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Effects of Ethephon and Abscisic Acid Application on Ripening-Related Genes in 'Kohi' Kiwifruit (*Actinidia chinensis*) on the Vine

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A B S T R A C T

The effects of ethephon and abscisic acid (ABA) application on ripening-related genes of pre-harvest 'Kohi' kiwifruit (*Actinidia chinensis*) were studied to clarify the possibility of the fruit ripening on the vine. The fruits were treated on the vine at mature stage with 250 μL·L^{-1} ethephon or 100 μmol·L^{-1} ABA, and the expression levels of *chlorophyll synthase* (*AcCLS*), *chlorophyllase1* (*AcCLH1*), *polygalacturonase* (*AcPG*), *expansin* (*AcEXP*), *β-amylase* (*Acβ-AM*), *sucrose synthase* (*AcSUSY*), *MADS-box SEPALLATA4/RIPENING INHIBITOR-like* (*AcSEP4/RIN*) and *FRUITFUL-like* (*AcTDR4/FUL*) genes were analyzed. The expression levels of *AcPG*, *AcEXP*, *Acβ-AM*, and *AcSUSY* increased in the ethephon-treated fruit, but those of *AcCLH1* at 6 and 9 days after treatment and *AcCLS* decreased. Moreover, the expression levels of *AcSEP4/RIN* and *AcTDR4/FUL*, the latter of which is associated with ethylene biosynthesis, were higher in the ethephon-treated fruit. The expression level of each gene in ABA-treated fruit was not significantly different from that of the untreated control. The results suggest that ethephon application increases the expression levels of *AcPG*, *AcEXP*, *Acβ-AM*, *AcSUSY*, *AcSEP4/RIN*, and *AcTDR4/FUL* in 'Kohi' kiwifruit on the vine.

Keywords: kiwifruit; *Actinidia chinensis*; ethephon; ABA; fruit ripening; ethylene

1. Introduction

Fruit ripening involves physiological, biochemical, and structural changes such as cell-wall degradation, pigment synthesis, and increases in both sugar and flavor (Seymour et al., 1933; Mworia et al., 2012). Molecular investigations of fruit development have concentrated mainly on fruit ripening (Gray et al., 1992). It is considered that fruit ripening commences with the synchronized expression of numerous genes, including various MADS-box transcription factors, and functional genes related to the degradation of cell walls, starch, chlorophyll and sucrose accumulation. For example, *EXP* and *PG* genes are involved with cell-wall degradation. Expansin influences cell wall degradation by disrupting noncovalent linkages in cellulose–hemicellulose (McQueen and Cosgrove, 1994). The *AcPG* gene is related to the depolymerization and solubilization of the pectic backbones of cell-wall polysaccharides in kiwifruit (*Actinidia chinensis*) in the ripening stage (Wang et al., 2000).

Furthermore, *β-AM* and *SUSY* genes are soluble sugar-related genes. *β*-amylase breaks starch into maltose during fruit ripening, resulting in a sweet flavor (Robyt and Whelan, 1968). *Maβ-AM* expression was strongly induced during the ripening of banana (*Musa acuminate*), and the increase in *Maβ-AM* expression levels was significantly correlated to the degradation of starch (Nascimento et al., 2006). Sucrose synthase (*SUSY* gene) catalyzes the reversible conversion of sucrose and a nucleoside diphosphate into the corresponding nucleoside diphosphate glucose and fructose (Edurne et al., 2012). Sucrose synthase plays an important role in sucrose accumulation in pear (*Pyrus pyrifolia*) (Zhang et al., 2014). The expression levels of the *CuSUSY* gene

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increased during fruit ripening in satsuma mandarin (*Citrus unshiu*) (Komatsu et al., 2002). In addition, *CLS* and *CHL* genes are chlorophyll related gene. Chlorophyll synthase (*CLS* gene) is a key enzyme in chlorophyll biosynthesis, and chlorophyllase (*CHL* gene) is a key enzyme in chlorophyll degradation. Finally, MADS-box transcription factors play important roles in the regulation of vegetative growth, flowering, floral organ development, seed development, senescence, fruit ripening, and organ abscission in plants (Smaczniak et al., 2012). MADS-box in kiwifruit includes *SEP/RIN*, *FRUITFUL TDR4/FUL*, *AGAMOUS* (*AG*)-*like TAGL1*, and *APETALA3* (*AP3*), all of which are associated with ethylene biosynthesis and ripening (McAtee et al., 2015).

Kiwifruit has two important market species: *Actinidia deliciosa* and *Actinidia chinensis*. In general, ethylene production in *A. chinensis* is higher than that in *A. deliciosa* in the ripening stage (Asiche et al., 2016). Ethylene and ABA are important plant hormones in the processes of fruit ripening. Ethephon is a widely used chemical replacing ethylene treatment when ethylene is not available and ethephon application accelerated ripening in kiwifruit after harvest (Mworia et al., 2010). In addition, ABA may also be associated with kiwifruit ripening, because it stimulated banana fruit ripening (*Musa sapientum*) (Jiang et al., 2000).

However, the effects of ethylene and ABA application on kiwifruit on the vine are not clear. It is considered that the ripening of kiwifruit on the vine may be more marketable. In this study, the effects of ethephon and ABA application on the ripening-related genes such as *AcPG*, *AcEXP*, *Acβ-AM*, *AcSUSY*, *AcCLS*, *AcCLH1*, *AcSEP4/RIN*, and *AcTDR4/FUL* in 'Kohi' kiwifruit on the vine were studied.

