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UV-B irradiation alleviates the deterioration of cold-stored mangoes by enhancing endogenous nitric oxide levels

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ABSTRACT

Effects of UV-B radiation on chilling injury, ripening and endogenous nitric oxide (NO) levels in mango fruit were evaluated. Chilling injury index, ion leakage, and malondialdehyde (MDA) content of the fruit pretreated with 5 kJ m^{-2} UV-B for 4 h were significantly lower than those of the control during fruit ripening at ambient temperature following cold storage at 6 °C for 10 days. Fruit firmness of the mangoes irradiated with UV-B was significantly higher than the control during the ripening period. Endogenous NO levels of the UV-B-irradiated fruit were rapidly increased after UV-B treatment. Pre-treatment of mangoes with the NO specific scavenger, not only abolished UV-B-triggered NO accumulation, but also suppressed the UV-B-reduced chilling injury, oxidative damage, and ripening delay of the fruit. Together, the results suggest that UV-B treatment may enhance chilling tolerance and delay fruit ripening of mangoes by triggering endogenous NO generation in the fruit.

1. Introduction

Cold storage of fruit is widely used to extend postharvest storage life. However, many tropical and subtropical fruit are extremely sensitive to low temperature, and this limits the maintenance of postharvest quality. Mangoes, like many other tropical and subtropical fruits, are susceptible to chilling injury when stored below 10 °C (Chaplin et al., 1991). The most common visual symptoms of chilling injury in mango fruit are dark, scald-like discolouration, pitting or sunken lesions on the peel and increased susceptibility to postharvest rot (Chaplin et al., 1991; Singh & Singh, 2012). The development of these chilling disorders greatly reduces consumer acceptance and increases postharvest losses of the fruit.

Plants from temperate and cold climates have evolved a mechanism to enhance their chilling tolerance when exposed to low temperature. Induction of chilling tolerance by chemical treatments or by biotic and abiotic stress is becoming a potential approach for protecting harvested fruit from chilling injury. Previous studies have reported that pre-storage exposure of mangoes and peaches to UV-C irradiation significantly reduces chilling injury and maintains better visual appearance after cold storage (González-Aguilar, Wang, & Buta, 2004; González-Aguilar, Wang, Buta, & Krizek, 2001). In comparison with UV-C, UV-B is a less harmful waveband. Studies showed that postharvest UV-B irradiation was effective in maintaining certain qualities in tomato and lime fruit, such as a high level of firmness and delay of colour development during storage at 14 °C and 25 °C (Castagna et al., 2013; Kaewsuksaeng, Urano, Aiamla-or, Shigyo, & Yamauchi, 2011; Liu et al., 2011), suggesting that UV-B may represent a new potential tool for alleviating quality deterioration in postharvested fruit during storage. However, so far, little information is available, in the literature, about the effect of postharvest UV-B irradiation on chilling injury of cold-stored mango fruit.

Activation of endogenous signalling has been well documented to be one of the mechanisms involved in acquisition of chilling tolerance in plants. Nitric oxide (NO) is an important signal molecule in plants (Neill, Desikan, & Hangcock, 2003). Genetic evidence from *Arabidopsis thaliana* mutant (nia1/nia2) has shown that endogenous NO production is essential for cold tolerance in the plants (Cantrel et al., 2011; Gupta, Hincha, & Mur, 2011). Recent studies have indicated that the chilling injury of mangoes pre-fumigated with NO was significantly less than that of a control after cold

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storage (Zaharah & Singh, 2011a), showing that NO may play an important role in modulating chilling tolerance in the cold-stored mangoes. Furthermore, it has been reported that a short period of UV-B irradiation markedly increases endogenous NO levels in plants (Zhang, Dong, Jin, Sun, & Xu, 2011). Based on these previous reports, we hypothesised that pre-storage UV-B treatment might enhance chilling tolerance of the cold-stored mango fruit by triggering endogenous NO production.

The objective of this work is to check the hypothesis by assaying the influence of UV-B irradiation on chilling tolerance and endogenous NO level of cold-stored mangoes and evaluating the possible role of UV-B-induced endogenous NO production in maintaining the postharvest quality of the fruit.

2. Materials and methods

2.1. Plant material and experimental design

Hard mature green mangoes (*Mangifera indica*, L. cv. Tainong) were obtained from a commercial orchard at Hainan, China. All the fruit for the experiments were collected in one harvest. Then, 720 homogeneous fruit, with uniform size and colour and free of visual blemishes and disease, were selected and randomly divided into 4 groups for the following treatments: control and irradiation with UV-B at 1, 5 and 10 kJ m^{-2} for 4 h at ambient temperature (29 °C). UV-B irradiation was supplied by fluorescent lamps (40 W/12, Beijing Lighting Research Institute, Beijing, China). The lamps were placed directly above the fruit and filtered with either 0.13 mm thick cellulose diacetate (transmission down to 290 nm) for UV-B irradiance or 0.13 mm polyester plastic films (absorbing all radiation <320 nm) which acted as the control. The desired irradiation was obtained by changing the distance between the lamps and the fruit (about 50 cm). The spectral irradiance from the lamps was determined with an Optronics Model 720 (Beijing Normal University Optronics Factory, Beijing, China) spectroradiometer. Following treatments, both control and UV-B-treated fruit were stored at 6 °C and approx. 95% relative humidity in darkness in a temperature-controlled chamber for up to 10 days. Then, the UV-B-irradiated and control fruit were transferred from cold storage and allowed to ripen at ambient temperature (29 ± 5 °C, relative humidity 51.7 ± 8.6%). During the ripening period, 3 replicates of 10 fruit were collected to determine Chilling injury index, fruit firmness, ion leakage levels, and MDA contents.

