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Comparative analysis of fruit ripening and associated genes in two kiwifruit cultivars ('Sanuki Gold' and 'Hayward') at various storage temperatures

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ABSTRACT

Kiwifruit exhibit a peculiar ripening pattern, as extensive softening is known to occur in the absence of any detectable ethylene. We previously demonstrated that this softening is regulated by low temperature independent of ethylene. However, there are no reports that provide comparisons of the ripening patterns among different kiwifruit cultivars at various storage temperatures. The purpose of this study was to compare the ripening responses and associated gene expression in 'Sanuki Gold' (*Actinidia chinensis* var. chinensis) and 'Hayward' ((*Actinidia chinensis* var. deliciosa) fruit, two kiwifruit cultivars differing in on–vine maturity dates and postharvest storability, during storage at 5 °C, 10 °C, 15 °C and 22 °C. Fruit softening, soluble solids concentration (SSC) increase and reduction of titratable acidity (TA) occurred in the absence of any detectable ethylene, and treatment with an ethylene inhibitor 1–methylcyclopropene (1–MCP) failed to suppress the changes, suggesting that they were independent of ethylene. 'Sanuki Gold' fruit showed a higher sensitivity to low temperature supported by accelerated fruit softening and TA reduction, and induction of several genes such as *AcACO3*, *AcXET2*, *AcPG*, *AcEXP1*, *AcPMEi*, *AcGA2ox1*, *AcMADS2*, *AcNAC5* and *AcbZIP2* at 5 °C, 10 °C and 15 °C within 28 d. By contrast, 'Hayward' fruit exhibited a lower sensitivity to low temperature as accelerated softening, TA reduction and induction of most ripening–associated genes were recorded only at 5 °C and 10 °C. These differences in sensitivity to low temperature, between 'Sanuki Gold' and 'Hayward' fruit, would account for the dissimilarities observed in on–vine maturity dates and postharvest storability.

1. Introduction

Fruit ripening encompasses highly coordinated physiological, biochemical and structural changes such as cell wall modifications, carbohydrate metabolism, pigment degradation and synthesis, and production of aroma volatiles (Klee and Giovannoni, 2011; Osorio et al., 2013). These changes are often orchestrated by the expression of several ripening–associated genes through a network of signaling pathways (Bouzayen et al., 2010). On the one hand, fruit ripening is considered beneficial since it results in soft edible fruit with desirable quality attributes. However, premature or excessive fruit ripening during storage is undesirable since it leads to huge postharvest losses (Golden et al., 2014). An understanding of the controllers of fruit ripening aspects is essential for successful maintenance of postharvest quality.

Climacteric fruit ripening is largely driven by ethylene–regulated changes in gene expression (Giovannoni, 2004; Klee and Giovannoni, 2011; Xu et al., 2012). In kiwifruit (*Actinidia* spp.), exogenous ethylene (or its analog, propylene) initiates rapid ripening–associated changes in a typical climacteric ripening pattern (Antunes et al., 2000; Mworia et al., 2010). Postharvest diseases (blossom–end rot, stem–end rot, and body rot) caused by *Botryosphaeria* sp., *Botrytis cinerea* and *Phomopsis* sp. are widely known for their devastating ethylene–related spoilage in kiwifruit during storage (Koh et al., 2005; Manning et al., 2016). Therefore, successful postharvest handling aims at ensuring no substantial ethylene accumulation in storage chambers (Atkinson et al., 2011). In addition, cold storage (0–4 °C) is universally used to extend the postharvest life of kiwifruit (Arpaia et al., 1987), based on the rationale that low temperature slows most cell metabolic activities, eventually delaying fruit ripening and senescence (McGlasson et al., 1979; Hardenburg et al., 1986).

