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Analysis of changes in volatile constituents and expression of genes involved in terpenoid metabolism in oleocellosis peel

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ABSTRACT

Oleocellosis is a serious physiological disorder in citrus fruit that mainly results in appearance and quality deterioration. It has been well established that the occurrence of oleocellosis is highly correlated with the release of peel oil from citrus fruit, while there is little information on the dynamic changes in the content of the volatile constituents and the expression of genes involved in terpenoid metabolism during oleocellosis development. In the present research, large changes in the volatile profiles and gene expression in terpenoid metabolism were observed in oleocellosis peels compared to healthy ones. Among volatiles, the decreased contents of α-pinene, D-limonene, β-myrcene, linalool, β-caryophyllene, α-terpineol, nonanal, neryl acetate and (−)-carvone played a major role in these changes. For gene expressions in terpenoid metabolism, the up-regulated genes aldehyde dehydrogenase (NAD+) (*ALDH*) and the down-regulated genes β-caryophyllene synthase 1 (*BCS1*), α-terpineol synthase 2 (*TES2*) and myrcene synthase (*MS*) were the main differences in oleocellosis peels.

1. Introduction

Citrus is one of the most widespread fruit crops in the world, based on fruit production and economical value. Oleocellosis is a physiological disorder in citrus fruit that occurs at harvest time and during postharvest storage. This disorder has been found in various citrus species and results in an unattractive surface blemish on the fruits, and, thus, important losses for growers. Oleocellosis has also been called oil spotting, with typical symptoms including rupture of the oil glands at the very beginning of oleocellosis development (Alferez, Burns, & Zacarias, 2004). With the deepening of the disorder, the adjacent epidermis of the oil glands shows tissue necrosis, the formation of irregularly shaped yellow, green or brown spots, and finally oil glands that prominently stand out from the skin (Ladanyia, 2008; Zheng et al., 2010). Oleocellosis considerably decreases external fruit quality and poses a threat to postharvest storage.

Numerous papers have described the causes of oleocellosis development, such as insect attack, mechanical damage, and climate change during fruit growth, maturation and storage (Cronje, Barry, & Huysamer, 2011; Knight, Klieber, & Sedgley, 2002). Among these causes, mechanical injury that induces the occurrence of oleocellosis may be the reason for the release of oil from the glands during injury (Scherrer Montero, Schwarz, Dos Santos, Dos Santos, & Bender, 2012). In addition, Fawcett (1916) and Knight, Klieber, and Sedgley (2001) have revealed a close relationship between oleocellosis in citrus and the release of citrus peel oil. Moreover, it has been proved that D-limonene, the major component of citrus peel oil and orange oil could induce oleocellosis (Knight et al., 2002; Liu, Ming, Zeng, & Liao, 2012). Therefore, it appears to be important to investigate the effects associated with the release of peel oil, which would be meaningful to unravel the mechanism underlying oleocellosis.

The peel essential oils consist mainly of monoterpenes (Zhang et al., 2017). They are mixtures of monoterpenes (including α-thujene, α-pinene, sabinene, β-myrcene and D-limonene), sesquiterpenes (including *cis*-β-ocimene, elixene, β-caryophyllene and β-farnesene), aldehydes (including octanal, citronellal, neral and geranial), alcohols (including terpineol, linalool, citronellol and geraniol), acetates (including citronellyl acetate, neryl acetate and geranyl acetate), monoterpene oxides (including caryophyllene oxide, (*Z*)-limonene oxide and (*E*)-limonene oxide) and ketones (including nootkatone, pulegone and (−)-carvone) (Luis Rambla et al., 2014; Yi, Dong, Liu, Yi, & Zhang, 2015). Terpenoid hydrocarbons have been reported to be the major chemical components of the volatile constituents of citrus peel oil, and accounted for 88.2% and 76.3% of total volatile material in orange and mandarin fruits, respectively (Espina et al., 2011).

A series of genes in citrus involved in terpenoid synthesis pathway have been identified in previous research. *CsTPS1*, which is expressed specifically during fruit maturation, was confirmed to be a key gene in

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the generation of the sesquiterpene aroma compound valencene (Sharon-Asa et al., 2003). *CitMTSE1*, *CitMTS3*, *CitMTS61* and *CitMTS61* were isolated from *Citrus unshiu* Marc and were shown to encode D-limonene synthase, γ-terpinene synthase, γ-terpinene synthase and β-pinene synthase, respectively (Shimada et al., 2004). One gene coding for sabinene synthase in rough lemon is *RlemTPS2*, which is responsible for sabinene emission from rough lemon (Kohzaki et al., 2009). Additionally, it has been suggested that the volatile metabolism pathways in citrus peel are affected by Huanglongbing disease and puffing disorder (Ibanez et al., 2014; Martinelli et al., 2012). Terpenoid metabolism often differs dramatically in citrus species, through the growing seasons and during disease or disorder (Ren et al., 2015; Zhang et al., 2017). The diversity of terpenoid metabolism stems mainly from the specific composition and expression of genes related to terpenoid metabolism (Sharon-Asa et al., 2003). However, there is no information about the expression of genes related to terpenoid metabolism in citrus peel, in response to oleocellosis disorder.

