Characterization and Transcript Profiling of PME and PMEI Gene Families during Peach Fruit Maturation

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ABSTRACT. Pectins are synthesized and secreted to the cell wall as highly methyl-esterified polymers and demethyl-esterified by pectin methylesterases (PMEs), which are regulated by pectin methylesterase inhibitors (PMEIs). PMEs and PMEIs are involved in pectin degradation during fruit softening; however, the roles of the PME and PMEI gene families during fruit softening remain unclear. Here, 71 PME and 30 PMEI genes were identified in the peach (Prunus persica) genome and shown to be unevenly distributed on all eight chromosomes. The 71 PME genes comprised 36 Type-1 PMEs and 35 Type-2 PMEs. Transcriptome analysis showed that 11 PME and 15 PMEI genes were expressed during fruit ripening in melting flesh (MF) and stony-hard (SH) peaches. Three PME and five PMEI genes were expressed at higher levels in MF than in SH fruit and exhibited softening-associated expression patterns. Upstream regulatory cis elements of these genes related to hormone response, especially naphthaleneacetic acid and ethylene, were investigated. One PME (Prupe.7G192800) and two PMEIs (Prupe.1G114500 and Prupe.2G279800), and their promoters were identified as potential targets for future studies on the biochemical metabolism and regulation of fruit ripening. The comprehensive data generated in this study will improve our understanding of the PME and PMEI gene families in peach. However, further detailed investigation is necessary to elucidate the biochemical function and regulation mechanism of the PME and PMEI genes during peach fruit ripening.

Received for publication 25 Jan. 2017. Accepted for publication 15 May 2017.

The plant cell wall is a highly organized structure composed of many different polysaccharides, proteins, and phenolic compounds. Fruit ripening involves extensive depolymerization of pectin, as well as other modifications to cell-wall components, including demethylation and removal of neutral sugar side chains (Brummell and Harpster, 2001). In current models, pectic polysaccharides are synthesized in the Golgi complex as highly methylesterified polymers [e.g., homogalacturonic acid (HGA)] that are secreted to the cell wall and partially demethylesterified by PMEs. During ripening, this methylesterification decreases dramatically, for example, the degree of pectic methylesterification drops from 90% in immature green fruit to 30% at the red-ripe stage of ripening in tomato [Solanum lycopersicum (Koch and Nevins, 1989)], and the de-esterified HGA backbone is then susceptible to cleavage by polygalacturonase (PG) (Prasanna et al., 2007).

The PME gene family was first described by Richard et al. (1996), and later classified in the Carbohydrate-Active Enzymes database as class 8 of the carbohydrate esterases (EC 3.1.1.11) (Cantarel et al., 2009). Active PME functions in two different ways: 1) a blockwise action by which blocks of demethylesterified HGA interact with Ca²⁺, promoting the formation of the so-called “egg-box” structure, thereby affecting the apoplastic potential of hydrogen (pH) and the mechanical and biochemical properties of the cell wall; and 2) a random demethylesterified action on HGA in which interaction with Ca²⁺ is not permitted, and HGA becomes the target for pectin-degrading enzymes, such as PGs, affecting the texture and rigidity of the cell wall (Micheli, 2001; Pelloux et al., 2007). PMEs are widespread in plants and microorganisms and belong to large multigene families with different roles in cambial cell differentiation and determination of fiber length in trees (Micheli et al., 2000; Siedlecka et al., 2008), microsporogenesis (Francis et al., 2006; Lacoux et al., 2003), organ initiation (Peaucelle et al., 2011), and fruit softening and ripening (Brummell et al., 2004; Deytieux-Belleau et al., 2008; Eriksson et al., 2004). PMEs are classified as either Type-1 PMEs (i.e., those with a proregion, similar to the PMEI domain) or Type-2 PMEs (no proregion). In Type-1 PMEs, the proregion operates as an effective retention mechanism, keeping unprocessed PME in the Golgi apparatus. Consequently, proprotein processing could constitute a posttranslational mechanism regulating PME activity (Wolf et al., 2009).

