Scientia Horticulturae 209 (2016) 255–260

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Effects of pre-harvest application of ethephon or abscisic acid on 'Kohi' kiwifruit (*Actinidia chinensis*) ripening on the vine

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ARTICLE INFO

Article history:
Received 20 April 2016
Received in revised form 15 June 2016
Accepted 18 June 2016
Available online 15 July 2016

Keywords:
ABA
Actinidia chinensis
Ethephon
Kiwifruit

ABSTRACT

The effects of ethephon, abscisic acid (ABA), and nordihydroguaiaretic acid (NDGA) application on the ripening of pre-harvest 'Kohi' kiwifruit (*Actinidia chinensis*) were studied. The fruits were treated on-vine at 155 days after full bloom (DAFB) (mature stage) with 250 μL/L ethephon, 100 μmol ABA, or 100 μmol NDGA. The fruits were sampled at 0, 3, 6, 9, and 12 days after treatment (DAT), and the following were analyzed at each time point: ethylene production, 1-aminocyclopropane-1-carboxylate (ACC) and ABA concentrations, ACC synthase (ACS) and ACC oxidase (ACO) activities, volatile compounds (*n*-hexanal and (*E*)-2-hexexnal), and the expressions of *AcACS1*, *AcACO1,* and 9-*cis*-epoxycarotenoid dioxygenase 1 (*AcNCED1*) genes. ABA concentrations and *AcNCED1* gene expression increased in ABA-treated fruit. Malic acid concentrations and fruit firmness decreased in ethephon-treated fruit, but soluble solids concentrations (SSC), ethylene biosynthesis, and both *AcACS1* and *AcACO1* gene expressions increased. The accumulated fruit drop rate in ethephon-treated fruit was 4% at the edible stage at 9 DAT. Moreover, the production of *n*-hexanal and (*E*)-2-hexexnal decreased in ethephon-treated fruit. These results suggest that 'Kohi' kiwifruit may be ripened by on-vine ethephon application at 9 DAT, thus obviating ripening treatment after harvest.

1. Introduction

Kiwifruit is classified as a climacteric fruit because it ripens in response to exogenous ethylene, and its ripening is characterized by a period of autocatalytic ethylene production (Park and Kim, 1995). The ripening of kiwifruit appears to be different from that of other typical climacteric fruit because fruit produces little ethylene while on the vine (Patterson et al., 2003). However, kiwifruit ripens with ethylene treatment after harvesting (Sfakiotakis et al., 1997; Mworia et al., 2010). There are two commercially important species of kiwifruit: *Actinidia deliciosa* and *Actinidia chinensis*. *A. deliciosa*, including cultivars such as 'Hayward', is widely known for its large fruit size, green flesh, and long storage life (Thompson et al., 2000). *A. chinensis*, including such cultivars as 'Hort 16A', 'Sanuki Gold', and 'Kohi', has yellow flesh, high soluble solids and low organic acid concentrations, but a short storage life (Xu et al., 1998, 2000). In general, *A. chinensis* produces more ethylene than *A. deliciosa* (Asiche et al., 2016). These results may hint that the fruit of *A. chinensis* could ripen more easily than that of *A. deliciosa* and also, the fruit has a shorter storage and shelf life than that of *A. deliciosa*. Ethylene is a kind of plant hormone that is involved in fruit ripening in many plants (Guo and Ecker, 2004). Ethephon has been applied to both *A. deliciosa* and *A. chinensis* commercially to accelerate ripening after harvest (Park et al., 2006; Mworia et al., 2010; Zhang et al., 2012; Pranamornkith et al., 2012). If kiwifruit can be ripened on the vine, the fruit may be more marketable.

ABA concentrations are very low in unripe climacteric fruit such as tomato (*Solanum lycopersicum*) (Zhang et al., 2009a), peach (*Pruns persica*) (Zhang et al., 2009b), and avocado (*Persea americana*) (Chernys and Zeevaar, 2000) but increase during fruit ripening. Therefore, ABA may also play an important role in regulating fruit ripening. The previous research suggested that ABA stimulated the processes of fruit ripening and promoted ethylene biosynthesis in climacteric fruits such as banana (*Musa sapientum* L.) (Jiang et al., 2000) and apple (*Malus domestica*) (Kongsuwan et al., 2012). However, the interaction between ABA and ethylene biosynthesis in 'Kohi' kiwifruit on the vine is unclear. Nordihydroguaiaretic acid (NDGA) is an ideal inhibitor of NCED enzyme and blocks ABA biosynthesis (Zhang et al., 2009a). NDGA was treated to clarify the roles of ABA in kiwifruit ripening. In this study, the possibility of

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http://dx.doi.org/10.1016/j.scienta.2016.06.026
0304-4238/

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

Gene		Forward/reverse primer (5′-3′)	Reference
AcNCED1	(F)	5′-GAACCATGGCCGAAAGTTTC-3′	Accession number
	(R)	5′-CAATTTGCAGTTCTGATTTCCA-3′	(LC123596)
AcACS1	(F)	5′-GCTCACGTTCATCACCTCGA-3′	Huang et al. (2013)
	(R)	5′-GACTGTATACAATGTGAACT-3′	
AcACO1	(F)	5′-GCTATGAAGGAATTTGCCGA-3′	Huang et al. (2013)
	(R)	5′-CAGCTCTGGCTGAGGACACG-3′	
Elongation factor-1α	(F)	5′-GCACTGTCATTGATGCTCCT-3′	Nieuwenhuizen et al.
	(R)	5′-CCAGCTTCAAAACCACCAGT-3′	(2009)

the fruit ripening on the vine and the effects of ethylene or ABA on the ripening of 'Kohi' kiwifruit on the vine were investigated.

