Available online at www.sciencedirect.com
ScienceDirect
Postharvest Biology and Technology 48 (2008) 84–91
Postharvest Biology and Technology
www.elsevier.com/locate/postharvbio

Different postharvest conditions modulate ripening and ethylene biosynthetic and signal transduction pathways in Stony Hard peaches

Maura Begheldo [a], George A. Manganaris [a], Claudio Bonghi [a], Pietro Tonutti [b,*]

[a] *Department of Environmental Agronomy and Crop Science, University of Padova, Viale dell'Università 16, 35020 Legnaro (Padova), Italy*
[b] *Sant'Anna School of Advanced Studies, Piazza Martiri della Libertà 33, 56127 Pisa, Italy*

Received 3 August 2007; accepted 18 September 2007

Abstract

Stony hard (SH) peaches are characterized, at ripening, by the maintenance of flesh firmness and the lack of ethylene production due to a reduced expression of *Pp-ACS1*. In a trial comparing melting flesh (MF, cv. 'Summer Rich') and SH ('IFF331' selection) fruit at two different postharvest temperatures (10 and 20 °C), unexpected behaviour was observed in SH peaches that displayed an increase in ethylene production and a decrease in flesh firmness when stored at 10 °C, a temperature regime basically ineffective in delaying ripening in MF fruit. This appeared to be the result of an induction of *Pp-ACS1* transcription, making this genotype of particular interest for studying temperature stress physiology and ethylene-related ripening processes in peaches. Comparative expression analyses of genes involved in cell wall metabolism pointed out the presence of a negative (*Pp-EG4*), positive (*Pp-endoPG*) or no (one member of the *PL* family) relationship with ethylene at ripening. Results clearly showed that the last stage of firmness decrease (melting) only occurs in fruit producing ethylene and is associated with *Pp-endoPG* transcript accumulation. The expression of genes involved in ethylene biosynthesis and signalling pathways was evaluated using QRT-PCR. *Pp-ACO1* appeared to be induced in SH kept at 10 °C but not at 20 °C. Transient increases in *Pp-CTR1* and *Pp-EIN2like* gene expression have only been detected at the early stages of ripening in samples producing ethylene, indicating that a causal relationship might exist between ethylene and elements of its transduction pathway during peach fruit ripening.

Keywords: CTR1; EIN2; Ethylene biosynthesis; Ethylene signal transduction; Firmness; Temperature stress; *Prunus persica* (L. Batsch)

1. Introduction

Fruit ripening is a complex genetically-programmed physiological syndrome, defined by concurrent processes not necessarily interrelated from a regulatory point of view in both climacteric and non-climacteric fruit, the former characterized by increases in respiration and ethylene biosynthesis. Both ethylene-dependent and ethylene-independent regulatory mechanisms co-exist (Lelievre et al., 1997; Giovannoni, 2004) and isolation of tomato mutants has been particularly useful for identifying ripening- and ethylene-related processes (Giovannoni, 2007).

Peach (*Prunus persica* L. Batsch) is a climacteric fruit in which the increase in ethylene production occurs at an advanced stage of ripening (Tonutti et al., 1991). There is a typical biphasic pattern of loss of firmness in melting flesh (MF) peach genotypes: the initial slow rate (softening) is followed by a rapid firmness decrease (melting) in correspondence to the onset of the ethylene climacteric (Tonutti et al., 1996). Stony hard (SH) fruit have crisp flesh at ripening and maintain high firmness values (both on- and off-tree), though they change colour normally and contain high soluble solids (Hayama et al., 2000; Haji et al., 2001, 2004). This behaviour is due to the lack of ethylene production and has been attributed to a single recessive gene (*hd*) (Haji et al., 2001, 2005). Working with SH peaches (cv. 'Yumyeong'), Tatsuki et al. (2006) showed that ethylene production at ripening is inhibited by a reduced expression of *Pp-ACS1*, a member of the 1-aminocyclopropane-1-carboxylic acid (ACC) synthase gene family, which is responsible for the conversion of *S*-adenosyl-L-methionine to ACC, the immediate precursor of ethylene (Adams and Yang, 1979). Interestingly, in the same SH genotype, ethylene production increases and *Pp-ACS1* transcripts accumulate in senescing flowers and in wounded immature and mature fruit (Tatsuki et al., 2006). The stony hard trait is inherited independently of the melting/non-melting flesh trait and is epistatic to this trait. When treated

* Corresponding author. Tel.: +39050883718; fax: +39050883210.
E-mail address: pietro.tonutti@sssup.it (P. Tonutti).

0925-5214/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.postharvbio.2007.09.023

with exogenous ethylene, mature SH peaches either ripen to the melting stage or just soften but never melt (Haji et al., 2005). The ethylene-promoted ripening is accompanied by increases in polygalacturonase (PG) gene expression and both *endo*- and *exo*-PG activity (Hayama et al., 2006a,b), but not by *Pp-ACS1* mRNA accumulation (Tatsuki et al., 2006), and autocatalytic ethylene production does not take place. These findings clearly indicate that the *stony hard* locus is related to the regulation of *Pp-ACS1* expression and not to any disturbance of the ethylene perception and signal transduction pathways.

