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Research article

Potential role of reactive oxygen species and antioxidant genes in the regulation of peach fruit development and ripening

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ABSTRACT

The roles of reactive oxygen species (ROS) as both toxic by-products and as signaling molecules have been reported in fruit development and ripening. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) play important roles in balancing the induction and removal of ROS in plants, and are respectively encoded by families of closely homologous genes. In the present study, we investigated the roles of ROS and the above-mentioned antioxidant genes during the development and ripening of peach fruit. The experimental results indicated that O_2^- and H_2O_2 acted as potential signaling molecules in the middle stage of fruit development, and only H_2O_2 might function as a main toxic molecule to stimulate lipid peroxidation and oxidative stress in the late stage of fruit ripening. *PpaCu/Zn-SODs* were the most abundant members in the *PpaSOD* gene family and they expressed steadily in peach fruit development and ripening. Low temperature (4 °C) postponed and suppressed the climacteric peaks of respiration and ethylene, significantly enhanced the activities of CAT and GPX, and up-regulated the expression of *PpaCAT1* and *PpaGPX6* in the late stage of fruit ripening. *PpaCAT1* and *PpaGPX6* were two key genes in alleviating oxidative stress in the late stage of fruit ripening.

1. Introduction

Peach (*Prunus persica* L.), of the *Rosaceae* family, is an important fruit worldwide. It is a typical climacteric fruit, and is highly perishable at ambient temperature. The development and ripening of climacteric fruits are oxidative processes, producing reactive oxygen species (ROS), such as superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2) (Mondal et al., 2004; Pandey et al., 2013). Fruit ripening can be effectively slowed down by low temperature. However, peach is not fit for long-term cold storage which causes physiological disorder in the fruit. The physiological disorder is caused by the imbalance between the production and removal of ROS (Sevillano et al., 2009). Therefore, ROS may be important regulators in peach fruit ripening at ambient and low temperature storage.

ROS in plants are inevitable by-products in normal metabolic processes and in response to biotic and abiotic stresses (Dasgupta et al., 2013). They are well known for their toxic roles in lipid peroxidation, in damage to membranes and in oxidization of protein and DNA, resulting in cell death (Liu and Wang, 2012). Recently, attention has been paid to the signaling roles of ROS in the development and ripening of fruit (Kocsy et al., 2013). However, the mechanism of these molecules in regulating the signaling network that controls fruit development and ripening is unclear.

Plant cells continuously generate ROS from the electron transport chains of photosynthesis and respiration in different organelles. To prevent the accumulation of ROS to the toxic level, plants develop enzymes and non-enzyme antioxidant chemicals as a defensive force against excess ROS (Sgherri et al., 2003). Superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6) and glutathione peroxidase (GPX; EC 1.11.1.9) scavenge ROS, such as O_2^- and H_2O_2, and prevent them from over-accumulation. SOD is the first line of defense against ROS, and it catalyzes the dismutation of O_2^- to molecular oxygen and H_2O_2. According to the metal species present at their active sites, SODs are classified into three types: the copper/zinc (Cu/Zn-SOD), the manganese (Mn-SOD) and the iron (Fe-SOD), which are located in different cellular compartments (Mittler, 2002). CAT decomposes H_2O_2 without the expense of other reductants and are indispensable for ROS detoxification in plants under stresses (Garg and Manchanda, 2009). GPX also protects organisms from oxidative damage by reducing harmful H_2O_2 and

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larger hydroperoxides (Huan et al., 2016). In fruit, these antioxidant enzymes are well known for their roles in regulating ROS under biotic and abiotic stresses (Melchiorre et al., 2009). Recent studies showed that they are also involved in regulating fruit development (Hu et al., 2011) and ripening (Pandey et al., 2013). Moreover, these antioxidant enzymes are encoded by families of closely homologous genes that usually display specific structure and expression pattern in different physiological processes. However, limited information about the functions of these genes is available in regulating ROS in peach fruit development and ripening.

Xiahui 5 is one of the most promising early-maturing peach fruit in Jiangsu Province, China, due to its rapid growth, high yield and delicious quality. The physiological changes during the ripening of some late-season peach cultivars have been reported (Ferrer et al., 2005), but no studies in this regard have been conducted, to our knowledge, on the early-maturing peach cultivars. The present study was to investigate the roles of ROS in the development and ripening of Xiahui 5 peach fruit, and the functions of antioxidant enzymes in regulating ROS in the above physiological processes at enzymatic and transcriptional levels. The research results would provide supports for monitoring the physiological processes of peach and for ways of extending the shelf-life of the fruit.

