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# Screening of cell wall-related genes that are expressed differentially during ripening of pears with different softening characteristics

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ABSTRACT

Postharvest softening is an important physiological process that influences pear fruit quality. 'Starkrimson' pears soften rapidly, resulting in fruits with a low tolerance for storage and transportation. To better characterize the mechanism regulating the rapid softening of pears, cell wall-related changes were compared among three soft-fleshed and rapid-softening cultivars ('Starkrimson', 'Bartlett', and 'Abbe Fetel') and a crisp-fleshed and non-softening cultivar ('Dangshansuli'). Cell wall polysaccharide content measurements revealed that rapid fruit softening was accompanied by extensive pectin degradation in the three soft-fleshed cultivars. Fifteen genes encoding two polygalacturonases (PG; EC 3.2.1.15), four pectin methylesterases (EC 3.1.1.11), four β-galactosidases (EC 3.2.1.23), one α-L-arabinofuranosidase (EC 3.2.1.55) and four cellulases (EC 3.2.1.4) were isolated from pear fruit flesh. Gene expression analyses indicated that almost all of the genes were involved in ripening. Furthermore, *PG2* exhibited the most significant expression-level differences between the two types of pears, suggesting it was responsible for the rapid softening of the three soft-fleshed pears. *PG1* was highly expressed only in 'Starkrimson' pears, which may explain the considerably faster softening rate of 'Starkrimson' fruits compared with those of other cultivars. Additionally, 1-methylcyclopropene (1-MCP) was used to control the softening rate of 'Starkrimson' pears, *PG1* and *PG2* expression was effectively inhibited by 1-MCP. The results of this study suggest that *PG genes* have crucial roles in the rapid softening of 'Starkrimson' pears.



## 1. Introduction

Fruit softening is a complex process that takes place during ripening. In addition to softening, fruits undergo changes to color, flavor, and aroma during this stage. Therefore, the regulation of the softening process has very important implications for fruit texture, shelf-life, and consumer acceptance. Fruit softening generally results from loosening of the cell wall, which involves the degradation of cell wall pectin and hemicellulose components. During the loosening of the cell wall, there is usually an increase in the amount of soluble pectin and a decrease in insoluble pectin abundance (Yoshioka et al., 1992; Muda et al., 1995; Sakurai and Nevins, 1997). Numerous enzymes and proteins associated with cell wall loosening have been identified in fruits, including pectin methylesterase (PME), polygalacturonase (PG), β-galactosidase (β-GAL), α-L-arabinofuranosidase (ARF), cellulase (Cel), expansin, and xyloglucan endotransglycosylase. Fruit softening is considered as the result of synergistic actions among these enzymes and proteins (Brummell and Harpster, 2001).

Pears are typical climacteric fruits with abundant ethylene being synthesized during the climacteric period (Wang et al., 1972; Ahmed and Labavitch, 1980). Thus, ethylene conditioning, including exogenous application of ethylene, propylene, and 1-methylcyclopropene (1-MCP; ethylene receptor inhibitor), can be used to regulate pear fruit softening and improve fruit quality. Ethylene and propylene have been used to induce ripening in winter pears, including 'D'Anjou' (Gerasopoulos and Richardson, 1996; Sugar and Einhorn, 2011; Sugar and Basile, 2013), 'Gebhard' Red 'D'Anjou' (Chen et al., 1993, 1997; Sugar and Basile, 2014), 'Comice' (Sugar and Basile, 2013), and 'Packham's Triumph' (Sugar and Basile, 2014). The application of 1-MCP can extend the shelf-life of summer pears such as 'Bartlett' (Trinchero et al., 2004) and 'Abbe Fetel' (Defilippi et al., 2011; Rizzolo et al., 2014). The efficiency of 1-MCP is affected by harvest maturity, dose, and storage conditions

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(i.e., holding time, temperature, and atmosphere) during treatment (Kubo et al., 2003; Rizzolo et al., 2014, 2015; Wang and Sugar, 2015). Additionally, 1-MCP can protect 'Abbe Fetel' (Rizzolo et al., 2014) and 'D'Anjou' (Xie et al., 2014) pears from superficial scald. Furthermore, 1-MCP has been used to identify factors that are ethylene-dependent and crucial for pear fruit ripening. In some cases, 'Conference' pears treated with 1-MCP are unable to ripen. However, cold storage can prevent this problem (Chiriboga et al., 2012, 2013).

Temperature is also an important factor that affects pear fruit softening. Generally, pears undergo ethylene and temperature conditioning simultaneously. Summer pears such as 'Bartlett' can soften to edible firmness after harvest without cold storage. In contrast, a critical period of cold treatment is required for winter pears such as 'D'Anjou' and 'Comice' to produce enough ethylene to initiate ripening (Chen et al., 1997). However, although 'Marguerite Marillat' and 'La France' fruits softened after a long period of cold storage, neither cultivar produced a desirable texture (Murayama et al., 2002a).

