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Impact of postharvest nitric oxide treatment on antioxidant enzymes and related genes in banana fruit in response to chilling tolerance

Bin Wu[a,c,1], Qin Guo[b,1], Qingpeng Li[b], Yiming Ha[b,*], Xueping Li[a,*], Weixin Chen[a]

[a] Guangdong Key Laboratory for Postharvest Science and Technology of Fruits and Vegetables, College of Horticulture, South China Agricultural University, Guangzhou 510642, China
[b] Institute of Agro-products Processing Science and Technology, Chinese Academy of Agricultural Sciences, Beijing 100193, PR China
[c] Institute of Agro-products Storage and Processing, Xinjiang Academy of Agricultural Sciences, Urumqi 830091, PR China

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ABSTRACT

Banana fruits harvested at the mature green stage were treated with 60 μL L^{-1} nitric oxide (NO) for 3 h at 22 °C, and then stored at 7 ± 1 °C with 90% RH for 15 days. The results showed that the application of NO at 60 μL L^{-1} was most effective in reducing chilling injury in banana fruit. The treatment reduced increases in electrolyte leakage and malondialdehyde content, and delayed increases in both superoxide anion ($O_2^{\bullet-}$) production rate and H_2O_2 content. Fruit treated with NO exhibited higher activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX), and significantly higher expression of *MaSOD*, *MaCAT*, *MaPOD* and *MaAPX* genes than control fruit during storage. These results indicate that NO treatment might enhance chilling tolerance of banana fruit via improving the activities of antioxidant enzymes and inducing the expression of antioxidant-related genes.

1. Introduction

Cold storage of harvested fruit is commonly used for extending postharvest life. However, many tropical and subtropical fruits are extremely sensitive to chilling injury (CI), and this reduces the overall quality and marketability of many fruits. Harvested banana fruits are susceptible to CI at temperatures below 12–13 °C, as characterized by pitting and browning of the peel and abnormal ripening pulp (Lu et al., 1999). Although various methods such as modified atmosphere (Nguyen et al., 2004), UV-C treatment (Pongprasert et al., 2011), and application of salicylic acid (Kang et al., 2003) have been demonstrated to reduce CI of banana, the mechanisms involved in the prevention of chilling injury are not clear.

Cell membrane integrity is the primary cell structure affected by CI (Rui et al., 2010). Though electrolyte leakage and malondialdehyde (MDA) content are widely used by researchers to indirectly evaluate cell membrane integrity, it is possible to assess the impact of CI in fruits and vegetables in a very precise way (Aghdam and Bodbodak, 2013). Oxidative stress from excess oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and superoxide anion ($O_2^{\bullet-}$) has been associated with the appearance of chilling damage in fruit (Hodges et al., 2004). The toxicity of ROS is due to their reactions with numerous cell components causing a cascade of oxidative reactions and the consequent inactivation of enzymes, lipid peroxidation, protein degradation, and DNA damage (Scandalios, 1993). To scavenge the chilling-induced ROS and combat oxidative damage, plants have evolved efficient antioxidant defense systems, including enzymatic and nonenzymatic scavenging systems such as antioxidative enzymes, lipid-soluble antioxidants and water-soluble reductants (Zhou et al., 2005). Previous studies have shown that a positive relationship exists between the antioxidant enzyme activities including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX), and chilling tolerance in harvested fruits (Yang et al., 2011; Xu et al., 2012). These results suggest that enhanced antioxidant enzyme systems and reduced peroxidation of membrane lipids may be involved in chilling tolerance in harvested fruits.

