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Effect of ethylene degreening on flavonoid pathway gene expression and phytochemicals in Rio Red grapefruit (*Citrus paradisi* Macf)

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ABSTRACT

Degreening early season grapefruits using ethylene helps to accelerate peel color change resulting in uniformly colored, attractive and marketable fruits. In current study, phytochemicals and flavonoid-pathway genes were studied in early season grapefruits degreened using 5 ppm and 10 ppm ethylene and stored for 35 days under market-simulated conditions. Ethylene treatment and concentration had significant influence during initial treatment and storage weeks on expression of genes involved in the flavonoid biosynthesis pathway, namely phenylalanine ammonia lyase, chalcone synthase, chalcone isomerase, and 1,2-rhamnosyltransferase. Ethylene treated fruits showed lower expression of flavonoid-pathway genes during three days of degreening treatment. Lycopene, vitamin C, and 6,7-dihydroxybergamottin were higher in non-degreened and 10 ppm treatment at the end of 35 days of storage. On the other hand, flavonoids and β- carotene were higher in 10 ppm degreened fruits. Significant effect of ethylene concentration was observed in flavonoids and furocoumarins at end of storage. At 7 days of storage after the fruits were removed from the ethylene treatment, flavonoids were significantly lower in degreened fruits. In conclusion, significant effect of ethylene degreening and ethylene concentration was observed on phytochemicals and flavonoid pathway genes in Rio Red grapefruit.

1. Introduction

Plants have developed complex mechanisms involving molecular signaling pathways, hormones, secondary metabolites, and programmed cell death for defense against biotic and abiotic stresses. Plant hormones play important roles in growth, development, and controlling metabolic activities. Abscisic acid, jasmonic acid, salicylic acid, and ethylene are the chief plant hormones involved in major biotic and abiotic stress induced signaling pathways. Ethylene regulates diverse metabolic processes such as ripening, senescence, and flower induction in plants. Ethylene biosynthesis increases in response to pathogen attack, hypoxia, and wounding (Boller and Kende, 1980; Ecker and Davis, 1987; Yang, 2014). In fruit ripening, an increase in ethylene biosynthesis induces key changes in fruit texture, aroma, and color. Ethylene accelerates chlorophyll degradation and accumulation of carotenoids. The use of ethylene to induce ripening and improve peel color is widely practiced in cultivation of various climacteric and non-climacteric fruits. Grapefruits (*Citrus paradisi* Macf) harvested in the early season (Oct – Dec) fetch higher prices compared with later in the season; however these fruits require degreening using ethylene to obtain a uniform, marketable peel color. Furthermore, ethylene can influence the biosynthesis of several secondary metabolites, including carotenoids and flavonoids.

Exogenous ethylene can affect several enzymes involved in plant metabolic pathways. Phenylalanine ammonia lyase (*PAL*), an important enzyme in the shikimate pathway, is the first enzyme in the phenylpropanoid pathway. A previous study reported that *PAL* expression increased in the flavedo in response to ethylene in citrus (Riov et al., 1969). Concurrent increases in ethylene and *PAL* occur under different stress conditions (wounding, radiation, chilling injury) (Hyodo and Nishino, 1981; Lafuente et al., 2001; Riov et al., 1970) and in response to pathogen elicitors (Marcos et al., 2005), confirming that ethylene significantly induces *de novo PAL* synthesis in citrus flavedo. Induction of genes involved in both ethylene and the phenylpropanoid pathway confer resistance in citrus fruits against pathogens (Ballester et al., 2011).

Chalcone synthase (*CHS*) catalyzes first committed step in flavonoid biosynthesis and is reported to be induced by ethylene in grape berries (El-Kereamy et al., 2003). Chalcone isomerase (*CHI*) and 1,2-rhamnosyl transferase (*2RT*) are downstream enzymes in the flavonoid pathway. However, the effect of ethylene on the genes encoding these key flavonoid enzyme in grapefruit remains unclear. Grapefruit is a rich source of flavonoids, mainly flavanones; therefore, the effect of ethylene treatment on the expression of *PAL*, *CHS*, *CHI*, and *2RT* requires further

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investigation, especially in the juice vesicles which are consumed.