The ethylene biosynthesis pathway has been studied in detail in peach (reviewed in Ramina et al., 2007), and an increasing body of information is becoming available on ethylene receptors and elements involved in the signal transduction pathway in this fruit species. Two peach ethylene receptor genes, *Pp-ETR1* and *Pp-ERS1*, have been isolated showing similar organization to that of the corresponding genes in *Arabidopsis* (Rasori et al., 2002). Unlike *Pp-ETR1*, *Pp-ERS1* appears to be induced by ethylene and repressed by 1-methycyclopropene (1-MCP), an antagonist of ethylene action (Rasori et al., 2002). Using a genomic approach, Trainotti et al. (2006a) identified a new member of the peach *ETR* family, named *Pp-ETR2*, which shows increased expression during the transition from pre-climacteric to climacteric stage. The last part of the ethylene receptor complex is CTR1, which acts as a negative key regulator of ethylene responses. In climacteric fruit, such as tomato (Leclercq et al., 2002) and pear (El-Sharkawy et al., 2003), *CTR1* genes are up-regulated during ripening and specific transcripts accumulate following exogenous application of ethylene. A decrease in *Pp-CTR1* transcript accumulation has been observed by Dal Cin et al. (2006) in ripening peaches treated with 1-MCP, suggesting that *CTR1* is also ethylene-inducible in this fruit species. With regard to EIN2, the first positive regulator in the ethylene signalling cascade acting downstream from CTR1 (Guo and Ecker, 2004), Zhu et al. (2006) observed no changes in *EIN2* transcript accumulation throughout tomato fruit ripening, whereas Wang et al. (2007) reported that an increase in *Le-EIN2* gene expression occurs at the mature green-breaker stages. In peach fruit, an induction of a putative ortholog of *EIN2* was observed during the transition from immature to mature stage (Trainotti et al., 2006a). However, preliminary microarray experiments did not clarify whether *EIN2* transcription is affected by ethylene during peach fruit ripening (Begheldo et al., 2007; Tonutti et al., 2007).

The fact that in SH peaches (cv. 'Yumyeong') *Pp-ACS1* is normally expressed except in ripening fruit has been ascribed to the disruption of a transcription factor that is specifically activated to induce *Pp-ACS1* mRNA and/or the presence of inhibitors effective in suppressing *Pp-ACS1* expression at ripening (Tatsuki et al., 2006). Considering this aspect, and the fact that stress conditions (e.g. wounding) are effective in overcoming *Pp-ACS1* inhibition and inducing ethylene production in 'Yumyeong' fruit (Tatsuki et al., 2006), SH peaches represent an interesting model to study factors controlling *Pp-ACS1* transcription, mechanisms modulating its expression, and, using comparative approaches, to better elucidate ethylene physiology in ripening peach fruit.

2. Materials and methods

2.1. Plant material

Peach fruit (*Prunus persica* L. Batsch) of MF cultivar 'Summer Rich' and an SH phenotype 'IFF331' ('Hacuto' × 'New Jersey 256') obtained by the Istituto Sperimentale per la Frutticoltura of Forlì (Italy) were used in the experiments. Fruit were harvested at their commercial maturity stage and, after eliminating defective fruit, they were divided in two different groups (50 fruit each) and stored in ethylene-free air at 20 °C and 10 °C until the end of the experiment.

Flesh firmness (*N*) was measured after removing a small disc of skin from each side of the fruit, using a penetrometer with an 8 mm probe. Epicarp and endocarp were removed and the mesocarp was frozen in liquid nitrogen and stored at −80 °C until required. Sampling days were selected based on the pattern of ethylene production.

2.2. Ethylene production

Ethylene biosynthesis was measured on 10 individual fruit at varying intervals, as described in Tonutti et al. (1991), by enclosing the fruit in 800 mL jars and withdrawing 1 mL of head space gas after 1 h incubation.

2.3. Transcript analyses

Total RNA was extracted as reported in Bonghi et al. (1998). Northern analysis (10 μg RNA) was performed as described in Ruperti et al. (2001), using molecular probes for pectate lyase (*PL*) (AJ532967) (Trainotti et al., 2003), *endo*-polygalacturonase (*endo-PG*) (AJ533395) (Trainotti et al., 2003) and *endo*-β-1,4-glucanase (*EGase*) (AJ890497.1) corresponding to *Pp-EG4* (Trainotti et al., 2006b).

Prior to quantitative real time-polymerase chain reaction (QRT-PCR), 30 μg of total RNA was treated with 10 units of RQ1 RNase-free DNase (Promega) and 1 unit of RNAguard (RNase INHIBITOR) (Amersham) for 30 min and then purified by phenol–chloroform. One microgram of total DNA-free RNA was reverse-transcribed with 200 units of M-MLV reverse transcriptase (Promega), 1 unit of RNAguard and 2.5 μM oligo-$dT_{12\text{-}18}$ primer at 37 °C for 90 min in a final volume of 20 μL, as described in Sambrook et al. (1989). The single strand cDNA obtained (100 ng) was subjected to real-time PCR in a final volume of 10 μL containing 2× Power SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, USA) and specific primers (3 pmol) for *Pp-ACO1, Pp-ACS1, Pp-CTR1,* and *Pp-EIN2like* (Table 1). Three technical replicates for each sample were run on an ABI 7500 Real Time PCR System Sequence Detection machine (PE Applied Biosystem) programmed to heat for 2 min at 50 °C then 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 30 s at 60 °C. Fluorescent detection was performed with an extra step of 35 s at temperatures varying in relation to each product's T_m (Table 1). The melting curves were checked for single peaks, and product size was confirmed in an agarose gel. The amplified cDNA fragments were cloned into