening by 1-MCP treatment agrees with previous reports for other fruits including apple (Watkins et al., 2000), banana (Pelayo et al., 2003), kiwifruit (Boquete et al., 2004) and plums (Menniti et al., 2004).

Total Soluble Solids (TSS) concentration at harvest was 18.1% for both treatments and changed to only 18.3% by day 3 (Fig. 3). After 7 days soluble solids decreased to 17.9%

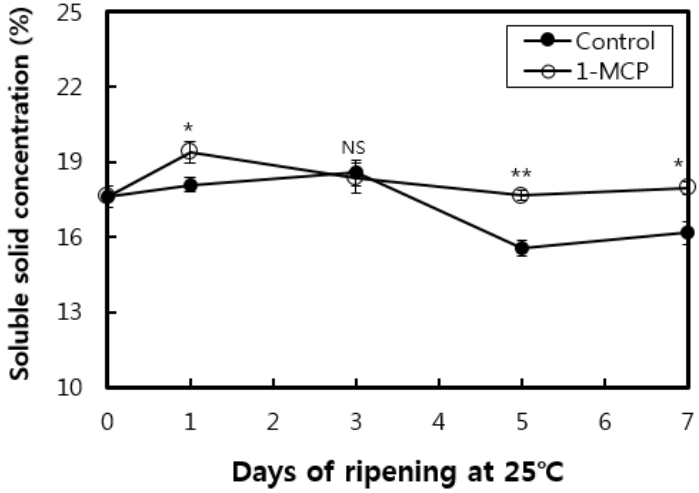

Fig 3. Effect of 1-MCP treatment on soluble solid contents in 'Bansi' persimmon fruit during ripening at 25°C. Vertical bars represent standard error of the means (n=15). NS,*,** indicate non significant at P>0.05, significant at P<0.05 and P<0.01 probability level, respectively.

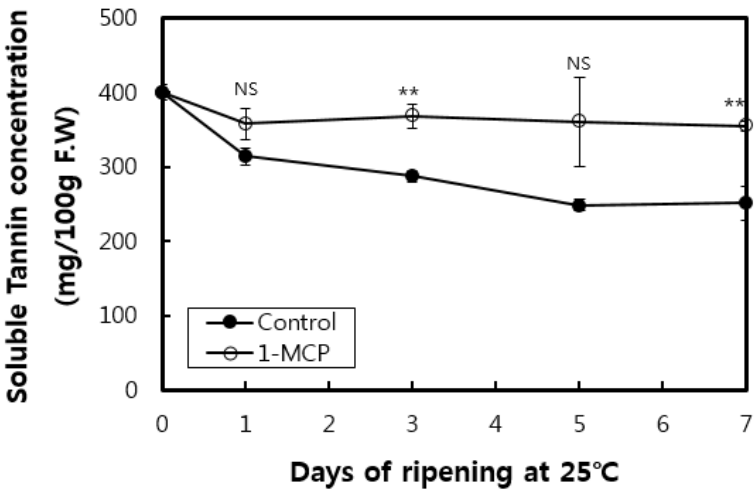

Fig 4. Effect of 1-MCP treatment on soluble tannin content in 'Bansi' persimmon fruit during ripening at 25°C. Vertical bars represent standard error of the means (n=15). NS,*,** indicate non significant at P>0.05, significant at P<0.05 and P<0.01 probability level, respectively.

and 16.1% for the 1-MCP and control fruit, respectively. Persimmon fruits contain free sugars such as fructose, glucose and sucrose, and Yoshihiro et al. (1985) reported that sucrose can break down to fructose and glucose through enzyme activities during storage. The reduction in TSS as the ripening period progressed is related to this breakdown. The 1-MCP group retained higher levels of TSS than the control group due to the inhibition of ripening by 1-MCP.