Nitric oxide (NO) is a bioactive molecule involved in regulation of many fruits physiological processes (Guo et al., 2011; Zheng et al., 2011). Recently, NO has emerged as a key signaling molecule in plant defense responses to both biotic and abiotic stresses (Besson-Bard et al., 2007), and protects plant cells against oxidative stress by reducing ROS accumulation (Xu et al., 2010; Zhu et al., 2010). NO has been applied to reduce the development of chilling injury symptoms during cold storage of many fruits species, including peach, mango, plum, cucumber and loquat (Singh et al., 2009; Zhu et al., 2010; Xu et al., 2012; Aghdam and Bodbodak, 2013). The alleviation of chilling injury by NO might be related to suppression of ethylene production and respiration (Singh et al., 2009; Zaharah and Singh, 2011), but evidence has also shown that bolstering the antioxidant enzyme or increasing proline levels can serve as an

* Corresponding authors. Tel.: +86 20 38294892; fax: +86 20 85288280.
E-mail addresses: hayiming2013@163.com (Y. Ha), lxp88@scau.edu.cn (X. Li).
[1] These authors contributed equally to this work.

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adaptive mechanism to chilling stress in higher plants (Zhang et al., 2011; Shang et al., 2011; Aghdam and Bodbodak, 2013). Thus, NO has a potential application in postharvest treatment by alleviating chilling injury and maintaining quality. We found that treatment with NO was effective in alleviating chilling injury of banana fruits during cold storage, although the mode of action of NO in reducing chilling injury has not been clearly elucidated in banana fruits.

The objective of this study was to determine how NO affects electrolyte leakage, MDA content, ROS production, the antioxidant enzymes activities and the expression of related genes in banana fruits during chilling stress, which may define specific roles of NO at the physiological and molecular biological levels to help us understand and improve chilling tolerance.

2. Materials and methods

2.1. Plant materials and treatment

Banana fruits (*Musa* spp., AAA group cultivar 'Brazil') were harvested at the mature green stage (respiration rate 0.53 mg kg^{-1} FW h^{-1}, firmness 42.10 N, soluble solids content 3.83, Hue angle value 118.73) in October of 2010 from an orchard at Panyu district, Guangzhou, China, and transported to the laboratory immediately after harvest. Fruits of uniform size, maturity and free from visual blemishes and disease were selected for the experiment and randomly divided into two lots of 180, comprising three replicates of 60. In a preliminary study, the fruits were treated for 3 h with NO (Source Gas Co., Ltd., Guangzhou, China) at concentrations between 10 μL L^{-1} and 100 μL L^{-1} as below, then stored at 7 ± 1 °C for 15 days. It was found that treatment with NO at 60 μL L^{-1} significantly alleviated subsequent CI in banana fruits and this concentration therefore was used (Supplementary Fig. 1). The first lot of fruit was treated with NO, which was injected into the sealed plastic containers (30.8 L) by controlling the released velocity with a flow meter to attain concentrations of 60 μL L^{-1} (60 μL L^{-1} NO), at 22 °C for 3 h, whereas the second lot of fruit was subjected to the same condition without exposure to NO (control). After treatment, banana fruits were placed in boxes and covered (not sealed) with 0.03 mm-thick PVC bags to minimize water loss and stored at 7 ± 1 °C with approximately 90% RH for 15 days. Fruit samples were taken from the peels (approximately 5 cm at the equatorial zone) after NO treatment (time 0) and at 3 day intervals during storage for measurements of CI, MDA, $O_2^{\bullet-}$ production rate, H_2O_2 content, activities of SOD, CAT, POD and APX, and the expression of *MaSOD*, *MaCAT*, *MaPOD* and *MaAPX* genes.

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.postharvbio.2014.01.017.

2.2. Measurement of chilling injury (CI) index

Surface pitting and browning were evaluated on a scale of 1–5 (Promyou et al., 2008). 1: no chilling injury; 2: mild injury (1–20% of fruit affected); 3: moderate injury (21–50% of fruit affected); 4: severe injury (51–80% of fruit affected); 5: very severe injury (81–100% of fruit affected). The CI index was calculated using the following formula:

$$\text{CI index} = \frac{\sum(\text{CI level}) \times (\text{number of fruit at that level})}{\text{total number of fruit}}$$

2.3. Assays of relative electrolyte leakage and malondialdehyde (MDA) content

Relative electrolyte leakage was determined as described previously by Li et al. (2012). Fifteen pieces of banana peel (1 mm) from one banana fruit were rinsed and incubated in 40 mL distilled water for 30 min. Then, the initial electrolyte leakage was monitored with a conductivity meter (DDS-307, Scientific Apparatus Ltd., Shanghai, China). Each sample was boiled for 5 min and then continually rinsed for 2 h. The final electrolyte leakage (total electrolyte) was again monitored. The relative electrolyte leakage was defined as relative to the initial electrolyte content.