The activity of PMEs is regulated by PMEIs (Balestrieri et al., 1990), which bind to the active site of the PME, generating a 1:1 complex (Di Matteo et al., 2005; Hothorn et al., 2004). PMEIs, which belong to a large family (Jolli et al., 2010; Wang et al., 2013), were originally discovered in kiwifruit [Actinidia chinensis (Balestrieri et al., 1990)] and subsequently identified in arabidopsis (Arabidopsis thaliana), pepper (Capsicum annuum), broccoli (Brassica oleracea), banana (Musa sapientum), tomato, and grape (Vitis vinifera) (An et al., 2008; Lionetti et al., 2015; Peaucelle et al., 2008; Raiola et al., 2004; Reca et al., 2012; Srivastava et al., 2012;
Zhang et al., 2010). Recent evidence shows the role of PMEIs in apical meristem development (Peaucelle et al., 2008), cell and organ size determination (Lionetti et al., 2007), cell growth acceleration (Pelletier et al., 2010), and pollen tube growth (Röckel et al., 2008; Zhang et al., 2010). In particular, the PMEIs from tomato are proposed to control the methylesterification of pectin during fruit softening and ripening (Reca et al., 2012).

Studies of the PME and PMEI gene families in several plant species provide a better understanding of this gene family (Pinzón-Latorre and Deyholos, 2013; Wang et al., 2013). To the best of our knowledge, no systematic investigations of the PME and PMEI gene families of peach have been reported to date. Peach cultivars can be divided into three groups based on characteristics such as fruit firmness and textural changes during ripening: MF, nonmelting flesh (NMF), and SH types (Haji et al., 2001). MF fruit exhibits sharply increasing auxin accumulation and ethylene production during the ripening process, resulting in rapid softening and a short shelf life, whereas SH fruit sustains low levels of auxin accumulation and ethylene production and barely softens on the tree or after harvest (Haji et al., 2005; Pan et al., 2015; Tatsuki et al., 2013). These two types are therefore good materials to identify the PME and PMEI genes involved in peach fruit ripening.

In the present study, the complete family of PMEs and PMEIs in peach was analyzed based on the peach genome accession no. 2.1 (Verde et al., 2013). A phylogenetic analysis was performed using PMEs and PMEIs from arabidopsis and peach. The expression profiles of all PME and PMEI genes were determined in MF and SH peaches during fruit ripening, and cis elements in the promoters of PMEs and PMEIs related to fruit ripening were analyzed.

Materials and Methods

Plant material and postharvest treatments. Representatives of four peach cultivars, Zhongyoutao 13 (CN13), Zhongyoutao 16 (CN16), Goldhoney 3, and Yumyeong, were obtained from the Institute of Zhengzhou Fruit Research, Chinese Academy of Agriculture Science, Zhengzhou, China. For mRNA extraction and analysis, ‘CN13’ (MF) fruit were collected at 81, 86, 92, and 97 d after flowering (DAF) (designated S3, S4 I, S4 II, and S4 III, respectively). ‘CN16’ (SH) samples were collected at 72, 77, 82, and 87 DAF (designated S3, S4 I, S4 II, and S4 III, respectively) were selected (Pan et al., 2015). The stages indicated as S3, S4 I, S4 II, and S4 III represent fruit from the end of stage S3 (second exponential growth phase) to stage S4 (climacteric).

‘CN16’ fruit collected at the S4 III stage (87 DAF) were subjected to auxin and ethylene treatments. For auxin treatment, whole fruit were dipped in 0.5 mM 1-naphthaleneacetic acid [NAA (Sigma-Aldrich, Darmstadt, Germany)] for 5 min with 100 μL·L⁻¹ of surfactant (Silwet L-77; Real-Times Biotechnology, Beijng, China) as a surfactant. For ethylene treatment, fruit were dipped in 1000 μL·L⁻¹ ethephon [an ethylene releaser (Solarbio, Beijng, China)] for 1 min and were then incubated in an airtight container. After treatment, the fruit were maintained at 25 °C and sampled at 12, 24, 36, 48, 72, and 96 h. Half of the sampled fruit were used to measure ethylene production, others were used to determine flesh firmness, then immediately frozen in liquid nitrogen, and stored at −80 °C for RNA extraction. Untreated fruit were used as the control. Each experimental and control group included at least 80 fruit.

