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Transcriptome analysis reveals a regulation of ethylene-induced post-harvest senescence in pear fruit

Huan-Yu Xu[1], Yang-Yang Chen[1], Li-Bin Wang, Zhi-Hua Xie, Chao Gu*, Shao-Ling Zhang*

Centre of Pear Engineering Technology Research, State Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing 210095, China

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ABSTRACT

Fruit senescence is induced by ethylene in pear, but it is unclear which ethylene responsive factor(s) is involved in. In this study, the ripening fruits of cv. Housui were respectively treated by ethephon and 1-MCP, and the treated fruits were used for transcriptome sequencing to reveal the genes associated with ethylene-induced senescence. As expected, ethylene biosynthesis and signal pathway genes were detected and had remarkably higher levels of expression in all the pre-decayed fruits than in the ripening fruits, but only one novel *ERF* gene (*Pbr022708.1*) was induced by ethylene in post-harvested fruits during storage. Moreover, based on the changes of fruit firmness, two ethylene-induced genes that individually encode polygalacturonase (*Pbr010853.1*) and xyloglucan endotransglucosylase/hydrolase (*Pbr040203.1*) were isolated to be associated with fruit softening during post-harvest storage. In addition, auxin signal and stress tolerance were likely involved into fruit senescence. These result will be available for understanding gene regulation of post-harvested fruits during storage.

1. Introduction

Pear is one of the popular fruits and has been cultivated in the world across Asia, Europe and America. Presently, pear cultivars are divided into five species (Bao et al. 2007; Katayama et al. 2007; Cao et al. 2012; Sehic et al. 2012), of these, the fruits of *Pyrus ussuriensis* and *P. communis* are edible after post-ripening, while the fruit of *P. pyrifolia*, *P. brestschneideri*, and *P. sinkiangensis* are directly edible at harvest (Hiwasa et al., 2003). Obviously, harvested fruit of the two ripening types have different progress during storage. The stored aim of post-harvested fruits of *P. pyrifolia*, *P. brestschneideri*, and *P. sinkiangensis* is to maintain freshness and to delay senescence, while that of *P. ussuriensis* and *P. communis* is to prolong shelf-life during fruit post-ripening.

Pear is respiratory climacteric fruit that is regulated by ethylene during ripening and post-harvest senescence (Cheng et al. 2012; Itai et al. 2000; Saltveit 1999). In the past decades, ethylene biosynthesis and signal pathway genes during fruit ripening are studied in *P. pyrifolia* (Itai et al. 1999, 2000) and has closely clarified in *P. ussuriensis* (Li et al. 2014) and *P. communis* (El-Sharkawy et al., 2003, 2004; Gao et al. 2007). The final component in ethylene signal pathway, ethylene responsive factor (ERF), which directly regulates down-stream effect genes, has recently to be shown in *P. brestschneideri* (Hao et al., 2018). However, little is known about ethylene biosynthesis and signal pathway genes during post-harvested fruit senescence in pear.

A powerful antagonist of ethylene, 1-methylcyclopropene (1-MCP), prevents down-stream physiological action of ethylene by binding to ethylene receptors more strongly. This inhibitor of ethylene action has been used to delay fruit senescence during post-harvest storage in pear (Dong et al. 2014; Mahajan et al. 2010), apple (Li et al. 2016; Mattheis and Rudell 2017), peach (Ortiz et al. 2010; Özkaya et al. 2016), and banana (Han et al. 2016a, 2016b; Xiao et al. 2013). In this study, to reveal the ethylene responsive factor involved in fruit senescence, 1-MCP was used to treat the ripening fruits of *P. pyrifolia* cultivar Housui, as well as ethephon. The ripening, ethephon- and 1-MCP treated fruits were used for transcriptome sequencing to acquire whole predicted gene expression database that used to isolate ethylene-induced differentially expressed genes. These result are available for understanding gene regulation of post-harvested fruits during storage.

2. Materials and methods

2.1. Plant material and treatment

Pear cultivar Housui (HS; *Pyrus pyrifolia*) is maintained at Jiangpu

* Corresponding authors.
E-mail addresses: guchao@njau.edu.cn (C. Gu), slzhang@njau.edu.cn (S.-L. Zhang).
[1] These authors have contributed equally to this work.

