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Research Paper

Effects of chlorine dioxide fumigation on redox balancing potential of antioxidative ascorbate-glutathione cycle in 'Daw' longan fruit during storage

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ABSTRACT

Redox status in ascorbate-glutathione (ASA-GSH) cycle plays a pivotal role in plant responses to physiological and environmental changes. The change of ASA-GSH redox potential is associate with oxidative stress leading to plant senescence. The present study was aimed to investigate the effects of gaseous ClO_2 on the redox status involved in delaying fruit senescence during storage of harvested longan fruit (*Dimocarpus longan* Lour. cv. Daw). Fresh longan fruit were fumigated with 10 mg/L ClO_2 for 10 min, packed into cardboard boxes and stored at room temperature for 7 d. The results show that redox status of the fruit determined by ASA/DHA, GSH/GSSG and NADPH/NADP ratios decreased steadily in ClO_2 untreated control group during storage. On the contrary, those ratios increased immediately after ClO_2 treatment before declining steadily afterwards. The activities of NADPH-generating dehydrogenases including glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, and ASA-GSH cycle enzymes including ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase were upregulated upon the ClO_2 treatment and remained stable for a few days. ClO_2 treatment decreased hydrogen peroxide content and fruit senescence index, including browning and disease symptoms in pericarp during storage. It was concluded that the imbalance of redox status of ASA-GSH cascade involved senescence in 'Daw' longan, and ClO_2 could maintain the redox balance, leading to the alleviation and delay fruit senescence.

1. Introduction

'Daw' longan is an important economic fruit in Thailand which is exported to the international markets more than 400,000 t with a value of $270 million per year (Chanrittisen and Chomsri, 2010). However, longan fruit undergo rapid pericarp browning, a symptom of fruit senescence, and develop disease after a few days at room temperature, resulting in a short storage life and reducing market value, as pericarp appearance is a key factor determining consumer selection (Jiang et al., 2002; Saengnil et al., 2014). Pericarp browning is caused by the oxidation of phenols to quinones, which are then polymerized to form brown pigments (Jiang et al., 2002). Moreover, longan fruit are susceptible to postharvest pathogens. High sugar and moisture induce microorganisms to rot the fruit rapidly, which also causes severe browning (Apai, 2010). Consequently, prevention of senescence including pericarp browning and fruit decay is important for improving fruit marketing for longan. Saengnil et al. (2014) reported that applications of gaseous chlorine dioxide (ClO_2) at 10 mg/L for 10 min on longan fruit before storage at room temperature significantly reduced pericarp browning and prolonged shelf life by reducing the oxidation of phenolic compound, delaying the occurrence of disease and maintaining higher fruit quality. Similarly, ClO_2 treatment of longan fruit reduced pericarp browning, enhanced the antioxidant defense system and reduced ROS and oxidative damage (Chomkitichai et al., 2014a, 2014b).

Fruit senescence involved the production and accumulation of reactive oxygen species (ROS) which cause detrimental effects through oxidative damage of macromolecules especially lipids, proteins and nucleic acids (Mittler, 2002; Chomkitichai et al., 2014a). However,

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plants have evolved free radical scavenging systems to minimize the accumulation of ROS and repair oxidative damage. The ascorbate-glutathione (ASA-GSH) cycle is one of those scavenging systems (Foyer and Noctor, 2011). Four antioxidative enzymes in ASA-GSH cycle including ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR), interplay with ascorbate (ASA) and glutathione (GSH) to dissipate hydrogen peroxide (H_2O_2) or other ROS (Noctor and Foyer, 1998). The ASA-GSH cycle controlled by ASA/dehydroascorbate (DHA) and GSH/glutathione disulfide (GSSG) ratios, is related to senescence in some horticultural crops (Potters et al., 2010). For example, the decrease in ASA/DHA and GSH/GSSG ratios increased senescence of broccoli during storage at 20 °C (Mori et al., 2009). In loquat fruit, decreased ASA/DHA and GSH/GSSG ratios were related to decreased activities of APX, MDHAR, DHAR and GR, while browning was found to increase during cold storage (Cai et al., 2011).

In ASA-GSH cycle, there is a complex relationship between ASA, GSH and reduced nicotinamide adenine dinucleotide phosphate (NADPH) which is regenerated from its oxidized form (oxidized nicotinamide adenine dinucleotide phosphate, NADP) by a group of NADPH-generating dehydrogenases such as glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) (Corpas and Barroso, 2014; Manai et al., 2014). The redox status of NADPH indicated by NADPH/NADP ratio, and activities of G6PDH and 6PGDH are affected by stresses. For example, NADPH redox balance in bean seedlings was declined during sulphur-deficient stress, while the decrease in G6PDH has been reported in rice under salt stress (Juszczuk and Ostaszewska, 2011; Zhang et al., 2013). Similarly, salt stress was shown to induce the decrease in NADPH/NADP ratio, G6PDH and 6PGDH activities in roots of tomato which coincided with a decrease in the growth of seedlings (Manai et al., 2014).

In order to delay or reduce senescence by mean of maintaining the redox status balance, various chemical methods have been employed on harvested fruit. For example, Mori et al. (2009) reported that an application of ethanol vapor on broccoli before storage at 20 °C delayed senescence by increasing ASA/DHA and GSH/GSSG ratios and activities of APX, MDHAR, DHAR and GR. The objectives of this research were to investigate how ClO_2 involves in the antioxidative redox homeostasis, especially the ASA-GSH cycle during storage longan fruit and to evaluate the effects of ClO_2 on the status of ASA, GSH and NADPH in the ASA-GSH cycle, as a possible mechanism of delaying senescence in 'Daw' longan.

