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Effects of chlorine dioxide treatment on respiration rate and ethylene synthesis of postharvest tomato fruit

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ABSTRACT

Tomato fruit at the mature green stage were treated with ClO_2 gas in a sealed container for 12 h, and then stored at 23 °C with 85% relative humidity (RH) for 23 d. Respiration rate, respiration-related enzymes including phosphohexose isomerase (PHI), succinate dehydrogenase (SDH), and glucose-6-phosphate dehydrogenase (G-6-PDH) and 6-phosphogluconate dehydrogenase (6-PGDH), ethylene production, and the expression of *LeAOX1a*, *LeCOX1*, *LeACS2*, *LeACS4* and *LeACO1* genes were measured. The results showed that application of ClO_2 gas was effective in reducing total respiration, cytochrome pathway respiration and the expression of *LeCOX1*, but no significant reduction in the activities of respiration-related enzymes was observed during storage. Fruit treated with ClO_2 resulted in lower ethylene production. Furthermore, the expression of ethylene biosynthesis related genes, including *LeACS2*, *LeACS4* and *LeACO1* was reduced by the ClO_2 treatment. These results indicate that ClO_2 treatment might delay the ripening of tomato fruit, possibly by a mechanism involving suppression of respiration rate and ethylene biosynthesis.

1. Introduction

Tomato (*Solanum lycopersicum* L.) fruit are important owing to their qualities for human nutrition and economic value (Yahia and Brecht, 2012). However, tomato fruit have a limited shelf-life due to rapid ripening, as characterized by an increase in respiration and ethylene (Alexander and Grierson, 2002; Paul et al., 2010; Xu et al., 2011). Although physiological control (Wang et al., 2010; Xu et al., 2011; Ali et al., 2013) and genetic modification (Kevany et al., 2007; Barry et al., 2000; Klee and Giovannoni, 2011) have been demonstrated to reduce respiration and ethylene production of tomatoes, the mechanisms involved in the suppression of respiration and ethylene are not clear.

Most tomato cultivars show a typical climacteric change in respiration, which accelerates their biochemical processes. Previous studies have found a positive relationship between respiration rate and ethylene levels in harvested fruit (Xu et al., 2012; Guo et al., 2013), and any elevation in respiration due to ethylene would increase storage losses and reduce postharvest quality. Sugar metabolism, including the glycolysis pathway (EMP), the tricarboxylic acid cycle (TCA) and pentose phosphate pathway (PPP), and the electron transport system, are the metabolic pathways of aerobic respiration, which are mainly regulated by phosphohexose isomerase (PHI), succinate dehydrogenase (SDH), glucose-6-phosphate dehydrogenase (G-6-PDH) and 6-phosphogluconate dehydrogenase (6-PGDH), (van Dongen et al., 2011; Guo et al., 2013; Vanlerberghe, 2013). In addition, the alternative oxidase (AOX) pathway which branches from the cytochrome c oxidase (COX) pathway at the level of ubiquinone (UQ), also contributes to respiratory metabolism (MØller et al., 2010). There is also evidence that AOX is involved in the respiratory climacteric and *LeAOX1a* plays a dominant role in the AOX respiratory pathway of tomato fruit (Xu et al., 2012), but limited information exists on the contribution of the COX pathway to fruit respiration.

Ethylene synthesis in tomato fruit is mainly regulated by different gene families encoding 1-aminocyclopropane-1-carboxylate (ACC) synthase (ACS) and ACC oxidase (ACO) (Nakatsuka et al., 1998). Studies have shown that suppression of expression of *ACO* and *ACS* resulted in a stronger inhibition of ethylene production (Hamilton et al., 1990; Oeller et al., 1991). Conversely, application of ethylene to climacteric fruit at the mature stage stimulates ethylene synthesis, accelerating fruit ripening and accelerates ethylene production and respiration, including the alternative pathway respiration. These results suggest that reduced respiration rates and ethylene production may be involved in ripening resistance in harvested fruit.

