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Low temperature conditioning combined with methyl jasmonate can reduce chilling injury in bell pepper

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ABSTRACT

Bell pepper is a highly perishable vegetable and chilling injury is a principal physiological factor that negatively impacts the postharvest quality of peppers during transportation and storage. This study parsed the effects of low temperature conditioning (LTC) combined with methyl jasmonate (MeJA) on chilling injury during the storage of bell pepper fruit. The results showed that treatments of LTC combined with MeJA effectively maintained quality of bell pepper, suppressed the development of chilling injury index, delayed increase of malondialdehyde (MDA) content, inhibited the decline of chlorophyll and Vitamin C content, enhanced the activities and relative gene expressions of Peroxidase (POD), Catalase (CAT) and Ascorbate Peroxidase (APX). The results obtained point to treatments LTC combined with MeJA can be used as a useful technology for enhancing tolerance of postharvest chilling injury in bell pepper fruit.

1. Introduction

Bell pepper (*Capsicum annuum* L.) is one of the most important horticultural plants grown in the tropical and sub-tropical regions of the world because of their nutritional value, flavor, and color (Lim et al., 2007). Bell pepper contains many nutritional compounds that have disease preventing and health promoting properties, such as ascorbic acid, antioxidants, vitamin E, carotenoids and other functional ingredients (Lee and Kader, 2000; Marín et al., 2004; Alvarez-Parrilla et al., 2011; Erin et al., 2017). Bell pepper is a highly perishable vegetable and needs appropriate handling and adequate care to maintain postharvest quality (Wang et al., 2016). The main problems that negatively affect the postharvest quality of peppers during transportation, short-term storage and marketing and sales are water loss (Lownds et al., 1993), softening (Pandey and Goswami, 2012) and chilling injury (Paull, 1990).

Up to now, different kinds of techniques have been used to alleviate the chilling injury in harvested bell peppers and extend postharvest quality, including modified atmosphere (Singh et al., 2014), heat shock (Ilic and Fallik, 2008), Intermittent warming (IW) (Biswas et al., 2012; Liu et al., 2015), irradiation with ultraviolet light (Vicente et al., 2005), and chemical treatments like gaseous ozone (Alwi and Ali, 2015; Glowacz et al., 2015), Brassinolide (Wang et al., 2012), Glycine betaine (Wang et al., 2016), Calcium chloride (Rao et al., 2011), 5-aminolevulinic acid (Korkmaz et al., 2010).

Low temperature conditioning (LTC), an effective method that can keeps cold sensitive fruit at temperatures just upon the cold-injured threshold to prompt tolerance to the succeeding lower temperatures (Woolf et al., 2003), has been reported to relieve chilling injury symptoms in lots of fruit and vegetables such as peach (Jin et al., 2009), pear (Wang et al., 2017a), kiwifruit (Yang et al., 2013), mango (Zhang et al., 2017) and zucchini (Carvajal et al., 2015). Methyl jasmonate (MeJA), exists naturally in numerous higher plants and reported as important regulation factors involved in many biological processes (Creelman and Mullet, 1997; Wang et al., 2015). In recent research, MeJA has been used to prevent the occurrence and development of chilling injury symptoms in a number of horticultural crops, including lemon, loquat fruit and tomato fruit (Wang et al., 2015; Siboza and

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Bertling, 2013; Cao et al., 2009).

Although several methods were used to maintain the postharvest quality of bell pepper, the effects of the combination of low temperature conditioning (LTC) and Methyl jasmonate (MeJA) were still unclear yet. Therefore, the aim of the study was to explore the effects of LTC treatment and MeJA on alleviating chilling injury in bell pepper fruit. In order to reach this goal, we examined and measured the MDA concentration, chilling injury index, chlorophyll content, Vitamin C content and antioxidant related enzymes such as ascorbate peroxidase (APX), peroxidase (POD) and catalase (CAT) as well as their encoding genes expression levels.

2. Materials and methods

2.1. Plant material and treatments

Bell pepper fruit (Champion) were harvested at commercial maturity from an organic vegetable farm in xiaotangshan, located in District Changping of Beijing, China, and directly transported to the laboratory. The samples were selected for uniform maturity and size, freedom from defects, and bright green color. The fruit were randomly divided into two groups (a) control, (b) low temperature conditioning (LTC) with methyl jasmonate (MeJA, Sigma, USA). The suitable concentration of methyl jasmonate (10 μmol L^{-1}) was determined by pre-experiment (no data shown). The treatments were as follows:

(a) Samples were directly stored at 4 °C (85%–90% relative humidity).
(b) Samples (dipping in 10 μmol L^{-1} MeJA for 10 min) were firstly stored at 13 °C for 2 d and then stored at 4 °C.

All fruit were packed in plastic trays and covered with polyethylene film (0.04 mm, the permeability rate for CO_2 was 1.175×10^4 mL m^{-2} d^{-1} atm^{-1}, the rate for O_2 was 3.575×10^3 mL m^{-2} d^{-1} atm^{-1}, and the moisture permeation rate was 2.76 g m^{-2} d^{-1}). Samples were taken and immediately analyzed or frozen with liquid nitrogen and stored at −80 °C for later analysis. The bell pepper fruit samples were used to determine the chilling injury index, chlorophyll, Vc content and MDA content, the activities of POD, CAT and APX and the genes relative expression. Each treatment had three replicates and every replicates were confirmed from eight fruits.

