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Antioxidant enzyme activities and exogenous ascorbic acid treatment of 'Williams' banana during long-term cold storage stress

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ABSTRACT

The effect of exogenous ascorbic acid (AA) application on chilling injury (CI) of banana fruit 'Williams' (Giant Cavendish AAA sub-group) harvested at the mature green stage. The investigation aimed to increase chilling tolerance of banana fruits under cold storage temperature. The experiment was carried throughout growth two seasons 2014 and 2015. Fruits were immersed in 0, 3, 6, and 9 mM ascorbic acid (AA) for 15 min at cold temperature. Thereafter, fruits were stored at cold temperature (6 ± 1 °C with 90% RH) for 27days. The results pointed out that the immersing banana fruits at 9 mM of AA were most effective in reducing chilling injury incidences in fruit peel. Also, the treatment reduced increases in cell membrane dysfunction at to lipid peroxidation (MDA) and protein oxidation (PCG) accumulation consequently, decreases ion leakage. Moreover, immersing in AA at 9 mM minimized superoxide anion ($O_2\cdot^-$) and H_2O_2 production. Also, it increased significantly antioxidant enzyme activities during cold storage stress of ascorbate peroxidase (APX; CE: 1.11.1.11), peroxidase (POD; EC: 1.11.1.7), catalase (CAT; EC:1.11.1.6), and superoxide dismutase (SOD; EC: 1.15.1.1) so, less chilling injury symptoms on fruit peel which it reflects on fruit appearing compared to control fruit and other AA treatments. These results pointed out that the AA treatments at 9 mM might increase chilling tolerance of banana fruit by increasing antioxidant enzyme activities. Consequently, decrease/alleviate $O_2\cdot^-$ and H_2O_2 generation during cold storage stress. Thus, exogenous AA treatment at 9 mM could be a useful application to reduce/delay/alleviate CI in banana fruit during cold storage.

1. Introduction

Banana (*Musa spp.* CV 'Williams' Giant Cavendish AAA sub-group) is one of the most commonly consumed and economically important fruit in the global market (FAO, 2013). Cold storage of harvested banana is usually used for extending postharvest life of fruits. Banana as many tropical and subtropical fruits are susceptibility to chilling temperature below 13 °C (Wu et al., 2014), which enhance chilling injury symptom (CI) of fruit peel and pulp (Jiang et al., 2004). Therefore, the storage, handling and transport potential of banana fruit are limited due to perishability (Duan et al., 2007). The most common visual CI symptoms include a dull gray peel color, ripening, flavor and increasing physical injury and peel and pulp browning (Hailu et al., 2013). The mechanism of CI is as results of disruption of normal physiological activities in fruit tissue that leads to metabolic imbalances. Therefore, changes in cell plasma membrane matrix such as decreases in unsaturation fatty acid and protein (Lo'ay, 2010). Chilling temperature stress leads an oxidative reaction by generating active oxygen species (AOS) during cold stress such as hydrogen peroxide (H_2O_2) and superoxide anion ($O_2\cdot^-$) which has been related to the appearance of chilling injury symptoms in fruit (Hodges et al., 2004). Low storage temperature provoked an imbalance between antioxidants and AOS production (homeostasis)' of susceptible plant tissue (Foyer and Noctor, 2000). Originally, a plant cell has a mechanism to quench AOS by synthesizing antioxidants which are considered to protect fruit tissue against AOS under low-temperature stress (Hodges et al., 2004). Some investigations have been focused on alleviating the symptoms of CI during cold storage of banana fruits using different techniques. Immersing banana fruit in hot water at a different time for 5, 10 and 15 min at 42 °C, or at 25 °C (control), before storing 4 days at 4 °C to delay chilled peel during low-temperature storage stress (Promyou et al., 2008). Another study applied the oxalic acid treatment and modified atmosphere packaging to observe the combined effects of them on CI symptoms and some quality characteristics of banana (Öz et al., 2016). Additionally, a procedure using UV-C at dosages of 0.03 kJm^{-2} to relieve CI appearance on banana fruit (Pongprasert et al., 2011). Also, in another way using n-

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propyl dihydrojasmonate (PDJ) and abscisic acid (ABA) treatment to reduce chilling injury in banana fruits at low-temperature stress (Chaiprasart et al., 2002), or immersing fruit on γ-aminobutyric acid to alleviate CI symptoms of banana (Wang et al., 2014).

