Fruit Ripening Process in Red Kiwifruit Cultivar ‘Rainbow Red’ 
(Actinidia chinensis) on Vines

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INTRODUCTION

In Japan, kiwifruit has been grown since the 1980s. Today, kiwifruit is a popular fruit crop owing to its high nutritional value. Kiwifruit is a climacteric fruit that shows an increase in ethylene production accompanied by an increase in respiration (Pratt and Reid, 1974). However, the ripening of kiwifruits appears to be different from that of other typical climacteric fruits. Kiwifruit does not readily produce ethylene autocatalytically after harvest unless ample exogenous ethylene is supplied (Yano and Hasegawa, 1993a, 1993b; Ikoma et al., 1995).

‘Rainbow Red’ (Actinidia chinensis) is a commercially important early-season cultivar in Japan. The fruits have a deep red color around the core, and the sweet taste when ripe. These desirable characteristics have led to its popularity; however, premature fruit ripening on the vines has been observed in this variety. The characterization of the fruit ripening process on vines is essential in achieving high fruit quality because postharvest management practice relies on the harvesting time. However, the factors involved in fruit ripening process on vines in ‘Rainbow Red’ remain unknown. Here we present the fruit ripening process of ‘Rainbow Red’ kiwifruits on vines.

MATERIALS AND METHODS

Fruit ripening process on vines

‘Rainbow Red’ kiwifruits, A. chinensis Planch., were obtained from an orchard in Shizuoka, Japan during the 2011 season. The full blooming time was on April 30. For fruit analysis, bulk harvests of 5 fruits from 5 vines were assessed. The fruits were carefully selected to exclude fruits with physical injury, disease, or pests. A portion of the fruit harvested on September 7 was used for ethylene treatment. Five fruits were sampled on that day and measured for ethylene production, core and flesh firmness, SSC, and TA rapidly increased. These results suggested that the ripening of ‘Rainbow Red’ on the vines is not associated with ethylene.

Keywords: ethylene, gene expression, open field, temperature

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1 ml of headspace gas was withdrawn and injected into a gas chromatograph (GC-8A, Shimadzu Co., Ltd., Japan). Core and flesh firmness was determined by measuring compression using Texture Analyzer (TA-XT2, Texture Technologies Corp., U.S.A.) with a 5-mm-diameter punch probe. Each fruit was subjected to a compression speed of 1 mm/s after contact and penetration to 10 mm. SSC of the fruit juice was measured using a digital refractometer (DBX-55A, Atago Co., Ltd., Japan) and expressed in Brix (%). TA of the fruit juice was determined by titration using 0.1 N NaOH and expressed as percentage citric acid equivalents. Evaluation of fruit quality was performed using 5 fruits at each time point.

**RNA extraction and quantitative reverse transcription polymerase chain reaction (qRT-PCR)**

Total RNA was extracted from the frozen samples using the method for polysaccharide-rich fruit tissues by Ikoma et al. (1996) and treated with DNease (RNeasy, Qiagen) to eliminate any DNA contamination. First-strand cDNA was synthesized from total RNA using a reverse transcription kit (TaqMan, Applied Biosystems) according to the manufacturer’s instructions and was used as a template for amplifying cDNA fragments encoding 5 ripening-related genes: 1-aminocyclopropane-1-carboxylate (ACC) synthase gene (ACS1), ACC oxidase gene (ACO3), Ethylene insensitive (EIN)-like gene (EIL4), ethylene response gene (ERF14), and polygalacturonase gene (PGB). Oligonucleotide primers were designed according to each gene's region. It was confirmed that there was only 1 PCR product for each primer pair. To confirm specific amplification of target genes by primers, all PCR products were resequenced. The sequences of all primers used for qRT-PCR are presented in Table 1.

qRT-PCR was performed using Real Time PCR System 7500 (Applied Biosystems) with a Power SYBR Green PCR (Applied Biosystems), as described in the manufacturer’s protocol. 18s rRNA was used as the internal standard in all experiments. The means of 3 individual PCR experiments were determined from separate but concurrent reactions.

**RESULTS**

**Fruit ripening process on vines**

A detailed study of ‘Rainbow Red’ during fruit development on vines from September 7 to October 24 was conducted (Fig. 1). Core and flesh firmness somewhat softened gradually during the investigation period. TA also decreased gradually. SSC gradually increased until October 11 and then increased rapidly. Ethylene production was at a basal level, and rapid production was not observed. Gene expression of ACS1, ACO3, EIL4, ERF14, and PGB was at the basal level, with no substantial change during the investigation period (Fig. 2). The average, maximum, and minimum temperatures in Shizuoka during the experimental period are shown in Fig. 3. There was a gradual drop in the average, maximum, and minimum temperatures during the experimental period.

