The Horticulture Journal *Preview*
doi: 10.2503/hortj.OKD-035

The Japanese Society for Horticultural Science
http://www.jshs.jp/

Characterization of Ripening-related Genes Involved in Ethylene-independent Low Temperature-modulated Ripening in 'Rainbow Red' Kiwifruit during Storage and On-vine

Oscar Witere Mitalo[1], William Olubero Asiche[1]**, Yuka Kasahara[1], Yasuaki Tosa[1], Willis Omondi Owino[2], Eric Gituma Mworia[3], Koichiro Ushijima[1], Ryohei Nakano[1] and Yasutaka Kubo[1]***

[1]*Graduate School of Environmental and Life Science, Okayama University, Okayama 700-8530, Japan*
[2]*Jomo Kenyatta University of Agriculture and Technology, Nairobi 62000-00200, Kenya*
[3]*Meru University of Science and Technology, Meru 972-60200, Kenya*

'Rainbow Red' kiwifruit have been reported to gradually ripen during low temperature storage and on the vine in the absence of detectable ethylene. This study was conducted to compare the expression of ripening-related genes during storage at different temperatures and on the vine. Fruit at 5°C and 10°C ripened faster to eating quality within four weeks accompanied with increased expression of ripening-related genes: *AcACO3*, *AcXET2*, *AcEXP1*, *AcPG*, *AcPMEi*, *AcSUS*, *AcβAMY1*, *AcβAMY2*, *AcGA2ox2*, *AcNAC3*, *AcNAC4*, and *AcMADS2*. Fruit at 15°C required a longer period of eight weeks to attain eating quality in concurrence with delayed accumulation of the ripening-related genes. Fruit at 22°C ripened at the slowest rate and did not attain eating quality even after eight weeks, with very minimal accumulation of ripening-related genes. On-vine ripening occurred slowly at the early stages when the average field temperature was ~20°C, but the rate increased as the temperature dropped to ≤15°C accompanied by increased expression of ripening-related genes. These results indicate that both ripening on-vine and during low temperature storage are modulated by low temperature independent of ethylene.

Key Words: field temperature, gene expression, ripening, transcription factor, 1-MCP.

Introduction

Fruit ripening is a complex, genetically programmed process that occurs during fruit development. Generally, fruit are classified as either climacteric or non-climacteric based on the presence or absence of a marked rise in respiration and ethylene production during ripening as well as their response to exogenous ethylene (McMurchie et al., 1972). Kiwifruit are considered climacteric fruit since exogenous ethylene accelerates fruit ripening characterized by softening, a decrease in titratable acidity (TA), an increase in soluble solids content (SSC), and development of aroma (Antunes et al., 2000; Asiche et al., 2016; Mworia et al., 2010; Pratt and Reid, 1974).

Commercially, kiwifruit are harvested at the pre-climacteric stage when the fruit are still firm, and have a high TA and low SSC content. The major postharvest problem in kiwifruit handling is the incidence of ripening rot caused by *Botryoshaeria* spp., *Phomopsis* spp., and *Diaporthe actinidia* (Kinugawa, 2000; Koh et al., 2005; Manning et al., 2016; Yano and Hasegawa, 1993). Infected fruit produce disease-induced ethylene that triggers a perceived climacteric-type ripening in healthy adjoining fruit (Asiche et al., 2016). Therefore, low temperature storage is used to extend kiwifruit storage life since it reduces the incidence of ripening rot (Arpaia et al., 1987; Pranamornkith et al., 2012). Furthermore, 1-methylcyclopropene (1-MCP) is a well-

Received; July 11, 2016. Accepted; December 6, 2016.
First Published Online in J-STAGE on April 19, 2018.
This study was supported in part by a Grant-in-Aid for Scientific Research (grant nos. 24380023 and 16H04873) from the Japan Society for the Promotion of Science, Japan.
Part of this study was presented at the 2015 Fall Meeting of the Japanese Society of Horticultural Science
* Corresponding author (Email: ykubo@okayama-u.ac.jp).
** These authors equally contributed to this work.

known potent ethylene perception inhibitor that has been shown to significantly suppress the initiation and onset of kiwifruit softening during storage (Crisosto and Garner, 2001; Ilina et al., 2010; Mworia et al., 2010).

Kiwifruit softening during low temperature storage has been observed in the absence of autocatalytic ethylene production (Hewett et al., 1999; Ilina et al., 2010; Kim et al., 1999; Yin et al., 2012). This softening has been attributed to basal "System 1" ethylene, as kiwifruit are believed to be extremely sensitive to ethylene (Jabbar and East, 2016; Pranamornkith et al., 2012). In other reports, kiwifruit ripening at low temperature is thought to be the result of a synergy between ethylene and low temperature (Minas et al., 2016). However, more recent studies have demonstrated that an alternative ethylene-independent ripening pathway that is modulated by low temperature exists in kiwifruit (Asiche et al., 2018; Mworia et al., 2012).

'Rainbow Red' is an important, early-maturing kiwifruit cultivar in Japan. The fruit have a high SSC of >18% and a TA of ~1% making it a premium cultivar (Asiche et al., 2016; Murakami et al., 2014). Gradual softening of 'Rainbow Red' fruit during low temperature storage has been previously reported (Asiche et al., 2018; Murakami et al., 2014). Although this softening was attributed to increased ethylene sensitivity of the fruit at low temperature (Murakami et al., 2014), it is postulated to be independent of ethylene and only due to responses to low temperature (Asiche et al., 2018). However, there is limited information on the expression of ripening-associated genes at various storage temperatures in the absence of ethylene.

On-vine ripening has been reported in kiwifruit without substantial ethylene production with many studies attributing it to basal "System 1" ethylene (McAtee et al., 2015; Richardson et al., 2011). Murakami et al. (2015) also reported that 'Rainbow Red' fruit ripened on the vine in the absence of detectable ethylene. Our previous study on various ripening-associated genes has indicated that on the vine ripening in 'Sanuki Gold' fruit is independent of ethylene and is similar to low temperature-induced ripening during storage (Asiche et al., 2018). It is therefore important to provide a more detailed examination into the relationship between on the vine ripening and expression of ripening-related genes in 'Rainbow Red' kiwifruit in order to deduce the low temperature-induced mechanisms.

