Food Chemistry 120 (2010) 864–872

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Peach fruit acquired tolerance to low temperature stress by accumulation of linolenic acid and *N*-acylphosphatidylethanolamine in plasma membrane

Changfeng Zhang [a,b], Shiping Tian [a,*]

[a] Key Laboratory of Photosynthesis and Environmental Molecular Physiology, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, PR China
[b] Graduate School of Chinese Academy of Sciences, Beijing 100039, PR China

ARTICLE INFO

Article history:
Received 28 April 2009
Received in revised form 27 September 2009
Accepted 13 November 2009

Keywords:
Chilling injury
Fatty acids
N-acylphosphatidylethanolamine
Peach fruit
Plasma membrane

ABSTRACT

Peach fruit (*Prunus persica* L. cv. Beijing 33) did not show symptoms of chilling injury in 0 °C-Air or 0 °C-CA, but did in 5 °C-Air after 21 d. The mechanisms by which 0 °C storage could activate chilling tolerance of peach fruit were investigated by analysing characteristics of plasma membrane. We found that peach fruit stored in 0 °C-Air and 0 °C-CA had much higher linolenic acid content and unsaturation degree of plasma membrane than did that in 5 °C-Air. In addition, the fruits stored in 0 °C-CA showed a higher membrane fluidity and membrane integrity than did that in 0 °C-Air, which was related to the accumulation of *N*-acylphosphatidylethanolamine (NAPE) of peach fruits stored in 0 °C-CA. Based on these results, it appears that a higher unsaturation degree of membrane lipid and NAPE accumulation are beneficial for maintaining membrane fluidity, leading to an enhanced tolerance of peach fruit to chilling stress.

1. Introduction

Many plant species, especially those of tropical or temperate origin, are severely injured by exposure to low but not freezing, temperatures (Saltveit & Morris, 1990). Exposure of these chilling-sensitive plants to unfavourable low temperatures often results in numerous cellular and metabolic dysfunctions, such as altered respiration rates, impaired photosynthetic activity, and changes in membrane permeability (Allen & Ort, 2001). Peach fruit is also sensitive to low temperature stress and chilling injury (CI) occurs easily when it is exposed to the low temperature for long periods (Saltveit & Morris, 1990). In a previous experiment, we found that peach fruits stored at 5 °C for 21 d usually showed CI, but no CI symptom occurred at 0 °C (Zhang & Tian, 2009). In addition, peach fruits kept at controlled atmosphere (CA), with 5% O_2 plus 5% CO_2 showed a stronger resistance to low temperature stress (Wang, Tian, & Xu, 2005) and application of methyl salicylate (MeSA) could effectively enhance tolerance of peach fruits to CI (Meng, Han, Wang, & Tian, 2009). A better understanding of the processes of chilling tolerance in fruit is now required as this may lead to important agricultural and economic benefits.

A biochemical basis to explain the CI mechanism of plant has not yet been established. Lyons (1973) proposes the existence of a primary event that results in a series of secondary events which, in turn, results in the symptoms of CI. The cell membranes are likely sites of primary effects of chilling. Mikami and Murata (2003) reported that unsaturated fatty acid (UFA) level in membrane lipids was positively correlated with plant chilling tolerance. Genetic manipulation of the level of UFAs led to the eventual modification of the cold sensitivity of tobacco plants (Murata et al., 1992). In cyanobacteria, sensitivity to cold is also closely correlated with the level of unsaturation of membrane lipids (Tasaka et al., 1996). Aside from the interest given to the responses of the UFA level to low temperature stress, the role of an unusual phospholipid class, *N*-acylphosphatidylethanolamines (NAPEs), in membrane protection and stabilisation has received considerable attention (Hansen, Moesgaard, Hansen, & Petersen, 2000). This compound is characterised by the presence of a third fatty acyl residue linked to the *N*-atom of the phosphatidylethanolamine headgroup by an amide bond and shows a propensity to accumulate under various stress conditions involving degenerative membrane changes (Schmid, Schmid, & Natarajan, 1990). For instance, NAPEs synthesis was observed in cultivated potato cells submitted to anoxia stress and the capacity to increase its NAPE level may confer some additional protection to the cell (Rawyler, Arpagaus, & Braendle, 2002; Rawyler & Braendle, 2001). However, studies on NAPEs biosynthesis and its involvement in the response of plant tissues to low temperature stress have, so far, been rare.