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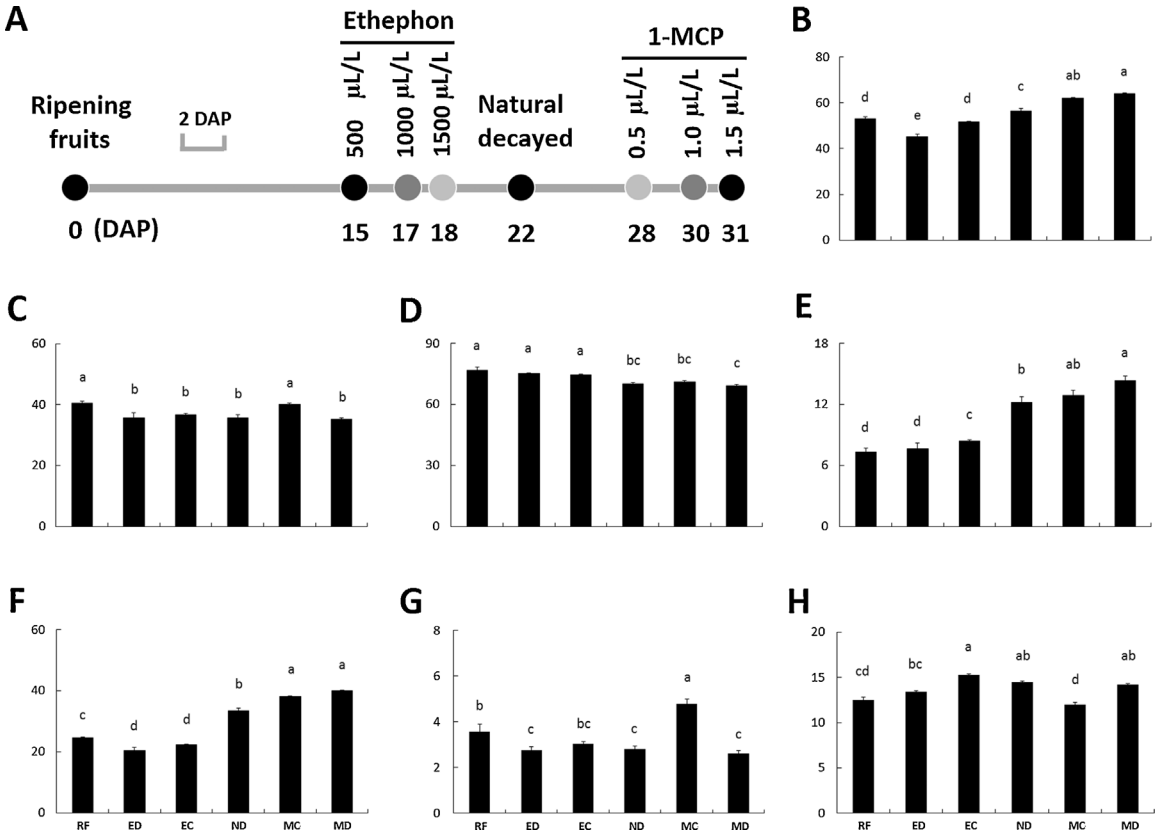

Fig. 1. Stored times and physiological indexes of post-harvested fruits. A, Stored times of ethephon and 1-MCP treated fruits. DAP, Days after post-harvest. Except the black circle of ripening fruits, the other black, gray, and silvery white circles were representing the decayed times of post-harvested fruits. Moreover, black circles are the time point for sampling pear fruits that were used for measurement of physiological indexes, including luminosity (B), chroma (C), hue (D), colors of red-green (E) and yellow-blue (F), flesh firmness (G), and soluble solids (H). The letters, a, b, c, d, and e, stand for the levels of significantly difference (P-value < 0.05) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

orchard, Nanjing Agricultural University (Nanjing, Jiangsu Province, China). Ripening fruits (RF) of cv. HS were harvested and divided into three groups that contain at least 40 fruits in each group. The first group was soaked into 500, 1000, and 1500 μL/L ethephon solutions for 10 min. After air-dried, the fruits were putted into plastic buckets to avoid water loss, and then stored at room temperature (approximately at 25℃). The second group was treated by 0.5, 1.0, and 1.5 μL/L 1-MCP for 24 h in a sealed plastic bucket at room temperature. The third group is the control of ethephon and 1-MCP treatment. When 1/2 of total fruits were decayed under ethephon treatment, the pre-decayed fruits (ED) were collected, as well as the control fruits (EC). Similarly, when 1/2 of total fruits were decayed in the control, the pre-decayed fruits (ND) were collected, as well as the 1-MCP treated fruits (MC). Moreover, when 1/2 of total fruits were decayed under 1-MCP treatment, the pre-decayed fruits (MD) were collected. The collected fruits were peeled, and the flesh were cut into small pieces and mixed together. The mixed samples were immediately frozen in liquid nitrogen and stored at -80℃ until use.

2.2. Measurements of physiological indexes

Luminosity, chroma, hue, and colors (red-green and yellow-blue) were determined by a chroma meter CR-400 (Konica Minolta Sensing, Japan). Flesh firmness of the collected fruits were probed by a handle penetrometer (Model GY-1, Hangzhou Scientific Instruments, Hangzhou, China), while soluble solids of from juice of each collected fruit were measured by a digital hand-held pocket refractometer (PAL-1; Atago, Itabashi-ku, Japan). The details are identical to our previous study (Hao et al., 2018).

2.3. Transcriptome sequencing and mapping of reads

Transcriptome libraries were constructed from samples of RF, ED, EC, ND, MC, and MD. Total RNAs of these samples were extracted using RNAprep Pure Plant Kit of Polysaccharides & Polyphenolics-rich (Tiangen, Beijing, China) according to the manufacturer's instructions. Process of RNA fragment, the first- and second-strand synthesis and A-tail supplementation, PCR enrichments, preparation and sequencing of cDNA libraries, base calling of raw data, achievement and mapping of clean reads were identical to the previous study (Hao et al., 2018).

2.4. Differential gene expression analysis

Number of clean reads mapped to each predicted gene, standardization of the read counts among samples, differential expression analysis, and *P*- and *Q*-values were determined as described in the previous study (Hao et al., 2018). A threshold *Q*-value of 0.005 and a log2-fold change of 1 were used to distinguish differentially expressed genes (DEGs) from non-differentially expressed genes. All DEGs were annotated by blasting in Swiss-Prot protein database (https://www.expasy.org/).

2.5. Quantitative real-time RT-PCR

Total RNA was extracted and subjected to synthesize the first-strand cDNA using TransScript One-Step gDNA Removal and cDNA synthesis Supermix (TransGen, Beijing, China) according to the manufacturer's instructions. Reaction mixture and condition of qRT-PCRs in LigthtCycler 480® II/96 Thermal Cycler (Roche Diagostics, Rotkreuz, Swizerland) were identical to the previous study (Hao et al., 2018). All