During low temperature storage, kiwifruit exhibit a peculiar ripening behavior in that extensive softening (to < 10 N) occurs in the absence of any detectable ethylene (Hewett et al., 1999; Kim et al., 1999). The mechanisms underlying the induction of fruit ripening in kiwifruit by low temperature remain elusive. Kiwifruit are considered highly sensitive to small ethylene concentrations (0.005–0.01 μL L^{-1}),

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which have been demonstrated to cause softening and postharvest losses during cold storage (Kim et al., 1999; Antunes, 2007; Pranamornkith et al., 2012; Jabbar and East, 2016). Most of the attempts to explain the atypical softening of kiwifruit during cold storage attribute it to ethylene signaling based on the extrapolation of the above finding; that is, kiwifruit are highly sensitive to basal levels of ethylene (system 1 ethylene) present in most fruit (Kim et al., 1999; Yin et al., 2009). However, this hypothesis is based only on studies that were conducted at temperatures near 0 °C. Experiments comparing the ripening behavior at different storage temperatures are required to uncover the extensive softening mechanisms in kiwifruit during cold storage.

There are two popular kiwifruit cultivars in Japan; 'Hayward' (*Actinidia chinensis* var. deliciosa) and 'Sanuki Gold' (*Actinidia chinensis* var. chinensis). 'Hayward' fruit are late–maturing, and are characterized by relatively low soluble solids concentration (SSC, 11–14%) and high titratable acidity (TA, ~1.5%) when fully ripe (Asiche et al., 2016). 'Hayward' is the leading cultivar on the international market, and this is attributed to the high storability of its fruit; up to six months at 0 °C (Arpaia et al., 1987; Ritenour et al., 1999). On the other hand, 'Sanuki Gold' fruit are early–maturing and are considered premium because of their large size (200 g), high SSC (> 16%) and low TA (1%) when fully ripe (Fukuda et al., 2007). However, 'Sanuki Gold' fruit have very short storage life (one to two months) which poses a major problem during postharvest handling (Mworia et al., 2012; Asiche et al., 2016). Until now, the mechanisms underlying the discrepancy in storage life, between 'Sanuki Gold' and 'Hayward' fruit, remain unknown.

Previously, Mworia et al. (2012) demonstrated that softening in 'Sanuki Gold' kiwifruit occurred faster during storage at 4 °C than at 25 °C in the absence of any detectable ethylene, which was linked to increased accumulation of *polygalacturonase* (*AcPG*), *pectate lyase* (*AcPL*) and *expansin* (*AcEXP*) mRNAs. These changes were not suppressed by repeated exposure of kiwifruit to 1–methylcyclopropene (1–MCP), a potent ethylene perception inhibitor, suggesting that the induction of kiwifruit ripening by low temperature was independent of ethylene signaling. This conclusion was further corroborated by our recent findings, through comparative transcriptome analyses in 'Sanuki Gold' fruit, that kiwifruit possess a distinct set of genes that are exclusively regulated by low temperature (5 °C) independent of ethylene (Asiche et al., 2018). While ethylene–independent modulation of ripening by low temperature has also been previously reported in other kiwifruit cultivars such as 'Rainbow Red' and 'Hayward' (Asiche et al., 2017; Mitalo et al., 2018), there are no reports that compare the ripening responses of different cultivars at various storage temperatures.

In this regard, the present study compared the ripening behavior of 'Sanuki Gold' and 'Hayward' kiwifruit cultivars during storage at four different temperature conditions (5 °C, 10 °C, 15 °C and 20 °C) in the absence of detectable ethylene. The expression pattern of selected ripening–associated genes in these two cultivars during storage was also studied. The overall objective was to determine whether there are dissimilarities that could account for the discrepancies observed in on–vine maturity dates and postharvest storability between 'Sanuki Gold' and 'Hayward' fruit.