Ascorbic acid (AA) in the plant cell is reverted to its functions as a precursor for oxalate and tartaric acid synthesis. It initiates in many processes, including photosynthesis, photoprotection, the cell cycle, cell-wall growth and cell expansion, synthesis of ethylene, gibberellins, anthocyanin and hydroxyproline, and resistance to environmental factors. The most important role of AA is scavenged directly/indirect of AOS in a plant cell (Smirnoff and Wheeler, 2000). It may rest the relative stability of the monodehydroascorbate radical. Moreover, AA also has another important photoprotective function because of its antioxidant capacity. It is one of two major soluble antioxidants in chloroplasts (Lo'ay, 2005) and it assumed to protect α-Tocopherol and recycle it (a major lipid-soluble antioxidant) from α-tocopheroxyl radical (Munné-Bosch and Alegre, 2002).

The objective of this study drew attention to the physiological roles of ascorbic acid as an antioxidant to increase chilling tolerance of banana under cold storage. in order to, delay/alleviate chilling injury during storage (27 days) at low temperature.

2. Materials and methods

2.1. Fruit material and experimental setup

Green banana 'Williams' fruit (Giant Cavendish AAA sub-group) grown in clay loam soil in a commercial orchard in Meet Ghamr, Dakahlia perviness, Egypt. The experiment was carried throughout two seasons 2014 and 2015. A sample of 60 hands was picked for regularity of size and color and soaked in 200 μL L^{-1} chlorine solution. Afterward, fruits were then dipped for 2 min in 2 g L^{-1} Benlate® solution to control fruit rots and were allowed to air dry before treatment. Fruits were immunized 30 min at ambient temperature in a solution of an ascorbic acid at different concentrations 0, 3, 6 and 9 mM. Each treatment has fifteen hands, five for color measurement and ten for chemical analysis. Fruits were stored for 27 days at 6 °C and 90% RH.

2.2. Measurements chilling injury index (CI-index), fruit peel color hue angle (h°), and total chlorophyll content (Ch_{ab})

Chilling injury was tested by determining the extent the browning area of fruit peel according to the following scale: 1 = no damage; 2 = very light damage; 3 = moderate damage (25% surface affected); 4 = severe damage (26–50% surface affected) and 5 = very severe damage (> 50% surface affected) as described (Lo'ay, 2005). The chilling injury index is then calculated using the following formula:

$$\text{Chilling injury index} = \sum_{1\rightarrow 5}^{5} \frac{(\text{chilling injury level}) * (\text{Number of fruits at the level})}{\text{Total number of fruit}}$$

Color hue angle was estimated on fruit peel (Lo'ay and El Khateeb, 2011). Thereafter, all images were interpreted by using software ImageJ Ver. 1.43u USA to get RGB signals to compute hue angle of fruit peel (Khojastehnazhand et al., 2010).

In term of total chlorophyll determination, 2 g of fruit peel from 6 fruits for each treatment in dipped10 ml N, N-dimethylformamide. Samples were stored at 4oC for 16 h to enable the DMF to extract the pigments from the sample. Finally, samples were centrifuged for 5 min at 16,000 rpm, and then samples were determined total chlorophyll using spectrophotometer (UV–vis spectrophotometer), the total Ch_{ab} content is presented in mg 100 g^{-1} FW (Lo'ay, 2005).