Ethylene production and flesh firmness. Ethylene production and flesh firmness were measured as previously described (Zeng et al., 2015). The ethylene concentration was measured with a gas chromatograph (GC2010; Shimadzu, Kyoto, Japan) equipped with a flame ionization detector. Flesh firmness was measured with a fruit-pressure tester (GY-4-J; TOP Instruments, Hangzhou, China) fitted with an 11-mm-diameter probe. Each measurement was performed in three replicates for each sample. Five to eight fruit per sample were measured.

Identification of PME and PMEI genes in peach. Predicted proteins containing PME (PF01095), PMEI (PF04043), or both domains were identified from the peach genome (Verde et al., 2013) (accession no. 2.1) using default parameters in hmmssearch/PfamScan (Punta et al., 2011). The predicted proteins were aligned to previously described PMEs and PMEIs from arabidopsis obtained from The Arabidopsis Information Resource (Lamesch et al., 2012), using BLASTp. Peach PMEs (PpPMEs) contained PF01095 and PF04043 domains, and PMEIs contained the PF04043 domain. The genome sequence (accession no. 2.1), identity number, and coding sequence length were downloaded from

| Genes* | Primers (forward/reverse) |
|--------|---------------------------|
| Prupe.1G113800 | 5’-TGCTCTTCTCTGTAACCTTTTGT-3’/5’-TGATGAAGCCCATAAAAGAACA-3’ |
| Prupe.1G114500 | 5’-GTGTATCCCTACCTTGTCTTC-3’/5’-CATAGTCTCCTGGTCTGT-3’ |
| Prupe.1G330900 | 5’-GTTGGTTTGCCTTGGGAAT-3’/5’-GATGAAAGACGGAGAAATGGA-3’ |
| Prupe.2G279700 | 5’-CCCGGAAGAAGGTTTCTTC-3’/5’-TGAGGCAAGTGGGAGGAGT-3’ |
| Prupe.2G279800 | 5’-CACACCCACCATACCCACT-3’/5’-TGAAACTACGGAGAGGC-3’ |
| Prupe.7G190300 | 5’-GCTCAGCCCTTCCCTCTCAAAAT-3’/5’-AGGCCCTAACAATAACCTTGAGT-3’ |
| Prupe.7G190400 | 5’-GGTGATGAGGATTTGTCTTGAT-3’/5’-TTGGTCAATGAGGAGGT-3’ |
| Prupe.7G192800 | 5’-GATTCCCCGTGCCGAAGTT-3’/5’-CCACGACCTTCCATCCAA-3’ |

*Five PMEI genes contain Prupe.1G113800, Prupe.1G114500, Prupe.1G330900, Prupe.2G279700, and Prupe.2G279800, and others are three PME genes. The housekeeping gene sequence (actin) has referenced by Tatsuki et al. (2013).
Table 2. A summary of accessions numbers, programmed frequency amplitude modulation (PFAM), open reading frame (ORF) length, position of chromosome, theoretical isoelectric point (pI), and molecular weight (M) of peach pectin methylesterase (PME) genes.

