Postharvest Biology and Technology 58 (2010) 185–193

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Degreening behavior in 'Fallglo' and 'Lee × Orlando' is correlated with differential expression of ethylene signaling and biosynthesis genes

Karthik-Joseph John-Karuppiah, Jacqueline K. Burns *

University of Florida, IFAS, Horticultural Sciences Department, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850, USA

ARTICLE INFO

Article history:
Received 12 February 2010
Accepted 28 July 2010

Keywords:
Ethylene sensitivity
ACC
MACC
Chlorophyllase
Total chlorophyll

ABSTRACT

Two citrus types ('Fallglo' and 'Lee × Orlando') exhibiting differential fruit degreening response when treated with ethylene were selected. Fruit were harvested at commercial maturity but at different developmental periods (Harvest I, II and III). Rate of color change was greater in 'Fallglo' than in 'Lee × Orlando' when fruit were treated with 5 $\mu L\,L^{-1}$ of ethylene for 24 h. After 24 h of transfer of fruit to ethylene-free storage, rate of change decreased in 'Fallgo' and exhibited varied response in 'Lee × Orlando' depending on harvest date. 'Fallglo' fruit from Harvests I and II were completely degreened at the end of storage for 7 d; however 'Lee × Orlando' were not and were green in color. No difference in seedling triple response was observed between 'Fallglo' and 'Lee × Orlando' and sequences of the four ethylene receptors were identical between them. Expression of genes involved in ethylene biosynthesis and signaling pathways were studied in flavedo to test if differences in these pathways were correlated with differential ethylene sensitivity of the citrus types. Basal levels of *ACS2* and *ACO* expressions declined as maturity progressed, and ethylene-induced expression of *ACS1* and *ACO* were influenced by fruit maturity. At Harvests I and II, ethylene-induced increase in *ACS1* and *ACO* expressions and ACC levels were greater in 'Fallglo' than in 'Lee × Orlando'. Ethylene treatment influenced MACC content only during Harvest I in 'Lee × Orlando'. MACC levels were generally higher in 'Lee × Orlando' than in 'Fallglo'. Expressions of *ETR1* and *ETR2* were ethylene responsive in 'Fallglo' and only *ETR1* expression was ethylene responsive in 'Lee × Orlando'. Ethylene had more impact on *ETR1* expression in 'Fallglo' than in 'Lee × Orlando'. Ethylene had a negative effect on *ETR3* expression which was more pronounced in 'Lee × Orlando' than in 'Fallglo'. Expressions of *ERS1*, *CTR1*, *EIN2*, *EIL1* and *EIL2* were not affected by ethylene in both citrus types. Expression of chlorophyllase gene and rate of total chlorophyll degradation were higher in 'Fallglo' than in 'Lee × Orlando' during ethylene treatment. Differential degreening behavior of 'Fallglo' and 'Lee × Orlando' correlated with peel maturity, and factor(s) downstream of ethylene signaling but upstream of ethylene biosynthesis play a role in the differential sensitivity.

1. Introduction

Degreening in citrus is a postharvest treatment during which fruit are exposed to ethylene to degrade the chlorophyll content of the peel. Under cool nights (<12.5 °C) fruit are subjected to mild stress that triggers endogenous ethylene production (Grierson et al., 1986), resulting in chlorophyll degradation and unmasking and/or synthesis of other natural pigments. Early season fruit in Florida are not subjected to cool nights and they remain green or partially green in color, while the internal quality has reached acceptable maturity standards for marketing. To enhance marketability, application of exogenous ethylene is required to degreen the fruit.

The response of citrus fruit to ethylene varies between cultivars. Though most of these differences are minor, some citrus types show extreme responses. Kitagawa et al. (1978) reported differences in degreening effectiveness in seven citrus cultivars. When treated with ethylene (1 $\mu L\,L^{-1}$) for 15 h, Satsuma mandarin had the highest chlorophyll loss followed by navel orange, 'Eureka' lemon, 'Hassaku', 'Natsudaidai', 'Trovita' orange and 'Valencia' orange. When 'Valencia' oranges and 'Duncan' grapefruit were removed from a short ethylene treatment, fruit continued to change color even after 1 d of ethylene-free conditions, but color change in 'Hamlin' oranges ceased within 2 h (Grierson and Newhall, 1960). Petracek and Montalvo (1997) showed that 'Fallglo' tangerine fruit after 24 h of ethylene treatment continued to change color in the absence of ethylene. For the first 6 h of ethylene

* Corresponding author. Tel.: +1 863 956 1151x1285; fax: +1 863 956 3579.
E-mail address: jkbu@ufl.edu (J.K. Burns).

