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Application of propyl gallate alleviates pericarp browning in harvested longan fruit by modulating metabolisms of respiration and energy

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ABSTRACT

Effects of propyl gallate on metabolisms of respiration and energy of harvested 'Fuyan' longans and its relationship to pericarp browning were investigated. Compared to control longans, propyl gallate could reduce ascorbic acid oxidase (AAO) activity, lower cytochrome *C* oxidase (CCO) activity during early-storage and mid-storage, increase NADK activity, elevate contents of NADP and NADPH, decrease contents of NAD and NADH, in addition, lower the decreases of ATP content and energy charge (E.C.), increase activities of mitochondrial H^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase during early-storage and mid-storage. Above results suggested that propyl gallate-retarded browning development in pericarp of harvested longans was resulted from decreases in activities of respiratory terminal oxidases like CCO and AAO, increase in proportion of pentose phosphate pathway (PPP) to Embden-Meyerhof pathway (EMP) and tricarboxylic acid (TCA) cycle, and maintenance of mitochondrial integrity via retaining higher levels of ATP content and energy charge, as well as higher activities of mitochondrial ATPase.

1. Introduction

Longan (*Dimocarpus longan* Lour.) is a characteristic fruit with high nutritional and medicinal value in southern China, also in many other countries in the world (Chen et al., 2014; Jiang et al., 2007; Lin, Chen, Chen, & Hong, 2001; Lin, Hu, et al., 2013; Lin et al., 2014; Lin, Lin, Lin, et al., 2016). However, it is vulnerable to pericarp browning, the main reason for restriction of transportation and loss of market value (Duan et al., 2007; Lin et al., 2001, 2014; Lin, Lin, Lin, et al., 2016; Lin, Lin, Lin, Ritenour et al., 2017; Su et al., 2005). Recently, there are mounting evidences showed that the disorder of energy metabolism might account for browning, chilling injury or other symptoms of senescence (Jin et al., 2015; Li, Yin, Song, & Zheng, 2016; Liu et al., 2007; Pan, Yuan, Zhang, & Zhang, 2017; Yang et al., 2009). Moreover, most energy (95%) is generated in the process of oxidative phosphorylation, the third stage of respiration metabolism conducted in the inner mitochondrial membrane (Qin, Wang, Liu, Li, & Tian, 2009). After several steps in the electron transport chain, oxygen is eventually reduced to hydrogen oxide, with the release of energy (Jin et al., 2013; Wang, Wang, Liang, & Zhao, 2008; Yang et al., 2009). The enzyme complexes like cytochrome *C* oxidase (CCO) on the inner mitochondrial membrane could use the released energy to generate ATP and to pump proton against electrochemical proton gradient into inter-membrane space of mitochondria (Jiang et al., 2007; Zhou et al., 2014). Although this process is efficient, there is still a small amount of electron may reduce oxygen prematurely to form superoxide reactive oxygen species (ROS) (Larsen, Schiffer, Weitzberg, & Lundberg, 2012). These substances can cause oxidative stress and mitochondrial recession, which, in turn, will block respiratory metabolism and energy production (Jiang et al., 2007; Li et al., 2016; Lin, Lin, Lin, Ritenour, et al., 2017).

Previous reports revealed that hydrogen peroxide (H_2O_2), the most consistent ROS, increased activities of CCO and ascorbic acid oxidase (AAO) with altered respiration metabolism pathway (Lin, Lin, Chen, et al., 2016). It also reduced ATPase activity and energy charge, and consequently aggravated longan pericarp browning (Lin, Lin, Lin, Ritenour, et al., 2017). In addition, propyl gallate was reported to decrease respiration rate, reduce generation of ROS, preserve unsaturated fatty acids of cell membrane lipid, and thus delay longan pericarp browning (Lin, Hu, et al., 2013; Lin, Lin, Chen, Chen, & Lin, 2013; Lin et al., 2015; Lin, Lin, Lin, Shi, et al., 2017). However, application of propyl gallate for alleviating pericarp browning of harvested longan fruit in association with the metabolisms of respiration and energy remains to be clarified. Therefore, effects of propyl gallate on activities of NAD kinase (NADK), CCO, AAO and adenosine triphosphatase (ATPase), contents of nicotinamide adenine dinucleotide phosphate (NADP), reduced nicotinamide adenine dinucleotide phosphate

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(NADPH), nicotinamide adenine dinucleotide (NAD), reduced nicotinamide adenine dinucleotide (NADH), adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP), and energy charge were conducted in this work.

