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The quality of Gold Queen Hami melons stored under different temperatures

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ABSTRACT

Low temperature storage prolongs the postharvest life of various fresh fruits and vegetables. However, the actual storage temperature may also significantly influence fruit and vegetable quality. In the present study, we stored Gold Queen Hami melons at three different temperatures [21 °C (control), 3 °C, and 0.5 °C (0 ± 0.5 °C)] for 36 days. The humidity for all three temperatures conditions was between 75% and 85%. The results indicated a correlation between chilling injury in Gold Queen Hami melons and storage temperature and time. Different degrees of chilling injury in Gold Queen Hami melons were observed after storage at 3 °C and 0.5 °C, whereas these were detected later when stored at 3 °C. In addition, the rotting rate, weight loss rate, and chilling injury index at 3 °C were significantly lower compared to that at 0.5 °C storage temperature. Compared to other temperatures, storage at 3 °C was associated with a delay in fruit rotting, sustained high fatty acid desaturase and adenosine triphosphatase (ATPase) activity, a decrease in lipoxygenase activity, enhanced cold resistance, and extended total storage period. At 21 °C, no chilling injury was observed, although extensive weight loss and high rotting rates were detected. Moreover, two highly expressed genes, namely, *JhMYB24* and *JhMYB48*, were screened and selected from the Gold Queen Hami melon transcriptome database. Real-time PCR analysis showed that the expression of *JhMYB24* and *JhMYB48* continuously increased under cold stress and peaked at the 18th day of cold storage. The expression of *JhMYB24* and *JhMYB48* at 3 °C was significantly higher than that at 0.5 °C or 21 °C. Together, our results demonstrate that regulating enzyme activity and upregulating the expression of JhMYB24 and JhMYB48 alleviate cold stress in fruits stored at 3 °C, thereby maintaining high fruit quality.

1. Introduction

Hami melon is a major economic crop of Xinjiang, China and comprises several varieties, including New Queen, 8601, Kalakusai, Jiashi, and Gold Queen (Yang et al., 2003). Low temperature storage is an effective and common method to prolong the postharvest life of fruit (Li et al., 2017). Honeydew melons stored at low temperature exhibit better quality and acquire fewer injuries (Fang et al., 2006). Gold Queen Hami melons stored at a low temperature exhibit delayed physiological aging, resistance to bacterial infections, and reduced rotting. However, Gold Queen Hami melons are sensitive to storage temperature. Low temperature storage of Gold Queen Hami melons leads to chilling injury, which in turn results in rotting. At the early stage of chilling injury, shallow brown specks appear on the surface of melons, whereas at the middle to later stages, these specks become bigger, darker, and undergo involution, which led to rotting (Cong et al., 2017). At different harvesting periods, muskmelon exhibits a different level of chilling injury during cold storage. Hami melons harvested in the summer are more susceptible to chilling injury. The lower the temperature, the more severe the chilling injury (Byungseon et al., 2010). A number of methods have been developed to alleviate chilling injury in Hami melons such as nitric oxide treatment (Zhang et al., 2017a), thereby inducing antioxidase activity during hot water washes (Fogelman et al., 2011; Jing et al., 2016). However, the specific technical requirements of these methods limit their application; therefore, a better storage temperature that retains the quality of Hami melons during cold storage is warranted.

Current studies on postharvest storage of Hami melons mainly focus on the effect of species varieties, hot water and shellac treatment, and hydrogen peroxide treatment on the fruit quality during storage (Guo et al., 2012; Zhang et al., 2017b; Zhou et al., 2015). Studies on the enzymatic characteristics of Gold Queen Hami melons in postharvest cold storage are limited. Several investigations have demonstrated that chilling injury starts from the cell membrane and lipoxygenase, fatty acid desaturase, and ATPase in fruits play important roles in maintaining the integrity and fluidity of the cell membrane (Chong et al., 2015; Selivanov et al., 2017; Tànczos et al., 2010). Changes in cell membrane permeability may be the primary response to adverse conditions, and a number of sequential responses are subsequently elicited. Alterations in the electrical conductivity of the cytoplasmic membrane

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reflect the extent of damage and cold resistance of the membrane (Chen, 1999). A relative electrical conductivity method has been employed to measure cold resistance in cucumber (Sobczyk et al., 1985), tomato (Domínguez et al., 2010), and Arabidopsis (Peng et al., 2007). Relative conductivity reflects changes in cell membrane permeability and directly indicates fruit injury level. However, the roles of these three enzymes in Gold Queen Hami melons with chilling injury are unknown.

The MYB transcription factor family is one of the largest transcription factor families in plants and plays a critical role in responding to non-biological stresses such as cold resistance (Agarwal et al., 2006; V et al., 2001; zhu et al., 2010). MYB transcription factors are involved in eliciting rapid responses to low temperature stress. These interact with gene promoters or other proteins to mediate signal transduction, induce gene expression for the synthesis of other protein or metabolites, thereby enhancing cold resistance in plants. Previous studies on MYB transcription factors and cold resistance have mainly focused on banana (Dou et al., 2016), Arabidopsis (Jung et al., 2008), rice (Vannini et al., 2004), apple (Pasquali et al., 2008), and watermelon (Kong et al., 2015). Several studies have demonstrated that the upregulation of MYB genes enhances cold resistance in plants. However, no research investigations on the role of the MYB gene on Gold Queen Hami melons have been conducted to date.