2. Materials and methods

2.1. Plant material

Three-year-old 'Kohi' kiwifruit (*A. chinensis*) vines top-grafted on 'Hayward' kiwifruit (*A. deliciosa*) vines at a Chiba University field, located at 35°N, Lat. 140°E, and elevation 37 m, were used in the experiment. Four hundred fruits were randomly divided into four groups at 155 DAFB (mature stage). The mature stage of kiwifruit has 95% black seeds and the color change of outer pericarp commences (Richardson et al., 2011). In the first group, the fruits were dipped into a 250 μL/L ethephon solution with 5% ethanol for 1 min on the vine. In the second and third groups, the fruits were dipped into either 100 μmol ABA or NDGA solution with 5% ethanol, similar to the ethephon treatment in the first group. In the fourth group, the fruits were dipped into 5% ethanol as an untreated control. The fruit drop rate was measured after treatment. Twenty fruits per treatment were harvested at 0, 3, 6, 9, and 12 DAT. Fruit firmness, soluble solids and malic acid concentrations, and ethylene production were measured immediately after harvest. The pulp was sampled and frozen by liquid N_2 at −80 °C for analysis of ACS and ACO activities, ACC and ABA concentrations, volatile compounds, and RNA extraction.

2.2. Fruit firmness and concentrations of soluble solids and malic acid

The firmness of 20 fruits was measured on each side of the fruit after peeling the skin using a penetrometer (FT 327, EFFEGI, Italy). Soluble solid concentration was determined using a refractometer (PL-1, ATAGO, Japan). Titratable acidity was calculated as malic acid concentration.

2.3. ACS and ACO activities, ACC concentrations, and ethylene production

ACS and ACO activities, as well as ACC and ethylene concentrations, were analyzed using gas chromatography with a flame ionization detector (model GC-2014; Shimadzu, Kyoto, Japan) according to the method of Kondo et al. (1991). Ethylene production was determined by incubating 20 fruits per treatment (5 replications of 4 fruits) in a 2 L plastic box at 25 °C for 2 h.

2.4. ABA concentrations

One-gram pulp samples (three replications) were homogenized in 20 mL of cold 80% methanol (v/v) with 0.3 μg ABA-d_6 as an internal standard. ABA was extracted and analyzed by the method of Setha and Kondo (2009). The methyl ester of ABA was analyzed by gas chromatography–mass spectrometry–selected ion monitoring (GC–MS–SIM; model QP5000; Shimadzu). The column temperature was a step gradient of 60 °C for 2 min, and thereafter was raised to 270 °C at 10 °C per min; it was held at 270 °C for 35 min. The ions were measured as ABA-d_0 methyl ester/ABA-d_6 methyl ester at m/z 190, 260, 194, and 264. The ABA concentration was determined from the ratio of peak areas for m/z 190(d_0)/194(d_6).

2.5. Isolation of NCED1 gene from 'Kohi' kiwifruit

RNA was extracted using the method reported by Kondo et al. (2012). First-strand cDNA was synthesized from total oligo (dT) according to the manufacturer's instructions (ImProm-II™ Reverse Transcription System, Promega, Madison, WI, USA). The cDNA was used as a template for amplifying the *NCED1* gene with degenerate primers (forward, 5′-GAACCRTGGCCRAAAGTTTC-3′; reverse, 5′-CAATYTGMAGYTCYGAYTTCCA-3′) designed from the conserved sequences of plant *NCEDs* (accession numbers KC816734 and AJ439079). PCR was performed under the following conditions: 94 °C for 3 min, followed by 30 cycles of 94 °C for 1 min, 50 °C for 2 min, and 72 °C for 3 min, with the final reaction being terminated at 72 °C after 10 min. The PCR product was transformed into *Escherichia coli* DH5α by TA cloning techniques (pGEM®-T Easy Vector Systems, Promega). Positive colonies were selected, amplified, and then sequenced. The phylogenetic tree was constructed using the Clustal W (http://clustalw.ddbj.nig.ac.jp/). The number for each interior branch is the percent bootstrap values calculated from 1000 replicates. The scale bar corresponds to 0.1 amino acid substitutions per residue.

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

RNA was extracted (300 mg FW; three replications) by the method reported by Henderson and Hammond (2013). cDNA synthesis was carried out according to the method of Kondo et al. (2014). Gene-specific primers for each gene (Table 1) were used for RT-PCR. The relative expression level of each gene was determined by a relative standard curve method. Quantitative real-time PCR (model: Step One Plus, Life Technology, Tokyo, Japan) was performed using a KAPA SYBR FAST Master Mix (Kapa Biosystems, Boston, MA, USA) according to the instruction manual. The expression level was normalized to that of the elongation factor-1α gene (Nieuwenhuizen et al., 2009).

2.7. Volatile compound analysis

Volatile compounds were analyzed according to a previous report (Wang et al., 2015). One-gram pulp samples (three replications) were put into a 5-mL vial to which was added 10 μL of 5 μg cyclohexanal as an internal standard. Volatile compounds were extracted by injecting a 10-μM polydimethylsiloxane solid-phase microextraction (SPME) fiber (Supelco, Bellefonte, PA, USA) into the vial and exposing it to the headspace for 30 min at 40 °C. Volatile compounds were analyzed using a gas chromatographer with a flame ionization detector (model GC-4000; GL Sciences,