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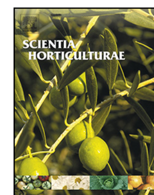

Transcriptome profiling reveals the candidate genes associated with aroma metabolites and emission of pear (*Pyrus ussuriensis* cv.)☆

Shuwei Wei[a,b], Shutian Tao[a], Gaihua Qin[a], Shaomin Wang[b], Jihan Tao[b], Jun Wu[a], Juyou Wu[a], Shaoling Zhang[a,*]

[a] *College of Horticulture, State Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing 210095, China*
[b] *Shandong Institute of Pomology, Taian 271000, China*

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ABSTRACT

Aroma is an important factor affecting pear fruit quality. However, the molecular basis for the development of aroma metabolites and their emission is poorly understood. Unripe 'Nanguoli' pear fruit has poor aroma, while the ripe fruit has an intense aroma, and calcium treatment can enhance the aroma, providing good material for transcriptome analysis.

Digital gene expression (DGE) analysis of 'Nanguoli' fruit was performed at three important developmental stages, with and without calcium treatment, six libraries in total. Of the total number of mapped reads (11.4–13.6 million), an average of 69.81% could be mapped to the pear genome. A total of 10,776 differentially expressed genes between the poor aroma and intense aroma were categorized into 36 functional groups and 37 pathways according to GO and KEGG databases, respectively. Genes more highly expressed in the more aromatic fruit include some from the fatty acid pathway such as *FAD, ADH, PDC, LOX,* and lipase genes. Some transcription factors were identified with high correlation to aroma biosynthesis in pear. Also, ABA may play an important role in the calcium-triggered improvement of 'Nanguoli' aroma. In addition, candidate genes were verified with quantitative real-time PCR.

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1. Introduction

Aroma is a decisive factor determining fruit quality and consumer acceptance (Zhang and Chen, 2007; Xi et al., 2012) and has gained increasing attention in recent years (Muna et al., 2013). 'Nanguoli', a variety of *Pyrus ussuriensis* – one of the four main cultivated species of pear in China – has a rich fragrance, but is very hard with little aroma when harvested at commercial harvest (unripe). Only after a post-harvest ripening process of about 8–10 d at 20 °C, the (ripe) fruit has intense aroma production, improving the final fruit quality.

The volatile aroma of pear has been studied since the report of Jennings and Sevenants, 1964, and many studies have investigated the characteristic aroma volatiles of different pear species or varieties (Qin et al., 2012a, 2012b), synthesis of aromatic compounds (Li et al., 2014) and also the effect of bagging and organic fertilizer (Wei et al., 2012), ripening stages (Kou et al., 2012) and storage (Zhang et al., 2013) on the aroma produced. The effects of calcium on fruit aroma have been reported: pre-harvest calcium spraying can improve volatile emission at commercial harvest of 'Fuji Kiku-8' apples (Ortiz et al., 2011); and emission of volatile esters of 'Golden Reinders' apple (Ortiz et al., 2010) after midterm storage can be improved by post-harvest calcium treatment. Our previous study also showed that spraying 'Nanguoli' fruit with 4% calcium chloride 15 d before harvest could promote the formation of aroma at harvest, in particular the formation of ester compounds that stimulate fruit flavor concentration.

Studies of enzyme activity, protein content, and expression of related genes indicated that production of aroma compounds was regulated by expression of key genes (Dudareva et al., 1998; Muna et al., 2013). Genes *PuLOX1*, *PuLOX8*, *PuADH3*, and *PuAAT* may also play important roles in ester formation of pear fruit (Li et al., 2014). However, the metabolic process and emission of aromatic compounds is very complex. The key genes that control or regulate metabolites and emission of aroma compounds in pear are

☆ Gene sequences of pear were obtained from the Pear Genome Project website (http://peargenome.njau.edu.cn/). Raw sequencing reads were deposited in the Sequence Read Archive (SRA) database in NCBI and accession numbers will be added when released.

* Corresponding author.
E-mail addresses: weisw2007@163.com (S. Wei), shutian_tao@hotmail.com (S. Tao), qghahstu@163.com (G. Qin), sdipwsm@163.com (S. Wang), sdtjh_69@163.com (J. Tao), juyouwu@njau.edu.cn (J. Wu), wujun@njau.edu.cn (J. Wu), slzhang@njau.edu.cn (S. Zhang).

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unknown, and an increased knowledge of gene expression and regulatory changes of ‘Nanguoli’ following post-harvest ripening and calcium treatment is necessary to understand the source of its strong aroma.

High-throughput sequencing is a revolutionary technological innovation, characterized by low cost and high data output, allowing practical genomics research (Elaine, 2008; Glenn, 2011). Digital gene expression (DGE) with high-throughput sequencing technologies is a novel and efficient approach to genome-wide expression profiling using next generation sequencing methodologies. This approach allows researchers to identify, quantify, and annotate expression at the whole genome level with or without prior sequence knowledge of any transcript from any organism, and has been used in many studies (Deng et al., 2014; Wang et al., 2014; Zhang et al., 2014). Overall, DGE dramatically enhances the identification of differentially expressed genes. Our laboratory previously completed whole genome sequencing (Wu et al., 2012) of pear ‘Dangshansuli’ (*Pyrus bretschneideri* Rehd.), which provided an invaluable new resource for biological research of Pyrus. In the present study, we established transcriptome datasets of poor and intense aroma pear fruit using DGE to gain a deeper insight into the molecular mechanism of pear fruit aroma biosynthesis and emission. We identified genes involved in fatty acid pathway, transcription factors, and other genes leading to improved aroma in ‘Nanguoli’ pear fruit. Our study presents a global survey of transcriptome profiles of aroma metabolites and emission in pear.