The objectives of this study are (1) to determine the effect of storage temperature on ripening-related genes and (2) to establish the role of low temperature in the regulation of on-vine ripening in 'Rainbow Red' kiwifruit.

Materials and Methods

Plant materials and treatments

'Rainbow Red' kiwifruit (*Actinidia chinensis*) were obtained from a commercial orchard in Takamatsu, Japan. Ethylene-induced ripening was conducted as previously reported (Asiche et al., 2018). Commercially mature fruit were harvested on September 11, 2012 and treated continuously with 5000 $\mu L \cdot L^{-1}$ of propylene at 20°C in a container fitted with soda lime to absorb the carbon dioxide produced by respiration. Untreated fruit labeled as control were also kept in air at 20°C.

For storage tests, healthy fruit were harvested on September 19, 2014, divided into four groups, and stored at 5°C, 10°C, 15°C, or 22°C for eight weeks using the individual separation technique as previously reported (Asiche et al., 2018; Mworia et al., 2012). Each storage temperature had a corresponding group of fruit that was treated with 1-MCP (SmartFresh™; Rohm and Hass, Philadelphia, PA, USA). 1-MCP treatment was done twice a week at 5 $\mu L \cdot L^{-1}$ of 1-MCP for 12 h as previously described by Mworia et al. (2012). To monitor for quiescent infections, ethylene production of all fruit was measured individually throughout the storage period. Fruit that started to produce ethylene were set aside and monitored in a separate room. These fruit developed ripening rot symptoms within a few days indicating that the ethylene was disease-induced. Healthy fruit with no ethylene production were sampled at harvest, and after four weeks and eight weeks.

On-vine sampling was from eight harvests: September 19, October 2, 16, 27, November 4, 25, and December 5, 17. Changes in environmental temperature were also monitored. Harvested fruit were sorted for uniform size, absence of defects or blemishes, and lack of ethylene production.

Measurement of ethylene production

Ethylene measurement was conducted using a gas chromatograph (model GC-8A; Shimadzu, Kyoto, Japan), equipped with a flame ionization detector (set at 200°C) and an activated alumina column (set at 80°C), as previously described by Mworia et al. (2010).

Evaluation of fruit quality parameters

Fruit firmness was measured as previously described (Asiche et al., 2016; Mworia et al., 2010) using a penetrometer (model SMT-T-50; Toyo Baldwin, Tokyo, Japan) fitted with a 5 mm plunger at a speed of 30 $mm \cdot min^{-1}$). SSC was measured from juice extracts using a digital refractometer (Atago Co. Ltd, Tokyo, Japan) and expressed as brix (%). TA was determined by titrating the juice extract against 0.1 N NaOH using phenolphthalein as the indicator and expressed as percentage citric acid equivalents. Each sampling point consisted of five fruit. The outer pericarp of three independent biological replicates for each sampling point was cut into pieces, frozen in liquid nitrogen, and stored at −80°C for further analysis.

Table 1. Kiwifruit primers used for Real-Time PCR analysis. Gene sequences were obtained from the Kiwifruit Genome Database and NCBI Database and primers designed using Primer3 software (version 0.4.0, http://bioinfo.ut.ee/primer3-0.4.0/).

	Gene	Accession No.	Name	Forward (5′ to 3′)	Reverse (5′ to 3′)
1.	AcACS1	Achn364251	ACC synthase	GAAAGGCTGCGTGCAATTCTC	CCTGAAAATGGACTGCCCATC
2.	AcACO2	Achn326461	ACC oxidase 2	TCTCAGAAATCCCCGATCTCG	TTGGAGCCACTGAAAGCCTTC
3.	AcACO3	Achn150611	ACC oxidase 3	CAGATGGCAACAGAATGTCG	AACTTGAGGCCAGCATAGAG
4.	AcPL2	HQ108112	Pectate lyase 2	AAGACGAGCCACTATGGATC	CTGGCTTGCAATCGTGTATG
5.	AcPG	AF152756	Polygalacturonase	TGGATTTGTTAGGGGTGTGC	CAACTTGTGTCGCTGATGAC
6.	AcEXP1	AY390358	Expansin 1	CGTGCTTCGAGCTAAAGTGC	CGGCGATCTTGAGGAACATG
7.	AcXET1	Achn349851	Xyloglucan endotransglycosylase 1	CGTTCGATCAACCGATGAAGA	TCTTGTAAGCAGCCACGAAGG
8.	AcXET2	Achn387971	Xyloglucan endotransglycosylase 2	GCTTCTCACGCTTTCTCTCG	GTTGACCCCAACGAAGACAG
9.	AcPMEi	FG458520	Pectin methyl esterase inhibitor	CTTTGCATCACCTCGCTCTC	ACTGGGACAGCTTCACCATC
10.	AcβAMY1	FG525163	β-Amylase 1	CCCCACATTGATGGAATGAC	GTTTGTGATGCTGCCACTCG
11.	AcβAMY2	Achn212571	β-Amylase 2	CAGAGAACGCAAACTGCTCG	GTTCCCGGAGTCTGATCTAC
12.	AcSUS	FG439911	Sucrose synthase	TGCCGAATTACAGGGTGTTC	CTCCAAGGCATGAGCAATGG
13.	AcGA2ox2	Achn218871	Gibberellic acid oxidase 2	CCTGCTCATGAACTTCCTCG	ATTCGTCGTAGGGTTTGGTG
14.	AcMADS2	Achn235371	MADS transcription factor	GGACAAGAACAGTCGCCAGG	GTATCTGTCGCCGGTGATG
15.	AcERF6	GQ869857	Ethylene response factor 6	ACGGCATCGAAAACCGTTC	TGCCGGATTCCTCTGTACTTG
16.	AcNAC3	Achn289291	NAC transcription factor 3	CGCTTATGTCCCTTCCATGTC	CAAAGCCCTGAGTGAATCCAG
17.	AcNAC4	Achn169421	NAC transcription factor 4	GAATTGCCCGAGAAAGCAGA	TGTCTTGATACCCTTCGGTGG
18.	AcACTIN	EF063572	Actin	TGGAATGGAAGCTGCAGGA	CACCACTGAGCACAATGTTGC

