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Activities of enzymes directly related with sucrose and citric acid metabolism in citrus fruit in response to soil plastic film mulch

Ni Jiang [a], Long-Fei Jin [a], Jaime A. Teixeira da Silva [b], MD Zahidul Islam [a], Hai-Wen Gao [a], Yong-Zhong Liu [a,*], Shu-Ang Peng [a]

[a] Key Laboratory of Horticultural Plant Biology (Huazhong Agricultural University), Ministry of Education, Wuhan 430070, People's Republic of China
[b] P.O. Box 7, Miki-cho Post Office, Ikenobe 3011-2, Kagawa-ken 761-0799, Japan

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ABSTRACT

Soil plastic film mulch is commonly employed in citrus production regions of East Asia to improve fruit quality. In the present study, Ponkan tangerine (*Citrus reticulata* Blanco) was mulched under the tree canopy with silver-black reflective film during fruit development. At about 12 days after mulching, total soluble sugar and citric acid contents in the segment membrane and/or juice sacs of fruit from mulched trees increased significantly relative to the control. In the segment membrane, the activities of acid invertase (AI) and sucrose synthase (SS; cleavage direction) increased significantly following mulch treatment. However, the activities of other enzymes, including neutral invertase, SS (synthetic direction) and sucrose phosphate synthase did not respond significantly under mulch treatment. In the juice sacs, SS activity (cleavage direction) from mulched treatments was significantly lower than that from control trees while SS activity (synthetic direction) from mulched trees was significantly higher than that from control trees. Moreover, the activities of cytoplasm aconitase (cyt-Aco) and isocitrate dehydrogenase (cyt-IDH) were significantly lower than those in the control fruits after 36 days of mulching. In conclusion, the activities of SS (synthetic direction) and AI were significantly enhanced while those of cyt-Aco and cyt-IDH were significantly reduced by soil plastic film mulch. A schematic model is present indicating the possible important roles that these key enzymes play in sugar and acid accumulation in citrus fruits under soil plastic film mulch.

1. Introduction

Citrus is one of the most important fruit crops in the world with an annual production exceeding 122.5 million tons in 2010 (FAOSTAT 2012). The period of fruit ripening is often in the rainy season in East Asian citrus production regions. Thus, soil plastic film mulch (SPFM) is commonly employed to control soil humidity by preventing rainwater from entering into the soil, and is a proven effective soil management practice for the improvement of fruit quality (Shi et al., 2011; Yakushiji et al., 1996).

SPFM is a soil management practice widely used in the production of vegetable and field crops (Kasirajan and Ngouajio, 2012; Lament, 1993), as well as fruit crops (Dusek et al., 2010; Glenn and Puterka, 2007; Layne et al., 2001; Yakushiji et al., 1996). The widespread application and importance of film mulch in agriculture is because it can improve the microclimate around a plant by regulating moisture, temperature, light and energy exchange (Heißner et al., 2005; Tarara, 2000). The use of film mulch has been shown to increase fruit soluble solids, total phenolics, flavanols, and anthocyanins in many fruit crops, including grape (*Vitis vinifera* L.) (Liu et al., 2008), strawberry (*Fragaria* × *aranassa* Duch.) (Loughrin and Kasperbauer, 2002; Wang and Millner, 2009), peach [*Prunus persica* (L.) Batsch] (Layne et al., 2001), apple (*Malus domestica* Borkh.) (Glenn and Puterka, 2007; Iglesias and Alegre, 2009) and pineapple (*Ananas comosus* L.) (Dusek et al., 2010). Fruit aroma was also increased by film mulch in strawberry (Loughrin and Kasperbauer, 2002) and pineapple (Liu et al., 2011). Citric acid content can also be increased in strawberry by film mulch with black plastic mulch (Wang and Millner, 2009) but when silver-black reflecting film was used, the titratable acid content decreased in loquat [*Eriobotrya japonica* (Thunb.) Lindl.] (Chen et al., 2010). In a citrus, Satsuma mandarin (*Citrus unshiu* Marc.), Yakushiji et al. (1996) found that mulch with micro-perforated vinyl sheets decreased soil water

