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ScienceDirect
Postharvest Biology and Technology 43 (2007) 28–35
Postharvest Biology and Technology
www.elsevier.com/locate/postharvbio

Influence of time from harvest to 1-MCP treatment on apple fruit quality and expression of genes for ethylene biosynthesis enzymes and ethylene receptors

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Received 10 March 2006; accepted 26 August 2006

Abstract

A strong potent inhibitor of ethylene action, 1-methylcyclopropene (1-MCP) maintains apple fruit quality during storage. To understand the influence of time after harvest until 1-MCP treatment, we studied expression patterns of genes for ethylene biosynthesis enzymes and ethylene receptors in two apple cultivars, 'Orin' and 'Fuji', which differ in ethylene production. Ethylene production and expression of *MdACS1*, *MdERS1*, and *MdERS2* were suppressed in all 1-MCP-treated 'Fuji' fruit, but in 'Orin', the later 1-MCP was applied after harvest, the less was the suppression of ethylene production and expression of these genes. In fruit in which 1-MCP had low efficacy (e.g., 'Orin' treated at 7 DAH), ethylene production and the level of *MdERS1* were briefly reduced by 1-MCP treatment at 2 days after treatment, then began to increase. Since ethylene receptors negatively regulate the ethylene signalling pathway, the increased levels of ethylene production and ethylene receptors after 1-MCP treatment might reduce 1-MCP efficacy.

Keywords: *Malus domestica* Borkh.; ACC synthase; ACC oxidase; Endopolygalacturonase

1. Introduction

Ethylene plays essential roles in multiple developmental processes, including seed germination, fruit ripening, abscission, and senescence (Abeles et al., 1992). In climacteric fruit, increased ethylene production is required for normal fruit ripening, as demonstrated in transgenic tomato fruit in which ethylene production is suppressed (Hamilton et al., 1990; Oeller et al., 1991; Picton et al., 1993) or ethylene sensing is inhibited (Wilkinson et al., 1997). The pathway of ethylene biosynthesis proceeds from *S*-adenosyl-L-methionine (SAM) via 1-aminocyclopropane-1-carboxylic acid (ACC) (Adams and Yang, 1979). The first step is catalysed by ACC synthase and the second by ACC oxidase. ACC synthase is generally the rate-limiting enzyme in the biosynthetic pathway. ACC synthase and ACC oxidase are encoded by multigene families, and their expressions are regulated by developmental and environmental factors (Kende, 1993; Zarembinski and Theologis, 1994). Ethylene perception and signal transduction have been extensively studied at the genetic and biochemical levels in *Arabidopsis* and tomato. Crossing experiments with *Arabidopsis* receptor knockouts indicate that ethylene receptors act as negative regulators of ethylene response (Hua and Meyerowitz, 1998). Ethylene receptor genes have been identified in many plant species, and their expression patterns have been examined; their expressions are regulated differentially according to tissue, developmental stage, and environmental stimuli (Lashbrook et al., 1998; Sato-Nara et al., 1999; Rasori et al., 2002; Cin et al., 2005).

A strong potent inhibitor of ethylene action, 1-methylcyclopropene (1-MCP), binds ethylene receptors more

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doi:10.1016/j.postharvbio.2006.08.010

strongly than ethylene and prevents the physiological action of ethylene. The application of 1-MCP has been shown to maintain the freshness of various fruit, vegetables, and flowers (Blankenship and Dole, 2003). Apple is a climacteric fruit, and harvested fruit quality is maintained by 1-MCP treatment (Fan et al., 1999a, 1999b; Rupasinghe et al., 2000; Watkins et al., 2000; DeEll et al., 2002). The efficacy of 1-MCP is influenced by cultivar, storage conditions, treatment temperature and duration, fruit maturity, and time from harvest (Blankenship and Dole, 2003). The effectiveness of 1-MCP declined slightly with later harvest times (Mir et al., 2001). Ethylene production in apple fruit at the time of 1-MCP treatment might influence 1-MCP efficacy (Watkins et al., 2000), although 1-MCP has a higher affinity for ethylene receptors than ethylene has. The molecular mechanism of 1-MCP efficacy has not yet been examined.

Ethylene biosynthesis in apple fruit differs considerably among cultivars (Abeles et al., 1992). Sunako et al. (1999) reported that an allele of *MdACS1*, which is expressed predominantly in climacteric fruit (Dong et al., 1991; Cin et al., 2005), accounts for the low level of ethylene production in apple cultivars such as 'Fuji', which is homozygous for *MdACS1-2*, which possesses a retroposon-like insertion in the promoter region. The ACC synthase allelotype has no effect on the fruit softening rate, although it defines the rate of fruit drop (Sato et al., 2004). Three apple ethylene receptor genes (*MdETR1*, *MdERS1*, *MdERS2*) have been isolated (Cin et al., 2005; Tatsuki and Endo, 2006), and their expression patterns were examined in abscising fruitlets (Cin et al., 2005) and in 1-MCP-treated apple fruit (Tatsuki and Endo, 2006). *MdETR1*, *MdERS1,* and *MdERS2* are expressed in ripening fruit, and expression of *MdERS1* and *MdERS2* is suppressed by 1-MCP treatment (Tatsuki and Endo, 2006).