MDA content was measured according to the method of Guo et al. (2013). MDA content was determined by adding 0.6% thiobarbituric acid (TBA) and 2 mL the extract. The mixture was boiled in a water bath for 15 min and then quickly cooled in an ice bath. The absorbance was measured at 532 nm with UV-2450 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) and corrected for nonspecific absorption at 600 nm. The MDA concentration was calculated using the extinction coefficient 155 mM cm^{-1}.

2.4. Determinations of $O_2^{\bullet-}$ production rate and H_2O_2 content

Banana peels (1.0 g) were homogenized in 3 mL of cold 50 mM potassium phosphate buffer (pH 7.8) containing 1% (w/v) polyvinylpyrrolidone, and then centrifuged at 5000 × *g* at 4 °C for 15 min. The $O_2^{\bullet-}$ production rate was measured by monitoring the nitrite formation from hydroxylamine in the presence of $O_2^{\bullet-}$ as described by Yang et al. (2011). A standard curve with NO_2 was used to calculate the $O_2^{\bullet-}$ production rate from the reaction equation of $O_2^{\bullet-}$ with hydroxylamine. The $O_2^{\bullet-}$ production rate was expressed as nmol min^{-1} g^{-1} FW.

The content of H_2O_2 was determined by the method of Xu et al. (2012). Banana peels (1.0 g) were homogenized in 6 mL of 100% acetone and then centrifuged at 5000 × *g* for 10 min at 4 °C. The supernatant (1 mL) was mixed with 0.1 mL of 5% $Ti(SO_4)_2$ and 0.2 mL of concentrated NH_4OH solution. The titanium-peroxide complex precipitated and this sediment was dissolved in 4 mL of 2 M H_2SO_4 after centrifugation at 3000 × *g* for 10 min. The absorbance was measured at 415 nm. H_2O_2 content was calculated from a standard curve prepared in a similar way and expressed as nmol g^{-1} FW.

2.5. Analysis of antioxidant enzymes

Banana peels (1.0 g) were homogenized in 4 mL of cold 50 mM phosphate buffer (pH 7.0, containing 1% PVP, 1 mM ascorbic acid and 0.1 mM EDTA) and centrifuged at 12,000 × *g* for 20 min at 4 °C. The supernatant was used for determination of enzyme activities.

Superoxide dismutase (SOD) activity was assayed by measuring its ability to inhibit the photoreduction of nitroblue tetrazolium (NBT) according to the method of Yang et al. (2011). One unit of SOD activity was defined as the amount of enzyme that would inhibit 50% of NBT photoreduction at 560 nm.

Catalase (CAT) activity was assayed by monitoring the disappearance of H_2O_2 according to the method of Tareen et al. (2012). One unit of CAT activity was defined as the amount of enzyme that decomposed 1 μmol H_2O_2 min^{-1} at 30 °C.

Peroxidase (POD) activity was measured by the increase in absorbance at 470 nm due to formation of tetraguaiacol from guaiacol in the presence of H_2O_2, according to Xu et al. (2012). One unit of POD activity was defined as the amount of enzyme that caused an increase in absorbance of 0.01 at 470 nm in 1 min.

Ascorbate peroxidase (APX) activity was carried out as described by the method of Zhang et al. (2013). One unit of APX activity was defined as the amount of enzyme that oxidized 1 μmol ascorbate min^{-1} at 30 °C.

The activity of each enzyme was expressed on a protein basis. Protein content was determined according to Bradford (1976), using bovine serum albumin as standard.