RNA extraction, cDNA synthesis, and Real-Time PCR

Total RNA was extracted from three biological replicates using a method for polysaccharide-rich fruit (Ikoma et al., 1996) with slight modifications. After RNA extraction, DNase treatment and RNA clean up were done using FavorPrep After Tri-Reagent RNA Clean up kit (Favorgen Biotech co., Ping-Tung, Taiwan) as previously described (Asiche et al., 2018). First strand cDNA was synthesized from 2.4 µg DNase treated RNA using RevTra Ace reverse transcriptase (Toyobo, Osaka, Japan) and random hexamer primer according to the manufacturer's instructions. Table 1 indicates the gene accessions and primers used for Real-Time PCR in this study. Gene expression was calculated by $2^{-\Delta Ct}$ and normalized using actin as the housekeeping gene. Mature fruit sampled at harvest were set as calibrators for calculating relative expression levels.

Results and Discussion

Ethylene-induced ripening in 'Rainbow Red' kiwifruit

The main changes that occur during kiwifruit ripening include softening, an increase in SSC, and a decrease in TA. The ripening changes are hugely accelerated by ethylene since kiwifruit are classified as climacteric fruit (McMurchie et al., 1972; Pratt and Reid, 1974). In the present study, 'Rainbow Red' kiwifruit treated with propylene depicted increased ethylene production, decreased in firmness and TA, and increased in SSC within 5 days, while control fruit remained unchanged even after 7 days (data not shown). These results were described in our previous study (Asiche et al., 2018) and are in agreement with other studies on different kiwifruit cultivars (Antunes et al., 2000; Asiche et al., 2016; Mworia et al., 2012).

Low temperature-modulated ripening in 'Rainbow Red' kiwifruit during storage

Postharvest kiwifruit are largely affected by ripening rot disease that is caused by *Botryoshaeria* spp., *Phomopsis* spp., and *Diaporthe actinidia* (Kinugawa, 2000; Koh et al., 2005; Manning et al., 2016; Yano and Hasegawa, 1993). In grouped storage, one infected kiwifruit produced disease-induced ethylene that affected adjoining fruit resulting in a perceived climacteric-type ripening of the whole batch (Asiche et al., 2018). The present study stored 'Rainbow Red' kiwifruit individually separated from each other coupled by frequent ripening rot screenings to reduce the effect of disease-induced ethylene. Using this technique, we managed to obtain healthy fruit with undetectable ethylene during storage for eight weeks.

In healthy 'Rainbow Red' kiwifruit with undetectable ethylene, ripening was observed (at least to some extent) at all storage temperatures irrespective of 1-MCP treatment (Fig. 1). This is in agreement with previous reports on low temperature-modulated ripening in kiwifruit (Asiche et al., 2018; Mworia et al., 2012). However, softening occurred fastest in fruit at 5°C and 10°C with fruit attaining eating quality firmness (~10N) within 4 weeks (Fig. 1A and D). Conversely, fruit at 15°C softened at a moderate rate and required eight weeks to attain eating quality while the ripening rate at 22°C was too slow for the fruit to achieve eating quality. This shows the dose-response effect of temperature on the ripening rate in kiwifruit with a temperature of 5°C and

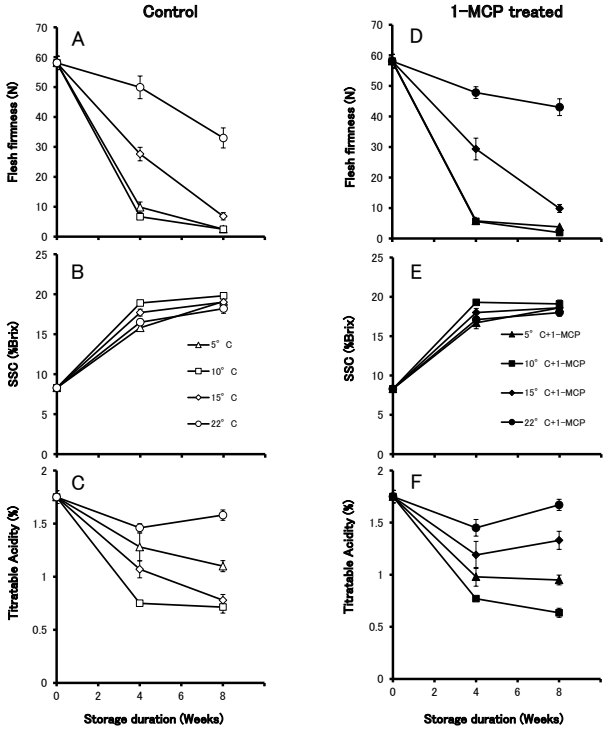

Fig. 1. Effect of storage temperature on ripening characteristics of control (A, B, C) and 1-MCP treated (D, E, F) 'Rainbow Red' kiwifruit. Only fruit with undetectable ethylene production were sampled. Data points represent means and vertical bars represent standard deviations (n = 5).

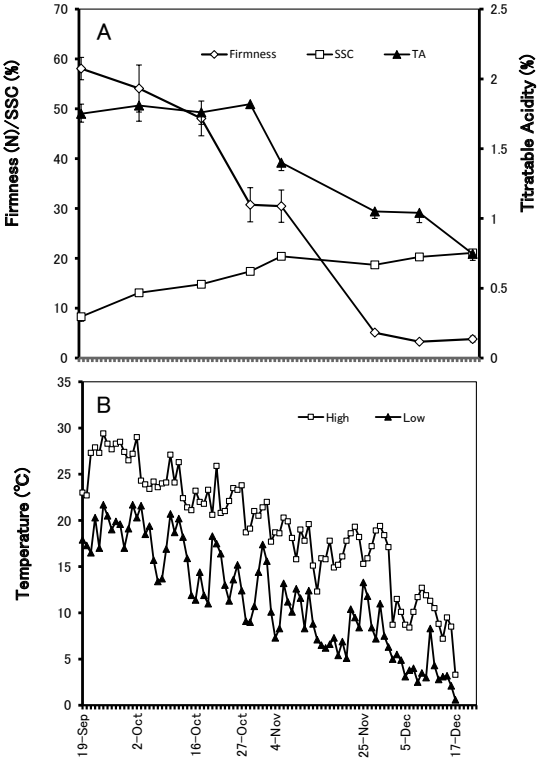

Fig. 2. (A) Fruit ripening characteristics of 'Rainbow Red' kiwifruit attached on the vine and (B) changes in environmental temperature during the experimental period. Only fruit with undetectable ethylene production were sampled. Data points represent means and vertical bars represent standard deviations (n = 5).

