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Low-temperature conditioning induces chilling tolerance in stored mango fruit

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ABSTRACT

In this study, mango fruit were pre-treated with low-temperature conditioning (LTC) at 12 °C for 24 h, followed by refrigeration at 5 °C for 25 days before removal to ambient temperature (25 °C) to investigate the effects and possible mechanisms of LTC on chilling injury (CI). The results showed that LTC effectively suppressed the development of CI in mango fruit, accelerated softening, and increased the soluble solids and proline content. Furthermore, LTC reduced electrolyte leakage, and levels of malondialdehyde, $O_2^{\cdot-}$ and H_2O_2, maintaining membrane integrity. To reveal the molecular regulation of LTC on chilling tolerance in mango fruit, a C-repeat/dehydration-responsive element binding factor (CBF) gene, *MiCBF1*, was identified and its expression in response to LTC was examined using RT-qPCR. LTC resulted in a higher *MiCBF1* expression. These findings suggest that LTC enhances chilling tolerance in mango fruit by inducing a series of physiological and molecular responses.

1. Introduction

Mango (*Mangifera indica* L.) is an important tropical fruit with a rich nutritional composition (fiber, minerals, organic acids, vitamins and polyphenolics) and high marketing value but a relatively short shelf life at ambient temperatures (Singh, Singh, Sane, & Nath, 2013). Cold storage is used extensively to extend the shelf life of crops; however, mangoes are susceptible to chilling injury (CI) when stored below 10–13 °C (Zaharah & Singh, 2011; Zhang et al., 2012). Chilled mangoes gradually develop CI symptoms, such as the blackening and pitting of the peel, pulp discoloration, reduced aroma and flavors, ripening failure, and pathogen proliferation (Zaharah & Singh, 2011). Symptoms of CI in mango fruit can become more severe when fruit were transferred from chilling to ambient temperatures (Chidtragool, Ketsa, Bowen, Ferguson, & van Doorn, 2011). Thus, the development of approaches to increase tolerance to chilling could reduce the deterioration of temperature-sensitive commodities during low-temperature storage.

A number of physical and chemical approaches, including heat treatment (Zhang et al., 2012), exposure to cold shock (Zhao, Jiang, Cao, Zhao, & Gu, 2006), nitric oxide fumigation (Zaharah & Singh, 2011), and dipping treatments with methyl salicylate (Han, Tian, Meng, & Ding, 2006), salicylic acid (Ding, Tian, Zheng, Zhou, & Xu, 2007), 2–4-dichlorophenoxyacetic acid (Wang et al., 2008), brassinolide (Li et al., 2012) and oxalate (Li, Zheng, Liu, & Zhu, 2014), have been tested to alleviate CI in mango fruit. Low-temperature conditioning (LTC), a technique that holds cold-sensitive plant tissues at temperatures just above the recognized cold-injured threshold to induce tolerance to subsequent lower temperatures (Woolf, Cox, White, & Ferguson, 2003), has also been reported to dampen CI in a number of harvested crops, such as grapefruit (Chaudhary, Jayaprakasha, Porat, & Patil, 2014; McDonald, McCollum, & Nordby, 1993), avocado (Woolf et al., 2003), loquat (Cai et al., 2006), peach (Jin, Wang, Shang, Tong, & Zheng, 2009), kiwifruit (Yang et al., 2013) and zucchini (Carvajal, Palma, Jamilena, & Garrido, 2015). These findings suggest that the application of LTC could be a valuable postharvest strategy to augment chilling tolerance and maintain product quality. Additionally, effective LTC durations and temperatures may vary depending on the species, the maturity or ripening stage and the storage conditions. Zhao, Jiang, Cao, Zhao, and Gu (2006) reported that a relatively severe LTC (i.e., a cold water-shock at 0 °C for 4 h) prior to refrigeration (2 °C) remarkably reduced the CI severity and increased the antioxidant capacity in 'Wacheng' mango, a late-maturing variety with a larger fruit size. In a preliminary investigation of 'Guifei' mango, an early-maturing variety with a smaller fruit size, we noted a more severe LTC at 2 °C (cold-air exposure

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for 24 h) prior to refrigeration at 5 °C caused more serious CI, in contrast to a significant alleviation of CI in fruit treated with a milder LTC at 12 °C. However, to the best of our knowledge, there is currently a lack of information regarding the physiological and molecular mechanisms of cold tolerance induced by mild LTC in early-maturing mango fruit.

C-repeat/dehydration-responsive element binding factors (CBF/DREBs) are transcription factors that mediate the response to cold stress in plants (Stockinger, Gilmour, & Thomashow, 1997). *CBFs* have been identified as the first wave of cold-responsive genes (Chinnusamy, Zhu, & Zhu, 2008). CBFs activate the transcription of large numbers of COR (cold-regulated) genes whose expression products mediate the changes needed for non-acclimated plants to acquire cold tolerance (Jaglo-Ottosen, Gilmour, Zarka, Schabenberger, & Thomashow, 1998). *CBF* genes were first isolated from *Arabidopsis* (Stockinger et al., 1997) and were recently identified from a few horticultural plants, such as apple (Yang et al., 2010), trifoliate orange (He et al., 2012), bilberry (Oakenfull, Baxter, & Knight, 2013) and kiwifruit (Ma et al., 2014).

