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Short communication

Antioxidant enzymes and fatty acid composition as related to disease resistance in postharvest loquat fruit

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ABSTRACT

Two cultivars of loquat fruit were stored at 20 °C for 10 days to investigate the relationship between disease resistance, and fatty acid composition and activities of endogenous antioxidant enzymes. The results showed that decay incidence increased with storage time in both cultivars. A significantly lower disease incidence was observed in 'Qingzhong' fruit than in 'Fuyang', suggesting 'Qingzhong' had increased disease resistance. Meanwhile, 'Qingzhong' fruit also had lower levels of superoxide radical and hydrogen peroxide, and lower lipoxygenase activity, but higher levels of linolenic and linoleic acids and higher activities of catalase (CAT) and ascorbate peroxidase (APX) compared with 'Fuyang'. These results suggest that the higher levels of linolenic and linoleic acids and the higher activity of CAT and APX have a role in disease resistance of postharvest loquat fruit.

1. Introduction

Loquat fruit has a very short shelf life, mainly due to anthracnose rot caused by the fungi of *Colletotrichum acutatum*. Traditionally, control of postharvest disease in this fruit relies heavily on the use of fungicides (Cao, Zheng, Yang, Tang, & Jin, 2008). However, consumer concern over the environmental pollution, chemical residues and the development of fungicide resistance in pathogens has urged the development of alternative approaches. Although various methods such as modified atmosphere packaging, low temperature storage, and applications of methyl jasmonate or 1-methylcyclopropene (1-MCP) have been demonstrated to reduce fruit decay and quality deterioration (Cao, Zheng, Yang, Tang, Jin, et al., 2008; Cao, Yang, Cai, & Zheng, 2011; Ding, Chachin, Hamauzu, Ueda, & Imahori, 1998; Ding, Chachin, Ueda, Imahori, & Wang, 2002), there is still considerable interest in the development of disease resistant cultivars, and the identification of appropriate markers for resistance.

It is well known that natural disease resistance in postharvest fruit declines with ripening and senescence (Prusky, Plumbley, & Kobiler, 1991; Prusky, 1996). Therefore, to reduce postharvest decay, it may be useful to slow or delaying fruit ripening and senescence. Reactive oxygen species (ROS) play a central role in mediating a variety of cellular responses in plant cells, including senescence (Shewfelt & Purvis, 1995). A increased ROS scavenging capacity is positively correlated with prevention of senescence and disease inhibition in postharvest fruit (Cao & Zheng, 2010; Castoria, Caputo, De Curtis, & De Cicco, 2003; Zheng, Yu, Chen, Huang, & Wu, 2007). In addition, unsaturated fatty acids such as linoleic acid (18:2) and linolenic acid (18:3) have been reported as a source of metabolites called oxylipins, some of which can inhibit growth of fungal pathogens (Blée, 2002; Howe & Schilmiller, 2002). Wang, Chin, and Chen (1998) have previously demonstrated that expression of the yeast Δ-9 desaturase gene in tomato resulted in sharp increases in unsaturated fatty acid contents and enhancement of its resistance to powdery mildew disease.

Our previous studies indicated that the oxidative stress and decrease of membrane lipid unsaturation were involved in the development of chilling injury in loquat fruit (Cao et al., 2011). However, whether the composition of fatty acids in membranes and/or the antioxidant system have a role in disease resistance in loquat fruit is unclear. The aim of the present study was to investigate the possible role of membrane fatty acid composition and antioxidant enzymes in disease resistance of loquat fruit using two cultivars with significant different susceptibilities to decay.

2. Materials and methods

2.1. Plant material and treatments

Loquat fruit (*Eriobotrya japonica* L.) from cultivars 'Qingzhong' and 'Fuyang' were hand-harvested when ripe stage from the same

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commercial plantation located in Suzhou (Jiangsu province) and transported within four hours to the laboratory. The fruit were selected for uniform size and color and the absence of visual defects, then randomly allocated to one of two groups, and stored at 20 °C and approx. 95% relative humidity for up to 10 days. There were three replicates of batches of fruit (5 kg) per treatment, and the experiment was conducted twice. Fruit samples were taken at two-day intervals during storage for measurements of decay incidence, superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2) content, fatty acid composition, and activities of lipoxygenase (LOX, EC 1.13.11.12), superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6) and ascorbate peroxidase (APX, EC 1.11.1.11).

2.2. Decay evaluation

The severity of fruit decay was evaluated visually on 10 fruit from each replicate according to a four-point scale, where 0 = no decay, 1 = slight decay covering ⩽25% of the fruit surface; 2 = moderate decay covering >25% but <50% of the fruit surface; and 3 = severe decay covering >50% of the fruit surface. Decay incidence expressed as a decay index was calculated using the following formula:

$$\text{Decay incidence } (\%) = [(1 \times N_1 + 2 \times N_2 + 3 \times N_3) \times 100/(3 \times N)]$$

where N was the total number of fruit measured and N_1, N_2, and N_3 were the numbers of fruit showing the different severities of decay.