10°C eliciting the strongest response. The changes in TA corresponded well with firmness changes (Fig. 1C and F) while the SSC increased at almost similar rates at all storage temperatures, although fruit at 10°C had the highest SSC (Fig. 1B and E).

On-vine ripening in 'Rainbow Red' kiwifruit

Kiwifruit are commercially harvested when they are still firm for the ease of postharvest handling. However, significant ripening of kiwifruit with undetectable ethylene production has been previously reported when fruit are left on vine after commercial harvest date (McAtee et al., 2015; Murakami et al., 2015; Richardson et al., 2011). However, the mechanism of the on-vine ripening has not been elucidated. It is not clear whether this ripening on the vine is due to basal ethylene or other factors. In the present study, 'Rainbow Red' kiwifruit gradually ripened on the vine with undetectable ethylene characterized by a decrease in firmness and TA, and an increase in SSC (Fig. 2A). It is interesting to note that between September 19 and October 16 (when the lowest field temperatures were 15–20°C), on-vine fruit ripening occurred slowly similar to the postharvest fruit at 15°C previously described (Fig. 2A and B). However, the decrease in firmness between October 16 and November 25 (when the lowest field temperatures were ≤10°C) occurred rapidly similar to postharvest fruit at 5°C and 10°C. Thus, our results, taken together with the accelerated ripening during low temperature storage, suggest that kiwifruit ripening on the vine is possibly modulated by low temperature. In order to verify the idea, we compared the expression pattern of ripening-related genes during ethylene-induced ripening, low temperature-modulated ripening in storage, and on-vine ripening.

Expression of ethylene biosynthesis and perception genes

Ethylene-induced kiwifruit ripening involves the increased expression of ethylene biosynthesis and perception genes (Giovannoni, 2004, 2007; Kim et al., 1999; McAtee et al., 2015; Sfakiotakis et al., 1997). In this study, transcript levels of *AcACS1* and *AcACO2* drastically increased in propylene-treated fruit at day 5 while no increases were observed during storage and on the vine (Fig. 3A and C). This accounts for the ethylene production in propylene-treated fruit and the lack of ethylene production in fruit during storage and on the vine. We previously reported that a kiwifruit ethylene biosynthesis-related gene, *AcACO3*, was exclusively responsive to low temperature during storage while propylene treatment had little effect on its expression in 'Sanuki Gold' and 'Hayward' cultivars (Asiche et al.,

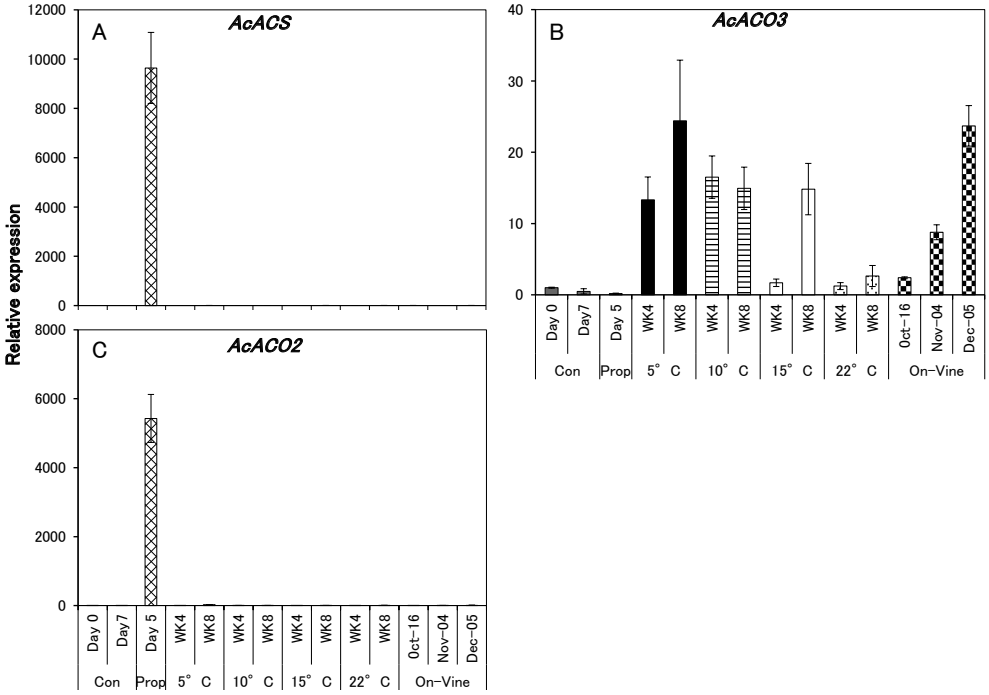

Fig. 3. Relative expression of ethylene biosynthesis and perception genes during propylene treatment, storage at different temperatures, and on-vine in 'Rainbow Red' kiwifruit. Actin was used as the housekeeping gene and Day 0 (harvest) samples were calibrated as 1. Data is the mean ± SD of three fruit (Con—control, WK—week, Prop—propylene).

2018). In the present study, increased expression of *AcACO3* was observed in fruit at 5°C and 10°C within only four weeks, while it took a longer time of eight weeks for it to increase in fruit at 15°C without detection of ethylene (Fig. 3B). This suggests that temperatures of 5°C and 10°C elicit the strongest response, correlating well with the changes in fruit ripening characteristics. Interestingly, *AcACO3* gradually increased on-vine from October 16, reaching the highest level on December 5 in parallel with the decrease in environmental temperature. This indicates that on-vine 'Rainbow Red' kiwifruit responded to the decrease in field temperatures in a similar way to fruit during storage.