**Ethylene-induced fruit ripening**

Fruit quality following ethylene conditioning was evaluated (Fig. 4). Core and flesh firmness rapidly decreased 4 days after ethylene conditioning. TA rapidly decreased, and SSC rapidly increased. Ethylene production increased and reached its maximum 4 days after ethylene conditioning. Gene expression of ACS1, ACO3, EIL4, ERF14, and PGB rapidly increased following ripening and ethylene production (Fig. 5).

**DISCUSSION**

In ‘Rainbow Red’ kiwifruit, many physiological changes associated with fruit ripening (starch conversion, gradual decline of core and flesh firmness, TA reduction) on vines occur in the absence of any marked increase in ethylene production (Fig. 1). Expression levels of the ethylene biosynthesis-related genes ACS1 and ACO3, ethylene signaling-related EIL4 and ERF14, and polygalacturonase gene PGB were at basal levels (Fig. 2). However, the application of exogenous ethylene rapidly accelerated fruit ripening with increasing ethylene production and expression of ripening-related genes (Figs. 4, 5). These results suggested that the ripening on vines in ‘Rainbow Red’ is not associated with ethylene.

In a previous study, it was shown that ‘Sanuki Gold’ kiwifruit softened to approximately 20% of firmness at har-
Fig. 1  Fruit quality characteristics and ethylene production of ‘Rainbow Red’ kiwifruits on vines from September 7 to October 24. 
A: Core firmness, B: Flesh firmness, C: Soluble solid content (SSC), D: Titratable acidity (TA), E: Ethylene production
DAA shows days after anthesis.

Fig. 2  Expression of 5 ripening-related genes on vines in ‘Rainbow Red’ kiwifruits from September 7 to October 24.
Steady-state levels were normalized to 18S rRNA. Data are mean±SD of 3 individual experiments.
DAA shows days after anthesis.
vest after 1 month of storage at 4°C under ambient conditions without any detectable ethylene production (Mworia et al., 2011). A similar observation has been reported for ‘Hayward’ kiwifruit (Manolopoulou et al., 1997). In this study, we confirmed that ‘Rainbow Red’ ripened on vines without exogenous ethylene. In particular, after around 140 days after anthesis (DAA), the degree of ripening progressed with decreasing temperature. Mworia et al. (2012) reported that low temperature modulates the ripening of ‘Sanuki Gold’ kiwifruit in an ethylene-independent manner, suggesting that kiwifruit ripening is inducible by low temperature signals. This phenomenon was also reported in ‘Rainbow Red’ (Asiche et al., 2012). Decreasing temperature was considered as a factor influencing the degree of ripening. In ‘Rainbow Red’, the ripening easily progressed together with a cool temperature modulator.

Core and flesh firmness somewhat softened gradually during the experimental period. However, invariable firmness remained at approximately 1500 N in the core on October 24. This softening pattern is similar to that of ‘Hort16A’ kiwifruit (A. chinensis) on vines (Richardson et al., 2011). However, the application of exogenous ethylene rapidly accelerates softening, and core firmness was less than 5 N. This result suggested that the process of fruit firmness reduction differs in an ethylene induction-dependent and an ethylene-independent manner.

In the kiwifruit variety ‘Hort16A’, physiological and biological analyses were performed from anthesis (0 DAA) until fruits were senescing on vines (Richardson et al., 2011). Compared with ‘Hort16A’, the ripening period in ‘Rainbow Red’ is short. These results indicate that ‘Rainbow Red’ ripens more easily than ‘Hort16A’ on vines.

In conclusion, ‘Rainbow Red’ fruits on the vine somewhat softened gradually. SSC increased whereas core and flesh firmness gradually decreased. However, significant increase in ethylene production was not observed, and gene expression of ACS1, ACO3, EIL4, ERF14, and PGB was at basal levels at each stage. Fruit quality following ethylene conditioning, core and flesh firmness, and TA rapidly decreased, whereas SSC and ethylene production rapidly increased. In addition, gene expression of ACS1, ACO3, EIL4, ERF14, and PGB rapidly increased. These results suggest that the ripening on the vine in ‘Rainbow Red’ is not associated with ethylene.

Fig. 3 The daily average, maximum, and minimum temperature from September 1 to October 31 in Shizuoka.

Fig. 4 Fruit quality characteristics and ethylene production of ‘Rainbow Red’ kiwifruits treated with ethylene. A: Core firmness, B: Flesh firmness, C: Soluble solid content (SSC), D: Titratable acidity (TA), E: Ethylene production.
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