| Number | Accession no. 1.0 | Accession no. 2.1 | PFAM* | Type† | ORF (aa) | Chromosome | pI  | M          |
|--------|------------------|-------------------|-------|------|---------|------------|-----|------------|
| 1      | ppa020682        | Pruepe.IG006800   | PF01095 | 2    | 346     | 1          | 7.13| 38,657.37  |
| 2      | ppa003528        | Pruepe.IG105400   | PF04043 PF01095 | 1    | 568     | 1          | 9.04| 62,478.95  |
| 3      | ppa017141        | Pruepe.IG116600   | PF01095 | 2    | 345     | 1          | 9.03| 38,034.05  |
| 4      | ppa003441        | Pruepe.IG123500   | PF04043 PF01095 | 1    | 574     | 1          | 8.16| 63,182.14  |
| 5      | ppa025631        | Pruepe.IG123700   | PF04043 PF01095 | 1    | 574     | 1          | 8.58| 63,475.56  |
| 6      | ppa026560        | Pruepe.IG123800   | PF04043 PF01095 | 1    | 575     | 1          | 8.74| 63,633.78  |
| 7      | ppa021815        | Pruepe.IG131900   | PF04043 PF01095 | 1    | 570     | 1          | 8.82| 62,139.08  |
| 8      | ppa003569        | Pruepe.IG132000   | PF04043 PF01095 | 1    | 564     | 1          | 8.91| 62,038.41  |
| 9      | ppa005208        | Pruepe.IG1330800  | PF04043 PF01095 | 1    | 542     | 1          | 8.57| 59,483.88  |
| 10     | ppa005184        | Pruepe.IG1340100  | PF04043 PF01095 | 1    | 539     | 1          | 8.62| 58,988.46  |
| 11     | ppa003581        | Pruepe.IG135900   | PF04043 PF01095 | 1    | 536     | 1          | 7.04| 39,034.98  |
| 12     | ppa003578        | Pruepe.IG136900   | PF04043 PF01095 | 1    | 535     | 1          | 8.16| 63,114.04  |
| 13     | ppa003433        | Pruepe.IG137900   | PF04043 PF01095 | 1    | 534     | 1          | 8.91| 62,038.41  |

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the Phytozome (Goodstein et al., 2012). The molecular weights (M) and isoelectric points (pIs) of the proteins were calculated from ExPASy (Gasteiger et al., 2003). Exon/intron structure was constructed using the Gene Structure Display Server web-based bioinformatics tool (Hu et al., 2015). For chromosome locations, genes were mapped to the chromosomes using MapInspect software (Van Berloo, 1999). A phylogenetic tree was generated with
molecular evolutionary genetics analysis software (MEGA 4.0) using the neighbor-joining (NJ) method, and edge support was estimated using 1000 bootstrap replicates (Tamura et al., 2007).

**LIBRARY CONSTRUCTION, DEEP SEQUENCING, AND DATA PROCESSING.** At least 5 μg of each isolated RNA from fruit samples (‘CN13’ S3, S4 I, S4 II, S4 III, and ‘CN16’ S3, S4 I, S4 II, S4 III) was sent to Gene Denovo Co. (Guangzhou, China) for the construction of eight libraries (two cultivars four stages). Libraries were established using an Illumina kit (Illumina, San Diego, CA), and each library was sequenced on the Illumina HiSeq 2500 sequencing platform.

Expression levels were measured in fragments per kilobase of exon per million fragments mapped [FPKM (Trapnell et al., 2010)], FPKM >1 was defined as the threshold of significant gene expression and was used to analyze the differences in gene expression between the two peach flesh types. The full list of normalized PME and PMEI gene expression was shown in Supplemental Tables 1 and 2.

**QUANTITATIVE REAL-TIME PCR VALIDATION.** RNA isolation and complementary DNA synthesis were performed as previously described (Zeng et al., 2015). Quantitative reverse-transcription PCR (qRT-PCR) was performed to investigate the expression of PME and PMEI genes in peach (Zeng et al., 2015). The relative gene expression levels were calculated using the 2^−ΔΔCt method (Livak and Schmittgen, 2001). All gene-expression analyses were performed with three independent biological replicates. Primers used for qRT-PCR were designed using PrimerExpress 3.0 software (Applied Biosystems, Foster City, CA) (Table 1).

**PROMOTER REGION ANALYSIS OF EIGHT PEACH PME AND PMEI GENES.** To investigate the motifs or cis elements in the promoter sequences of the eight peach PME and PMEI genes related to hormone treatment, 1.5 kb of genomic DNA sequences upstream of the initiation codon (ATG) were obtained from the peach database. The motifs or elements in the promoter sequences were analyzed in the plant cis-acting regulatory DNA elements (PLACE) database (Higo et al., 1999) and the PlantCARE database (Lescot et al., 2002). The fruit-specific cis elements, the motifs TCCAAAA and TGTCACA, were searched manually in the promoter sequence (Yamagata et al., 2002; Yu et al., 2014).

