Scientia Horticulturae 237 (2018) 311–317

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Exogenous nitric oxide treatment impacts antioxidant response and alleviates chilling injuries in longkong pericarp

Karthikeyan Venkatachalam

Department of Food Technology, Faculty of Science and Industrial Technology, Prince of Songkla University, Makhamtia, Muang, Surat Thani 84000, Thailand

ARTICLE INFO

Keywords:
Longkong
Pericarp
Chilling injury
Nitric oxide
SNP
Browning
ROS
Antioxidant enzymes

ABSTRACT

Nitric oxide (NO) is an important exogenous signaling molecule used to protect plants against abiotic stress-induced damages. Sodium nitroprusside (SNP) is a key exogenously applied NO donor to plants. Longkong fruit is susceptible to chilling injuries (CI) during prolonged low-temperature stress and causes the higher economic losses. The present study was aimed to control the CI and assess the ROS production and antioxidant responses in longkong pericarp by treating with SNP at various concentrations (0, 10, 20, 30 mM/L). The fruit was immersed in SNP solution for 20 min at room temperature and was then stored at 13 °C and at an 85% RH for 18 days. Chilling injury index, electrolytic leakage, MDA content, $O_2\cdot^-$ and H_2O_2 contents, and activities of the enzymes such as phenylalanine lyase (PAL), polyphenol oxidase (PPO), peroxidase (POD), superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (GPX) were measured at 3-day intervals. The results show that increasing the SNP concentration effectively increased the NO level and decreased the chilling injuries to longkong fruit pericarp. Treatment at 30 mM/L controlled the CI index, electrolytic leakage and regulated the production of MDA, $O_2\cdot^-$ and H_2O_2. NO treated fruit pericarp had lower activities of browning related enzymes (PAL and PPO) and higher activities of antioxidant enzymes (POD, SOD, CAT, and GPX) than the control. The SNP treatment of longkong fruit could significantly control the chilling injuries and prolong the resistance against chilling stress.

1. Introduction

Longkong is a unique tropical and non-climacteric fruit with abundant nutritional benefits. It has a very short shelf life (3–7 days) at ambient temperature, due to rapid pericarp browning and chemical and microbial deterioration. Its pericarp has sensitive epidermal trichomes that activate polyphenol oxidase (PPO) enzyme to produce browning pigments upon external stress. In addition, rapid weight loss also promotes the browning on the fruit pericarp (Venkatachalam, 2016; Venkatachalam and Meenune, 2015). Low-temperature storage is widely practiced to control the quality deterioration of various plant products. It reduces the enzymatic activities and respiration, and consequently also reduces the weight loss. Normally, plants have cold-induced genes that help them adapt when exposed to a cold environment (Thomashow, 2001). However, chilling injuries (CI) remain a major drawback of low-temperature techniques with tropical crops, as these are naturally poor in adapting to cold. Normally, CI in plants damages and oxidizes the membrane phospholipids, and gelates the saturated lipids in the membrane, further inducing leakage of ions from the cells (Gwanpua et al., 2018). Furthermore, also reactive oxygen species (ROS) indicate CI in plants and participate in the cell destruction associated with CI.

Longkong fruit stored at 13 °C for two weeks tend to have chilling injuries, especially surface browning, ion leakage, pitting and scalding. The economic value of longkong fruit mainly depends on the external appearance, and the chilling injuries during postharvest operations will negatively impact the appearance (Venkatachalam, 2016). Nitric oxide (NO) plays various regulatory roles in fruits, particularly as a signaling molecule protecting plants against several stress-induced adverse biochemical reactions (Fan et al., 2015). Studies have reported that NO affecting the responses to biotic and abiotic stresses, and particularly protecting against oxidative stress by ROS, by limiting the activity of lipoxygenase (LOX) enzyme (Maccarrone et al., 1996; Yang et al., 2011). NO regulates the CI symptoms in plants by strengthening (lignification) the subcellular compartments, by reducing lipid oxidation, and by activating antioxidant-related enzymes (Yang et al., 2011). Duan et al. (2007) reported sodium nitroprusside (SNP) as suitable NO donor for treating and protecting fruits against oxidative stresses. In several studies, the application of NO and/or NO donor has alleviated CI symptoms in plants (Barman et al., 2014; Ghorbani et al., 2017; Lichanporn and Techavuthiporn, 2013). Lichanporn and Techavuthiporn (2013) observed that the exogenous application of