The softening mechanism of European pears has been well-studied and the relationship between cell wall polysaccharides and European pear softening has been characterized. Murayama et al. (1998) determined that softening of 'Marguerite Marillat' and 'La France' pears was accompanied by an increase in water-soluble polyuronides (WSP) abundance and a decrease in HCl-soluble polyuronides. Expression profiles of genes associated with cell wall modification during the softening of European pears have also been studied. In 'Rocha' pears, *PcPG1*, *PcPG2*, *PcβGAL*, *PcXYL*, and *PcEXPA1* were reported to be likely involved in cell wall loosening (Fonseca et al., 2005). *PcExp2*, *PcExp3*, *PcExp5*, and *PcExp* 6 were up-regulated during 'La France' pear fruit ripening, and the expression patterns of these genes were consistent with the rate of softening (Hiwasa et al., 2003b). The *PpGAL1* and *PpGAL4* genes, which are two members of the β-galactosidase gene family, were observed to be regulated by ethylene and associated with fruit softening in 'La France' pears (Mwaniki et al., 2005). Among the 13 genes associated with cell wall-degradation isolated from 'La France' pear, *PcPG1* was considered to be the only one that more involved in fruit softening than in melting texture development (Sekine et al., 2006).

'Starkrimson' is an attractive soft-fleshed pear cultivar because of the special coloration and aroma of its fruits. However, the fruits cannot soften normally after harvest because they rot at the higher temperatures in the harvesting season. Excessive rotting influences their edibility and commercial value. Moreover, 'Starkrimson' pears soften quickly even following a period of cold storage. Therefore, these fruits exhibit a lower tolerance for storage and transportation. To better characterize the mechanism regulating the rapid softening of 'Starkrimson' fruits, two other soft-fleshed and rapid-softening pear cultivars ('Bartlett' and 'Abbe Fetel') and a crisp-fleshed and non-softening pear cultivar ('Dangshansuli') were also analyzed. Cell wall-related changes were investigated during the fruit softening process. Additionally, we assessed the utility of 1-MCP in controlling the softening of 'Starkrimson' and attempted to identify genes that are crucial for pear fruit ripening.

## 2. Materials and methods

### 2.1. Plant materials and treatment

Fruits of three soft-fleshed pear (*Pyrus communis* L.) cultivars ('Starkrimson', 'Bartlett', and 'Abbe Fetel') and one crisp-fleshed pear (*Pyrus bretschneideri* Rehd.) cultivar ('Dangshansuli') were harvested at the commercial maturity stage in 2014 from a pear orchard in Meixian, Shaanxi, China. The fruits were harvested on July 10 ('Starkrimson'), August 11 ('Bartlett' and 'Abbe Fetel'), and September 3 ('Dangshansuli'). We collected 300 defect-free fruits of uniform size and maturity for each cultivar. Half of the 'Starkrimson' fruits were treated with 1 μL $L^{-1}$ 1-MCP (SmartFresh™, Rohm and Hass, Shanghai, China) for 12 h at ambient temperature in an airtight container. Treated and untreated fruits were stored at 1 °C for 30 d.

Fruits were softened at ambient temperature. At 2-d intervals, we measured the firmness of 12 fruits for each cultivar. The mesocarp tissue of another three fruits were flash-frozen in liquid nitrogen and stored at −80 °C.

### 2.2. Flesh firmness

Flesh firmness was measured using a GS-15 Fruit Texture Analyzer (GUSS, South Africa) equipped with an 8 mm probe. The skin on the two sides of the equatorial region of each pear was removed, and firmness was measured on each side.

### 2.3. Determination of pectin and cellulose contents

Pectin and cellulose were extracted and measured according to the method of Bu et al. (2013) with some modifications. For pectin, the holding time in darkness was modified to 1 h. For cellulose, the mass of flesh tissue was modified to about 1.0 g. We used D-(+)-galacturonic acid (Gal A, Solarbio, Beijing, China) and sodium carboxymethylcellulose (Sigma, Xi'an, China) to prepare standard curves. The absorbances were measured using an automated M200 Pro microplate reader (Tecan, Switzerland). Pectin and cellulose concentrations were expressed as mg $kg^{-1}$ of Gal A and g $kg^{-1}$ on a fresh weight basis, respectively.

### 2.4. Identification of candidate genes encoding cell wall hydrolases

Candidate genes encoding PG, PME, β-GAL, ARF, and Cel enzymes were identified using a hidden Markov model search of protein domains obtained from the Pfam database (http://pfam.xfam.org/) and a local BLASTN analysis of genes included in the NCBI database (http://www.ncbi.nlm.nih.gov/) in the Pear Genome Database (http://peargenome.njau.edu.cn/default.asp?d=4&m=2). Accession numbers of the identified candidate genes in the Pear Genome Database are listed in Table S1.

### 2.5. Total RNA extraction and reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was extracted using the sodium dodecyl sulfate-phenol method of Fonseca et al. (2004) and quantified using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE, USA). The RNA quality was evaluated by agarose gel electrophoresis. We used 1 μg total RNA for cDNA synthesis using the PrimeScript RT Reagent Kit with gDNA Eraser (TaKaRa, Dalian, China).

### 2.6. Full length cloning of cDNAs and sequence analysis

A mixed cDNA sample of 'Starkrimson' was used as the cloning template. The PCR was completed using PrimeSTAR® Max DNA Polymerase (TaKaRa) following the manufacturer's instructions. The PCR products were poly (A)-tailed using DNA A-Tailing Kit (TaKaRa) and inserted into the pGEM®-T Easy vector (Promega, Beijing, China). Positive single colonies were rechecked by PCR and sequenced by the Sangon Biotech Corporation. All cloned genes were renamed according to their similarities to reported genes. The designations and primer sets used for all cloned genes are summarized in Table S2.

Motif scanning was completed by searching for amino acid sequences in the Pfam database of EMBL-EBI (http://pfam.xfam.org/)