The major aim of the present study was to investigate the changes in the concentration of volatile constituents and the expression profiles of the related genes in the oleocellosis citrus peel, with a final goal of deciphering the molecular mechanism underlying oleocellosis. Therefore, gas chromatography–mass spectrometry (GC–MS) was employed to investigate the changes in the volatile constituents, and RNA-Seq technology was applied to probe the expression of genes related to terpenoid metabolism in citrus fruit during oleocellosis development. Comparisons of the volatile content and gene expression were performed between healthy and oleocellosis citrus fruits from three varieties, including Jincheng orange, Navel orange and Ponkan mandarin.

2. Materials and methods

2.1. Plant materials

Mature fruits of the 'Beibei 447' Jincheng orange (*C. sinensis* Osbeck), Navel orange (*C. sinensis* Osbeck) and Ponkan mandarin (*C. reticulata* Blanco), were collected at 220 days after flowering in an orchard located in the Beibei District of Chongqing, China, in 2015.

After washing with tap water and air drying at room temperature, the healthy and oleocellosis-affected peels of citrus fruits were separated by cutting around the equator of the fruit without touching the inner part (edible segment) (Fig. 1). The peels (only flavedo) were ground immediately in liquid nitrogen and stored at −80 °C until extraction.

2.2. Standards and reagents

Internal standards of ethyl decanoate and a standard series of C_7-C_{30} saturated alkanes were purchased from Sigma (St. Louis, MO). Methyl *tert*-butyl ether (MTBE) (HPLC grade) from TCI Development Co., Ltd (Shanghai, China) was used for the extraction of volatiles. Anhydrous sodium sulfate and dichloromethane were purchased from Kelong Co., Ltd (Sichuan, China). The sources of the volatile standards, the linear regression range, equations for the standard curves, regression coefficient (r^2), and correction factor (CF) for each standard compound are listed in Table 1, and the characteristic fragment (CF), retention index (RI) and identification approach for each identified compound are listed in Supplementary Table S1.

2.3. Volatile extraction and GC–MS analysis

The extraction of volatiles from 1.00 g of citrus peel was performed using 5 mL MTBE with 42.50 μg of ethyl decanoate added as an internal standard. The extractions from the three biological replicates were carried out in an ultrasonic cleaner model KQ5200DE from Kunshan Ultrasonic Instruments Co. Ltd (Kunshan, China) for 1 h, and the organic layer was collected, dried over Na_2SO_4, and concentrated to a

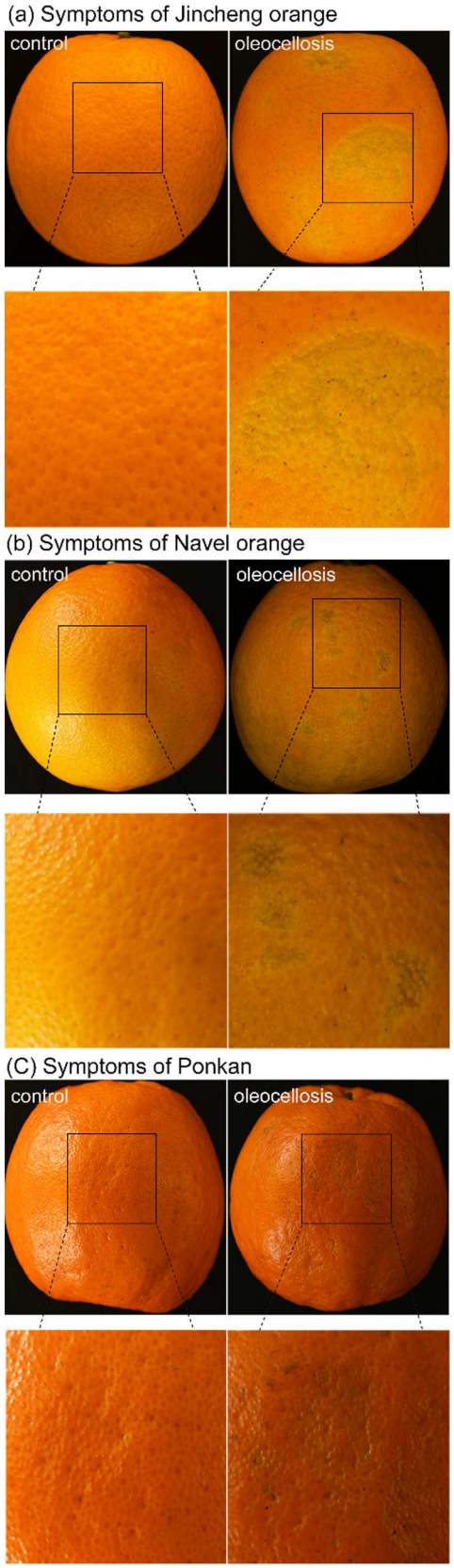

Fig. 1. Oleocellosis in citrus fruit. Controls are healthy fruit compared alongside citrus fruits with oleocellosis. Citrus fruit were photographed immediately after harvest.