Expression of cell wall-modifying genes

Kiwifruit ripening is majorly characterized by a decrease in firmness that is due to stimulation of genes encoding several cell wall-modifying enzymes: xyloglucan endotransglycosylase (XET), polygalacturonase (PG), pectate lyase (PL), expansins (EXP), and pectin methyl esterase (PME) among others (Atkinson et al., 2009; Redgwell and Percy, 1992; Wang et al., 2000; Yang et al., 2007). Based on studies on 'Sanuki Gold' kiwifruit, cell wall-modifying genes have been classified as either ethylene-responsive only, low temperature-responsive only, or responsive to both stimuli (Asiche et al., 2018). In the present study, *AcXET1* and *AcPL2* drastically increased exclusively in propylene-treated fruit, indicating that they are exclusively regulated by ethylene (Fig. 4A and C). Conversely, *AcXET2* transcripts did not respond to propylene treatment but highly accumulated in fruit at 5°C with very minimal increases in fruit at other storage temperatures and on vine (Fig. 4B). This indicates that *AcXET2* is exclusively regulated by low temperature and this gene requires lower temperatures to induce its expression. The transcripts of *AcEXP1*, *AcPG*, and *AcPMEi* accumulated in response to propylene treatment, storage at 5°C, 10°C, and 15°C and during on-vine ripening, suggesting that these genes are regulated by both ethylene and low temperature stimuli (Fig. 4D, E and F). However during storage, *AcPG* transcript accumulation was very prominent in fruit stored at 5°C and moderate in fruit at 10°C while it was very limited in fruit at 15°C, further suggesting that it requires lower temperatures to induce its expression. The expression of *AcEXP1* and *AcPMEi* depicted similar patterns as their transcripts accumulated highly in fruit stored at 5°C and 10°C, with moderate but significant accumulation in fruit at 15°C. Generally, the moderate expression of cell wall-modifying genes during storage of fruit at 15°C accounted for the slow softening rate. Furthermore, significant accumulation of these genes was observed in on-vine fruit, accounting for the softening observed. Our results therefore indicate that low temperature exposure could be involved in on-vine softening since cell wall-modifying genes accumulated as the field temperature decreased.

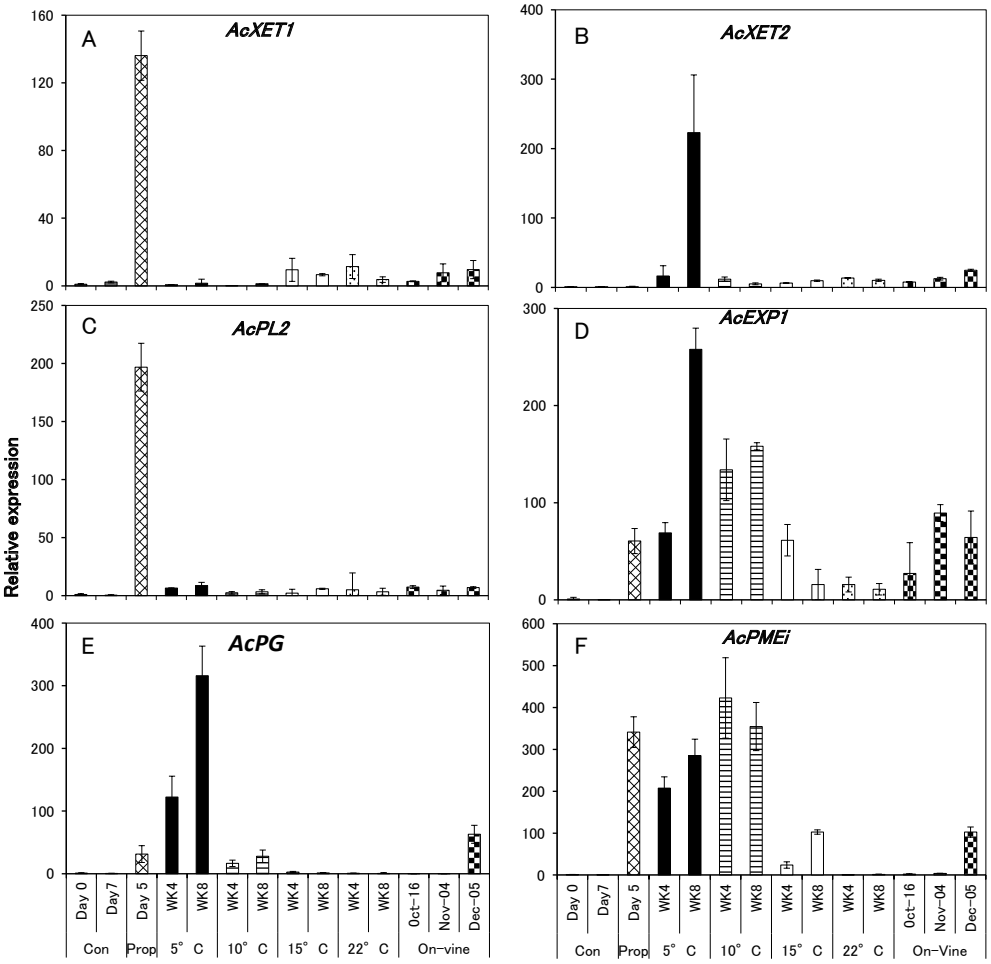

Fig. 4. Relative expression of cell wall modifying genes during propylene treatment, storage at different temperatures, and on-vine in 'Rainbow Red' kiwifruit. Actin was used as the housekeeping gene and Day 0 (harvest) samples were calibrated as 1. Data is the mean ± SD of three fruit (Con—control, WK—week, Prop—propylene).