2. Materials and methods

2.1. Plant materials and treatments

For the characterization of aroma emission, ‘Nanguoli’ (*Pyrus ussuriensis* cv.) was selected for research. Fruits were provided by the National Germplasm Repository of Pear in the Research Institute of Pomology, Chinese Academy of Agricultural Sciences (CAAS), Xingcheng, Liaoning Province, China. On September 5, 2013 (15 days before harvest), six uniform ten-year-old ‘Nanguoli’ trees were selected and divided into two groups: one was treated with calcium, while the second one was maintained as control. Same size and color fruits from different sides of experimental trees were sprayed with calcium chloride 4% (W/V) on fruit surface, and the other three trees were sprayed with water as control (CK). Orchard management procedures, such as fertilization and irrigation, were the same for all treatments. Healthy fruits were harvested in commercial ripening period (135 days after pollination) and five days before harvest (130 days after pollination) by hand-picking, with all fruits picked from the southern or western crown, about 2 m above the ground. Individually packed fruit were taken to the laboratory immediately after harvest. Half of the fruits harvested in commercial ripening were cored and seeded and the skin and flesh were frozen with liquid nitrogen and stored in −80 °C freezer. The other half of the fruits harvested in commercial ripening were kept in 20 °C for five days of post-harvest ripening, at the end of which they were cored and seeded, while the skin and flesh was frozen with liquid nitrogen and stored in a −80 °C freezer. For each treatment, 3–5 pears were combined for each of three replicates.

2.2. Extraction and concentration of volatile aroma compounds

GC–MS and HS-SPME were used for the extraction and concentration of volatile aroma compounds, following the methods reported by Qin et al. (2012a, 2012b). SPME fibres coated with a 65 μm thickness of polydimethylsiloxane–divinylbenzene (65 μm PDMS/DVB; Supelco Co., Bellefonte, PA, USA) were used in this study and activated before sampling according to the manufacturer’s instructions. The core and seeds of each pear were removed and discarded; the skin and flesh was ground with a commercial blender to pulp for the extraction and concentration of volatile aromatic compounds. For each extraction, 10 g of pulp were placed into a 20 ml screw-cap vial containing 3.6 g of NaCl to facilitate the release of volatiles compounds. Prior to sealing of the vials, 50 μl of 0.04 g ml^{-1} 3-nonanone was added as internal standard, and mixed with a glass rod. A magnetic follower was added to each vial, which was placed into a constant-temperature water bath at 40 °C with stirring. The SPME fibre was exposed to the head space of the sample for 30 min to adsorb the analyte, then introduced into the heated injector port of the chromatograph for desorption at 250 °C for 5 min in splitless mode.

Table 1
Description of ‘Nanguoli’ fruit samples used in this study.

Sample code	Description
F5B_CK	Fruits without calcium treatment 5 days before commercial harvest
F5B_Ca	Fruits with calcium treatment 5 days before commercial harvest
F5_CK	Fruit without calcium treatment in commercial harvest
F5_Ca	Fruit with calcium treatment in commercial harvest
F5L_CK	Fruit (without calcium treatment) post-harvest ripening for 5 days in 20 °C
F5L_Ca	Fruit (with Calcium treatment) post-harvest ripening for 5 days in 20 °C

2.3. GC–MS conditions

The volatile constituents were analyzed with an Agilent 5973B mass selective detector coupled to an Agilent 7890A gas chromatograph, equipped with a 30 m × 0.25 mm × 1.0 mm HP-5 MS (5% phenyl-polymethylsiloxane) capillary column. A constant column flow of 1.0 ml/min helium was used as carrier gas. The injector and detector temperature were 250 and 280 °C, respectively. The oven temperature program was 35 °C for 8 min, increased at 2 °C/min to 140 °C, 140 °C for 2 min, then increased at 10 °C/min to 260 °Cand held for 5 min. Mass spectra were recorded at 70 eV in electron impact (EI) ionization mode. The temperatures of quadrupole mass detector and ion source were 150 and 230 °C, respectively. The temperature of transfer line was 280 °C. Mass spectra were scanned in the *m*/*z* range 33–350 amu at intervals of 1 s. Tentative identification of the volatile components was done by comparing the mass spectra of the samples with the data system library (NIST 98). Whenever it was possible, MS identification was confirmed with authentic References

Quantification was done by the internal standard method, where the concentration of each volatile aromatic compound was normalized to that of 3-nonanone.

2.4. DGE-tag profiling

Total RNA was isolated from all the samples and libraries were named (Table 1). DGE-tag profiling was carried out as described by Qi et al. (2013). Total RNA was extracted from the samples using Plant RNA Isolation Kit (AutoLab), followed by RNA purification with RNeasy MiniElute Cleanup Kit (Qiagen), according to the manufacturer’s instructions. RNA degradation and contamination was monitored on 1% agarose gels. RNA purity was checked using the NanoPhotometer® spectrophotometer (IMPLEN, CA, USA). RNA concentration was measured using Qubit® RNA Assay Kit in Qubit® 2.0 Flurometer (Life Technologies, CA, USA). RNA integrity was assessed using the RNA Nano 6000 Assay Kit of the Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA).

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