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Changes of polyamines and CBFs expressions of two Hami melon (*Cucumis melo* L.) cultivars during low temperature storage

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ABSTRACT

Hami melon (*Cucumis melo* L. var. inodorus Jacq) is susceptible to chilling injury (CI) during low temperature storage, which causes changes in physiological mechanisms and gene expression. In this study, cold tolerance of two Hami melon cultivars, namely Xinmi No. 3 and Xinmi No. 11 was evaluated. We assessed changes of membrane permeability, the content of polyamines (PAs), and the expression of C-repeat/dehydration-responsive element (CRT/DRE)-binding factors (CBFs) in different section of fruit of each cultivar stored at 3 °C under 75–80% relative humidity. The results showed that Xinmi No. 11 was more cold tolerant than Xinmi No. 3, the content of PAs and the expression of CmCBFs in the cold-tolerant cultivar were significantly higher than in the cold-sensitive cultivar during storage at 3 °C. CI was observed in the peel tissue, but not in the pulp tissue. The content of PAs and the expression of CmCBFs in the peel tissue were significantly higher than in the pulp tissue. The membrane permeability, putrescine (Put), spermine(Spm), and the expression of CmCBF1 and CmCBF3 in the peel tissue of Hami melon fruit increased with the degree of CI. Our results provide novel evidence about the positive correlation between the content of PAs, the expression of CmCBF1 and CmCBF3 and cold tolerance. The CI had major impact on Put, Spm and membrane permeability. The assessed parameters could provide sensitive indicators reflecting cultivar cold tolerance of Hami melon fruit under chilling stress.

1. Introduction

Cold storage is an effective strategy for ensuring the maintenance of postharvest quality and for extending the shelf life of fruits and vegetables especially those of tropical and subtropical origin (Paull, 1990; Sevillano et al., 2009), are highly vulnerable to chilling injury (CI) when exposed to low,but non-freezing temperatures (Sevillano et al., 2009). Hami melon (*Cucumis melo* L. var. inodorus Jacq) is susceptible to CI during low temperature storage. Previous studies have reported CI symptoms, including discoloration, browning, and pitting of fruits as well as the appearance of pits on the fruit peel of Hami melon fruit in cold storage (Bi et al., 2003; Evensen, 1983; Fogelman et al., 2011; García et al., 2005; Krarup et al., 2009; Krarup and González, 2005). Chilling injury leads to substantial degradation of the quality of the produce. For controlling CI, it is important to understand the process of its induction as well as that of the development of cold tolerance in some cultivars. In addition, knowledge of the changes in physiological mechanisms and gene expression in response to CI is necessary.

To date, not much is known regarding of cold tolerance of Hami melon cultivars (Bi et al., 2003; Krarup et al., 2009; Krarup and González, 2005), Moreover, besides the association of antioxidant activity with cold tolerance of melon fruit (Fogelman et al., 2011), no other physiological mechanism has been related to CI.A growing body of evidence suggests that polyamines (PAs), in addition to their role in the regulation of plant development and physiology (Matin-Tanguy, 2001; Paschalidis and Roubelakis-Angelakis, 2005), also assay important roles in modulating the defense response of plants to diverse environmental stresses (Bibi et al., 2010; Cuevas et al., 2008; Gill and Tuteja, 2010; Kusano et al., 2007; Sfakianaki et al., 2006; Tassoni et al., 2000). It has been observed that plants significantly accumulate PAs under environmental stresses. This accumulation constitutes a mechanism that may confer adaptive and protective functions under abiotic stresses (Galston and Sawhney, 1990). Hence, the increase in the endogenous content of PAs is suggested to reverse the damaging effects and enhance the tolerance of plants to various stresses (He et al., 2002; Unal et al., 2007). It has been observed that chilling-tolerant plants increase their endogenous PAs in response to the chilling response to a greater extent than the chilling-sensitive ones (Bouchereau et al., 1999; Groppa and Benavides, 2008; Lee et al., 1997; Navakoudis et al., 2003; Shen et al., 2000). These data indicate the involvement of

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PAs in the cold tolerance of plants. However, the role of PAs in enhancing the cold tolerance of Hami melon fruit is not known. Thus, it is fundamental to understand whether there is any correlation between PAs and cold tolerance.