Expression of carbohydrate metabolism and gibberellins degradation genes

During ripening, kiwifruit accumulate significant amounts of glucose, fructose, and sucrose that culminate in an increase of SSC (Nishiyama et al., 2008). Sugar accumulation is attributed to hydrolysis of starch by β-amylase (Nardozza et al., 2013; Richardson et al., 2011), and metabolism of sucrose by sucrose synthase (Moscatello et al., 2011). In the present study, the expression of *AcSUS* and *AcβAMY1* increased in propylene-treated fruit and both during storage and on-vine ripening, accounting for the increase in SSC observed (Fig. 5A and B). Low expressions of *AcSUS* at 15°C and *AcβAMY1* at 10°C, 15°C, and 22°C could be compensated by higher protein accumulation and metabolic rate, accounting for the similar rates in SSC increase at all storage temperatures. The expression of *AcβAMY2* increased only in fruit during storage and on-vine, suggesting that this gene is independent of ethylene (Fig. 5C). It is worth noting that during storage, fruit at 5°C and 10°C had high expression of these genes within only four weeks while fruit at 15°C had increased transcript levels after eight weeks. Similarly, a gradual increase in expression of these genes was observed in parallel with the progression of on-vine ripening further denoting the role of low temperature.

The degradation of gibberellins, an ethylene antagonist, has been shown to occur during kiwifruit ripening (Atkinson et al., 2011). Our previous study demonstrated that the expression of gibberellic acid oxidase genes (*AcGA2ox1* and *AcGA2ox2*) increased during low temperature storage independent of ethylene in 'Sanuki Gold' and 'Hayward' fruit (Asiche et al., 2018). In the present study, *AcGA2ox2* transcripts highly accumulated in fruit at 5°C and 10°C within only four weeks while in fruit at 15°C, a longer exposure time of eight weeks was required to increase its expression (Fig. 5D). Furthermore, the transcript levels of *AcGA2ox2* accumulated gradually as on-vine ripening progressed.

Expression of ripening-associated transcription factors

Fruit ripening (both for climacteric and non-climacteric fruit) is under the control of transcription factors, specific proteins that bind to the promoter sites

of ripening-related genes repressing or promoting the transcription process (Giovannoni, 2007; Yin et al., 2012). We have previously demonstrated that several transcription factors are induced during kiwifruit ripening at a low temperature despite undetectable ethylene levels (Asiche et al., 2016a). In the present study, the expression of *AcERF6* drastically increased in propylene-treated fruit with very slight increases during

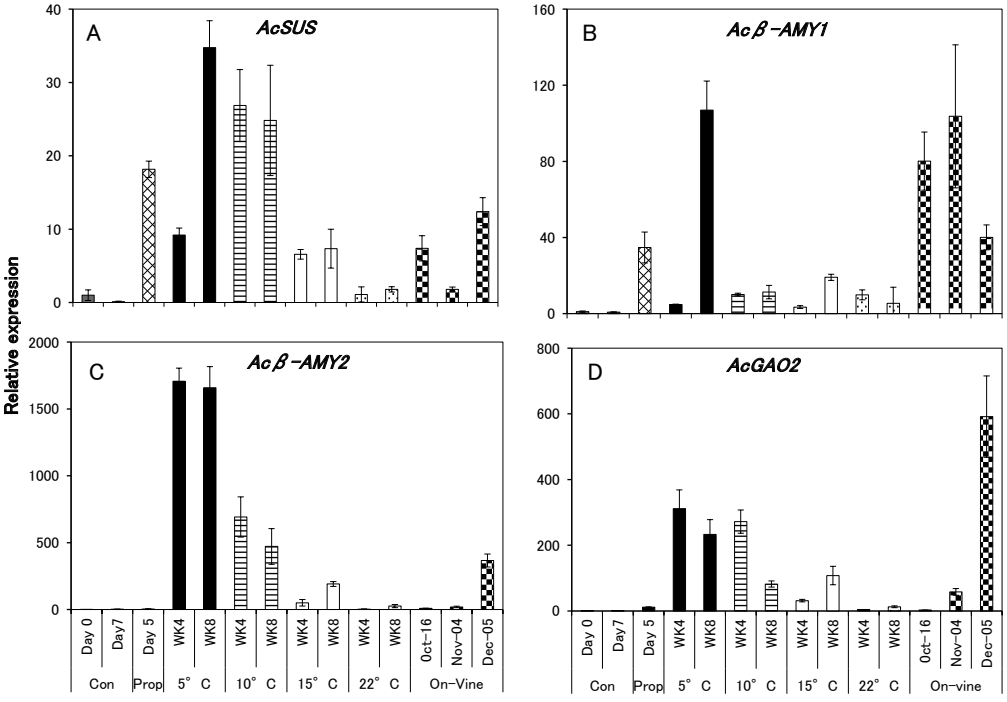

Fig. 5. Relative expression of carbohydrate metabolism and gibberellins degradation-associated genes during propylene treatment, storage at different temperatures, and on-vine in 'Rainbow Red' kiwifruit. Actin was used as the housekeeping gene and Day 0 (harvest) samples were calibrated as 1. Data is the mean ± SD of three fruit (Con—control, WK—week, Prop—propylene).