Abbreviations: Aco, aconitase; AI, acid invertase; CS, citrate synthase; cyt-Aco, cytoplasm aconitase; cyt-IDH, cytoplasm isocitrate dehydrogenase; IDH, isocitrate dehydrogenase; mit-Aco, mitochondrial aconitase; NI, neutral invertase; PEPC, phosphoenolpyruvate carboxylase; SPS, sucrose phosphate synthase; SPFM, soil plastic film mulch; SS, sucrose synthase; SS-CD, sucrose synthase-cleavage direction; SS-SD, sucrose synthase-synthetic direction; TSS, total soluble sugar.

* Corresponding author. Tel.: +86 27 87281897; fax: +86 27 87282010.
E-mail addresses: liuyongzhong@mail.hzau.edu.cn, liuyz05@126.com (Y.-Z. Liu).

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potential, but increased sugar content and fruit acidity. Shi et al. (2011) also found that vapor-permeable reflective film mulch and silver-black reflecting film mulch increased soluble solids significantly, but had no significant effect on fruit acidity of Ponkan tangerine (*Citrus reticulata* Blanco).

Although film mulch has a positive effect on fruit quality, only few studies have investigated the possible reason for this impact. For example, Liu et al. (2008) suggested that an increase in acid invertase (AI; EC 3.2.1.26) activity plays an important role in the increase in sugar accumulation in grape fruit under blue plastic film mulch. Improvement of the microclimate, including an increase in light intensity and canopy air temperature as well as a reduction in canopy relative humidity was attributed to the improvement of apple (Layne et al., 2001) and peach (Glenn and Puterka, 2007) skin coloration. The contents of soluble sugar, titratable acid and their ratio are important for citrus fruit flavor quality (Zhou et al., 1985). Even though citrus fruit sugar and acid contents can also be affected by film mulch (Shi et al., 2011; Yakushiji et al., 1996), knowledge of the underlying mechanism(s) remains scant.

It is well known that citrus fruit sugar is transported in the form of sucrose from source leaves and that pulp acidity is synthesized in fruit cell mitochondria through the incorporation of acetyl-CoA with oxaloacetate, a reaction catalyzed by citrate synthase (CS; EC 2.3.3.1), and then translocated to and stored in the vacuole (Sinclair, 1984). Sucrose partition into fruit is mainly determined by sink strength, which is the competitive ability of an organ to attract assimilates (Marcelis, 1996) and is mainly related to the ability of sucrose-metabolizing enzymes, such as sucrose synthase (SS; EC 2.4.1.13) and invertase (EC 3.2.1.26), to hydrolyze sucrose (Koch, 2004). On the other hand, fruit pulp acidity is directly related with citric acid content (Sinclair, 1984), which is determined by the balance in activity of phosphoenolpyruvate carboxylase (PEPC; EC 4.1.1.31), CS, aconitase (Aco; EC 4.2.1.3) and isocitrate dehydrogenase (IDH; EC 1.1.1.41). Aco activity plays an important role in determining the accumulation of citric acid in the vacuole (Cercós et al., 2006; Sadka et al., 2000).

In this paper, we investigated the activities of all the enzymes directly related with sucrose and citric acid metabolism in citrus fruit juice sacs and/or segment membrane under SPFM. These enzymes are sucrose phosphate synthase (SPS; EC 2.4. 1.14), SS, AI, neutral invertase (NI), PEPC, CS, mitochondrial Aco (mit-Aco), cytoplasmic Aco (cyt-Aco) and cytoplasmic IDH (cyt-IDH). The objective of this study was to identify enzymatic factors involved in increasing soluble carbohydrate and acid accumulation in citrus fruit in response to SPFM during fruit development and ripening.