2. Materials and methods

2.1. Plant material and treatments

Mature longan (*Dimocarpus longan* Lour. cv. Fuyan) fruit were harvested from an orchard in Anxi County of Fujian province, China, and transported under ambient conditions within 3 h to the laboratory of Institute of Postharvest Technology of Agricultural Products, Fujian Agriculture and Forestry University in Fuzhou, Fujian province, China. Fruits were selected on the basis of the uniformity of maturity, color, shape and size. Any blemished or diseased fruits were excluded.

The selected fruits were separated into two groups. The fruits of group one were dipped in distilled water for 20 min and used as control. Another group of fruits were dipped with 0.5 mM propyl gallate solution for 20 min. The selection of suitable concentration of propyl gallate (0.5 mM) in this experiment was based on our previous published works (Lin, Hu, et al., 2013; Lin et al., 2015), which reported that 0.5 mM propyl gallate-treated longan fruit exhibited a significant ($P < 0.01$) lower pericarp browning index as compared to the control fruit (Appendix 1). The treated fruits were then dried under ambient condition for about one hour, packed in 0.015 mm thick polyethylene bags (50 fruits per bag), stored at (15 ± 1) °C and 80% humidity. Samples were taken initially and at 2-days interval during storage for determining the following physiological and biochemical indices in the process of browning development in pericarp of harvested longan fruit.

2.2. Measurement of activities of cytochrome C oxidase (CCO), ascorbic acid oxidase (AAO), NAD kinase (NADK) and mitochondrial ATPase activity

The methods of Pignocchi, Fletcher, Wilkinson, Barnes, and Foyer (2003), Qin et al. (2009), Jin et al. (2013), Lin, Lin, Chen, et al. (2016) and Zhang et al. (2017) were applied to extract and determine the activities of CCO, AAO and NADK. One unit of CCO, AAO and NADK activity was defined as the amount of enzyme that oxidized 1 μg of cytochrome *C*, ascorbic acid or catalyzed 1 μmol of NADP production in 1 min, respectively.

The method described in our previous study (Lin, Lin, Lin, Ritenour, et al., 2017) was applied to determinate the activity of H^+-ATPase, Mg^{2+}-ATPase and Ca^{2+}-ATPase in mitochondria. One unit of ATPase activity was defined as the amount of enzyme that catalyzed 1 μmol of inorganic phosphate (Pi) production by ATP decomposition per hour

The method of Bradford (1976) was used to determine protein content. The unit of $U \cdot mg^{-1}$ protein was used to express the activities of CCO, AAO, NADK and ATPase.

2.3. Assay of contents of NADP(H) and NAD(H)

The methods of Chen et al. (2015) and Zhang et al. (2017) were applied to determine the contents of NADP(H) and NAD(H). 1 g longan pericarp tissue from 10 fruit was homogenized with mortar and pestle at 4 °C in 5 mL of 0.1 M HCl for NAD or NADP determination or in 5 mL of 0.1 M NaOH for NADH or NADPH determination. The homogenates were then heated within boiling water bath for 5 min, cooled in an ice bath, and centrifuged for 10 min at 4 °C and 10,000 × *g*. Supernatants were neutralized with respectively 0.1 M NaOH or HCl and centrifuged for 10 min at 4 °C and 10,000 × *g*. Final supernatants were kept on ice for the coenzyme assays.