0925-5214/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.postharvbio.2010.07.013

treatment, fruit were similar in color as that of control fruit but after 8 d in ethylene-free storage, treated fruit were completely degreened.

Chlorophyllase is the first enzyme in chlorophyll degradation which converts chlorophyll a and b to chlorophyllide a and b, respectively. Increase in chlorophyllase activity during degreening of citrus fruit has been reported in several studies (Shimokawa et al., 1978; Purvis and Bramore, 1981; Amir-Shapira et al., 1987; Trebitsh et al., 1993). Application of 1-MCP (an ethylene perception inhibitor) was shown to inhibit or delay chlorophyll loss during degreening (Goldschmidt et al., 1993; Porat et al., 1999; McCollum and Maul, 2007), suggesting ethylene perception plays an important role. When mature green 'Valencia' orange fruit were treated with 80 $\mu L\,L^{-1}$ ethylene, chlorophyllase activity increased 5- and 12-fold after 24 and 72 h of ethylene treatment, respectively (Trebitsh et al., 1993). Synthesis of chlorophyllase protein was observed after 24 h of ethylene treatment, whereas no chlorophyllase protein was detected for up to 7 d in fruit not exposed to ethylene, indicating that exogenous ethylene plays an important role in triggering chlorophyllase gene expression and protein synthesis. Although increase in chlorophyllase activity varied among different citrus species; 18-fold in *Citrus unshiu* (Shimokawa et al., 1978), 3-fold in calamondin (Purvis and Bramore, 1981), 4-fold in *Citrus reticulata* (Amir-Shapira et al., 1987) and 5-fold in *Citrus sinensis* cv. Valencia (Trebitsh et al., 1993), differential induction of chlorophyllase between citrus species could not be compared because of the varied concentrations and durations of ethylene treatment. However, differential ethylene-induced degradation of chlorophyll content in seven cultivars under the same degreening conditions (Kitagawa et al., 1978) suggested that chlorophyllase enzyme synthesis and activity respond to ethylene differentially in different cultivars.

Little is known about the basis of differential ethylene sensitivity between citrus species or cultivars during degreening. Differences in ethylene sensitivity were observed between young fruitlets and mature fruit of 'Valencia' oranges (Katz et al., 2004). Exogenous ethylene (20 $\mu L\,L^{-1}$) application induced expression of *ACS1* (1-amino-cyclopropane-1-carboxylate synthase 1), *ACO1* (1-amino-cyclopropane-1-carboxylate oxidase 1) and *ERS1* (ethylene response sensor 1) genes in detached young fruitlets but not in detached mature fruit, indicating a decrease in ethylene sensitivity during fruit maturation. Differential ethylene sensitivity between cultivars was observed in chrysanthemum (Narumi et al., 2005) and muskmelon (Sato-Nara et al., 1999). We hypothesized that differential expression of ethylene biosynthesis or perception genes is correlated with differences in rate and amount of degreening between citrus species or cultivars. In this study, two citrus types with disparate degreening behavior were selected. 'Fallglo' tangerine degreens rapidly after exposure to ethylene, whereas 'Lee × Orlando' tangerine does not respond or responds poorly. We show differences in peel maturity between the two citrus types and an impaired response in 'Lee × Orlando' with respect to ethylene biosynthesis and perception genes.