Recent studies have revealed that several exogenous treatments, including ethylene (Zhao et al., 2009) and nitric oxide (Zhao et al., 2011), as well as hot water (Ma et al., 2014), triggered *CBF* expression in tomato fruit and kiwifruit, respectively, which enhanced cold tolerance during low-temperature storage. Nevertheless, studies investigating the response of *CBF* expression to LTC and its regulating role for cold tolerance of mango fruit are unavailable.

The current study aimed to investigate the effect of mild LTC (12 °C, 24 h) treatment on cold tolerance in 'Guifei' mango fruit at ambient temperature following the removal of fruit from chilling temperature. Included were analyses of CI index, firmness, soluble solids and proline contents, membrane permeability, malondialdehyde level, and reactive oxygen species (ROS) production. In addition, we cloned a full-length *CBF* gene (*MiCBF1*) from chilled mango fruit and examined whether its expression involved in cold tolerance.

2. Materials and methods

2.1. Plant material

Mature green mango (*M. indica* L. cv. Guifei, an early-maturing cultivar) fruit, each weighing 242 ± 4.5 g, with a firmness of 108.1 ± 2.9 N and a soluble solids content of 8.3 ± 0.2% of (n = 15), were harvested from a commercial orchard located in Dongfang City, Hainan Province of China. The fruit were packed in cartons and transported to the postharvest laboratory within six hours in an air-conditioned cargo van at 25 °C. Fruit of uniform size, shape, and ripeness with an absence of visible symptoms of disease and mechanical injury were selected for the experiments.

2.2. Low-temperature conditioning (LTC) treatment and sampling

Mango fruit were disinfected with 0.5% (v/v) sodium hypochlorite for 3 min, rinsed with tap water, air-dried and then assigned randomly to 2 treatment groups, with 600 fruit in each group. The first group was air-preconditioned at 12 ± 1 °C for 24 h in a cold room, and then stored at 5 ± 1 °C and 85–90% RH for 25 d before being transferred to ambient conditions (25 ± 1 °C and 85–90% RH) for 5 d. The second group (control) was first exposed to ambient temperature (25 ± 1 °C) for 24 h before being stored using the same procedure as the first group. The conditioning temperature (12 °C) was selected as optimal based on preliminary experiments at 2, 7 and 12 °C (data not shown).

The CI severity, firmness, SSC and relative leakage rate were analyzed daily during storage at ambient temperature after 25 d of cold storage at 5 °C, during which the exocarp tissue (peel) was sampled to determine the malondialdehyde, proline and ROS contents. *CBF* gene expression was analyzed in fruit exocarp tissues sampled at three stages: i) at 6-h intervals during 24 h of preconditioning treatment at 12 °C; ii) at 5-d intervals during 25 d of cold storage at 5 °C; and iii) daily sampling at 25 °C after removal from cold storage. All of above-mentioned exocarp samples were rapidly frozen in liquid nitrogen, pounded into small particles and then stored at −80 °C until use. Each treatment contained three replicates at each sampling time, the CI was investigated in 30 fruits per replicate, and the other parameters were analyzed in 6 fruits per replicate.

2.3. Chilling injury (CI) index

CI index was evaluated on a rating scale ranging from 0 to 4 based on visual symptoms of black or brown lesions and wrinkles on the peel of the affected fruit (Zaharah & Singh, 2011). The scale executed was as follows: 0 = absence of injury; 1 = very slight injury; 2 = slight injury; 3 = moderate injury; and 4 = severe injury. The CI index was calculated according to the following formula: $\sum$(CI scale × number of fruit in each scale)/(number of total fruit × 4) × 100%.

2.4. Firmness

A pressure tester (Model FT327; Effegi, Milan, Italy) equipped with an 8-mm diameter probe was used to determine the firmness. Two measurements were carried out at 2 equidistant points on the equatorial axis of each peeled fruit.

2.5. Soluble solids content (SSC)

Fruit flesh tissue (30 g) was homogenized using a FSH-2A homogenizer (Liangyou Instrument Co., Ltd., Changzhou, China), followed by centrifugation at 8000×*g* for 20 min at 4 °C. The supernatant was filtered through four layers of cheese cloth. One drop of the supernatant was dispensed onto a hand-held refractometer (Master-M, ATAGO Co. Ltd., Tokyo, Japan) to determine the SSC. The SSC was expressed as a percentage.

2.6. Proline content

The proline content was determined by the acid ninhydrin method as described by Cao, Cai, Yang, and Zheng (2012) with some modifications. Frozen exocarp tissues were homogenized using a FW-100 grinder (Yongguangming Medical Equipment Co., Ltd., Beijing, China). The grinder chamber was pre-chilled with liquid nitrogen prior to processing of each tissue. One gram of powder was mixed with 15 ml of sulfosalicylic acid (3%, v/v), in boiling water for 10 min, with shaking at 100 rpm using a TS-110X50 water bath shaker (Tensuc Lab Instrument & Equipment Co., Ltd., Shanghai, China). After cooling, the homogenate was filtered through four layers of cheesecloth, and the filtrate was obtained as proline extract. The extract (2 ml) was mixed with an equal volume of glacial acetic acid ninhydrin reagent and boiled for 30 min. After cooling, the reaction mixture was mixed with 4 ml of toluene, shaken and stewed. The organic phase was subjected to centrifugation at 3000×*g* for 5 min, and the absorbance of the supernatant was read at 520 nm. The proline content was determined based on a standard curve built using known amounts of proline and expressed as μg proline g^{-1} fresh weight (FW).