At harvest, the Hunter 'L' value was 63.68 for control fruit and decreased rapidly to 42.51 by day 7 (Fig. 5). 1-MCP treated fruit decreased slightly to 60.37 within 7 days. The Hunter 'a' value at harvest was 24.86. The control fruit showed a color development value up to 32.87 within 3 days, which declined to 14.67 by day 7 day, and only a slight change in the Hunter 'a' value during the ripening period (Fig. 6). Ethylene treatment of persimmon before harvest can

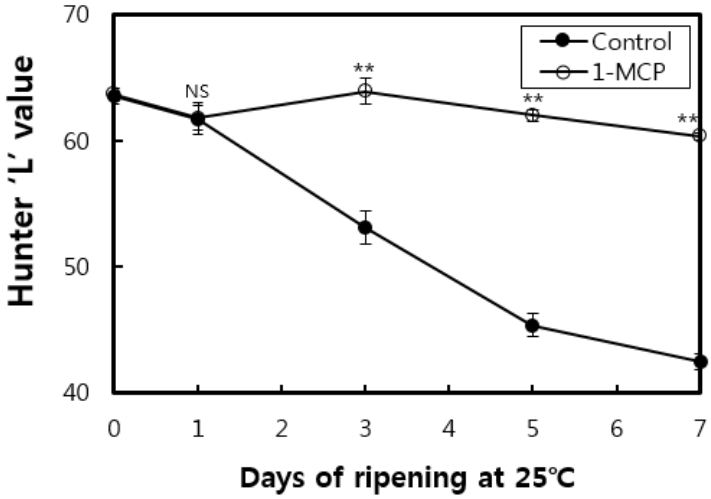

Fig 5. Effect of 1-MCP treatment on Hunter 'L' value in 'Bansi' persimmon fruit during ripening at 25°C. Vertical bars represent standard error of the means (n=15). NS,*,** indicate non significant at P>0.05, significant at P<0.05 and P<0.01 probability level, respectively.

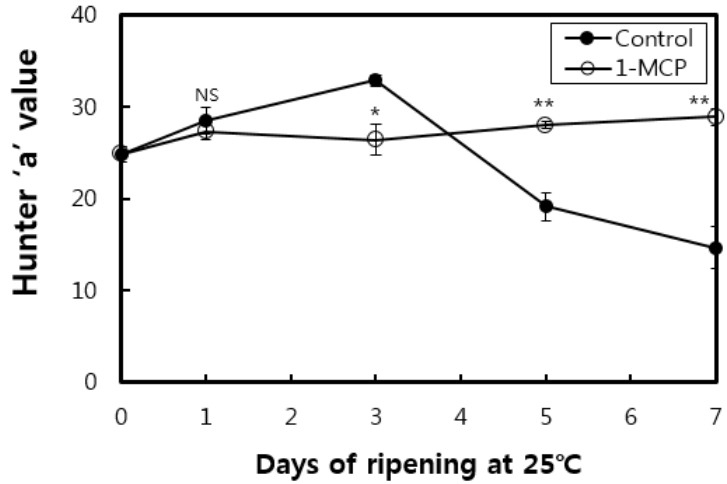

Fig 6. Effect of 1-MCP treatment on Hunter 'a' value in 'Bansi' persimmon fruit during ripening at 25°C. Vertical bars represent standard error of the means (n=15). NS,*,** indicate non significant at P>0.05, significant at P<0.05 and P<0.01 probability level, respectively.

therefore promote maturation and pigment development, resulting in increased Hunter 'a' values, which explains the results for control fruit (Lee and Chujo, 1991; Park and Kim, 2002a, 2002b). According to No et al. (2014), a longer astringency removal treatment period decreases the Hunter 'a' value, probably due to the dissolution of water-soluble tannins. Our results were similar, as the control fruit had lower Hunter 'a' values by day 3.

Immediately after harvest, the soluble tannin concentration was 399.5 mg/100g. For control fruit, the tannin level decreased significantly to 248.5 mg/100g by day 5, with no significant subsequent change (Fig. 6). The 1-MCP fruit declined to 357.8 mg/100g at day 1, and did not change during the subsequent ripening period. Tannins are mainly associated with astringency; the loss of astringency is due to reactions between the acetaldehyde produced in the fruit and the soluble tannins (Seo et al., 1999; Plaza et al., 2012). Treatment of astringent persim-