For the effects of UV-B induction time on chilling injury in mangoes, 720 homogeneous fruit were randomly divided into 4 groups for the following treatments: control and irradiation with 5 kJ m^{-2} UV-B for 1, 4, and 8 h at ambient temperature (29 °C). After treatment, fruit were stored and ripened as in the procedures described above. Chilling injury index was determined every day during the ripening period.

2.2. Effect of an NO-scavenger on UV-B-induced cold tolerance and ripening delay

The NO-specific scavenger 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO) has been widely used to suppress NO accumulation in plants (Delledonne, Zeier, Marocco, & Lamb, 2001). Therefore, cPTIO was used in this work to diminish endogenous NO accumulation in mango fruit. For the effects of the NO-scavenger on UV-B-induced chilling tolerance and ripening delay, fruit were selected and randomly divided into 4 groups for the following treatments: control, 5 kJ m^{-2} UV-B, 5 kJ m^{-2} UV-B plus 0.5 mM cPTIO (cPTIO + UV-B), and 0.5 mM cPTIO (cPTIO). Treatments were performed according to the procedures described above. Fruit were treated with 0.5 mM cPTIO 40 min before UV-B radiation. Chilling injury index, fruit firmness, ion leakage levels, and MDA contents were determined daily during the ripening period. The concentration of cPTIO was chosen according to reports in the literature and in our preliminary experiments (Delledonne et al., 2001; Xu, Dong, & Zhu, 2005).

2.3. Measurement of chilling injury index

Chilling injury index was evaluated using a scale of 0–4, based on the common visual symptoms of dark colouration and prominence of lenticels on the skin area of affected fruit as follows: 0 = no damage; 1 = very light damage; 2 = light damage; 3 = moderate damage; 4 = severe damage, as previously described by Nair and Singh (2004). The chilling injury index was calculated by the following formula: $[\Sigma(A \times B)/5C$, in which A presents the injury score of individual fruit, B is the number of fruit affected, 5 is the total number of scores (0–4) used and C is the total number of fruit recorded.

2.4. Quantification of NO by haemoglobin assay

NO accumulation was assayed and calculated by following the conversion of oxyhaemoglobin (HbO_2) to methaemoglobin (MetHb) spectrophotometrically at 401 nm and 421 nm, using an extinction coefficient of 77 mM^{-1} cm^{-1} (A_{401} HbO_2, A_{421} MetHb) (Pasqualini et al., 2009). Oxyhaemoglobin was prepared as detailed previously (Clarke & Higgins, 2000). Fresh tissues were excised from the fruit, using different treatments with a 20 mm diameter stainless steel cork borer. Two grammes of samples were collected from the excised tissues and immediately frozen in liquid nitrogen. Then, the samples were homogenised in a mortar with 100 mM K-phosphate buffer (pH 7.0) and 0.6% (w:v) insoluble polyvinylpolypyrrolidone. The extract was clarified by adding powdered activated carbon and centrifuged at 11,000*g* for 10 min at 4 °C. The supernatant was filtered through a PTFE Millipore membrane (0.45 μm) and immediately assayed for NO. Five minutes before oxyhaemoglobin addition, samples were pretreated with catalase (100 U) and superoxide dismutase (100 U) to remove ROS. To evaluate the percentage of recovery of NO during extraction, 1 mM SNP, which releases 5 μM NO at room temperature, was added to the sample and the measured recovery after Millipore filtration ranged from 75% to 80%.

2.5. Ion leakage measurement

Ion leakage was determined according to the method reported previously (Song, Ding, Zhao, Sun, & Zhang, 2006) with minor modifications. Cylinders of fresh tissue were excised from the middle of mango fruits with a 10 mm diameter stainless steel cork borer. Two pieces of 5 mm thickness were cut from each cylinder. After being rinsed two times (2 min) with an isotonic solution of 0.2 M mannitol, 10 pieces were placed into 50 ml plastic tubes containing 16 ml isotonic solution of 0.2 M mannitol at 25 °C. After 2 h, the conductivity was measured ($C1$) and the samples were boiled for 30 min to achieve 100% electrolyte leakage ($C2$). Relative ion leakage was expressed as a percentage of the total conductivity after boiling (Relative ion leakage % = $C1/C2 \times 100$).

2.6. Analysis of lipid peroxidation

Lipid peroxidation was determined by measuring the content of malondialdehyde (MDA) according to the method of Guo et al. (2006). Five fresh samples that were collected from the excised tissues, as in the procedure described above, were homogenised with a mortar and pestle in 5% trichloroacetic acid (TCA) and then centrifuged at 1000*g* for 10 min. The supernatant was mixed with an equal volume of 5% TCA containing 0.67% thiobarbituric acid (TBA). The mixture was heated at 100 °C for 30 min and quickly