E-mail address: karthikeyan.v@psu.ac.th.

https://doi.org/10.1016/j.scienta.2018.04.032
Received 1 February 2018; Received in revised form 14 April 2018; Accepted 16 April 2018

nitrous oxide reduced the browning of longkong fruit during cold storage (13 °C). Furthermore, the application of NO has possessed a strong inhibitory effect against the microbial activities in various fruits (Liu et al., 2012; Lalithya et al., 2017). The present study examined the protective effect of exogenous application of SNP as a NO donor, at various concentrations, on controlling the chilling injuries of longkong fruit pericarp, assessing ROS and antioxidant enzyme activities.

2. Materials and methods

2.1. Plant material and treatments

Longkong fruit (*Agalaia dookkoo* Griff) at a commercial maturity level were freshly obtained from a garden in Surat Thani province of Thailand and were transported to the laboratory within 4 h. Fruit for the experiments was screened based on color, uniform size (~3–4 cm in diameter) and has no apparent damage. The fruit was separated from racemes and washed thoroughly with clean tap water, and thereafter subjected to the treatments. There were four treatment groups (*control (no SNP), SNP 10* mM/L, *SNP 20* mM/L *and SNP 30* mM/L), and treatment was by immersion into a SNP solution for 20 min at room temperature. After treatment, the fruit was air-dried and then kept (approx. 500 g) in perforated polyethylene bags during storage at 13 °C and at an 85% relative humidity. Storage was terminated when severe browning and/or visible microbial growth on the pericarp surface was observed. At 3-day intervals, fruit pericarp was sampled from the stored fruit for analyses after it moved from cold storage and kept for 10 h at room temperature.

2.2. Analyses

2.2.1. Determination of NO content in the fruit pericarp

NO concentration in the pericarp was determined in accordance with the method of Murphy and Noack (1994). A 2 g sample of pericarp tissue was homogenized using 20 ml of extraction buffer (50 mM HEPES-KOH buffer (pH 7.6), 0.5 M sorbitol, 5 mM ethylene diamine tetra acetic acid (EDTA), 5 mM ethylene glycol bis (2-aminoethyl ether) tetra acetic acid, 10 μM flavin adenine dinucleotide (FAD), 10 mM dithiothreitol and 10 μM (p-amidinophenyl) methyl sulfonyl fluoride). After extraction, the homogenate was centrifuged at 10,000 × *g* for 15 min at 4 °C. Then, the supernatant was collected and incubated with 100 U of CAT and 100 U of SOD for 10 min to remove the ROS. The supernatant was incubated with oxyhemoglobin (5 mM) for 15 min at room temperature, and then the conversion of oxyhemoglobin to methemoglobin was measured via absorbance at 421 nm using a spectrophotometer. NO content was calculated using the standard curve, and the results are expressed in μmol g^{-1} fresh weight (FW).

2.2.2. Determination of chilling injury (CI) index

The symptoms of CI in longkong fruit pericarp included browning, scalding, and pitting, and were measured in sixty individual fruit. Pericarp was assessed for CI index after it has been transferred from cold storage to room temperature storage for 10 h. CI assessment was done using the following visual appearance scoring scale: 0, no symptoms (good quality); 1, slight symptoms; 2, < 1/4 symptoms; 3, 1/4 – 1/2 symptoms; 4, > 1/2 browning (poor quality). The CI index was determined as:

Σ (symptom scale x percentage of corresponding fruit within each class)

2.2.3. Determination of relative ion leakage and MDA content

Relative electrolyte leakage was determined in accordance with the method of Chen et al. (2008). Pericarp was cut into discs using 10 mm diameter stainless steel borer. The discs were thoroughly washed in distilled water three times and wiped using absorbent paper. After that, the discs were placed in test tubes, and 20 ml of distilled water was added, before incubation at 25 °C for 30 min in a water bath shaker. The electrical conductivity (L_0) was measured for the samples after incubation, and then they were incubated at 100 °C for 20 min, after which the electrical conductivity (L_1) was measured again. The rate of electrolyte leakage was calculated using the following formula.

