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Amelioration of postharvest chilling injury in sweet pepper by glycine betaine

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ABSTRACT

The reduction in the quality of harvested green peppers due to physiological disorders resulting from chilling injury (CI) results in significant economic losses. In the current study, the ability of glycine betaine (GB, a stress-ameliorating compound) to reduce CI in sweet pepper was investigated. GB at 1 mmol L^{-1} significantly reduced CI in sweet pepper fruit during a sixteen-day period of storage at 3 °C followed by an additional 3 days at 20 °C. CI index, membrane permeability, chlorophyll content, ascorbic acid (ASA), and malondialdehyde (MDA) were assessed. The activities of a variety of antioxidant enzymes, including peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), and lipoxygenase (LOX) were determined along with transcript levels. Results indicated that amelioration of CI in sweet pepper by GB was associated with a reduction in cellular leakage, MDA content, and lipid peroxidation in sweet pepper. Both gene expression and enzyme activity of POD, CAT, APX, and GR in GB-treated fruit were higher than levels in control fruit. These data suggest that GB increases chilling tolerance in pepper fruit by inducing antioxidant gene expression and enzyme activity, thus alleviating the potential injury resulting from CI.

1. Introduction

Pepper (*Capsicum annuum L.*) has many nutritional compounds that are found to have disease preventing and health promoting properties, such as vitamin C (Lee and Kader, 2000; Alvarez-Parrilla et al., 2011). Low temperature storage is a common method used to extend the postharvest quality of fruits and vegetables, allowing them to be transported to distant markets and remain available to consumers for an extended period. Pepper fruit, however, are very susceptible to chilling injury (CI) at temperatures below 7–10 °C, depending on the cultivar, stage of maturity, and duration of exposure to low temperature (Mercier et al., 2001; Lim et al., 2007). The main symptoms of CI are sunken lesions and pitting on the fruit surface (Fung et al., 2004). CI limits the storage life and leads to significant degradation of produce quality. Different methods have been used to alleviate CI in harvested peppers and extend postharvest quality, including modified atmosphere (Singh et al., 2014), UV-C (Vicente et al., 2005), hot water treatment (Gonzalez-Aguilar et al., 2000), and chemical treatments like diphenylamine (Purvis, 2002), 5-aminolevulinic acid (Korkmaz et al., 2010), salicylates, and jasmonates (Fung et al., 2004).

Glycine betaine (GB, *N,N,N*-trimethylglycine), is a common compatible solute, that plays a crucial role in osmotic adjustment in various organisms, including bacteria, fungi, algae, plants, animals, and humans (Rhodes and Hanson, 1993; de Zwart et al., 2003; Craig, 2004). Exogenous application of GB has been demonstrated to improve stress tolerance, growth, and survival in a wide variety of plants subjected to various conditions of abiotic stress (Diaz-Zorita et al., 2001; Ashraf and Foolad, 2007; Chen and Murata, 2008). Some reports have highlighted the effect of exogenously applied GB on chilling injury. Park et al. (2006) reported that exogenous application of GB improved chilling tolerance in tomato (*Lycopersicon esculentum*). Nayyar et al. (2005) found that GB reduced CI in chickpea (*Cicer arietinum*), increased cellular respiration and improved the final yield. Tolerance to low temperatures was also improved by exogenous application of GB in other plant species, such as maize (Farooq et al., 2008), strawberry (Rajashekar et al., 1999), and tobacco (Holmstrōm et al., 2000). To the best of our knowledge, however, little information is available pertaining to the effect of GB on chilling tolerance in pepper.

GB has been shown to protect the structure of proteins and macromolecules, as well as the integrity of membranes directly

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against abiotic stresses (Rajashekar et al., 1999; Niu et al., 2015). GB has also been found to enhance antioxidant enzyme activity such as ascorbate peroxidase (APX), catalase (CAT), and superoxide dismutase (SOD) under stress conditions (Park et al., 2006; Ahmad et al., 2013). The objectives of the present study were to evaluate the effect of GB on CI, fruit quality, and the antioxidant enzymes present in harvested green peppers, including peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), and lipoxygenase (LOX). The levels of gene expression and the levels of enzyme activity of these proteins were measured.

2. Materials and methods

2.1. Pepper fruit and experimental treatments

Green bell peppers (*Capsicum annuum* L. cv. Mutianqiushuo) were harvested 46 d after blooming during June to August in 2011 and 2012, from an organic orchard in Miyun, Beijing, China, and immediately transported to the laboratory. Two harvests were conducted during June/August in 2011 and one during July in 2012. Pepper fruit used in the experiments were selected for bright green color, uniform size, and freedom from defects, and then randomly divided into two lots: fruit that were dipped in water only (control) and those dipped in 1.0 mmol L^{-1} GB for 20 min. This GB concentration was selected as optimal based on preliminary experiments utilizing 1, 5, 10, 20 mmol L^{-1} (data not shown). The surface of the fruit was then dried in cool air and the fruit were stored at 3 °C. After 16 days of cold storage, fruit were moved to a controlled environment chamber and maintained at 20 °C for 3 days. Fruit CI index was measured every day and membrane permeability was assessed every 2 days. Samples of sweet pepper flesh (approximately 80 g) were collected and frozen in liquid nitrogen and stored at −80 °C for measurements of chlorophyll, ascorbic acid (ASA), and malondialdehyde (MDA), as well as LOX, POD, CAT, APX and GR gene expression and enzyme activity.