Chlorine dioxide (ClO_2), as an effective antimicrobial agent, has been applied to reduce respiration and ethylene during storage of many fruit species, including apricot, pepper, gooseberry, melon

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and apple (Zhong et al., 2006; Du et al., 2007; Tian et al., 2011; Zhao et al., 2011; Guo et al., 2013). However, little research has been done on respiration and ethylene of tomato fruit in response to ClO_2. Also, the mechanisms involved in regulation of respiration and ethylene of postharvest fruit by ClO_2 have not been elucidated in detail. Therefore, the aim of this study was to investigate the effects of ClO_2 on postharvest respiration and ethylene of tomato fruit and the possible mechanisms involved.

2. Materials and methods

2.1. Plant material and treatment

Tomato fruit (*Solanum lycopersicum* L. cv. Messina) were harvested at the mature green stage in October of 2012 from an orchard at 'Wujia' district, Xinjiang, China, and immediately transported to the laboratory after harvest. Fruit of uniform size, maturity and free from visual blemishes and diseases were selected and randomly divided into two lots. The preparation of gaseous ClO_2 was based on our previous study (Guo et al., 2010). Gaseous ClO_2 was generated by combining 4.0 g of sodium chlorate, 4.0 g of oxalic acid and stabilizer into a sachet (prepared by our laboratory) to generate an expected concentration of 60 mg L^{-1} after 12 h, mixing and shaking for activation. The first lot of fruit was treated with gaseous ClO_2 in sealed plastic containers (30.8 L) at 23 °C for 12 h according to the method reported previously (Guo et al., 2013), whereas the second lot of fruit was subjected to the same conditions without exposure to ClO_2 (control). After treatment, tomato fruit were placed in boxes and stored at 23 ± 1 °C with approximately 90% relative humidity for 23 days. Sixty fruit served as an experimental unit and six fruit were collected at each sampling time point. For each sample, pulp of tomato fruit was cut into small cubes and frozen with liquid nitrogen, and stored at −80 °C until analysis. All experiments were conducted in three replicates.

2.2. Measurement of respiration rate

Respiratory oxygen consumption was measured using Clark-type electrodes (Hansatech, King's Lynn, UK) based on the method of Xu et al. (2012). Fruit pulp (0.05 g; adjacent to the peel) was weighed and cut into small pieces, then pre-treated with 5 mL of deionized water for 15 min in order to minimize the effect of wound-induced respiration. Measurements were performed at 23 °C in a final volume of 2 mL of 2 mM phosphate buffer (pH 6.8), and the cuvette was tightly closed to prevent diffusion of oxygen from the air. Total respiration (V_t) is defined as O_2 uptake rate without any inhibitor. The capacity of the alternative pathway (V_{alt}) is defined as O_2 uptake rate in the presence of 1 mM KCN. Residual respiration (V_{res}) is defined as O_2 uptake in the presence of both 1 mM KCN and 3.0 mM salicyclohydroxamic acid (SHAM). Cytochrome pathway capacity (V_{cyt}) was calculated by the formula: $V_{cyt} = V_t - V_{alt} - V_{res}$.

2.3. Measurement of respiration-related enzyme activity

The activity of phosphohexose isomerase (PHI) was determined by the method of Dannel et al. (1995), with slight modifications. Fruit tissues (1.0 g) were homogenized in 5 mL cold 0.05 mol L^{-1} Tris–HCl (pH 8.0) and centrifuged at 5000 × *g* for 30 min at 4 °C. The supernatant was used for assaying the enzyme activities. The reaction mixture contained 50 mmol L^{-1} Tris (pH 8.0, 5 mM $MgCl_2$, 1 mM NaCl, 0.39 mM NADP, 460 U G-6-PDH and 1.4 mM F-6-P). The absorbance was measured at 340 nm with UV-2450 spectrophotometer (Shimadzu, Japan). Enzyme activity was expressed as μg fructose per gram fresh weight.

The activity of succinate dehydrogenase (SDH) was assayed according to the method of Guo et al. (2013). Tissues (1.0 g) were homogenized in 3.2 mL of cold 0.05 mol L^{-1} phosphate buffer (pH 6.8, 0.25 M sucrose, 5 mM EDTA and l mg bovine serum albumin) and centrifuged at 13,000 × *g* for 15 min at 4 °C. Supernatant was discarded, pellet was re-suspended in 5 mL of 0.06 mol L^{-1} Tris–HC1 (pH 7.6) for assaying the enzyme activities. The substrate solution contained 0.l mL of 1.5 mol L^{-1} phosphate buffer (pH 7.4), 0.l mL of 1.2 mol L^{-1} succinic acid (pH 7.4), 0.l mL of 0.9 mmol L^{-1} 2,6-dichloroindophenol (DCIP) and 2.5 mL distilled water, incubating at 30 °C for 10 min. The activity was determined by adding 0.1 mL of enzyme extract and 0.l mL phenazine methosulfate (PMS). The absorbance was measured at 600 nm. One unit (U) of enzyme activity was defined as an increase of 0.01 in absorbance per minute per gram.

The total activity of glucose-6-phosphate dehydrogenase (G-6-PDH) and 6-phosphogluconate dehydrogenase (6-PGDH) was based on the method of Guo et al. (2013). Tissues (1.0 g) were homogenized in 5 mL of cold 0.05 mol L^{-1} potassium phosphate buffer (pH 6.8, 0.25 M sucrose, 5 mM EDTA, and l mg bovine serum albumin) and centrifuged at 13,000 × *g* for 15 min at 4 °C. Supernatant was discarded, pellet was re-suspended in 5 mL of 0.06 mol L^{-1} Tris–HC1 (pH 7.6) for assaying the enzyme activities. The mixture contained 9 mL of 0.1 mol L^{-1} Tris–HCl (pH 7.4, 5 mM 6-P-G, 5 mM $MgCl_2$ and 5 mM NADP) and 0.1 mL of enzyme extract. The activity was determined by measuring absorbance at 340 nm. One unit (U) of enzyme activity was defined as an increase of 0.01 in absorbance per minute per gram.

2.4. Measurement of ethylene production

Ethylene production was determined as follows. Each subsample of three fruit was placed in a hermetically sealed container. After 2 h, 1 mL the internal atmosphere of the container was extracted with a syringe and the ethylene was quantified using a gas chromatograph (GC-2014C, Shimadzu Co., Ltd., Kyoto, Japan). The temperatures of the column, injector, and a flame ionization detector were 80, 140, and 150 °C, respectively. The ethylene production was expressed as μL h^{-1} kg^{-1} FW.

2.5. RNA extraction and cDNA synthesis

Total RNA was extracted from tomato fruit using TRIzol Reagent (Invitrogen, Shanghai, China). The cDNA was synthesized by using AMV-reverse transcriptase (Promega Madison, WI, USA). The RT reaction mixture contained 4.0 μL of RNA sample, 1.0 μL of dNTPs (2.5 mmol L^{-1}), 1.0 μL of Oligo dT_{18} Primer and 5.0 μL of ddH_2O, incubating at 65 °C for 10 min, then added 4.0 μL of 5× First stand buffer, 1 μL of 0.1 M DDT (40 U μL^{-1}), 0.5 μL of Super-RI (10 U μL^{-1}), and 0.5 μL of AMV reverse transcriptase in a total volume of 20 μL. The mixture was incubated at 37 °C for 2 h, heated to 70 °C for 15 min to denature the reverse transcriptase, and then cooled to 4 °C. After all resulting cDNAs were tested by electrophoresis, they were stored at −20 °C until further analysis.

2.6. Semi-quantitative reverse transcription polymerase chain reaction (sqRT-PCR) analysis

sqRT-PCR was carried out to verify the pattern of expression of respiration related genes, including *LeAOX1a* and *LeCOX1*, and ethylene biosynthetic genes, including *LeACO1*, *LeACS2* and *LeACS4* during fruit ripening. Specific primers (Table 1) were designed from their nucleotide sequences selected from National Center for Biotechnology Information (NCBI), and synthesized by Invitrogen Company (Shanghai, China).