2. Materials and methods

2.1. Plant materials and ClO_2 treatments

Mature longan fruit were harvested from a commercial orchard in Lamphun province, Thailand in June to December 2016 (rainy and winter seasons of Thailand) and transported to the Postharvest Physiology and Technology Research Laboratory at Chiang Mai University within 2 h. Fruit were selected for uniformity in shape, color, size and lack of defects and divided into 2 groups of 720 fruits each. ClO_2 gas was prepared according to Saengnil et al. (2014). A 10 min fumigation with ClO_2 (0 and 10 mg/L) at 25 ± 1 °C in a chamber (75 L capacity) was done in triplicate. After fumigation, the chamber was ventilated for 30 min to remove any residual ClO_2. The fumigated fruit in each group were placed in a cardboard box (25 cm (L) × 17 cm (W) × 9 cm (H)) for 30 fruit per box. All boxes were stored in a storage room at 25 ± 1 °C with a relative humidity of 82 ± 5% for 7 d. A single cardboard box (30 fruit) of each replication was randomly taken every day to determine redox status of ASA, GSH and NADPH, ASA-GSH cycle enzyme activities, NADPH regenerating enzyme activities, H_2O_2 level and senescence index.

2.2. Determination of ASA and DHA contents

ASA and DHA contents were assayed by the procedure of Kampfenkel et al. (1995) with some modifications. Pericarp of 30 fruit in each treatment was cut into small pieces and mixed. Then 1 g was sampled and homogenized in 5 mL of 6% (w/v) trichloroacetic acid (TCA) at 4 °C for 1 min. The homogenate was then centrifuged at 15,600*g* and 4 °C for 5 min and the supernatant was immediately assayed for ASA and DHA contents.

For ASA determination, 0.2 mL of the supernatant was added into 3.8 mL of reaction mixture containing 0.2 M potassium phosphate buffer (pH 7.4), 10% (w/v) TCA, 42% (v/v) *ortho*-phosphoric acid, 4% (w/v) 2,2′-dipyridyl and 3% (w/v) $FeCl_3$. The mixed solution was placed at 42 °C for 40 min before measuring the absorbance with a spectrophotometer (Thermo Spectronic model Helios Epsilon, USA) at 525 nm.

Total ASA was determined by the reduction of DHA into ASA using dithiothreitol (DTT). Briefly, 0.2 mL of the supernatant was added to 0.8 mL of 10 mM DTT. The reaction was incubated at 42 °C for 15 min and stopped by adding 0.2 mL of 0.5% (w/v) *n*-ethylmaleimide. The reduced samples were then assayed for ASA as described above. ASA and total ASA contents were determined from the linear equation of a standard curve prepared with ASA. The concentration of DHA was determined by subtracting ASA from the total ASA. The concentrations of ASA and DHA were expressed on a fresh weight basis (g/kg).

2.3. Determination of GSH and GSSG contents

GSH and GSSG contents were assayed by the 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB)-glutathione reductase (GR) recycling method as described by Hodges and Forney (2000) with some modifications. One gram of pericarp (from 30 fruit) was sampled and homogenized in 15 mL of 5% (w/v) TCA at 4 °C for 1 min. The homogenate was then centrifuged at 12,000*g* and 4 °C for 15 min and the supernatant was immediately assayed for GSH and GSSG content.

For total glutathione (GSH + GSSG) determination, the supernatant (0.2 mL) was neutralized with 0.5 M potassium phosphate buffer (pH 7.0) with the ratio of 1:25 (supernatant per buffer). The reaction medium consisted of 2.2 mL of 0.1 M potassium phosphate buffer (pH 7.5) containing 5 mM ethylenediaminetetraacetic acid (EDTA) and 0.2 mL of 1 mM NADPH. The reaction was initiated by adding 0.2 mL of 6 mM DTNB prepared in 0.1 M potassium phosphate buffer (pH 7.5) and 0.2 mL of supernatant to the reaction medium. The change in absorbance of the mixture was measured with a spectrophotometer at 412 nm for 5 min.

For determination of GSSG, 0.2 mL of the supernatant was neutralized with 0.5 M potassium phosphate buffer (pH 6.5) at the ratio of 1:25. GSH was first sequestered by incubating 5 mL of the supernatant with 0.1 mL of 2-vinylpyridine at 25 °C for 1 h, and then subjected to a similar reaction as described above for total glutathione. Total glutathione and GSSG contents were determined from the linear equation of a standard curve prepared with GSH. For each sample, GSH concentration was obtained by subtracting GSSG from total glutathione. The concentrations of GSH and GSSG were expressed on a fresh weight basis (μg/kg).

2.4. Determination of NADP and NADPH contents

NADP and NADPH contents were assayed by the procedure of Brugidou et al. (1991) with some modifications. One gram of pericarp (from 30 fruit) was sampled and ground in liquid nitrogen and the powder was then homogenized in 10 mL of 0.5 M perchloric acid in 10% (v/v) methanol for NADP extraction, or 0.5 M NaOH in 10% (v/v) methanol for NADPH extraction at 4 °C for 1 min. The homogenate was then centrifuged at 5000*g* and 4 °C for 15 min. The supernatant was adjusted to either pH 5.0 with 6 M KOH for the NADP extraction or pH