2.3. Lipid peroxidation and protein oxidation and cell membrane permeability

Fruit peel sample 2 g was used to estimate Malondialdehyde (MDA) content determine, and homogenized with 25 mL of 5% (w/v) metaphosphoric acid, 500 μL of 2% (w/v) butylated hydroxytoluene in ethanol. The standard curves established by measuring 1,1,3,3-tetraethyoxypropane (Sigma) in the range 0–2 mM (TBARS) which was equivalent to 0–1 mM malondialdehyde (MDA). 1,1,3,3-Tetraethyoxypropane transformed into MDA during the acid-heating step of the assay. The MDA presented ηM g^{-1} FW (Iturbe-Ormaetxe et al., 1998).

Samples from fruit peel about 2.5 g protein carbonyl group (PCG) was measured (Levine et al., 1994). Fruit peel sample 2.5 g was ground in a mixer with 10 mL of 20 mM K3PO4 buffer (pH 7.0) to extract soluble proteins. Subsequently, the spectrum was measured spectrophotochemically (UV–vis spectrophotometer) against the corresponding blank in case of cured (without sample) samples. The PCG was calculated from the absorbance of the 2,4-dinitrophenylhydrazine measured at 390 nm and assuming an extinction coefficient of 22,000 M-1 cm-1

Samples of 6 peel disks (5 mm diameter), collected with a puncher from the peel tissue. The disks were washed three times with demineralized water and placed in 10 mL 0.4 M mannitol in demineralized water at 24 °C for 3 h (Lo'ay, 2005). The electrical conductivity of the aqueous phase was recorded using conductivity meter, thereafter, samples killed by heating in water bath at 100 °C for 20 min. Once samples cooled to room temperature the conductivity recorded and the relative electrolyte leakage from the uncooked peel samples was calculated as a percentage as follows:

$$\text{IL\%} = \frac{\text{conductivity after 3hours}}{\text{the conductivity of boiling}} \text{x } 100$$

2.4. Total phenol (TP), flavonoids content (FL), and polyphenol oxidase (PPO)

Total phenolic (TP) in banana fruit peel was estimated by a colorimetric assay. Presently, 1 mL of sample was mixed with 1 mL of Folin–Ciocalteu reagent. 3 min Later, 1 mL of saturated Na_2CO_3 solution was added to the mixture and the sample volume was increased up to 10 ml by distilled water. The reaction mixture was kept in the dark for 90 min, thereafter, TP was recorded at 725 nm. Gallic acid was used to measure the standard curve (0.01– 0.4 mM). TP amount was defined as mg of gallic acid per 100 g^{-1} FW (Singleton and Rossi, 1965).

Polyphenol oxidase (PPO) activity was measured in 2.5 g of fruit peel. The soluble proteins were extracted by homogenizing the powder in 5 mL of buffer consisting of 0.1 M sodium phosphate (pH 7.5), 1 mM ethylenediaminetetraacetic acid (EDTA), 10 mM dithiothreitol (DTT), 5 mM ascorbate, 10% (v/v) glycerol, 0.1% (v/v) Triton X-100, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM p-amino benzamidine, and 10 μM leupeptin. The homogenate was centrifuged at 8000 rpm for 5 min at 4 °C and the supernatant was three times as replicates used for measuring PPO enzyme (Lo'ay and Dawood, 2017).

PPO activity was assayed (Huang et al., 2016) by using 100 μL extraction supernatant with 2.9 mL sodium phosphate buffer (0.05 M pH 7.0) containing 10 mM catechol. One unit of PPO activity was expressed as the amount of enzyme causing 0.001 absorbance increase min^{-1} at 398 nm.

2.5. Determinations of $O_2\cdot^-$ production rate and H_2O_2 content

one gram of fruit peel was mixed with 3 mL of 50 mM potassium phosphate buffer (pH 7.8) under cooling at 4 °C containing 1% (w/v) polyvinylpyrrolidone (PVP) and then centrifuged at 10,000 rpm at 4 °C for 15 min. The $O_2\cdot^-$ production was marked by observing the NO2