



# Exogenous $\gamma$ -aminobutyric acid treatment affects citrate and amino acid accumulation to improve fruit quality and storage performance of postharvest citrus fruit



Ling Sheng, Dandan Shen, Yi Luo, Xiaohua Sun, Jinqiu Wang, Tao Luo, Yunliu Zeng, Juan Xu, Xiuxin Deng, Yunjiang Cheng\*

Key Laboratory of Horticultural Plant Biology (Ministry of Education), Key Laboratory of Horticultural Crop Biology and Genetic Improvement (Central Region), MOA, PR China, College of Horticulture and Forestry Science, Huazhong Agricultural University, Wuhan 430070, People's Republic of China

## ARTICLE INFO

### Article history:

Received 6 June 2016

Received in revised form 8 August 2016

Accepted 8 August 2016

Available online 9 August 2016

### Keywords:

Citrate

Storage quality

GABA treatment

Glutamate

GAD

Citrus fruits

## ABSTRACT

The loss of organic acids during postharvest storage is one of the major factors that reduces the fruit quality and economic value of citrus. Citrate is the most important organic acid in citrus fruits. Molecular evidence has proved that  $\gamma$ -aminobutyric acid (GABA) shunt plays a key role in citrate metabolism. Here, we investigated the effects of exogenous GABA treatment on citrate metabolism and storage quality of postharvest citrus fruit. The content of citrate was significantly increased, which was primarily attributed to the inhibition of the expression of glutamate decarboxylase (GAD). Amino acids, including glutamate, alanine, serine, aspartate and proline, were also increased. Moreover, GABA treatment decreased the fruit rot rate. The activities of antioxidant enzymes and the content of energy source ATP were affected by the treatment. Our results indicate that GABA treatment is a very effective approach for postharvest quality maintenance and improvement of storage performance in citrus production.

© 2016 Elsevier Ltd. All rights reserved.

## 1. Introduction

Postharvest citrus fruits are subjected to a series of biotic and abiotic stresses, which cause physiological and biochemical changes and eventually lead to fruit quality deterioration, nutrient loss, water loss and decay. Consumers of fresh fruits are chiefly concerned about the flavor and the nutrients beneficial to human health. Thus, quality maintenance has always been an important issue in fruit postharvest production. As a result, postharvest treatments aimed to maintain fruit quality have been widely investigated (Ma et al., 2014; Matsumoto & Ikoma, 2012). Although some effects can be achieved through these treatments, it is difficult and costly to meet the requirements of a complete cold chain system in the process of postharvest storage, transportation and sales; moreover, the residue of chemical preservative agent after treatment will pose a threat to human health and environmental security. Therefore, more and more research is focused on the application of GRAS (Generally Recognized as Safe) compounds in fruit postharvest production (Lu, Joerger, & Wu, 2014; Palou, Pérez-Gago, & Valencia-Chamorro, 2014). As one of the GRAS

compounds,  $\gamma$ -aminobutyric acid (GABA) is widely used in postharvest treatments of horticultural products to alleviate fruit chilling injury, oxidative damage and induce resistance against pathogens (Shang, Cao, Yang, Cai, & Zheng, 2011; Song, Xu, Wang, Wang, & Tao, 2010; Yu et al., 2014).

GABA is mainly metabolized via a short pathway called GABA shunt because it bypasses two steps of the TCA cycle (Bouche & Fromm, 2004). In recent years, molecular biology studies have suggested that GABA participates in citrate degradation, and GABA shunt is closely related to the central carbon and nitrogen metabolism (Cercós et al., 2006; Michaeli et al., 2011). GABA is firstly biosynthesized through the consumption of proton and glutamate, which is irreversibly catalyzed by glutamate decarboxylase (GAD) (Bown & Shelp, 1997). The generated GABA in cytosol is then transported into the mitochondria by a mitochondrial GABA permease (GABP) and is subsequently metabolized to produce succinate which flows back to the TCA cycle (Fait, Fromm, Walter, Galili, & Fernie, 2008). GAD is the key enzyme in this metabolism pathway (Bouche & Fromm, 2004; Fait et al., 2008). In citrus fruit, transcript analyses of two GAD genes have revealed a close relationship between GAD and citrate utilization (Liu et al., 2014). More recently, the study of GABP in Arabidopsis also indicated the connection between GABA shunt and TCA cycle and the importance of GABA shunt for normal

\* Corresponding author.