2. Materials and methods

2.1. Plant material

'Sanuki Gold' kiwifruit (500 fruit) were harvested on October 9, 2014 corresponding to 151 days after full bloom (DAFB) from a commercial orchard in Takamatsu, Japan. 'Hayward' kiwifruit (500 fruit) were harvested at 169 DAFB (October 27, 2014) from an experimental orchard at Okayama University, Okayama, Japan. Upon harvesting, fruit were immediately transported to the Laboratory of Postharvest Physiology at Okayama University and sorted to ensure uniform size, absence of defects, and no detectable ethylene production.

2.2. Treatments

All fruit were dipped in a mixed fungicide solution containing 0.015 g L^{-1} of oxytetracycline (Pfizer Co., Ltd., Japan), 0.15 g L^{-1} of streptomycin (Pfizer), 0.5 g L^{-1} of iprodione (FMC Chemicals Ltd., Japan), *Bacillus subtillis* HAI0404 spores (1×10^{10} cfu/L, Nippon Soda Co., Ltd., Japan), and 0.5 g L^{-1} of benomyl (Sumitomo Chemical Ltd., Japan). After air–drying, fruit were divided into four groups for storage at either 5 °C (40 fruit), 10 °C (40 fruit), 15 °C (40 fruit) or 22 °C (130 fruit) in air (ethylene–free chambers). Separately, four other groups (containing the same number of fruit as the corresponding temperature) were treated with 1–MCP (5 μL L^{-1}, 12 h) twice a week to keep the fruit insensitive to ethylene (Mworia et al., 2012). 1–MCP was released by dissolving 1–MCP powder (SmartFresh^{SM}, AgroFresh, PA, USA) in water.

2.3. Storage technique

During storage, fruit were individually wrapped in perforated polythene bags to reduce water loss, and then placed separately (10 cm apart) in plastic crates (Supplementary Fig. 1; Asiche et al., 2018). This storage technique allowed for the monitoring of ethylene production pattern of each fruit, unlike the conventional technique where ethylene emanating from a single fruit can influence adjoining fruit. To avoid accumulation of ethylene in the storage chambers, fruit that produced detectable ethylene (≥0.003 ng kg^{-1} s^{-1}) were removed and monitored separately.

2.4. Determination of ethylene production

Ethylene production was measured by incubating individual fruit in 440 − mL airtight containers. After about 1 h, 1 mL of headspace gas was withdrawn and injected into a gas chromatograph (Model GC8 CMPF; Shimadzu, Kyoto, Japan) equipped with a flame ionization detector (set at 200 °C) and an activated alumina column set at 80 °C (Mworia et al., 2010). This procedure has a minimum ethylene detection capacity of 0.003 ng kg^{-1} s^{-1}.

2.5. Fruit quality assessments

Firmness, SSC and TA were monitored using fruit that did not produce any detectable ethylene (five independent biological replicates with three technical replicates) at harvest (0 d) and after 28 d and 56 d of storage. Core and outer pericarp firmness was measured using a penetrometer (model SMTT50; Toyo Baldwin, Tokyo, Japan) fitted with a 5-mm plunger (Mworia et al., 2012). SSC of the fruit juice was measured using a digital Atago PR1 refractometer (Atago Co. Ltd, Tokyo, Japan), and the value was expressed as a percentage. TA was determined by titrating the fruit juice against 0.1 N NaOH using phenolphthalein as a pH indicator, and the value was expressed as percentage citric acid equivalents. The outer pericarp of fruit (three independent biological replicates for each treatment) was then cut into small pieces and stored at − 80 °C until further analysis.

2.6. Effect of propylene on kiwifruit ripening

The effect of propylene exposure on kiwifruit ripening was reported in our previous study (Asiche et al., 2018). Kiwifruit at commercial maturity were continuously exposed to 5000 μL L^{-1} propylene at 20 °C to induce ripening–associated changes, and endogenous ethylene production (McMurchie et al., 1972; Mworia et al., 2010).

2.7. RNA extraction, cDNA synthesis and RT–qPCR analysis

Total RNA was extracted from the outer pericarp (three biological replicates) of fruit at 0 d (harvest), 5 d (for propylene treatment), and