2.3. Measurements of O_2^- and H_2O_2

For O_2^- production measurement, 5 g of flesh was ground in 5 ml of 50 mmol/L phosphate buffer (pH 7.8) using the method of Elstner (1976). For H_2O_2 determination, 2 g of tissue was homogenized with 5 ml of chilled 100% acetone according to the method of Patterson, Mackae, and Ferguson (1984). The extracts were then homogenized and centrifuged at 10,000*g* for 20 min at 4 °C. The supernatants were used for the O_2^- and H_2O_2 assays. O_2^- production was calculated against the standard curve and expressed as nmol g^{-1}FW min^{-1}. H_2O_2 content was expressed as nmol g^{-1} FW.

2.4. Fatty acid quantification

Total lipids were extracted according to Valero, López-Frías, Llópis, and López-Jurado (1990). Briefly, 20 g of tissue were homogenised in 10 ml chloroform:methanol:0.1 mol/L HCl (200:100:1) then 10 ml of 0.1 mol/L HCl were added before centrifugation at 4000*g* for 10 min. The organic phase was collected and evaporated to dryness under nitrogen. Methylation of fatty acids was carried out by adding 1 ml boron trifluoride/methanol at boiling for 10 min. Methylated fatty acids were extracted with hexane, evaporated to dryness under nitrogen and redissolved in 200 μl chloroform before injection. Fatty acids were separated and quantified according to Mirdehghan et al. (2007) by gas chromatography (GC, Hewlett-Packard model 6890) equipped with flame ionization detector (FID). Identification and quantification of fatty acids were performed by comparing retention times and peak areas with authentic standards (Sigma Chemical Co., St. Louis, MO, USA).

2.5. Enzyme assays

All enzyme extract procedures were conducted at 4 °C. For LOX, 2 g of flesh was ground with 5 ml of 50 mmol/L Tris–HCl (pH 8.0) containing 10 mmol/L KCl, 500 mmol/L sucrose and 0.5 mmol/L phenylmethylsulfonylfloride at 4 °C. For SOD and CAT, 2 g of flesh tissue was ground with 5 ml of 50 mmol/L sodium phosphate buffer (pH 7.8 or 7.0), respectively. For APX, flesh tissue (2 g) was ground with 5 ml of 50 mmol/L sodium phosphate buffer (pH 7.0) containing 0.1 mmol/L EDTA, 1 mmol/L AsA and 1% (w/v) polyvinyl-pyrrolidone. The extracts were then homogenized and centrifuged at 20,000*g* for 20 min at 4 °C. The supernatants were used for the enzyme assays.

LOX activity was assayed using the method of Todd, Paliyath, and Thompson (1990). One unit of LOX was defined as the amount of enzyme that caused an increase in absorption at 234 nm of 0.01 min^{-1} at 25 °C when linoleic acid is used as the substrate.

SOD activity was determined by the method of Rao, Paliyath, and Ormrod (1996). One unit of SOD activity was defined as the amount of enzyme that caused a 50% inhibition of nitro blue tetrazolium.

CAT activity was assayed according to the method of Chance, and Maehly (1955). One unit of CAT was defined as the amount of enzyme that decomposed 1 μmol of H_2O_2 min^{-1}.

APX activity was carried out as described the method of Nakano, and Asada (1989). The activity was calculated from the change in absorbance at 290 nm for 1 min when the extinction coefficient was 2.8 $mmol^{-1}$ cm^{-1}.

Protein content in the enzyme extracts was estimated using the Bradford (1976) method, using bovine serum albumin as a standard. Specific activity of the enzymes was expressed as units per milligram protein.

2.6. Statistical analysis

The results were analyzed using one-way analyses of variance (ANOVA) with the statistical software of SPSS (SPSS Inc., Chicago, IL, USA). Differences between means were studied with Duncan's test and difference at $p < 0.05$ was considered to be significant.

3. Results

3.1. Changes of decay incidence of 'Fuyang' and 'Qingzhong' loquat fruit during storage

Fruit decay incidence increased in 'Fuyang' and 'Qingzhong' loquat fruit during storage (Fig. 1). However, a significantly ($p < 0.05$) lower incidence was observed in 'Qingzhong' fruit compared with 'Fuyang' fruit. At the end of the storage, the decay incidence in 'Qingzhong' fruit was 45.8% lower than that in 'Fuyang'.

3.2. Changes of O_2^- and H_2O_2 contents of 'Fuyang' and 'Qingzhong' loquat fruit during storage

Levels of O_2^- and H_2O_2 increased with storage time in both cultivars. However, significantly ($p < 0.05$) lower concentrations occurred in 'Qingzhong' (Fig. 2). O_2^- and H_2O_2 concentrations in 'Fuyang' were 112% and 125%, respectively, higher than those in 'Qingzhong' after 10 days of storage.

3.3. Changes of LOX, SOD, CAT and APX activities of 'Fuyang' and 'Qingzhong' loquat fruit during storage

LOX activity in 'Fuyang' increased with storage time. However, a decline occurred in 'Qingzhong' after eight days of storage (Fig. 3A). SOD activity in both cultivars decreased initially and then increased rapidly after the fourth day. The activity declined again subsequently with storage time. There was no significant difference in SOD activity between the two cultivars (Fig. 3B). CAT activity in 'Qingzhong' increased rapidly during the first four days of storage and then declined gradually. APX activity increased with