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Enhanced chilling tolerance of banana fruit treated with malic acid prior to low-temperature storage

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ABSTRACT

The effects of postharvest malic acid (MA) treatment on alleviating the occurrence of chilling injury (CI) symptoms in banana (*Musa spp.*, AAA group, cv. Brazil) fruit under 6 °C were evaluated. Application of 80 mM MA alleviated CI symptoms (surface browning), delayed the decrease in chlorophyll fluorescence (Fv/Fm) and chlorophyll content. The activities of peroxidase (POD) and polyphenol oxidase (PPO) were also suppressed by MA. Furthermore, compared with the control group, fruit that were treated with MA showed lower levels of reactive oxygen species, but higher antioxidant activities. The results suggest that the application of MA, as an organic acid, exhibited the potential for alleviating chilling injury symptoms of banana fruit by reducing skin browning and inducing antioxidant activities under low temperature.
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1. Introduction

Banana is highly sensitive to storage temperatures below 13 °C that induce chilling injury (CI) of fruit. The CI symptoms are usually observed on the fruit skin, which exhibits delayed yellow color development, browning or blackness spotting of the skin; and finally induce the failure of fruit soften (Jiang et al., 2004).

Malic acid (MA) has been found to play pivotal roles not only in affecting starch metabolism and increasing soluble substance and chlorophyll contents during the growth period, but also in regulating maturation and senescence of fruit or flowers (Centeno et al., 2011; Darandeh and Hadavi, 2011). In addition, according to biochemical, molecular or proteomics approaches, the application of exogenous organic acids such as MA, oxalic acid (OA), citric acid (CA) and salicylic acid (SA) has been found to affect fruit quality and induce stress tolerance (Darandeh and Hadavi, 2011; Shoor, 2010; Zheng et al., 2011). These organic acids mainly function in maintaining the ability to inhibit O_2^- accumulation, delaying H_2O_2 decrease (Ding et al., 2007; Huang et al., 2013a), enhancing antioxidant enzyme (PPO and POD) activities (Cao et al., 2010) and increasing the expression of senescence-related proteins or defense proteins (Wang et al., 2009) to keep the fruit in good quality during storage. However, little is known about the application of malic acid on CI tolerance of banana fruit during storage.

The objectives of this study were to investigate the effects of exogenous malic acid in the production of ROS and the antioxidant activities in skin tissues of banana fruit during storage at low temperatures. Knowledge gained from this study will help to understand the role of this organic acid in reducing chilling injury of banana fruit and developing appropriate postharvest technologies accordingly.

2. Materials and methods

2.1. Fruit material and treatments

Green mature banana fruit were harvested from a commercial orchard in Guangzhou. Fruit were washed and selected for uniformity of shape, color, and size; then placed into two groups in a completely randomized design: immersion in water only (control) or 80 mM MA for 5 min at 25 °C. After treatment, fruit were air dried, then packed in plastic polyethylene bags (200 × 150 mm, 0.03 mm thickness and 3 fruit per bag), and stored at 6 °C, 85–90% relative humidity. Six fruit from each treatment were randomly selected at 0, 2, 4, and 6 d during storage and the chilling index and chlorophyll fluorescence (Fv/Fm) were evaluated. Then the skin tissues were sliced, frozen in liquid N_2, and stored at −20 °C prior to analyses of enzymatic activity and antioxidant ability.

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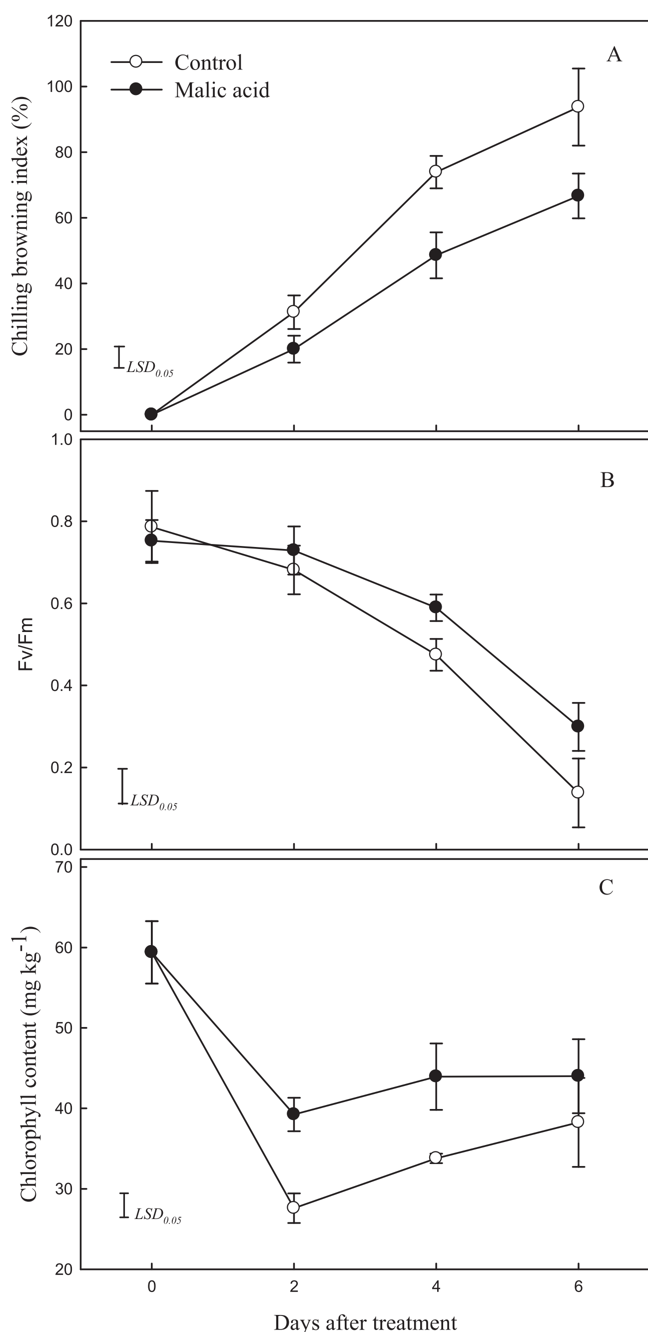