2. Materials and methods

2.1. Plant material and seedling triple response

'Lee × Orlando' (USDA 6-15-150; [*C. reticulata* cv. Clementine × (*C. reticulata* cv. Dancy × *C. paradisi* cv. Duncan)] × [*C. reticulata* cv. Dancy × *C. paradisi* cv. Duncan]) fruit were harvested from experimental groves at Water Conserv II (Winter Garden, FL) and 'Fallglo' (Bower citrus hybrid [*C. reticulata* Blanco × (*C. reticulata* Blanco × *C. paradisi*)] × 'Temple' [purported *C. reticulata* Blanco × *C. sinensis*]) fruit were harvested from groves at the Citrus Research and Education Center (Lake Alfred, FL). For citrus seedling triple response assay, seeds were collected from 'Fallglo' and 'Lee × Orlando' fruit, surface sterilized with 20% bleach for 15 min and rinsed with sterile distilled water. Seeds were germinated on Murashige and Skoog medium (Murashige and Skoog, 1962) with 0.8% agar in polycarbonate boxes (Magenta vessel, Sigma–Aldrich, St. Louis, MO). The boxes were kept in chambers at 27 ± 1 °C under dark conditions. Seeds in magenta boxes were germinated in chambers maintained at a concentration of 10 $\mu L\,L^{-1}$ of ethylene. Control seedlings were germinated in ethylene-free air held at the same temperature. Root and shoot lengths were measured on seedlings 18 d after sowing.

Fruit were harvested on three dates (early- [October 3, 2007], mid- [October 24, 2007] and late-season [December 14, 2007], designated Harvest I, II and III, respectively) for 'Lee × Orlando' and at two dates (early- [October 3, 2007] and mid-season [October 24, 2007], designated Harvest I and II, respectively) for 'Fallglo'. Late-season 'Fallglo' fruit abscised and no fruit remained in the tree, consequently, no Harvest III fruit for this cultivar were available for testing. 'Fallglo' harvest dates were selected based on marketable % soluble solids/% citric acid juice ratio and color break (the appearance of orange color on the peel), the optimal being that of Harvest II (Table 1). The date for Harvest I of 'Lee × Orlando' was selected to closely match marketable juice ratio of 'Fallglo'. 'Lee × Orlando' and 'Fallglo' fruit of similar size and shape (224 pieces) were harvested at random canopy locations from 6 and 5 trees, respectively, at each harvest stage and transported to Lake Alfred. Juice ratio and peel color were measured in 24 fruit separated into 6 fruit/replication. The remaining fruit were randomly assigned to 4 replications of 25 fruit each for control and ethylene treatments. Total soluble solids was measured using a Hand Held Refractometer (Fisher Scientific, Pittsburgh, PA) and titratable acidity (% citric acid) was measured by titrating juice samples with NaOH using phenolphthalein as indicator (Boland, 1990). Peel color at equidistant locations along the fruit equator, was measured as a^* and b^* using Chroma Meter CR-400 (Konica Minolta Sensing, Inc., Japan) and expressed as a^*/b^* ratio. Measurements were averaged for each replicate fruit.

2.2. Ethylene treatment

Fruit were treated with 5 ± 0.5 $\mu L\,L^{-1}$ ethylene for 24 h at 27 ± 0.04 °C. After 24 h, ethylene-treated fruit were transferred to ethylene-free storage at 27 ± 0.06 °C for 1 week. Control fruit were

Table 1
Juice quality (% soluble solids/% acid) and peel color (a^*/b^*) for 'Lee × Orlando' and 'Fallglo' fruit at different harvest stages on the indicated dates. The – indicates that sampling was not done. Values within each parameter followed by different letters are significantly different by Duncan's multiple range test at $P \leq 0.001$.

Parameter	Citrus type	First year			Second year		
		Harvest I (10/3/2007)	Harvest II (10/24/2007)	Harvest III (12/14/2007)	Harvest I (9/25/2008	Harvest II (10/28/2008	Harvest III (12/16/2008)
Juice quality (% soluble solids/% acid)	Fallglo	5.80 d	9.79 c	–	6.07 d	9.62 c	–
	Lee × Orlando	9.03 c	12.44 b	15.39 a	9.56 c	12.73 b	14.92 a
Peel color (a^*/b^*)	Fallglo	−0.48 d	−0.32 c	–	−0.39 c	−0.21 b	–
	Lee × Orlando	−0.61 e	−0.58 e	0.02 a	−0.63 e	−0.57 e	0.04 a