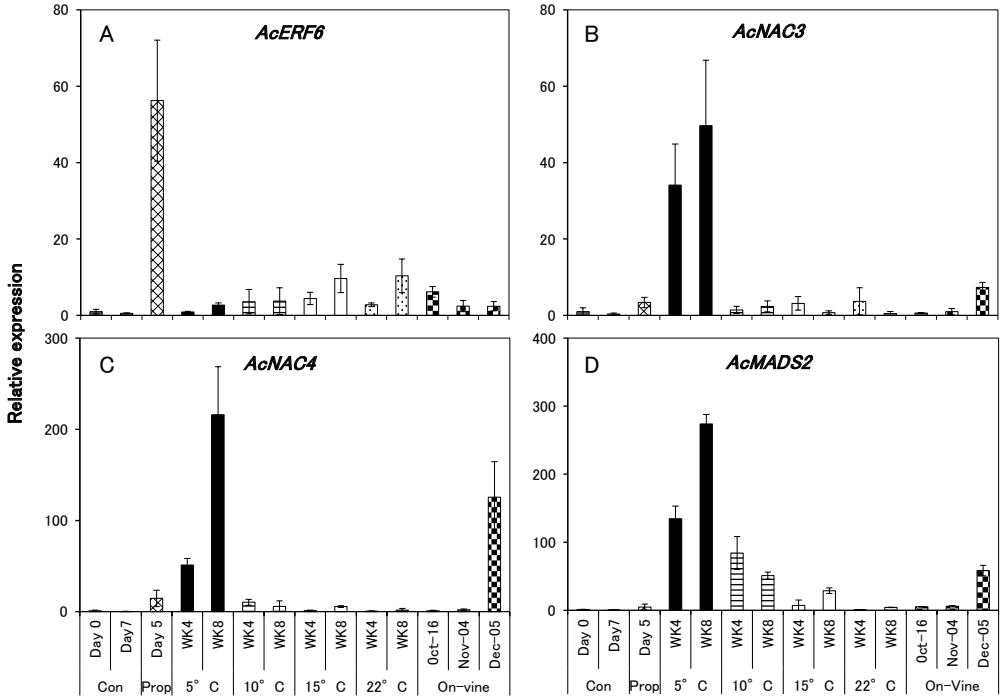

Fig. 6. Relative expression of ripening-associated transcription factor genes during propylene treatment, storage at different temperatures, and on-vine in 'Rainbow Red' kiwifruit. Actin was used as the housekeeping gene and Day 0 (harvest) samples were calibrated as 1. Data is the mean ± SD of three fruit (Con—control, WK—week, Prop—propylene).

storage and on-vine ripening (Fig. 6A). However, the transcripts of *AcNAC3* and *AcNAC4* accumulated markedly in fruit at 5°C and on-vine on December 5 suggesting that this gene responds to much lower temperatures only (Fig. 6B and C). Conversely, *AcMADS2* accumulated markedly in fruit at 5°C and 10°C within only four weeks, while it took eight weeks for the gene to accumulate in fruit at 15°C (Fig. 6D). Furthermore, the expression of this gene increased gradually with progression of on-vine ripening indicating that it responded to decreases in field temperature.

In conclusion, our results indicate a dose-response effect of low temperature on ripening in 'Rainbow Red' kiwifruit. Fruit stored at 5°C and 10°C required only four weeks to ripen while fruit at 15°C required a longer exposure period of eight weeks to ripen. The expression of ripening-related genes corresponded well with this dose-response effect as transcripts of most genes accumulated within four weeks of storage at 5°C and 10°C, while they were only induced after eight weeks at 15°C. Furthermore, progression of 'Rainbow Red' ripening on the vine, accompanied with accumulation of several ripening-related genes, is in parallel with the gradual decrease in field temperature. Therefore, the present study suggests the possible role of low temperature in modulating on-vine kiwifruit ripening independent of ethylene.

Literature Cited

Antunes, M. D. C., I. Pateraki, A. K. Kanellis and E. M. Sfakiotakis. 2000. Differential effects of low-temperature inhibition on the propylene induced autocatalysis of ethylene production, respiration and ripening of 'Hayward' kiwifruit. J. Hort. Sci. Biotech. 75: 575–580.

Arpaia, M. L., J. M. Labavitch, C. Greve and A. A. Kader. 1987. Changes in cell wall components of kiwifruit during storage in air or controlled atmospheres. J. Amer. Soc. Hort. Sci. 112: 474–481.

Asiche, W. O., Y. Kasahara, O. W. Mitalo, Y. Tosa, E. G. Mworia, W. O. Owino, K. Ushijima, R. Nakano, K. Yano and Y. Kubo. 2018. Comparative transcriptome analysis reveals distinct ethylene-independent regulation of ripening in response to low temperature in kiwifruit. BMC Plant Biol. 18: 47.

Asiche, W. O., E. G. Mworia, C. Oda, O. W. Mitalo, W. O. Owino, K. Ushijima, R. Nakano and Y. Kubo. 2016. Extension of shelf life by limited duration of propylene and 1-MCP in three kiwifruit cultivars. Hort. J. 85: 76–85.

Atkinson, R. G., K. Gunaseelan, M. Y. Wang, L. Luo, T. Wang and C. L. Norling. 2011. Dissecting the role of climacteric ethylene in kiwifruit (*Actinidia chinensis*) ripening using a 1-aminocyclopropane-1-carboxylic acid oxidase knockdown line. J. Exp. Bot. 62: 3821–3835.

Atkinson, R. G., S. L. Johnston, Y. Yauk, N. N. Sharma and R. Schröder. 2009. Analysis of xyloglucan endotransglucosylase/hydrolase (XTH) gene families in kiwifruit and apple. Postharvest Biol. Technol. 51: 149–157.

Crisosto, H. C. and D. Garner. 2001. 1-MCP inhibits kiwifruit softening during storage. Perishables Handling Quarterly 108: 19–20.

Giovannoni, J. J. 2004. Genetic regulation of fruit development and ripening. Plant Cell 16 (Suppl 1): S170–S180.

Giovannoni, J. J. 2007. Fruit ripening mutants yield insights into ripening control. Curr. Opin. Plant Biol. 10: 283–289.

Hewett, E. W., H. O. Kim and N. Lallu. 1999. Postharvest physiology of kiwifruit: the challenges ahead. Acta Hortic. 498: 203–216.

Ikoma, Y., M. Yano, K. Ogawa, T. Yoshioka, Z. C. Xu, S. Hisada, M. Omura and T. Moriguchi. 1996. Isolation and evaluation of RNA from polysaccharide-rich tissues in fruit quality by cDNA library construction and RT-PCR. J. Japan. Soc. Hort. Sci. 64: 809–814.

Ilina, N., H. J. Alem, E. A. Pagano and G. O. Sozzi. 2010. Suppression of ethylene perception after exposure to cooling conditions delays the progress of softening in 'Hayward' kiwifruit. Postharvest Biol. Technol. 55: 160–168.

Jabbar, A. and A. R. East. 2016. Quantifying the ethylene induced softening and low temperature breakdown of 'Hayward' kiwifruit in storage. Postharvest Biol. Technol. 113: 87–94.

Kim, H. O., E. W. Hewett and N. Lallu. 1999. The role of ethylene in kiwifruit softening. Acta Hortic. 498: 255–261.