$$\text{Electrolyte leakage (\%)} = (L_0/L_1) \times 100$$

MDA content in fruit pericarp was measured in accordance with the method of Wang et al. (2013). Fruit pericarp (2 g) was homogenized with 8 ml of phosphate buffer (50 mM, pH 7.8). Then, the homogenate was centrifuged at 12,000 g for 20 min at 4 °C. The supernatant (2 ml) was transferred to a test tube and 3 ml of 5 g/l TBA in 100 g/l TCA was added. Then, the reaction mixture was mixed well and held in a boiling water bath for 15 min, cooled rapidly under running cold water, and centrifuged at 12,000 × *g* for 10 min to clarify the solution that was measured for absorbance at 532, 600 and 450 nm. The MDA concentration was calculated using the following formula:

$$\text{MDA content (nmol/g)} = [6.45(A_{532}\text{-}A_{600}) - 0.56A_{450}]*V_tV_r/(V_sm)$$

Vt is the total volume of the extract solution, *Vr* is the total volume of the reaction mixture solution, *Vs.* is the volume of extract solution from the reaction mixture solution, and *m* is mass of the sample. The content of MDA is expressed in nmol g^{-1} FW.

2.2.4. Determination of $O_2\cdot^-$ production rate and H_2O_2 content

Superoxide anion ($O_2\cdot^-$) production rate in longkong pericarp was measured in accordance with the method of Wang and Luo (1990). Pericarp tissue (4 g) was homogenized with 12 ml of cold potassium phosphate buffer (50 mmol/l, pH 7.8) containing 1% (w/v) polyvinylpyrrolidone, and was followed by centrifugation of the homogenate at 5000 × *g* for 15 min at 4 °C. The supernatant (1 ml) was collected and mixed with 0.9 ml of potassium phosphate buffer (50 mmol/l, pH 7.8) and 0.1 ml of hydroxylamine hydrochloride (10 mmol/l) and then incubated for 30 min at 25 °C. The incubated solution (1 ml) was added to 1 ml of 3-aminobenzenesulphonic acid (17 mmol/l) and 1 ml of 1-naphthylamine (7 mmol/l) and then further incubated for 20 min at 25 °C. The absorbance was observed at 530 nm. A standard curve with NO_2 was used to identify the $O_2\cdot^-$ production rate from the reaction equation of $O_2\cdot^-$ with hydroxylamine. The $O_2\cdot^-$ production rate was expressed as nmol g^{-1} min^{-1} FW.

Hydrogen peroxide (H_2O_2) content in the pericarp was measured following the method of Patterson et al. (1984). Pericarp tissue (2 g) was homogenized with 15 ml of acetone under cold environment, and then an aliquot was centrifuged at 6000 × *g* for 15 min at 4 °C. Then, supernatant (1 ml) was collected and mixed with 0.1 ml of titanium sulphate (5%) and 0.2 ml of ammonia, and then the reaction mixture was centrifuged at 6000 × *g* for 10 min at 4 °C. After centrifugation, the pellet was collected and mixed with 3 ml of H_2SO_4 (10% v/v) and again centrifuged for 10 min at 5000 g. The supernatant was collected for measuring the absorbance at 410 nm. H_2O_2 content in the sample was calculated using the standard curve for H_2O_2, and the results are expressed in μ mol g^{-1} FW.

2.2.5. Enzyme assay

2.2.5.1. Extraction and browning related enzymes. For phenylalanine ammonia lyase (PAL) enzyme extraction, pericarp tissue (2 g) was homogenized in 20 ml of sodium borate buffer (0.1 M, pH 8.0) containing 0.2 g PVP, 5 mM MCP and 2 mM EDTA. After homogenization, the extract was centrifuged at 19,000 × *g* for 20 min at 4 °C. The supernatant was collected and used for PAL activity determination by the method of Jiang and Joyce (2003). One unit of enzyme activity was defined as increasing the absorbance by 0.01 per