Fig. 1. Changes in chilling injury index (A), chlorophyll fluorescence (Fv/Fm) (B), and chlorophyll content of banana fruit treated with malic acid during storage at 6 °C. Values are the means of three replicates ± SE (n = 3). Vertical bars represent the standard errors of the means.

2.2. Determination of chilling injury index

Chilling injury was assessed by estimating the extent of browning of the fruit surface using the following scale: 1, almost no browning; 2, 0–1/4 browning of the fruit surface; 3, 1/4–1/2 browning area; 4, 1/2–3/4 browning area; and 5, >3/4 browning area. The chilling injury index was calculated as: $\sum$ (chilling injury scale (corresponding fruit with each class)/number of total fruit (the highest scale)) × 100.

2.3. Measurement of chlorophyll fluorescence and chlorophyll content

Chlorophyll fluorescence was determined using a chlorophyll fluorometer (FAM 2100, Walz, Germany). Fo and Fm were measured at three equidistant points around the middle position of each fruit after being dark-adapted for 30 min. The maximal variable fluorescence (Fv = Fm–Fo) and PSII quantum yield (Fv/Fm) were calculated.

Chlorophyll was extracted by grinding 2 g of peel tissue from six fruit from each treatment in 80% (v/v) cold acetone at 4 °C with quartz sand for 30 min. The amount of chlorophyll (a and b) was determined by the absorbance at 663 and 645 nm using a spectrophotometer (UVmini-1240, Shimadzu Corp., Japan), and calculated into mg kg^{-1} on fresh weight basis.

2.4. Assays for enzyme activities of PPO and POD

For analyses of enzyme activities, peel tissue (2.0 g) from six fruit from each treatment were homogenized with 10 mL of 0.05 M sodium phosphate buffer (pH 7.0) containing 0.2 g PVPP (Dingguo, Beijing, China) and then centrifuged at 15,000 × g for 20 min (Sigma Laborzenfrifugen, 3K15, Germany) 4 °C. The supernatant was collected for enzyme assays.

PPO (EC 1.10.3.2) activity was measured by incubating 0.1 mL of enzyme extractionin 2.9 mL of sodium phosphate buffer(0.05 M, pH 7.0) containing 10 mM catechol. One unit of PPO activity was defined as the amount of enzyme causing 0.001 absorbance increase per minute at 398 nm.

POD (EC 1.11.1.7) activity was assayed by reaction mixture of 3 mL contained 0.05 mL of enzyme extraction, 0.1 mL of 4.0% guaiacol, 0.1 mL of 0.46% H_2O_2 and2.75 mL of sodium phosphate buffer (0.05 M, pH 7.0). One unit of POD was defined as the amount of enzyme causing 0.01 absorbance increase per minute at 470 nm.

2.5. Assays of reactive oxygen species (ROS)

Superoxide anion (O_2^-) generation rate was measured by the method of Wang and Lou (1990) with modifications. Frozen peel tissues (2.0 g) from six fruit were extracted with 10 mL of extraction buffer (pH 7.8) containing 1 mM EDTA, 2% polyvinylpyrrolidone (PVP, w/v) and 0.3% Triton X-100, centrifuged at 12,000 × g for 30 min at 4 °C. The 1 mL of supernatant was incubated with 1 mL of 1 m Mhydroxylammoniumchlroride for 30 min at 25 °C. Then 1 mL of above solution was mixed with 1 mL of 17 mM 3-amino-benzene-sulfonic acids (Sigma, USA) and 1 mL of 7 mM 1-naphthylamine (Sigma, USA) for 20 min at 25 °C. The absorbance of the solution was monitored at 530 nm. The O_2^- production rate was expressed as nmol s^{-1} kg^{-1} on a fresh weight basis.

H_2O_2 content was determined by homogenized frozen peel tissues (2.0 g) from six fruit with 10 mL of cold acetone, centrifuged at 12,000 × g for 30 min at 4 °C. The supernatant (1 mL) was mixed with 0.1 mL of 5% titanium sulphate and 0.2 mL ammonia, centrifuged at 12,000 × g for 10 min at 4 °C. The precipitates were dissolved in 3 mL of 10% (v/v) H_2SO_4, centrifuged at 12,000 × g for 10 min. The absorbance of the supernatant was measured at 420 nm and the H_2O_2 content was expressed on nmol kg^{-1} on a fresh weight basis.

2.6. Assay of DPPH radical scavenging activity and reducing power

Frozen peel tissues (3.0 g) from six fruit were firstly crushed into powder and extracted with 30 mL of methanol for 30 min, centrifuged at 15,000 × g for 20 min at 25 °C. The supernatants were collected.

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