Kinugawa, M. 2000. Studies on infection source and control of kiwifruit ripe rot. 2. The role of old bark as infection source. Bull. Kagawa Agric. Exp. Stn. 52: 41–54 (In Japanese with English abstract).

Koh, Y. J., J. S. Hur and J. S. Jung. 2005. Postharvest fruit rots of kiwifruit (*Actinidia deliciosa*) in Korea. N. Z. J. Crop Hort. Sci. 33: 303–310.

Manning, M., J. Burdon, N. De Silva, X. Meier, P. Pidakala, M. Punter and D. Billing. 2016. Maturity and postharvest temperature management affect rot expression in 'Hort16A' kiwifruit. Postharvest Biol. Technol. 113: 40–47.

McAtee, P. A., A. C. Richardson, N. J. Nieuwenhuizen, K. Gunaseelan, L. Hoong, X. Chen, R. G. Atkinson, J. N. Burdon, K. M. David and R. J. Schaffer. 2015. The hybrid non-ethylene and ethylene ripening response in kiwifruit (*Actinidia chinensis*) is associated with differential regulation of MADS-box transcription factors. BMC Plant Biol. 15: 304.

McMurchie, E. J., W. B. McGlasson and I. L. Eaks. 1972. Treatment of fruit with propylene gives information about biogenesis of ethylene. Nature 237: 235–236.

Minas, I. S., G. Tanou, E. Karagiannis, M. Belghazi and A. Molassiotis. 2016. Coupling of physiological and proteomic analysis to understand the ethylene and chilling induced kiwifruit ripening syndrome. Front. Plant Sci. 7: 120.

Moscatello, S., F. Famiani, S. Proietti, D. Farinelli and A. Battistelli. 2011. Sucrose synthase dominates carbohydrate metabolism and relative growth rate in growing kiwifruit (*Actinidia deliciosa*, cv Hayward). Sci. Hortic. 128: 197–205.

Murakami, S., Y. Ikoma and M. Yano. 2014. Low temperature increases ethylene sensitivity in *Actinidia chinensis* 'Rainbow Red' kiwifruit. J. Japan. Soc. Hort. Sci. 83: 322–326.

Murakami, S., Y. Ikoma and M. Yano. 2015. Fruit ripening process in red kiwifruit cultivar 'Rainbow Red' (*Actinidia chinensis*) on vines. Environ. Control Biol. 53: 159–163.

Mworia, E. G., T. Yoshikawa, N. Salikon, C. Oda, W. O. Asiche, N. Yokotani, D. Abe, K. Ushijima, R. Nakano and Y. Kubo. 2012. Low-temperature-modulated fruit ripening is independent of ethylene in 'Sanuki Gold' kiwifruit. J. Exp. Bot. 63: 963–971.

Mworia, E. G., T. Yoshikawa, N. Yokotani, T. Fukuda, K. Suezawa, K. Ushijima, R. Nakano and Y. Kubo. 2010. Characterization of ethylene biosynthesis and its regulation during fruit ripening in kiwifruit, *Actinidia chinensis* 'Sanuki Gold'. Postharvest Biol. Technol. 55: 108–113.

Nardozza, S., H. L. Boldingh, S. Osorio, M. Höhne, M. Wohlers, A. P. Gleave, E. A. MacRae, A. C. Richardson, R. G. Atkinson and R. Sulpice. 2013. Metabolic analysis of kiwifruit (*Actinidia deliciosa*) berries from extreme genotypes reveals hallmarks for fruit starch metabolism. J. Exp. Bot. 64: 5049–5063.

Nishiyama, I., T. Fukuda, A. Shimohashi and T. Oota. 2008. Sugar and organic acid composition in the fruit juice of different Actinidia varieties. Food Sci. Technol. Res. 14: 67–73.

Pranamornkith, T., A. East and J. Heyes. 2012. Influence of exogenous ethylene during refrigerated storage on storability and quality of *Actinidia chinensis* (cv. Hort16A). Postharvest Biol. Technol. 64: 1–8.

Pratt, H. K. and M. S. Reid. 1974. Chinese gooseberry: seasonal patterns in fruit growth and maturation, ripening respiration and the role ethylene. J. Sci. Food Agric. 25: 741–757.

Redgwell, R. J. and A. E. Percy. 1992. Cell wall changes during on-vine softening of kiwifruit. N. Z. J. Crop Hort. 20: 453–456.

Richardson, A. C., H. L. Boldingh, P. A. McAtee, K. Gunaseelan, Z. Luo, R. G. Atkinson, K. M. David, J. N. Burdon and R. J. Schaffer. 2011. Fruit development of the diploid kiwifruit, *Actinidia chinensis* 'Hort16A'. BMC Plant Biol. 11: 182.

Sfakiotakis, E. M., M. D. Antunes, G. Stavroulakis, N. Niklis, P. Ververidis and D. Gerasopoulos. 1997. Ethylene biosynthesis and its regulation in ripening 'Hayward' kiwifruit. p. 44–56. In: A. K. Kanellis, C. Chang, H. Kende and D. Grierson (eds.). Biology and biotechnology of the plant hormone ethylene. Kluwer Academic Publishers, Boston, Dordrecht.

Wang, Z. Y., E. A. MacRae, M. A. Wright, K. M. Bolitho, G. S. Ross and R. G. Atkinson. 2000. Polygalacturonase gene expression in kiwifruit: relationship to fruit softening and ethylene production. Plant Mol. Biol. 42: 317–328.

Yang, S., C. Xu, B. Zhang, X. Li and K. Chen. 2007. Involvement of both subgroups A and B of expansin genes in kiwifruit fruit ripening. HortScience 42: 315–319.

Yano, M. and Y. Hasegawa. 1993. Ethylene production in harvested kiwifruit with special reference to ripe rot. J. Japan. Soc. Hort. Sci. 62: 443–449 (In Japanese with English abstract).

Yin, X. R., A. C. Allan, C. J. Xu, J. Burdon, S. Dejnoprat, K. S. Chen and I. B. Ferguson. 2012. Differential expression of kiwifruit ERF genes in response to postharvest abiotic stress. Postharvest Biol. Technol. 66: 1–7.