A major step towards the understanding of regulation of gene expressionunder low temperature storage conditions in Hami melon. It has been shown that C-repeat/dehydration-responsive element (CRT/DRE) −binding factors (CBFs) play an important role in the cold response pathway in plants, CBFs have been identified as the first wave of cold-induced genes (Akhtar et al., 2012; Chinnusamy et al., 2007; Thomashow, 2010). Indeed, the mechanisms through which low temperature induces gene expression involve a CBF family of transcriptional activators. The CBF family is conserved in a broad range of species in both cold-tolerant and sensitive species (Champ et al., 2007; Dubouzet et al., 2003; Gao et al., 2002; Vagujfalvi et al., 2003), including Arabidopsis (Medina et al., 2011), tomato (Pennycooke et al., 2008; Zhang et al., 2004), grape (Takuhara et al., 2011),apple (Wisniewski et al., 2011), citrus (He et al., 2012), peach (Liang et al., 2013), and kiwifruit (Ma et al., 2014). However, different CBF gene members of this family exhibit distinct expression patterns, that result in different functional responses to low-temperature stress (Gilmour et al., 1998; Karimi et al., 2015; Liang et al., 2013; Medina et al., 1999; Zhang et al., 2004). Previous studies have demonstrated different temporal regulation of CBF transcription factors in different plants (Karimi et al., 2015; Novillo et al., 2004), which suggests that the expression of the CBF genes might be relevant in low-temperature stress. The expression of CBFs is positively correlated with cold tolerance (Karimi et al., 2015; Liang et al., 2013; Ma et al., 2014; Zhang et al., 2011; Zhao et al., 2009). However, the above-mentioned studies focused mainly on evaluating the roles of CBF genes in response to low temperature during plant growth and development. The possible involvement of CBF genes in changes in fruit during postharvest cold storage warranted further study.

In the present study, two Hami melon cultivars (Xinmi No.3 and Xinmi No.11) were used to investigate the difference in cold tolerance and for the evaluation of changes in membrane permeability, the content of PAs, and the expression of CBFs in different sections of each cultivar during storage at 3 ˚C at a relative humidity (RH) of 75–80%. The purpose was to establish a relationship between the content of PAs,CBFs expressions, and cold tolerance in Hami melon fruit during low temperature storage.

2. Materials and methods

2.1. Plant materials and treatments

Two Hami melon (*Cucumis melo* L. var. inodorus Jacq.) cultivars (Xinmi No.3 and Xinmi No.11) with clear differences in their sensitivity to CI during storage at 3 ˚C were used in this study. Mature fruit of Xinmi No.3 (with a soluble solid content of 13.20 ± 0.21%) were harvested on 26 July 2014 from an orchard in Hami (latitude 43.46˚ N and longitude 95.01˚ E), in the eastern Xinjiang Province, China. Mature fruit of Xinmi No.11 (with a soluble solid content of 13.65 ± 0.32%) were harvested on 1 September 2014 from an orchard in Altay (latitude 47.85˚ N and longitude 88.33˚ E), in the northern Xinjiang Province, China. Maturity was determined based on the time of development,the net of the fruit's skin, and the soluble solid content which was measured using a hand-held refractometer (Model 10481 S/N,USA).The fruits were sorted for the uniform size and absence of any obvious injury, packed individually in 35 cm long net bags made of foam plastic, and palced in standard melon shipping boxes (4 melons/box). Fruit of each cultivar were transported to the store room of Xinjiang Academy of Agricultural Sciences within 12 h, respectively.The fruit were selected for uniform size and colour, and for the absence of mechanical damage, After shorting, they were placed in boxes and stored at 3 ˚C for 49 days. For each cultivar, 81 fruit were used for the estimation of CmCBF1 and CmCBF3 expression after short-term storage (for 0, 1, 2, 4, 6, 8, 12, 24, and 48 h) at 3 ˚C, three fruit of each cultivar were used in triplicates.In addition, three replicates with five fruit of each cultivar, stored at 3 ˚C and subsequently kept at 25 ˚C for 3 days, were used to evaluate CI index at 7 day intervals. Furthermore, 120 fruit were used to measure membrane permeability, the content of PAs and the long-term expression of CmCBF1 and CmCBF3 during low temperature storage (five fruits in triplicate for each date).The peel and pulp tissues of Hami melon fruit from the equator area were sampled using the method previously described by Moing et al. (2011), cut into small pieces, and immediately transferred to liquid nitrogen and stored at −80 ˚C until further analyses.

