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Differential expression of iron–sulfur cluster biosynthesis genes during peach fruit development and ripening, and their response to iron compound spraying

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ABSTRACT

Iron (Fe) is required for the iron–sulphur (Fe–S) cluster biosynthesis, which occurs in chloroplasts, mitochondria and cytosol that needs dozens of genes. However, molecular mechanisms of Fe metabolism in fruit trees are largely rare, especially towards fruit development and ripening. In this work, we characterized 'Xiahui8' peach development, and analyzed expression profiles of 44 Fe–S cluster biosynthesis genes during different fruit development stages. The development of 'Xiahui8' peaches last for an average period of 130 days, followed by a rapid on-tree ripening during 7 days. Notably, the Fe accumulation in peach flesh was negatively correlated to fruit weight and volume, but positively correlated to flesh firmness. Quantitative Real-Time PCR (qRT-PCR) analysis showed that Fe–S cluster biosynthesis genes were differentially expressed during distinct fruit development stages. A majority of plastid SUF and mitochondrial ISC machinery genes were gradually enhanced along with fruit growth, and began to decrease since commercial harvest phase. The highest expression level of all cytosolic CIA machinery genes were appeared in early fruitlet formation stage, and gradually reduced until fruit ripening. Moreover, foliar spraying of amino acid-Fe compound fertilizer significantly enhanced the total soluble solid (TSS) content in peaches, accompanied by strengthened flesh firmness, Fe concentration, and succinate dehydrogenase (SDH) and aconitase (ACO) activities. Thirteen Fe–S cluster biosynthesis genes were responsive to fertilizer treatment, whose expression level were significantly induced, with the exception of *HSCA2* that was reduced. Our findings provided molecular basis for Fe metabolism in peach fruit development, and revealed potential genes for further functional verification.

1. Introduction

In orchards, iron (Fe) is an indispensable cation required as mineral nutrition of fruit trees (Tagliavini et al., 2000; Pestana et al., 2005). Fe deficiency is a major constraint for many fruit trees, especially those grown on calcareous and/or alkaline soils, and it negatively affects fruit size, yield and quality (Chen and Barak, 1982; Tagliavini et al., 2000; Tagliavini and Rombolà, 2001; Álvarez-Fernández et al., 2003; Pestana et al., 2005; Barton and Abadia, 2006). Foliar spraying of different compound Fe preparations favorably improved fruit quality and enhanced fruit yield in pear (Tagliavini et al., 2000), grape (Tagliavini and Rombolà, 2001), nectarine (Ren et al., 2009), kiwifruit (Che et al., 2011; Wang et al., 2011) and pear-jujube (Niu et al., 2015). However, such reports mainly focused on physiological and biochemical studies. Reports on the molecular basis of Fe metabolism in fruit crops are limited.

In *Arabidopsis*, a great majority of Fe usage occurs in the form of iron-sulfur (Fe–S) proteins, which contain Fe–S clusters as cofactors, and are involved in many important metabolic pathways and cellular processes (Johnson et al., 2005; Lill, 2009; Balk and Pilon, 2011; Couturier et al., 2013). For example, the Fe–S protein nitrite

Abbreviations: ACO, aconitase; CIA, cytosolic iron–sulfur cluster assembly; DAFB, day after full bloom; Fe–S, iron–sulfur; ICP-AES, inductively coupled plasma atomatic emission spectrometry; H, harvest; ISC, iron–sulfur cluster; NiR, nitrite reductase; qRT-PCR, quantitative real-time PCR; SDH, succinate dehydrogenase; SUF, sulfur mobilization.

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reductase (NiR) plays a key role in chloroplastic nitrogen assimilation, while aconitase (ACO) and succinate dehydrogenase (SDH) are crucial Fe–S proteins involved in the mitochondrial citric acid cycle of glycometabolism. Notably, a highly conserved Fe–S cluster biosynthesis process includes a sulfur donor (NFS), an iron donor, and a number of scaffolds (SUFB, SUFC, SUFD, NFU, etc.) and delivery proteins (ISA, GRX, HSCA, etc.) (reviewed in Balk and Lobreaux, 2005; Balk and Pilon, 2011; Couturier et al., 2013). To date, more than 40 genes have been identified as Fe–S cluster biosynthesis genes in *Arabidopsis* (Balk and Pilon, 2011), rice (Liang et al., 2014) and soybean (Qin et al., 2015), and they are located in chloroplasts, mitochondria, and cytosol, respectively. In particular, the plastids harbor the sulfur mobilization (SUF) pathway and the mitochondria organelles independently use the iron–sulfur cluster (ISC) assembly pathway, whereas the cytosolic Fe–S cluster assembly depends on the emerging cytosolic iron–sulfur cluster assembly (CIA) pathway and mitochondria (Balk and Lobreaux, 2005; Bernard et al., 2013). Understanding Fe–S cluster biosynthesis in model plants contributes to practical applications in perennial woody plants.