2.2. Chilling injury index

Symptoms of chilling injury (CI) in pepper manifest as surface pitting, seed browning, shriveling caused by moisture loss, tissue discoloration, especially in the calyx (Concellón et al., 2005). The CI index was calculated over 24 fruit using a 6 stage scale: 0 = no damage (no signs of CI); 1 = trace (0% < CI ≤ 10%); 2 = slight damage (10% < CI ≤ 20%); 3 = regular damage (20% < CI ≤ 30%); 4 = moderate damage (30% < CI ≤ 50%); 5 = severe damage (CI ≥ 50%). The CI index was estimated by Wang et al. (2016) as follows: CI index (%) = Σ [(CI level) × (number of fruit at this level)] / (highest level × number of total fruit) × 100.

2.3. Chlorophyll and vc content

Chlorophyll content was determined according to the method of Shi et al. (2016) with a minor modification. The frozen sample of 1 g was homogenized in cold acetone: ethanol (2: 1) (− 20 °C) using a mortar. The homogenate was placed at −20 °C for 30 min and then centrifuged at 13,000 × *g* for 5 min at 4 °C. The supernatant was used to determine chlorophyll content. The absorbance was recorded at 645 nm and 663 nm in a UV-spectrophotometer (Shimadzu UV-1800, Japan) with proper calibration.

Vitamin C content was estimated as described by Jiang et al. (2010) with moderate modification. Frozen sample (1.0 g) with 5 mL of extract solution was centrifuged at 13,000 × g for 20 min at 4 °C. The extract solution was made up of 0.05 M oxalic acid and 0.2 mM ethylenediaminetetraacetic acid (EDTA). The reaction system, including 2 mL of supernatant, 3 mL of oxalic acid, 1 mL of 5% sulfuric acid, 0.5 mL of metaphosphoric acid and 2 mL of 5% (w/v) ammonium molybdate, was heated at 80 °C for 10 min. Thereafter the mixed solution was cooled to the room temperature (25 °C) quickly and diluted with sterile deionized water to 10 mL. The vitamin C content was measured at 760 nm in a UV-spectrophotometer (Shimadzu UV-1800, Japan) with proper calibration and was recorded as g kg^{-1}.

2.4. Malondialdehyde (MDA) assay

The content of MDA was determined using the thiobarbituric acid (TBA) method according to the described by Zhang et al. (2017). The frozen samples were homogenized in trichloroacetic acid (TCA, 100 g L^{-1}), and then centrifuged at 13,000 × *g* for 30 min at 4 °C. The supernatant of 2 mL was mixed with 2 mL TBA (0.67%), incubated in boiling water for 20 min. Then the mixture was used to measure the MDA content, the absorbance was at 532 nm, 450 nm and 600 nm in a UV-spectrophotometer (Shimadzu UV-1800, Japan) with proper calibration. MDA content was calculated using the formula, (mass of MDA per mass of sample, mmol kg-1) = [6.45 × (OD532-OD600)-0.56 × OD450] × Vt × Vr/(Vs × m), where Vt, Vr and Vs are the total volume of the extract solution, the total volume of the reaction mixture solution, and the volume of the extract solution contained in the reaction mixture solution, respectively, and m is the mass of the sample.

2.5. POD, CAT and APX activities

About 50 g frozen sample per group were grounded in a mill. The resulting powder was used to enzyme assay. For peroxidase (POD) about 1 g frozen powder was suspended in 5 mL 0.1 mol L^{-1} phosphate buffer (pH 7.8) containing polyvinylpyrrolidone (0.5%), then centrifuged at 13,000 × *g* for 20 min at 4 °C. The extract was measured based on the increase of guaiacol oxidation in absorbance at 470 nm by H_2O_2 in a UV-spectrophotometer (Shimadzu UV-1800, Japan) with proper calibration. For catalase (CAT), the enzyme extract was prepared as POD, and the activity was determined based on the decline of H_2O_2 consumption in absorbance at 240 nm in a UV-spectrophotometer (Shimadzu UV-1800, Japan) with proper calibration. For ascorbate peroxidase (APX), the activity was measured based on the reduction in absorbance of vitamin C at 290 nm (Shi et al., 2016). The reaction system was made up of 0.1 mL of above supernatant, 0.3 mL of 2 mM H_2O_2 and 2.6 mL of PBS. The PBS solution contained 0.1 mM EDTA and 0.5 mM ASA. The assay is based on the decrease in absorbance at 290 nm as ascorbate is oxidized. One unit of APX was defined as the amount required to oxidize 1 mol L^{-1} of ascorbate in one minute.

2.6. RNA extraction and RT-qPCR analysis

The frozen samples per group were ground in liquid nitrogen, and ~0.1 g of the resulting powder was mixed with 1 mL Trizol reagent (Invitrogen, US) to extract the total RNA. The total RNA was reversed transcription using SuperScript® RT according to manufactures instruction (Invitrogen, US). Then the cDNA was used as a template for qPCR. The qPCR was performed with a total volume of 20 μL, including 0.8 μL cDNA template, 0.4 μL each primer (10 mM), 10 μL 2 × UltraSBR Mixture (Kangwei, China) and 8.4 μL RNase-Free water on a Roche LightCycler® 480 machine (Roche, Bazel, Switzerland) according to the manufacturer's instructions. The primers were designed using Primer 5.0 (Applied Biosystem). Primers specific to the following genes were used: *UBI-3*, forward: TGTCCATCTGCTCTCTGTTG (5′- -3′), reverse: CACCCCAAGCACAATAAGAC (5′- -3′); *POD* (accession no. FJ596178), forward: TTCCTCCTCCTACTTCTAACCT (5′ - -3′), reverse: AACAGAC CTCTTTTGCTCACTA (5′- -3′); CAT (accession no. AF227952), forward: