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Research Paper

Sucrose degradation is regulated by 1-methycyclopropene treatment and is related to chilling tolerance in two peach cultivars

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ABSTRACT

In order to reveal how sucrose degradation is related to chilling tolerance, two varieties of peach fruit, 'Zajiao' and 'Yulu', were treated with 1-methylcyclopropene (1-MCP) vapor at 1 μL/L for 24 h before storage at 5 °C for 35 and 28 d respectively. 'Yulu' peaches had higher sucrose content than 'Zajiao' peaches at harvest, but a higher rate of sucrose degradation in untreated 'Yulu' fruit may have resulted greater sensitivity to chilling stress compared with untreated 'Zajiao' fruit. 1-MCP treatment significantly inhibited chilling injury (CI) and preserved higher firmness values in both varieties. This treatment reduced the activities and expression of enzymes related to sucrose degradation, and increased activity and expression of sucrose synthase synthesis, resulting in a lower rate of sucrose degradation and the increase of glucose and fructose content in both varieties during cold storage. These results suggest that lower degradation rates of sucrose during cold stress, rather than the higher content of sucrose at harvest time, enhances chilling tolerance in peach fruit.

1. Introduction

Peaches are sought by consumers around the world for their good taste and high nutritional value. Although peach fruit are climacteric and have a short shelf life when stored at ambient temperature, cold storage is a practical method to slow ripening (Cantín et al., 2010). However, the storage temperature is critical, and peaches are susceptible at 5 °C, but not at 0 °C or 10 °C (Wang et al., 2013; Yu et al., 2015) to chilling injury (CI), manifested by internal browning, flesh mealiness, and loss of flavor(Lurie and Crisosto, 2005). These symptoms can be reduced by hot air (Wang et al., 2014), methyl jasmonate (Jin et al., 2009), and oxalic acid (Jin et al., 2014).

The greatest capacity of the peach/nectarine fruit to produce ethylene after cold storage was associated to lower CI (Giné-Bordonaba et al., 2016). Maintaining the ability to produce ethylene or adding exogenous ethylene can prevent CI of nectarine fruit (Zhou et al., 2001). 1-Methylcyclopropene (1-MCP) is an ethylene perception inhibitor that is widely used to reduce ethylene production, slow the ripening process and prolong shelf life in horticultural crops (Watkins, 2006). Interestingly, studies have confirmed that 1-MCP treatment has inhibitive effects on CI of loquat (Cao et al., 2009), pear (Cheng et al., 2015), persimmon (Salvador et al., 2004) and citrus fruit (Dou et al., 2005). Puig et al. (2015) demonstrated that although ethylene is related to tolerance to cold storage of peach, differential auxin subcellular accumulation and signaling may play a role in determining chilling sensitivity/tolerance. This maybe the reason that 1-MCP inhibits the release of ethylene but not resulted in the reduction of chilling tolerance in some fruits. However, the effect of 1-MCP treatment on CI of peaches and nectarines is contrary. Jin et al. (2011) reported that 0.5 μL/L 1-MCP treatment retarded internal browning and flesh mealiness in 'Baifeng' peaches. The CI index was also inhibited by two doses (0.5 and 1 μL/L) of 1-MCP in nectarines (Özkaya et al., 2016). In contrast, other researchers found that treatments with 0.5 μL/L or 0.9 μL/L1-MCP increased CI symptoms in 'Elberta' or 'Chiripá' peaches (Fan et al., 2002; Girardi et al., 2005). These contrary results maybe related with the different fruit cultivars and 1-MCP concentration.

Sucrose and reducing sugars (glucose and fructose) are the major soluble sugars in peach fruit at harvest. The dominant sugar is sucrose, representing about 75% of total sugar (Aubert et al., 2014). Soluble sugars are closely linked to flavor and nutritional value, and also help support resistance to low-temperature and other osmotic stresses by regulating osmotic pressure, protecting cell membranes from fusion and leakage (van den Bogaart et al., 2007), providing oxidation resistance (Bolouri-Moghaddam et al., 2010) and controlling gene expression (Wang et al., 2013).

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Recently, soluble sugars have been shown to reduce CI development of postharvest fruits during cold storage. Higher levels of reducing sugars improve CI tolerance in loquat (Cao et al., 2013; Shao et al., 2013) and apricot fruit (Wang et al., 2016). However, in other fruit such as peach (Wang et al., 2013) and mandarin (Holland et al., 2002), sucrose, rather than reducing sugars, is related to enhanced CI tolerance. Our previous study showed that chilling stress stimulates the activities and transcription levels of enzymes involved in sucrose metabolism, resulting in increased sucrose cleavage in peach fruit (Yu et al., 2015). The increased sucrose levels that occur during cold storage enhance the chilling tolerance in peach fruit treated with hot air and methyl jasmonate (MeJA) (Yu et al., 2016).

The ability of 1-MCP to alleviate CI in peach fruit has been attributed to antioxidant enzyme activities (Jin et al., 2011). 1-MCP treatment reduced pectin methylesterase and polygalacturonase activities, leading to lower CI incidence in nectarine fruit (Özkaya et al., 2016). Similar observations have been reported in other fruits treated with 1-MCP, such as pears (Cheng et al., 2015), persimmon (Salvador et al., 2004) and loquat (Cao et al., 2009). However, the relationship between 1-MCP treatment and soluble sugar metabolism has not been explored. To examine sucrose degradation and the development of CI during cold storage (5 °C), we treated two varieties of peach fruit with1-MCP and monitored CI index, soluble sugar content, soluble sugar metabolism-related enzyme activity, and gene expression.