2. Materials and methods

2.1. Plant materials and treatment

Experiments were performed on 6-year-old Ponkan (*C. reticulata* cv. 'Egan 1′) trees grafted on *Poncirus trifoliata* at the citrus orchard of Huazhong Agricultural University in 2011. Citrus fruit development was divided into three stages: cell division, rapid growth period and maturation (Bain, 1958). The beginning of the rapid growth period of Ponkan is about early August in the research area. During the rapid fruit growth period (August 18, 2011), six healthy, approximately uniform and fruitful trees were selected and irrigated well with about 50 L of water per tree. Then, a plot containing three trees (i.e., three replicates) was fully overlaid with silver-black reflective film (Qingdao Aolong plastic products Co., Ltd. China) while another plot, also containing three other trees not covered with film, served as the control. Two guard rows of citrus trees separated the two plots. The silver-black reflective film covered the soil under the tree crown tightly to protect rainfall from permeating into the mulched soil. After film mulching, irrigation was paused for mulched trees while control trees were irrigated normally, i.e., once (about 50 L of water per tree) a week if no rain fell. A total of 25–30 mature leaves per tree were collected randomly from spring shoots every 24 days while 6–10 fruits were randomly collected from the outer crown of each tree every 12 days. The segment membrane and juice sacs of fruits from each tree were separated and the same tissue was then pooled. Some juice sacs were used fresh to determine total soluble solid content. The remaining samples were frozen in liquid nitrogen, then ground into granules and stored at −80 °C to determine sugar and acid content and to analyze related enzyme activity.

2.2. Measurement of water status

Soil water potential was measured by a soil tension meter (TEN-30, Zhejiang Top Instrument Co., Ltd., Hangzhou, China). The soil tension meter, one per tree, was buried 30 cm under the soil surface 10 cm from the tree canopy drip line. Data was collected between 9:00 and 10:00 a.m. Leaf water potential was measured by a plant pressure chamber (ARIMAD-3000, Israel) according to the manufacturer's instruction manual. Six mature leaves were randomly collected from the spring shoots of the outer canopy of each tree. Leaves sampled from the six trees were located at almost the same height and orientation. They were collected at predawn (about 6:00 a.m.) to minimize the effect of transpiration on the water status of plants.

2.3. Measurement of net photosynthetic rate

The net photosynthetic rate of leaves was measured with a portable photometer (Li-6400, USA). Measurements were made on healthy mature leaves from the middle of spring shoots of each plant (10 random leaves per tree) 48 days after mulch treatment. All measurements were carried out between 09:00 and 11:00 a.m. under an air CO_2 concentration of 385 ± 10 μmol mol^{-1}.

2.4. Determination of leaf proline

Proline was determined every 24 days according to a spectrophotometric method (Li, 2000). Approximately, 0.5 g of leaf granules was homogenized in 10 mL of 3% aqueous sulfosalicylic acid. The homogenate was filtered through Whatman #2 filter paper into a clean 15-mL centrifuge tube. 2 mL of filtrate was reacted with 2 mL of glacial acetic acid and 2 mL of acid ninhydrin in a 15-mL centrifuge tube for 30 min at 100 °C. After the reaction was terminated in an ice bath, the filtrate was extracted with 4 mL of toluene and mixed vigorously for about 30 s and centrifuged at $3000 \times g$ for 5 min. The supernatant was used to detect proline at 520 nm with a UV-1600 Shimadzu spectrophotometer (Japan).

2.5. Determination of total soluble solids, starch, soluble sugar, citric acid and malic acid contents

Fruit total soluble solids content (expressed as a percentage) was determined using a common laboratory refractometer (Chengdu Tech. Co., Ltd., China). Leaf total soluble sugar (TSS) and starch were determined using the phenol-sulfuric acid method and acid-hydrolytic method (Li, 2000), respectively. Soluble sugars (glucose, fructose and sucrose) and citric acid were determined by gas-liquid chromatography (Bartolozzi et al., 1997).

2.6. Enzyme activity assay

All procedures related to enzyme extraction were carried out at 4 °C or lower. The extraction of sucrose-metabolizing enzymes and activity assays were determined by the method of Lowell