In addition to flavonoids, ethylene also influences other phytochemicals in grapefruit, such as carotenoids, vitamin C, and limonoids. The effect of ethylene on carotenoids in citrus peels has been extensively studied (Matsumoto et al., 2009; Rodrigo and Zacarias, 2007; Zhou et al., 2010). The main objective of the current study was to investigate the effect of ethylene on phytochemicals and flavonoid-related genes in grapefruit juice sacs during market-simulated storage conditions, as well as to study the correlation between gene expression profiles and flavanone accumulation. Genes involved in flavonoid biosynthesis such as *PAL*, *CHS*, *CHI*, and *2RT* were studied for the first time in grapefruit. Key phytochemicals, namely vitamin C, carotenoids, limonoids, flavonoids, and furocoumarins were quantified using high performance liquid chromatography and confirmed by HR-MS.

2. Results and discussion

2.1. Peel color measurement

Degreening using ethylene is one of the most common postharvest treatment practiced in citrus industry. In current study, degreening fruits with ethylene for three days had significant effect on peel color. Fruits under both degreening treatments developed reddish yellow, while non-degreened fruits retained their green peel color (Fig. 1). Hue angle measurements showed significant differences between non-degreened and degreened fruits throughout the storage period (Table 1). As the hue angle decreases the peel color appears to be more orange/red. After three days of degreening treatment (D3) sharp decrease in hue angle was observed from ~107° to ~85° in both degreening treatments. Where as in non-degreened fruits hue angle gradually decreased throughout storage and reached ~85° after 21 days (S21) of storage. At end of storage at S35 significant differences were observed only between non-degreened and fruits under 10 ppm ethylene treatment. No significant differences were observed between the peel color of both degreening treatments throughout the degreening and storage period. Several studies have reported changes in citrus peel color after ethylene treatment (Matsumoto et al., 2009; Rodrigo and Zacarias, 2007; Stewart and Wheaton, 1972). Current study confirms that increasing ethylene concentration has no significant effect on improving peel color or reducing degreening time as reported previously (Cohen, 1978).

Table 1
Peel color readings measured in °hue of non-degreened and degreened fruits (5 ppm and 10 ppm ethylene) stored under market simulated conditions for three weeks at 11 °C and then transferred to 21 °C for two weeks.

Days	Non-degreened	Deg 5 ppm	Deg 10 ppm
D0	106.33 ± 0.84 a	105.58 ± 0.84 a	107.88 ± 0.84 a
D3/S0	100.25 ± 0.78 a	84.63 ± 0.78 b	85.27 ± 0.78 b
S7	94.32 ± 0.76 a	81.19 ± 0.76 b	81.90 ± 0.77 b
S14	90.94 ± 0.72 a	80.33 ± 0.72 b	81.06 ± 0.72 b
S21	85.14 ± 0.72 a	77.95 ± 0.72 b	78.51 ± 0.72 b
S28	81.74 ± 0.79 a	76.69 ± 0.78 b	76.79 ± 0.77 b
S35	79.38 ± 0.96 a	76.34 ± 0.96 ab	75.75 ± 0.89 b

*The data represent means ± S.E. of 30 fruits (n = 30, except at S28, n = 27 and at S35 n = 18). Means with different letters denote significant differences ($P < 0.05$) between treatments at each storage period.

2.2. Effect of degreening on expression of genes in flavonoid-pathway and flavonoid content

Degreening treatment has been studied in different citrus crops to investigate their effect on flavonoid content and *PAL* gene expression (Riov et al., 1969). Citrus fruits contain five groups of flavonoids such as flavones, flavanones, flavonols, flavans, and anthocyanins (Tripoli et al., 2007). Of these, flavanones are most abundant in grapefruit and present in both aglycone and glycoside forms (Zhang, 2007). In the current study, narirutin, naringin, neohesperidin, didymin, and poncirin were detected in grapefruit juice vesicles, with naringin having the

Fig. 1. Peel color changes in non-degreened and degreened fruits under 5 ppm and 10 ppm ethylene treatment at beginning (Day 0) and after three days of ethylene treatment. Non-degreened fruits were held under air at same conditions as ethylene degreeneed fruits.