E-mail address: [yjcheng@mail.hzau.edu.cn](mailto:yjcheng@mail.hzau.edu.cn) (Y. Cheng).

carbon metabolism (Michaeli et al., 2011). However, the effects of exogenous GABA treatment on citrate content and fruit storage quality are still unknown.

In citrus fruit, organic acids, sugars and amino acids are the most important compounds for fruit inner quality, especially organic acids (Sun et al., 2013). The changes of organic acids can not only influence the fruit flavor, but also affect the fruit senescence process and storage performance. Generally, a high concentration of organic acids or a low pH value is an indication of delayed fruit senescence (Angioni & Schirra, 2011). Citrate is the main organic acid in citrus fruit, which accounts for about 70–90% of the total organic acids (Sinclair, 1984). Amino acids are major nitrogenous compounds in citrus fruit and are important for human health, and the contents of amino acids can affect several aspects of fruit quality, such as the taste and aroma. Therefore, the changes of these components directly affect the fruit flavor and nutrition, and it is highly necessary to explore the effects of postharvest treatments on the accumulation of these metabolites. However, there have been no reports about the effects of postharvest treatments on citrate accumulation in citrus.

In the present study, we performed exogenous GABA treatment on the postharvest fruits of two citrus cultivars: Olinda Valencia orange and 'Newhall' navel orange (*C. sinensis* Osbeck), aiming to investigate the changes of citrate during postharvest storage, and to assess the fruit quality after treatment. The expression of GAD and GABP was also detected, which partly elucidated the mechanism by which GABA treatment affects the accumulation of citrate. Our results are expected to provide an effective approach to reduce postharvest quality loss and improve the storage performance of citrus fruit.

## 2. Materials and methods

### 2.1. Plant materials and treatments

Commercially mature fruits of Olinda Valencia orange and 'Newhall' navel orange (*C. sinensis* Osbeck) were harvested from a commercial orchard in Yichang, Hubei Province, PR China, in May 2013 and December 2014 respectively, and then were immediately transported to our laboratory. Only the fruits of uniform size and color and free from any visible damage or defects were chosen as the samples for the study. For Valencia fruit, about 300 kg of fruits were evenly divided into three groups, which were respectively dipped in distilled water (as control), 0.5 mM and 10 mM GABA aqueous solution for 2 min. For Newhall fruit, about 200 kg of fruits were evenly divided into two groups, which were respectively dipped in distilled water (as control) and 0.5 mM GABA aqueous solution for 2 min. After being air dried, the fruits were uni-packed and stored at room temperature with a relative humidity of 85–90% for about three months.

Juice sacs were separated from 5 fruits in each group at 0, 10, 20, 50, 80 days after storage (DAS) with three replicates, to measure the levels of organic acids and amino acids, the activities of antioxidant enzymes and the expression of related genes. Samples were immediately frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ . For each treatment, there were three biological replications, with each consisting of 5 citrus fruits.

### 2.2. Primary metabolite analysis by GC–MS

Primary metabolites were detected with the approach of GC–MS analysis as described by Sun et al. (2013) with minor modifications. Exactly 0.2 g of mixed sample were ground in liquid nitrogen, and then extracted with 2.7 mL of chromatographic grade methanol, followed by the addition of 300  $\mu\text{L}$  of internal standard

(0.2  $\text{mg mL}^{-1}$  ribitol). After drastic shaking and ultrasonic treatment for 30 min, the mixture was put into a thermostatic water bath at  $70^{\circ}\text{C}$  for 15 min and centrifuged at 5000g for 15 min. Then, 100  $\mu\text{L}$  supernatant were obtained and vacuum-concentrated.