Because of the light sensitivity of phenazine ethosulfate (PES) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), manipulations were carried out in low light. Equal volumes of 1 M Tricine–NaOH buffer, 40 mM ethylene diamine tetraacetic acid (EDTA), 4.2 mM MTT, 16.6 mM PES, and 25 mM glucose-6-phosphate (for determination of NADP and NADPH) or 5 M ethanol (for determination of NAD and NADH) were mixed just before the assays, and 300 μL of this mixture was transferred to 1.5-mL microtubes. 50 μL supernatants were added to the mixture and the volume was brought to 800 μL with 0.1 M NaCl. Tubes containing the assay media were incubated for 5 min at 37 °C water bath. Enzyme cycling was initiated by adding either 50 μL glucose-6-phosphate dehydrogenase (G6PDH) solution [for NADP(H) determination] or alcohol dehydrogenase (ADH) solution [for NAD(H) determination]. ADH or G6PDH reactions were stopped by adding 600 μL 6 M NaCl stock solution after incubated for 40 min at 37 °C. After centrifugation at 10,000 × *g* for 10 min at 4 °C, absorbance of the precipitant dissolved by 4 mL 95% ethanol was measured. The result was expressed with the unit of $\mu mol \cdot g^{-1}$.

2.4. Measurement of contents of ATP, ADP and AMP, and energy charge

Contents of ATP, ADP and AMP, and energy charge were determined by applying the methods of Chen et al. (2014) and Lin, Chen, et al. (2017).

2.5. Statistical analyses

All assays were repeated in triplicate. Data were presented as means ± standard errors. Analytic variance was tested by SPSS version 17.0. Difference at $P < 0.05$ or $P < 0.01$ was considered significantly or extremely significantly, respectively.

3. Results and discussion

3.1. Effects of exogenous propyl gallate treatment on activities of CCO and AAO in pericarp of harvested longan fruit

Cytochrome *C* oxidase (CCO), also called as cytochrome oxidase or mitochondrial complex IV, is located in the end of the mitochondrial respiratory electron transport chain. It can bond with cytochrome *C* to transport electron, from mitochondrial complex I, II and III, to oxygen for forming H_2O (Larsen et al., 2012). When a couple of electrons are transported, two protons will be consumed in mitochondrial matrix. Meanwhile, two protons will be transported from mitochondrial matrix to inter-membrane space of mitochondria (Li et al., 2016). Alteration in CCO activity can reflect respiratory activity and functional characteristics of mitochondria (Zhou et al., 2014). It was presumed that restricted transfer of electrons in cytochrome pathway respiration in fresh-cut 'Hami' melon fruit treated by chlorine dioxide made a contribution to the regulation of respiration rate and the extended shelf life (Guo et al., 2013). Exposure to ultraviolet-C illumination could delay senescence of peach fruit via inhibiting respiratory activity due to reduced CCO activity (Yang, Cao, Su, & Jiang, 2014). The change of CCO activity was shown in Fig. 1A, which illustrated that, for propyl gallate-treated longan fruit, CCO activity decreased rapidly in the first 2 d of storage, increased rapidly during day 2 to the day 8 of storage, and then changed little. Meanwhile, propyl gallate-treated fruit showed significantly ($P < 0.05$) lower CCO activity than the control fruit on days 2, 4 and 6 of storage, coinciding with the lower respiratory rate (Lin, Lin, Chen, Chen, & Lin et al., 2013) and lower pericarp browning index published in our previous study (Lin et al., 2015, Appendix 1). These results together indicated that through the inactivation of CCO, propyl gallate treatment would inhibit the respiratory electron transport chain of mitochondria and reduce the transfer and leak of electron, which might preserve mitochondrial structure, to delay pericarp browning and senescence of longan fruit.

Ascorbic acid oxidase (AAO) containing copper is located in cytoplasm or cell wall. It couples with redox reaction to serve as respiratory terminal oxidase (Aloni, Karni, Deventurero, Turhan, & Aktas, 2008). It