2. Materials and methods

2.1. Plant material

Three-year-old 'Kohi' kiwifruit (*A. chinensis*) vines top-grafted on 'Hayward' kiwifruit (*A. deliciosa*) vines were used in the experiment in 2014. The vines were grown in an open field at Chiba University, located at 35°N latitude, 140°E longitude, and at an elevation of 37 m. Three hundred fruits from the vines (100 fruits per vine) were randomly divided into 3 groups (100 fruits per group) at mature stage (155 days after full bloom during the commercial harvest period for general ripening in the room following harvest, and soluble solids content was 10%–11% on the vine). In the first group, the fruits were dipped into 250 $\mu L \cdot L^{-1}$ ethephon solution with ethanol with a 5% concentration for 1 min on the vine. The fruits in the second group were dipped into 100 $\mu mol \cdot L^{-1}$ ABA solution with 5% ethanol similarly with ethephon treatment. In the third group, the fruits were dipped into 5% ethanol as an untreated control. The concentrations of ethephon and ABA that were used in our study were effective to promote the ripening in some fruits (Abeles et al., 1992). Twenty fruits from each group were sampled at 0, 3, 6, 9, and 12 days after treatment (DAT). The pulp was sampled and frozen by liquid N_2 at –80 °C for the analysis of gene expression.

2.2. RNA extraction, cDNA synthesis, and quantitative real time RT-PCR (qRT-PCR) analysis

Total RNA was extracted from a sample (500 mg FW; 3 replications) with a modification of the MagExtractor PCR & Gel Clean Up (Toyobo, Osaka, Japan) method reported by Vogelstein and Gillespie (1979). cDNA synthesis for *AcPG*, *AcEXP*, *Acβ-AM*, *AcSUSY*, *AcCLS*, *AcCLH1*, *AcSEP4/RIN*, and *AcTDR4/FUL* genes was performed according to the instruction manual for ReverTra Ace® qPCR RT Master Mix (FSQ-201, Toyobo). Quantitative RT-PCR (Step One Plus, Life Technologies, Tokyo, Japan) was performed using a KAPA SYBR FAST Master Mix (Kapa Biosystems, Boston, MA, USA) according to the instruction manual. Gene-specific primers for each gene (Table 1) were used for qRT-PCR analysis. The expression level of each gene (3 replications) was determined by a comparative $2^{-\Delta\Delta CT}$ method. The expression level was normalized to that of the *Elongation factor-1α* gene (Nieuwenhuizen et al., 2009).

Table 1 Primers used for quantitative real-time RT-PCR

Gene	Forward/Reverse primer (5′–3′)	Reference
AcSEP4/RIN	F: GAGGCTCAAGACAAGGGTTG R: AAGCTGCTCAAGCTCCTTTG	HQ113364.1 (Varkonyi-Gasic et al., 2011)
AcTDR4/FUL	F: GGAGAGTGCAGCTGAAGAGG R:AGATCTCGCGAGCTTTCTTG	Achn247791 (Huang et al., 2013)
AcCLH1	F: TGTAAGCCACCGTGTTGGTA R: GTCAGCGTCTCCTTCCCATA	Achn035481 (Huang et al., 2013)
AcCLS	F: GGCCCGTGTCTTACTGGATA R: CCTGACGGAATAGGACGGTA	Achn001951 (Huang et al., 2013)
Acβ-AM	F: GAAACCCTCTCTGGGGACTC R: CACGATCCTCCATGTTCCTT	Achn387071 (Huang et al., 2013)
AcSUSY	F: GTGTACGGGACCATCGATTT R: TCTGGTTCTCGTTCGGTTTC	Achn064451 (Huang et al., 2013)
AcPG	F: GCAGAATGCCTGACTTCCTC R: CAAAGCTCGGAATGAAGGAG	Achn144321 (Huang et al., 2013)
AcEXP	F: TCCTGCTTTGAGCTGATGTG R: GCACCAGATGTCTTGGGTCT	Achn194511 (Huang et al., 2013)
Elongation factor-1α	F: GCACTGTCATTGATGCTCCT R: CCAGCTTCAAAACCACCAGT	Nieuwenhuizen et al., 2009

2.3. Statistical analysis

Data (presented as means ± *SE* of 3 replications) were subjected to analysis of variance procedures and separated by the Tukey–Kramer test at $P \leq 0.05$ using the SAS statistical analysis package (SAS Institute, Cary, NC, USA).

3. Gene expression analysis

3.1. AcPG and AcEXP

In the ethephon-treated fruit, the expression of *AcPG* was significantly high at 9 and 12 DAT (Fig. 1, A), while that of *AcEXP* was significantly high at 12 DAT (Fig. 1, B). The ABA-treated fruit showed no significant difference with the control.

3.2. Acβ-AM and AcSUSY

The expression levels of *Acβ-AM* and *AcSUSY* were significantly high in the ethephon-treated fruit after treatment (Fig. 2). In the ABA-treated fruit, the expression levels of *Acβ-AM* showed no significant difference except for 12 DAT (Fig. 2, A). The expression levels of *AcSUSY* in ABA-treated fruit were lower than the control at 12 DAT, but were higher at 6 DAT (Fig. 2, B).