2.2. Evaluation of CI index

The CI index of Hami melon fruit was evaluated using a subjective scale of visual symptoms based on brownish pitting,as previously described (Bi et al., 2003). Fruits were ranked according to the following scale: 0 = normal (no CI); 1 = trace CI (0% < CI area ≤ 10%); 2 = slight CI (11% < CI area ≤ 25%); 3 = moderate CI (26% < CI area ≤ 50%); 4 = severe CI (CI area ≥ 50%). The CI index (between 0 and 1) was measured according to the following formula: Σ[(CI scale) × (number of fruits with that CI scale)]/[(total number of scales) × (total number of fruits)].

2.3. Determination of membrane permeability

Membrane permeability was measured using the methods of Li et al. (2011). Cylinders of the peel and pulp tissues of Hami melon fruit were excised from the flesh of five fruits from the equatorial region using a 10 mm-diameter stainless steel cork borer. Thirty small discs were immersed in 50 mL double-distilled water in glass vials for 2 h, and the initial conductivity of the sample solution was determined with a conductivity meter (DDS-11A, Shanghai, China). Next, the disk solution was boiled for 30 min and then cooled to room temperature to obtain a final conductivity measurement. Membrane permeability was expressed as relative conductivity: (initial conductivity/final conductivity) × 100.

2.4. Polyamine isolation, identification, and analysis

Polyamines in the frozen peel and pulp tissue of Hami melon fruit of each cultivar sampled at different time points (0–49 d) were extracted according to the method of Kakkar and Nagar (1997) with some modifications. The frozen tissues (1 g) were homogenised in 2 mL 5% (v/v) cold perchloric acid (PCA) and incubated at 4 ˚C for 2 h. The homogenate was centrifuged at 12000*g* at 4 ˚C for 30 min (Eppendorf 5810R centrifuge, Hamburg, Germany).The resulting supernatant was used to quantify free and soluble polyamines.

To 1.0 mL of 2 N NaOH, 0.5 mL of extract was added followed by 15 mL of benzoyl chloride.After vortexing for 30 s, the samples were incubated for 20 min at 30 ˚C,The reaction was stopped by adding 2.0 mL of saturated NaCl. Benzoylamines were extracted with 2.0 mL chilled diethylether; 1.0 mLof ether phase was collected and evap-orated to dryness under a stream of air, and the residue was finally suspended in 0.20 mL of methanol (HPLC grade) for HPLC analysis.The standards were treated in a similar way taking known concentrations of putrescine (Put), spermidine (Spd) and spermine(Spm).

Separation and quantification of PAs were performed using reverse phase HPLC (Agilent 1260, USA).The benzoylated fractions were filtered through Millipore filters (pore size 0.46 μm) and injected (10 μL) into an Agilent C18 column (250 mm × 5 mm) protected by a guard column. The elution was carried out in an isocratic mode at a flow rate of 0.8 mL min^{-1} with a mixture of methanol: acetonitrile: water (60.5:2.5:37, v/v) for 40 min at aconstant temperature of 30 ˚C. The solvents were filtered through Millipore membranes (pore size 0.22 μm)