2.2. CI index

Symptoms of CI include sunken lesions and pitting on the fruit surface. The CI index was estimated based on the percentage of total fruit surface area containing sunken lesions or surface pitting (Fung et al., 2004): Grade 0 (no signs of CI), Grade 1 (<25% of the fruit area showing CI), Grade 2 (25–50% of the fruit area showing CI) and Grade 3 (>50% of the fruit area showing CI). The CI index was expressed as: CI index (%) = $\sum$[(CI level) × (number of fruit at this level)]/(highest level × total number of fruit in the treatment) × 100. Three replicates for each treatment were performed, and each replicate contained 30 fruit.

2.3. Determination of chlorophyll and vitamin C

Total chlorophyll content was measured on a fresh weight basis using the method of Zhang et al. (2008). Five grams of green pepper were homogenized in 20 mL of 80% acetone with a tissue homogenizer at 2000 × *g* for 30 s. The homogenate was then filtered through filter paper and centrifuged at 2000 × g for 15 min. The absorbance of the filtered homogenate was measured at 645 and 663 nm in a UV-spectrophotometer (Shimadzu UV-1800, Japan).

Vitamin C content was determined using the method of Li (2000), with a slight modification. Pepper fruit tissue (1.0 g) was ground in 15 mL of 0.05 mol L^{-1} oxalic acid-EDTA. The mixture was centrifuged at 13,000 × *g* for 30 min and the supernatant was collected. Subsequently, 2 mL of supernatant was mixed with 3 mL oxalic acid-EDTA, 0.5 mL metaphosphoric acid-acetic acid, 1 mL 5% H_2SO_4, and 2 mL 5% ammonium molybdate solution. The mixture was left to settle at 80 °C for 10 min, cooled to room temperature, and then absorbance was read at 760 nm, using ascorbic acid as a standard. Results were reported as mass of ascorbic acid per fresh weight mass of tissue, g kg^{-1}. Each treatment consisted of three replicates and the experiment was repeated three times.

2.4. Membrane permeability and lipid peroxidation

Membrane permeability, determined by electrolyte leakage, was estimated using the method described by Mao et al. (2007). Discs of fruit tissue were removed from the pepper fruit with a 9 mm diameter cork borer. They were briefly rinsed twice with deionized water and blotted dry on a filter paper. Twelve pieces were put into 20 mL of deionized water in a 35-mL test tube and shaken for 20 min. Conductivity was measured with a conductivity meter (Model EC 215, HANNA Instruments). After measuring initial conductivity, tubes were then boiled in water for 20 min, cooled to room temperature, and conductivity was measured once again. Electrolyte leakage was expressed as a percentage of the initial electrolyte leakage. Each treatment consisted of three replicates and the experiment was repeated three times.

2.4.1. MDA analysis

The MDA concentration was measured according to the method of Meng et al. (2012). Tissue samples (3.0 g) were homogenized in 15 mL of 5% (w/v) trichloroacetic acid (TCA) and centrifuged at 13,000 × *g* for 30 min. The supernatant (2.0 mL) was mixed with 2.0 mL of 0.67% thiobarbituric acid (TBA), heated at 100 °C for 30 min, quickly cooled, and then centrifuged at 10,000 × *g* for 10 min. The supernatant was collected and absorbance at 532 nm, 600 nm and 450 nm was measured in a spectrophotometer (Shimadzu UV-1800, Japan). MDA content was calculated using the formula, (mass of MDA per mass of sample, mmol kg^{-1}) = $[6.45 \times (OD_{532} - OD_{600}) - 0.56 \times OD_{450}] \times V_t \times V_r/(V_s \times m)$, where V_t, V_r and V_s 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.

2.4.2. LOX analysis

Two grams of fruit tissue was ground in 6 mL of 0.1 mmol L^{-1} ice-cold, sodium phosphate buffer (pH 6.8). The extracts were then homogenized and centrifuged at 13,000 × *g* for 30 min at 4 °C. The supernatants were used for the enzyme assays. The substrate used in the enzyme assay was made by mixing 0.5 mL linoleic acid, 0.25 mL Tween-20, and 10 mL deionized water. This mixture was clarified with 1 mol L^{-1} NaOH, and the resulting solution was diluted to 100 mL with deionized water. LOX activity was assayed using the method of Gonzalez-Aguilar et al. (2004). LOX activity was assayed by mixing 2.75 mL of 0.1 mmol L^{-1} acetic acid-sodium acetate buffer (pH 5.5) with 0.05 mL of the substrate solution. The resulting mixture was kept at a temperature below 30 °C for 10 min, after which 0.2 mL of the enzyme extract was added. The change in absorbance at 234 nm was measured in a spectrophotometer (Shimadzu UV-1800, Japan). One unit of LOX was defined as the amount of enzyme that caused an increase in the absorbance at 234 nm of 0.01 unit in one minute.

2.5. Antioxidant enzyme activity

For POD and CAT, 2 g (fresh weight) of fruit tissue was ground in 6 mL of 0.1 M ice-cold, sodium phosphate buffer (pH 7.8). For the analysis of APX, fruit tissue (2 g) was ground in 6 mL of 0.1 mol L^{-1} ice-cold, potassium phosphate buffer (pH 7.5), containing 0.1 mmol L^{-1} EDTA, 1 mmol L^{-1} ascorbic acid and 1% polyvinyl-pyrrolidone (PVP). For GR, 2 g of fruit tissue was ground in 6 mL of 0.1 mol L^{-1}