Endopolygalacturonase (PG) catalyses the hydrolytic cleavage of α-(1-4) galacturonan linkages and is a key enzyme involved in the large changes in pectin structure that accompany the ripening of many fruit (Hadfield and Bennett, 1998). In tomato, accumulation of *PG* mRNA is induced by ethylene (Maunders et al., 1987; Bird et al., 1988; Sitrit and Bennett, 1998), and continuous ethylene perception is required for *PG* expression (Lincoln et al., 1987; Davies et al., 1988). *MdPG1* cDNA (L27743) has been isolated in apple (Atkinson, 1994); it is up-regulated in ripening apple fruit (Atkinson et al., 1998) and is controlled by ethylene (Wakasa et al., 2006).

In this study, to understand the influence of the timing of 1-MCP treatment on its efficacy, we treated apple fruit at 1, 3, or 7 days after harvest, and examined fruit quality and the expression patterns of genes for ethylene biosynthesis enzymes (ACC synthase, *MdACS1* and *MdACS3*; and ACC oxidase *MdACO1*), ethylene receptors (*MdETR1*, *MdERS1*, *MdERS2*), and endopolygalacturonase (*MdPG1*) as a ripening indicator. We used two apple cultivars, 'Fuji' and 'Orin', which differ in ethylene production. The *MdACS1* genotype of 'Orin' is *Md-ACS1-1/1-2*, and 'Orin' produces more ethylene than 'Fuji'.

2. Materials and methods

2.1. Plant materials and treatment

'Orin' and 'Fuji' apple (*Malus domestica* Borkh.) fruit were harvested at commercial maturity in Fukushima Prefecture. All apples were stored at 20 °C for 1, 3, or 7 days until 1-MCP treatment. For 1-MCP treatment, fruit were placed in 117-L plastic containers and treated with 1 μL L^{-1} of 1-MCP (SmartFresh, AgroFresh Inc., Springhouse, PA, USA) for 12 h at 22 °C. For the control, fruit that were stored for 1 day after harvest (DAH) were left in air for 12 h at 22 °C. After 1-MCP treatment, fruit were stored in air for 2, 4, 8, 15, 31, 46, 61, or 91 days at 20 °C. Five fruit per treatments on each day were sampled and used for ethylene measurement, and then fruit firmness and acidity were determined. Part of each fruit was frozen in liquid nitrogen, and stored at −80 °C until RNA extraction.

2.2. Assessment of fruit quality

For measurement of ethylene, fruit were placed in a 1.2-L air-tight glass chamber for 1 h at 20 °C. One millilitre of headspace gas was withdrawn from the chamber for each measurement and injected into a gas chromatograph (model GC-14B, Shimadzu, Kyoto, Japan) equipped with an activated alumina column and flame ionization detectors. Firmness was determined on opposite sides of each fruit by using a penetrometer (Italtest, FT011, 8-mm diameter) and expressed in newtons (N). After measuring firmness, we divided each apple into eight equal-sized segments, then measured titratable acidity (TA) in two segments that were diametrically opposite from each other by using an autotitrator (Foodstat FS-51, Toko Chemical Laboratories, Co., Ltd., Tokyo, Japan).

2.3. RNA extraction and isolation of cDNA fragments

Total RNA of fruit was extracted from frozen fruit samples by the hot borate method (Wan and Wilkins, 1994). First-strand cDNA was synthesized by reverse transcriptase (SuperScript II, Invitrogen, Carlsbad, CA, USA) from 2 μg of the total RNA from ripening fruit of each cultivar. The cDNA fragments of *MdACS1* (L31347), *MdACS3* (U73816), *MdACO1* (X61390), *MdETR1* (AF032448), *MdERS1* (AY083169), *MdERS2* (AB213028), and *MdPG1* (L27743) were amplified by RT-PCR (reverse transcription PCR) with specific primer sets (Table 1) using cDNA templates from 'Orin' (*MdACS1*, *MdACO1,* and *MdPG1*) and 'Fuji' (*MdACS3*, *MdETR1*, *MdERS1,* and *MdERS2*) fruit. The PCR conditions for *MdACS1*, *MdACO1,* and *MdACS3* were 95 °C for 12 min, followed by 35 cycles of 94 °C for 0.5 min, 55 °C for 1 min, and 72 °C for 2 min, with a final extension of 7 min at 72 °C. AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA) was used. The PCR conditions for *MdETR1*, *MdERS1,* and *MdERS2* were 94 °C for 1 min, followed by 30