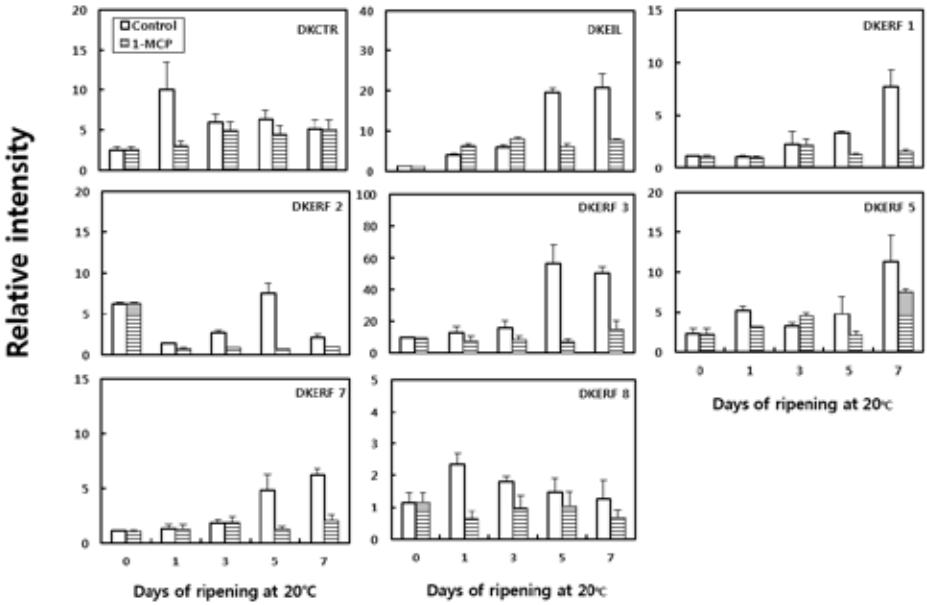

Fig. 7. Effect of 1-MCP treatment on transcript accumulation of targeted genes in 'Bansi' persimmon fruit.

mons with ethylene reduced the soluble tannin concentration from 1.45% to 0.39% in 3 days (Xue-ren et al., 2012); a similar trend occurred in the present study for the control fruit. The changes in soluble tannins were smaller in the 1-MCP treatment fruit than in the control fruit, possibly because ripening of persimmon is controlled by ethylene action, which is inhibited by 1-MCP treatment.

Ethylene action is achieved by regulating ethylene receptors and triggering of signal transduction reactions, and ultimately by controlling relevant gene expression in the fruits (Solano et al., 1988; Bleecker and Kende, 2000). Therefore, real-time PCR was used to evaluate the expression of eight ethylene receptor genes to determine the molecular mechanism of 1-MCP on ethylene production and fruit quality. Expression of all the ethylene response genes during ripening was lower in the 1-MCP treatment group than in the control group (Fig. 7). The control fruit showed strong expression increases during ripening, but the 1-MCP treated fruit showed strong suppression of the increases in DkERF1, DkERF3 and DkERF7 transcript levels toward the end of the ripening period. The increase in some transcript levels, such as DkCTR and DkERF8, found in the control fruit at day 1 was also inhibited by 1-MCP. The expression of the DKEIL and DkERF5 ethylene receptor genes was significantly inhibited by 1-MCP treatment at days 5 and 7, again confirming a likely association between the transcript increases and ethylene production.

We also found that DkERF1and DkERF3 expression was associated with soluble tannin content, while DkERF8 expression was associated with fruit firmness and ethylene production. Xue-ren et al. (2012) reported that fruit ripening and softening in astringent persimmon were associated with the DkCTR, DkEIL, and DkERF1-8 gene families. In addition, DkERF8 expression was highly related to fruit ripening and softening. These results indicate that the inhibition of expression of ethylene receptor genes by

1-MCP treatment resulted in extended shelf life of astringent persimmons. The 1-MCP treatment reduced ethylene production and delayed ripening, as indicated by inhibition of fruit softening and expression of ethylene response genes. The ethylene response genes mainly associated with this 1-MCP effect appear to be DKERF1, DKERF3, and DKERF8.

This result suggests that 1-MCP application blocks ethylene receptors, resulting in the reduction of the softening during postharvest of astringent persimmon as does on non-astringent persimmon (Kim and Lee, 2005).

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