2. Materials and methods

2.1. Plant material and experimental design

1-MCP formulation (Lubo Fresh) was purchased from Xinyi Institute of Fruit and Vegetable Quality, Jiangsu Province, China. 'Zajiao' and 'Yulu' peach fruits (*Prunus persica* L. Batsch) were harvested at optimum commercial maturity (about 80% maturity levels, accordance to growers recommendations) from a commercial orchard in Fenghua, Zhejiang Province, China. After harvest, the fruits were immediately transported to the laboratory and precooled at 5 °C for 3 h to remove field heat. Fruits were selected for uniform size and shape and lack of physical or mechanical damage. Peach fruits from each cultivar were randomly assigned to 1-MCP treated or control groups. Each group consisted 270 fruits divided into three biological replicates. The experimental group was treated with 1 μL/L 1-MCP vapor in a sealed container for 24 h at 5 °C. After treatment, the container was opened and ventilated for 1 h, the 'Zajiao' peaches were stored at 5 °C for 35 d, the 'Yulu' peaches, were stored at 5 °C for 28 d. The untreated control peaches were stored at 5 °C; 35 d for 'Zajiao', 28 d for 'Yulu'. Fifteen fruits per replicate were analyzed at 7-day intervals during cold storage. CI indexes and firmness were assessed immediately after removal from cold storage, and then peaches were pooled and immediately frozen in liquid nitrogen. All frozen samples were stored at −40 °C for further analysis.

2.2. Determination of CI index and firmness

Internal browning is a frequent postharvest disorder that can be observed when peach and nectarine fruits are maintained in cold storage for long periods (Cáceres et al., 2016). Thus, internal browning was estimated visually to calculate as the CI index, using the brown area observed after cutting each fruit along its axial diameter (Wang et al., 2013). CI level was graded using the following scale: 0 = none; 1 = browning area less than 5%; 2 = browning area between 5% and 25%; 3 = browning area between 25% and 50%; 4 = browning area more than 50%. CI index was calculated as [(CI level) × (the number of fruits at this CI level)]/(4 × total number of fruits in each treatment).

Firmness was determined using a digital fruit hardness tester (GY-4, Dongwan City Zhiqu Precision Instrument Co., Ltd, China) fitted with an 8 mm diameter probe. The puncture distance was 10 mm. Firmness was recorded in Newtons (N).

2.3. Measurement of soluble sugars content

Soluble sugar content was analyzed using the method described by Shao et al. (2013). 5 g samples of frozen peach tissue were finely ground in a solution containing 0.5 mL 300 mM zinc acetate and 0.5 mL 63 mM potassium ferrocyanide. The homogenate was diluted to 25 mL with deionized water and then passed through a 0.22 μm membrane filter. Soluble sugars were measured using a high performance liquid chromatography (HPLC) system (Model 2695, Waters, USA), an XBrige™ Amide Column (3.5 μm, 4.6 × 250 mm, USA), and a refractive index (RI) detector (Model 2414, Waters, USA). A 20 μL aliquot was injected into the HPLC system for analysis. The mobile phase composition was acetonitrile/water (80:20, v/v). The total flow rate was 1 mL/min and the column temperature was 35 °C.

2.4. Extraction and assays of sucrose metabolism-related enzyme activities

Enzymes were extracted using the method of Zhang et al. (2012). Frozen peach tissue (1 g) was homogenized on ice in 5 mL buffer containing 100 mM sodium phosphate (pH 7.5), 5 mM $MgCl_2$, 1mMEthylenediaminetetraacetic acid (EDTA), 2.5 mM dithiothreitol (DTT), and 0.1% (v/v) Triton X-100. The homogenates were centrifuged at 10,000 × *g* for 20 min at 4 °C. The supernatant was dialyzed immediately in a 10-fold volume of extraction buffer (without Triton X-100) at 4 °C for 20 h. The crude enzyme extracts were used for measurements of the following enzyme activities.

Acid invertase (AI) activity was assayed using the method of Sun et al. (2011). The reaction consisted of 100 mM sodium citrate buffer (pH 4.5), 1% (m/v) sucrose, and crude enzyme extract. The mixture was incubated for 30 min at 37 °C. 3, 5-dinitrosalicylic acid was then added and the sample placed in a boiling water bath for 5 min to terminate the reaction. Absorbance at 540 nm was measured after cooling. Neutral invertase (NI) activity was assayed similarly, but using 100 mM sodium phosphate buffer (pH 7.5). Sucrose synthase-cleavage (SS-cleavage) activity was assayed in a mixture of 80 mM Hepes-NaOH (pH 5.5), 5 mM NaF, 5 mM uridinediphosphate (UDP), 100 mM sucrose and crude enzyme extract. Subsequent steps were as described for AI. AI, NI and SS-cleavage activities were expressed as micromoles glucose per hour per gram fresh weight (FW).

SS-synthesis and sucrose phosphate synthase (SPS) activities were assayed using the method of Solomakhin and Blanke (2010). The SS-synthesis assay consisted of 100 mM Hepes-NaOH (pH 8.0), 4 mM uridinediphosphate glucose, 15 mM $MgCl_2$, 60 mM fructose and crude enzyme extract. The SPS assay consisted of 100 mM HEPES-NaOH (pH 8.0), 10 mM UDP-glucose, 5 mM fructose-6-phosphate, 15 mM glucose-6-phosphate, 15 mM $MgCl_2$ and crude enzyme extract. The mixture was incubated for 30 min at 37 °C. The reaction was terminated by adding 5 mM NaOH and then placing the sample in boiling water for 10 min. After cooling, anthrone was added to the mixture and the sample was incubated for 10 min at 80 °C. The samples were allowed to cool, and absorbance was measured at 620 nm. SS synthesis and SPS activities were expressed as micromoles sucrose per hour per gram FW.