Prior to GC–MS analysis, metabolites were derivatized with the approach of Zhao et al. (2014) with minor modifications. Firstly, samples were dissolved in 80  $\mu\text{L}$  methoxamine hydrochloride (20  $\text{mg mL}^{-1}$  in pyridine), incubated for 90 min at  $37^{\circ}\text{C}$ , and then reacted with 80  $\mu\text{L}$  of MSTFA (*N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide) for 30 min at  $37^{\circ}\text{C}$ . After microporous filtering, the samples were analyzed by GC–MS.

Exactly 1  $\mu\text{L}$  of each sample was injected into the gas chromatograph onto a fused-silica capillary column (30 m  $\times$  0.25 mm i.d., 0.25  $\mu\text{m}$  DB-5MS stationary phase). The MS operating parameters were as follows: 70 eV ionization voltage,  $200^{\circ}\text{C}$  ion source temperature and  $250^{\circ}\text{C}$  interface temperature. Total ion current spectra were recorded over a mass range of  $m/z$  45–600 in scan mode.

For individual metabolites, we quantified the final concentrations ( $\mu\text{g g}^{-1}$  FW) using a ribitol internal standard. The final data were used for statistical analyses.

### 2.3. Enzyme activity assays

For POD activity analysis, 0.5 g of flesh tissue were ground in triplicate with 4.5 mL of 0.1 M sodium phosphate buffer (pH 7.2). The extracts were then homogenized and centrifuged at 3500 rpm for 10 min at  $4^{\circ}\text{C}$ . The supernatants were then used for the enzyme assays with a peroxidase (POD) assay kit (Nanjing Jiancheng Bioengineering, Nanjing, China).

For superoxide dismutase (SOD) activity analysis, 0.5 g of flesh tissue were ground with 5 mL of 50 mM sodium phosphate buffer (pH 7.8). Flesh tissue (0.5 g) was ground with 5 mL of 50 mM sodium phosphate buffer (pH 7.0) for CAT. The extracts were then homogenized and centrifuged at 12,000g for 20 min at  $4^{\circ}\text{C}$ . The supernatants were used for the enzyme assays. SOD activity was determined using a T-SOD assay kit (Nanjing Jiancheng Bioengineering, Nanjing, China). Catalase (CAT) activity was determined by spectrophotometrically monitoring the degradation of absorbance per minute at 240 nm, and was given as  $\text{U min}^{-1} \text{g}^{-1}$  fresh weight.

### 2.4. Measurement of ATP contents

An ATP assay kit (Nanjing Jiancheng Bioengineering, Nanjing, China) was used to analyze the ATP content. Exactly 0.5 g of the sample were ground into powder in the presence of liquid nitrogen, and then 5 mL of boiled water were added. The samples were put into a boiling water bath for 10 min, mixed with vortex for 1 min, and centrifuged for 10 min at 1500g. The supernatants were used to detect ATP contents.

### 2.5. RNA isolation and real-time quantitative PCR

Total RNA of each sample was independently isolated according to the method described by Liu, Liu, Tao, and Deng (2006), and was used for real-time quantitative PCR. The related genes were selected and validated by real-time quantitative PCR (qRT-PCR). The RNA samples were reverse transcribed using a ReverAid<sup>TM</sup> M-MuLV reverse transcriptase Kit (MBI, Lithuania). The gene encoding actin was used as the endogenous control, as its expression was not influenced by exogenous treatment. The primers for actin were: forward 5'-CCAAGCAGCATGAAGATCAA-3' and reverse 5'-ATCTGCTGGAAGGTGCTGAG-3'. The detailed operating procedure was depicted by Sun et al. (2013). The specific primer pairs of the selected genes were designed using Primer Express 3.0 (Applied Biosystems, Foster City, CA), and are listed in Supplementary Table 1.

Download English Version:

<https://daneshyari.com/en/article/1183059>

Download Persian Version:

<https://daneshyari.com/article/1183059>

[Daneshyari.com](https://daneshyari.com)