2. Materials and methods

2.1. Fruit materials and storage condition

Early-maturing peach fruit (*Prunus persica* L. cv. Xiahui 5) were grown in the orchard at Jiangsu Academy of Agricultural Sciences in Nanjing, Jiangsu Province, China. The developing peach fruits were harvested at 10-day intervals, beginning at day 60 after full bloom. Thirty fruits were selected for quality attribute measurement at each time point and the results were shown in Table 1. According to the classical double-sigmoid curve described by Chalmers and van den Ende (Chalmers and Ende, 1975), the first samples were obtained during the second rapid fresh weight increase (SIII), when a dramatic increase in fruit size occurs. Therefore, we defined the selected time points during peach fruit development as SIII-1, SIII-2, SIII-3 and SIII-4.

For postharvest experiment, approximately six hundred fruits, uniform in size, disease-free, with no mechanical damage were harvested at phase SIV, when fresh weight was steady. The harvested fruits (SIV-0) were randomly divided into two groups. One was directly stored at ambient temperature (25 ± 1 °C) with 85–90% humidity for 5 day at 2-day intervals (SIV-1, SIV-3 and SIV-5). The other group was stored at 4 ± 0.5 °C with 85–90% humidity for 5 weeks (SIV-7, SIV-14, SIV-21, SIV-28 and SIV-35).

To describe the characteristics of peach fruit, the fruit development was divided into two stages based on the changes in fruit diameter and fresh weight (Supporting Information Fig. S1). The middle stage: from SIII-1 to SIII-3 (Stage I); and the late stage: from SIII-3 to SIV-0 (Stage II). Fruit ripening was divided into four stages based on the changes in respiration rate. Two early stages: from SIV-0 to SIV-1 at ambient temperature (Stage III) and from SIV-0 to SIV-7 at low temperature (Stage V); two late stages: from SIV-1 to SIV-5 at ambient temperature (Stage IV) and from SIV-7 to SIV-35 at low temperature (Stage VI).

At each time point, 30 fruit samples in three replicates of 10 fruits were taken for analysis, and only the mesocarp was used for analysis.

2.2. Respiration rate and ethylene production

Five fresh fruits enclosed in 2-L glass jars at 25 °C for 1 h were treated as an experiment unit and six replicates were obtained from a total of 30 fruit samples at each time point. CO_2 levels were measured by an infrared CO_2 gas analyzer (GXH-3010E, Nuoji Instruments Inc, Changzhou, China). The results were expressed as CO_2 mg kg^{-1} h^{-1}. For the ethylene production analysis, a 1-mL headspace gas sample was injected into a gas chromatograph (Agilent GC7890A) equipped with a column and a flame ionization detector (FID). The ethylene production rate was expressed as μL kg^{-1} h^{-1}.

2.3. Superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2) contents

The O_2^- content was measured according to the method of Xu (Xu et al., 2012) with slight modifications. Two grams of samples was homogenized in 6 mL of 65 mM sodium phosphate buffer (pH 7.8) and was centrifuged at 10,000*g* for 15 min. The incubation mixture contained 1 mL of 65 mM sodium phosphate buffer (pH 7.8), 0.1 mL of 10 mL of 10 mM hydroxylammonium chloride and 1 mL of supernatant. After incubation at 37 °C for 1 h, 1 mL of 58 mM sulphanilic acid and 7 mM α-naphthyl amine were separately added to the incubation mixture. The mixture reacted at 37 °C for 20 min, and then the absorbance was read at 530 nm. A standard curve with $NaNO_2$ was used to calculate the O_2^- content which was expressed as micromoles per gram of fresh weight.

To determine H_2O_2, 2 g of fresh tissue was homogenized with 5 mL of chilled 100% acetone and then centrifuged at 10,000*g* for 20 min at 4 °C. The supernatant was collected immediately for H_2O_2 analysis according to the method of Patterson (Patterson et al., 1984). The H_2O_2 content was expressed as micromoles per kilogram of fresh weight.

2.4. Lipid peroxidation and electrolyte leakage

Lipid peroxidation was determined by measuring the content of malondialdehyde (MDA) according to the method of Shah (Shah

Table 1
Quality attributes in peach fruit development.

Quality attributes					
Diameter (mm/fruit)	28.76 ± 0.76e	37.42 ± 0.98d	43.62 ± 1.17c	65.74 ± 1.58b	72.19 ± 2.43a
Fresh weight (g/fruit)	25.57 ± 1.23e	37.81 ± 0.69d	61.65 ± 1.67c	140.81 ± 1.95b	202.15 ± 2.93a
Color a* (Peel)	−7.82 ± 1.09b	−9.20 ± 2.05b	−14.65 ± 0.75c	−8.86 ± 2.79b	11.38 ± 3.74a
Color a* (Pulp)	−17.30 ± 0.32e	−15.02 ± 0.62d	−13.96 ± 0.79c	−7.52 ± 0.92b	−2.92 ± 1.15a

SIII-1, SIII-2, SIII-3, SIII-4 and SIV-0 represent the 60 day to the 100 day at 10-day intervals after full bloom respectively.
a* means color intensity changing from green to red.
All the values are expressed as means ± SE of 30 fruit samples and the different normal letters indicate significant differences at 0.05 level (Duncan's test).