* Corresponding author. Address: Institute of Botany, Chinese Academy of Sciences, Nanxincun 20, Xiangshan, Beijing 100093, PR China. Tel.: +86 10 62836559; fax: +86 10 82594675.
E-mail address: tsp@ibcas.ac.cn (S. Tian).

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.11.029

As compared with plant responses to chilling stress, there are a number of papers describing that the development of CI of fruit is caused by an imbalance between oxidative and reductive processes due to metabolic gas gradients inside the fruit (Franck et al., 2007; Wang et al., 2005). Accumulation of reactive oxygen species may induce loss of membrane integrity which becomes macroscopically visible through the enzymatic oxidation of phenolic compounds to brown-coloured polymers. However, plasma membrane involvement in the process of CI development has received little attention to date. In order to fully understand whether the biochemical and biophysical characteristics of plasma membrane lipids are correlated with chilling-resistance of peach fruit, we investigated the function of linolenic acid (C18:3) and unsaturation degree of the plasma membrane in enhancing tolerance of peach fruit to low temperature stress. Here, we present the first evidence that peach fruit acquired chilling-tolerance by accumulation of C18:3 and NAPEs in plasma membrane, and explain why peach fruit stored at 0 °C showed a higher chilling tolerance than did that at 5 °C.

2. Materials and methods

2.1. Fruit and treatments

Peach fruits (*Prunus persica* L. cv. Beijing 33) were harvested at commercial maturity from an orchard in the Pinggu district of Beijing, China, and transported to the laboratory, within 2 h, after harvest. Fruit used for experiment were selected for uniform size and for the absence of physical injuries or infections. There were 360 fruits in each group. One group was stored in air at 5 °C (5 °C-Air) and served as control. A second group was stored in air at 0 °C (0 °C-Air) and a third group was stored in controlled atmosphere (5% O_2 + 5% CO_2) at 0 °C (0 °C-CA). Fruits were placed in plastic boxes ($40 \times 30 \times 25$ cm), wrapped in polyethylene film bags (0.04 mm thickness, with 5 holes of 20 mm diameter on upper and side surfaces) to maintain relative humidity (RH) at approximately 95%. At 7 day intervals, CI incidence, CI index and electrolyte leakage of fruit were measured on the same sample consisting of 30 fruits per replicate (30×3) for each treatment. Then these samples were cut into small pieces and frozen in liquid nitrogen, then stored at −80 °C for other assays.

2.2. Evaluation of CI

The CI incidence and CI index were estimated on flesh browning according to the method of Wang et al. (2005). The score of CI was assessed by measuring the browning area in each fruit based on the following scale: 0 = no browning; 1 = less than 1/4 browning; 2 = 1/4–1/2 browning; 3 = 1/2–3/4 browning area; 4 = more than 3/4 browning. The CI index was calculated from the following formula: $(1 \times N_1 + 2 \times N_2 + 3 \times N_3 + 4 \times N_4) \times 100/(4 \times N)$, where N = total number of fruit measured and N_1, N_2, N_3 and N_4 were the numbers of fruit showing the different degrees of browning.