Peach (*Prunus persica*) is a popular stone fruit worldwide, which has been genetically sequenced (Jung et al., 2008; Layne and Bassi 2008). 'Xiahui8′ is a new late-ripening and melting flesh Chinese peach cultivar that ripens in early-mid August in Nanjing, China (Yu et al., 2014). The natural soil fertility in Nanjing area is relatively high, and the total soil Fe content of the surface soil is sufficient (Liu et al., 2012; Wang et al., 2012). However, both the soil available Fe (0.17 g kg^{-1}) and available Fe activation rate (0.54%) in the Nanjing area are relatively low, and far less than the other trace elements (Wang et al., 2012; Wang et al., 2013). Thus, we wondered whether there would be effects of additional Fe fertilizer on peach fruit quality, and whether Fe–S cluster biosynthesis genes would respond to Fe fertilizer applications. In previous studies, we identified 44 putative Fe–S cluster biosynthesis genes in peach (Song et al., 2014). Here, we characterized 'Xiahui8′ peach fruit development and ripening, and determined the expression profiles of these Fe–S cluster biosynthesis genes during the whole fruit development process and their responses to Fe spray treatments. Our findings revealed the molecular basis of Fe metabolism in fruit development and ripening, especially for perennial woody trees.

2. Materials and methods

2.1. Plant material and growth condition

Seven-year-old 'Xiahui8' peach trees growing at the National Peach Germplasm Repository (Nanjing, China) were used throughout this study. The peach orchard was divided into two plots in which six trees per plot, growing under the same common field conditions, were selected for sample collection. One plot was chosen as the control for normal peach fruit growth, and 20 peaches with no visible defects were randomly collected from trees at 10-day intervals, starting from 10 days after full bloom (DAFB) through to ripening. The other plot was subjected to a preharvest foliar spray of an amino acid-Fe compound fertilizer (with a final 1000 mg kg^{-1} concentration of Fe_2SO_4), which was made according to the description of the National Agricultural Standard for Water Soluble Fertilizer Liquid Products of China (NY 1429-2007). Target trees were sprayed with 2 l of the compound fertilizer twice, the first time on June 13th (80 DAFB, second exponential growth phase) and the second on July 13th (110 DAFB, 10 d before commercial harvest), as in previous reports (Che et al., 2011; Wang et al., 2011; Niu et al., 2015). The control trees were sprayed with clear water. To determine the effects of the fertilizer spray on the fruit quality and the Fe nutrition status, 20 'Xiahui8′ peaches (total of 60) were collected 100 (20 d after the first spraying), 130 (20 d after the second spraying) and 137 (27 d after the second spraying) DAFB.

2.2. Physiological analysis

Fruits were collected, and the fresh weights, and vertical and transverse diameters were calculated. Flesh firmness was checked using a TA.XTplus Texture Analyser (Stable Micro System, London, UK), by inserting 5 mm of the detector into the middle area beside the fruit suture line, at the rate of 1 mm s^{-1}. Then, the mesocarps were isolated for further physiological and molecular analyses. For the Fe concentration analysis, samples were dried and then digested using the HNO_3-$HClO_4$ method, and subjected to ICP-AES systems (IRIS Advantage; Thermo Electron, Waltham, MA, USA). ACO activity was determined as described by Liang et al. (2014). The activity assays of NiR and SDH were carried out using relevant detection kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), according to the manufacturer's descriptions. The total soluble solid (TSS) of peach flesh juice was assayed using WZS-1 Abbe Refractometry (Shanghai, China). Titratable acidity (TA) was determined by titration with 0.1 N NaOH to an endpoint of pH 8.3 and expressed as meq per 100 ml flesh juice.

2.3. Chromosome distribution analysis

The starting position of all of the Fe–S cluster biosynthesis genes on each chromosome were confirmed using the BLASTN algorithm to search local databases of the complete nucleotide sequences of the peach genome (https://www.rosaceae.org/species/prunus/prunus_persica). MapInspect software was used to determine the physical locations of Fe–S cluster biosynthesis genes (http://www.plantbreeding.wur.nl/uk/software_mapinspect.html).

2.4. Phylogenetic analyses of ISU1 and HSCA1 homologs from different species

The full-length amino acid sequences of *ISU1* and *HSCA1* homologs in peach (Song et al., 2014), rice (Liang et al., 2014), soybean (Qin et al., 2015) and *Arabidopsis* (Balk and Pilon 2011) were obtained from the Phytozome genome database (http://www.phytozome.net). An unrooted phylogenetic tree was created by the bootstrap option of the ClustalW multiple alignment package using the neighbor-joining method. The scale indicates the genetic distance. The gene locus IDs are as follows: *PeISU1* (ppa012356m), *OsISU1* (Os01g47340), *GmISU1* (Glyma05g02260), *AtISU1* (At4g22220), *PeHSCA1* (At4g37910), *OsHSCA1* (Os02g53420), *GmHSCA1* (Glyma13g32790) and *AtHSCA1* (ppa002402 m).

2.5. RNA extraction and quantitative real-time PCR

Total RNA of fruit samples were extracted using RNAprep Pure Plant Kit for Polysaccharides & Polyphenolics-rich (TianGen, Beijing, China), and then were reverse transcribed into cDNA using PrimeScript™ RT reagent Kit (TaKaRa, Kyoto, Japan). qRT-PCR was carried out on 7500 Real Time PCR System (Applied Biosystems, New York, USA), using SYBR Premix Ex Taq (TaKaRa, Kyoto, Japan) reaction kit. The *Ubiquitin* (Genebank No. KJ598788) and *Actin* (KP690196) genes in peach were used as reference genes, according to the studies of Tong et al. (2009) and Song et al. (2015). Specific primers of Fe–S cluster biosynthesis genes and the control gene were listed in Supplemental Table 1. PCR reaction procedure was as follows: 95 °C for 30 s, 40 cycles of 95 °C for 5 s and 60 °C for 34 s, and then 72 °C for 60 s. To calculate the starting template concentration and PCR efficiency for each sample, the linear regression of the log (fluorescence) per cycle number data was used according to the