The present study investigated the effect of different storage temperatures on the quality of Gold Queen Hami melons. We tested several variables, including rotting rate, weight loss rate, firmness, chilling injury index, relative conductivity, and lipoxygenase, fatty acid desaturase, and ATPase activity. Moreover, we screened and selected two highly expressed genes, JhMYB24 and JhMYB48, from the Gold Queen Hami melon transcriptome database. Our results provide a physiological and molecular biological basis for improving cold storage of Hami melons.

2. Materials and methods

2.1. Materials and processing

This study utilized Gold Queen Hami melons that were collected from 121 regiment farms in Shihezi, Xinjiang, China. Fruits showing 80% ripe levels, with similar sizes, and free of insect injury or mechanical damage were collected. The soluble substance in the fruits was about 11%. All fruits were stored at the Food College of Shihezi University, Shihezi, Xinjiang at the indicated temperatures. Previous studies have demonstrated that in early-middle ripe Hami melons, chilling injury occurs at the temperatures below 3 °C (Zhang et al., 2015; Xu et al., 2007). Our previous studies showed that the freezing point of the Gold Queen Hami melon was about 3 ± 0.5 °C. Thus, in this study, Gold Queen Hami melons were stored at three different temperatures, namely, 21 °C, 3 °C, and 0.5 °C (0 ± 0.5 °C), respectively. The relative humidity of the storage conditions was set within the range of 75–85%. Three replicates were used at each temperature, and each replicate contained 70 individual melons. Among these melons in each replicate, 35 melons were used to test physical and chemical indexes, including rotting rate, weight loss rate, and cold injury index. After physical and chemical index testing, the melons were returned to their original storage. For the remaining 35 melons in each replicate, five melons were collected every six days, from which a piece of rind was collected from the equatorial region and cut into small pieces, as described by A et al. (2011). The rind was then used to test firmness, relative conductivity, and lipoxygenase, fatty acid desaturase, and ATPase activity. The samples were snap frozen in liquid nitrogen and transferred to −80 °C freezers for future analysis.

2.2. Evaluation of rotting rate, weight loss rate, and firmness

The following equations were used to calculate rotting rate and weight loss rate:

$$\text{Rotting rate (\%)} = (\text{Number of rotting fruits/Total number of fruits}) \times 100\%$$

$$\text{Rate of weight loss (\%)} = [(\text{Original weight} - \text{Current weight})/\text{Original weight}] \times 100\%$$

To evaluate firmness, the Gold Queen Hami melons were cut longitudinally and then transversely. The thickness of each slice was about 1.5 cm. Samples from the equatorial region were then prepared using a 1.6-cm diameter puncher and were modified into cylinders with a thickness of 1 cm. Firmness was tested using an analyzer (SMSTA.XTplus, Stable Micro System, UK) in the puncturing mode. Five repeats were performed for each melon, and the average was calculated.

2.3. Evaluating chilling injury index

Symptoms of chilling injury in Gold Queen Hami melon include small specks on the surface, browning, and denting (Yu et al., 2015). Chilling injury was graded based on the size of the dents and specks as described by Meng et al. (2009), as follows: grade 0: no chilling injury symptoms; grade 1: area of chilling injury ≤25%; 2 grade: area of chilling injury is between 25% and 50%; grade 3: area of chilling injury between 50% and 75%; and grade 4: area of chilling injury ≥75%. Chilling injury index was calculated using the following formula:

$$CII = \sum (Chilling\_injury\_\text{grade} \times fruit\_number)/(\ \text{n} \times N)$$

where n is the highest chilling injury index; and N is the total number of fruits.

2.4. Assessment of relative conductivity

A puncher with a diameter of 8 mm was used to collect samples from the equatorial region of Gold Queen Hami melons. A slice with the thickness of 3 mm and an approximate weight of 2 g was collected and dried using absorbance papers. The sample was incubated in a beaker with 40 mL of distilled water for 30 min at 20–25 °C. After incubation, conductivity (P_1) was evaluated. The sample was then boiled for 15 min and allowed to cool down to room temperature, followed by measure of conductivity (P_2). The whole process was repeated for thrice, and the average was calculated using the following formula:

$$\text{Relative\_conductivity\%} = \frac{P1}{P2} \times 100$$

2.5. Evaluating lipoxygenase, fatty acid desaturase, and ATPase activity

ELISA is an immunoassay in which the labeled enzyme is absorbed onto a solid phase surface together with the antigen and incubated with the sample containing the targeted enzymes. To measure lipoxygenase activity, ELISA was performed according to Dauk et al. (2007), in which an ELISA kit (from Kenuodi Biotechnology) was applied. To measure fatty acid desaturase activity, ELISA was performed according to Contreras et al. (2016). To assess ATPase activity, ELISA was performed according to Ghasemnezhad et al. (2008).

2.6. Real-time PCR analysis

A column-based plant total RNA extraction kit (SK8661) was used to extract total RNA from Hami melons. cDNA was synthesized using a reverse transcription kit (RevertAid Premium reverse transcriptase) according to the product manual. LightCycler480 Software Setup (Roche) was used to study the expression of *JhMYB24* and *JhMYB48* in Hami melons. cDNA from different samples were PCR amplified, which was repeated for thrice. Primers for the target genes were designed and