2.3. Isolation of plasma membrane

Plasma membrane (PM) was isolated, as described by Quartacci, Cosi, and Navari-Izzo (2001), using the two-phase aqueous polymer partition system. Frozen peach fruits (120 g) were homogenised (using an ordinary kitchen homogenizer) for 3×20 s in 300 ml of an extraction medium consisting of 0.33 M sucrose, 80 mM Tris, 5 mM Bis-Tris Propane (BTP)-Mes, pH 8.9, 10 mM ascorbic acid, 5 mM dithiothreitol (DTT), 5 mM Na_2-EDTA, 10% glycerol, 0.4% BSA, 0.4% casein, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 0.15% (w/v) polyvinylpolypyrrolidone (PVPP). The homogenate was filtered through four layers of nylon cloth and centrifuged at 12,000*g* for 1 h. A microsomal pellet was obtained from the supernatant by centrifugation at 120,000*g* for 1 h. This pellet was suspended in a total volume of 10 ml in 0.25 M sucrose, 3 mM KCl, 5 mM potassium phosphate, pH7.8, and 9.0 g of the suspension was added to the 27.0 g aqueous two-phase polymer system to give a 36.0 g phase system with a final composition of 6.2% (w/w) dextran T500, 6.2% (w/w) polyethylene glycol 3350, 0.33 M sucrose, 3 mM KCl, 5 mM potassium phosphate, pH 7.8. The PM was further purified using a two-step batch procedure. The resulting upper phase was diluted 4-fold with 50 mM Tris–HCl (pH 7.5), containing 0.25 M sucrose, and centrifuged for 30 min at 120,000*g*. The resultant PM pellet was resuspended in the same buffer containing 30% ethylene glycol and stored at −80 °C for lipid analyses. All steps of the isolation procedure were carried out at 4 °C. To check the purity of the PM, the activity of the vanadate-sensitive ATPase as a marker enzyme was determined. NO_3-sensitive ATPase activities and azide-sensitive ATPase were used as markers of mitochondria and tonoplast, respectively. The chlorophyll assay was performed to determine the level of contamination from chloroplast (Quartacci et al., 2001). The marker enzyme assay for plasma membrane (vanadate-sensitive ATPase activity) suggested a 24-fold enrichment in the final plasma membrane fraction compared with the microsomal fraction, whilst the proportion of tonoplast, mitochondria, and chloroplast contamination decreased. In addition, chlorophyll was not detected in the PM fraction.

2.4. Spin labelling and measurement of electron paramagnetic resonance (EPR)

Plasma membrane fluidity was investigated by EPR, according to the method of Zhang and Tian (2009), with a slight modification. Stock solution of the fatty acid spin probes, 5- and 16-doxylstearic acid (5- and 16-DSA, Aldrich), was made up in ethanol (10 mg ml^{-1}). The probes were added to 200 μl samples of plasma membrane at a label to lipid ratio of 1:20 (v:v). After incubation for 1 h at ambient temperature and under nitrogen atmosphere, the sample was washed three times in 0.1 M potassium phosphate buffer, pH 7.0, by centrifugation at 200,000*g* for 30 min. Free spin probes were not detected in the supernatant following the third washing. For EPR analysis, the plasma membrane suspension was transferred to a 100 μl glass capillary tube, which was sealed and inserted into a quartz sample holder and put in the microwave cavity of the spectrometer. EPR measurements were performed on an ER-200D Bruker spectrometer. EPR spectra were obtained at X-band (9.80 GHz) with microwave power of 20 mW, modulation frequency 100 kHz and amplitude 1G The sweep time was 100 s and magnetic field scan 200 G. In the case of 5- and 16-DSA, the fluidity of the lipid chain can be estimated from the order parameter S and the rotational correlation time τ_c, respectively.

2.5. Extraction and analysis of plasma membrane lipids

Lipids were extracted from the PM suspension by the addition of boiling 2-propanol followed by $CHCl_3$: MeOH (2:1, v/v) containing butylhydroxytoluene (50 μg ml^{-1}) as an antioxidant. The solvent mixture was then washed with 0.2 volume of 0.88% KCl to separate the $CHCl_3$ phase. The upper H_2O phase was re-extracted with $CHCl_3$, than the $CHCl_3$ phases combined and dried under a stream of N_2. The individual polar lipid was purified by two-dimensional thin-layer chromatography (TLC) on activated silica gel plates (Silica gel 60, 0.25 mm thickness, Qingdao, China) according to the method described by Zhang and Tian (2009).

Fatty acids of the individual polar lipid classes were identified and quantified by gas chromatography (GC) after conversion to