**Results**

**IDENTIFICATION OF THE PEACH PME AND PMEI FAMILY GENES.** A search of predicted transcripts of the peach whole-genome assembly (accession no. 2.1) for the programmed frequency amplitude modulation (PFAM) domains pectinesterase (PF01095) and PMEI (PF04043) identified 71 putative PMEs and 30 putative PMEIs (Tables 2 and 3). Independent alignment of 66 arabidopsis PME and 71 PMEI protein
sequences in the TBLASTN program to the peach genome did not identify any additional peach genes other than those identified by the Pfam domain alignment. Among the predicted 71 PpPMEs, 36 were Type-1 [i.e., encoding both a PMEI (PF04043) and PME (PF01095) domains] and 35 were Type-2 (i.e., encoding a PME domain, but no PMEI domain). Only one of the genes (Prupe.3G031000) contained two PME and PMEI domains. Detailed information on the PpPME and PpPMEI genes is showed in Tables 2 and 3, respectively, including accession no. 1.0, accession no. 2.1, the Pfam domain, the open reading frame (ORF), the theoretical pI and M.

The ORF lengths of the Type-1 PME family members except Prupe.3G031000 ranged from 1473 bp (Prupe.6G344600) to 1827 bp (Prupe.2G310600), encoding peptides of 409–608 aa. The predicted M of these genes varied from 54.16 to 67.66 kDa. The ORF lengths of the Type-2 PME family members ranged from 477 bp (Prupe.2G210900) to 1443 bp (Prupe.1G377100), encoding peptides of 158–480 aa. The predicted M of these

Fig. 2. Phylogenetic analysis of the pectin methylesterase (PME) and pectin methylesterase inhibitor (PMEI) protein sequences from peach and arabi
dopsis. (A) Phylogenetic relationships among 71 peach PMEs and 66 arabidopsis PMEs determined based on amino acid sequences. (B) Phylogenetic relationships among 30 peach PMEIs and 71 arabidopsis PMEIs determined based on amino acid sequences. Black circle = arabidopsis PMEs and PMEIs, square frame = peach PMEs and PMEIs.
genes varied from 17.26 to 51.48 kDa, and the theoretical pIs of the PME family ranged from 4.93 (Prupe.6G312200) to 9.63 (Prupe.2G278300). The ORF lengths of the PMEI family members ranged from 297 bp (Prupe.3G008000) to 1356 bp (Prupe.8G267500), encoding peptides of 98–451 aa. The predicted M of these genes varied from 11.06 to 49.98 kDa, and the theoretical pIs of the PMEI family ranged from 4.44 (Prupe.5G112600) to 10.02 (Prupe.5G076900).

**Genomic distribution and sequence analysis.** Of 71 peach PME genes, 69 were unevenly distributed among the eight peach chromosomes varying from 1 to 18. Chromosome (Chr) 6, Chr 4, and Chr 8 were anchored by 1, 2, and 3 PpPME genes, respectively; Chr 3 × 6; Chr 1 and Chr 2 × 18; Chr 7 × 10; and Chr 6 × 11 genes. Prunus1005000 and Prunus1005200 belonged to the Type-2 PME family and were not mapped to the peach chromosome. Thirty-six PpPME genes were distributed in 15 clusters on peach chromosomes, including tandem-duplicated genes, such as Prunus.6G128000, Prunus.6G128100, Prunus.6G128200, and Prunus.6G128300 (Fig. 1). Similarly, 30 PMEI genes were nonrandomly distributed on eight peach chromosomes (Fig. 1). One PMEI gene was distributed on Chr 7; two on Chr 4 and Chr 8; three on Chr 6; five each on Chr 1, Chr 2, and
Chr 3; and six on Chr 5. A total of 13 PpPMEI genes were located in five clusters on four peach chromosomes, including four clusters of tandem-duplicated genes, such as Prupe.3G007700, Prupe.3G007800, Prupe.3G007900, and Prupe.3G008000. Several PME and PMEI genes were located in four clusters on peach chromosomes, such as Prupe.1G330800 (PME family member) and Prupe.1G330900 (PMEI family member).

**Phylogenetic and gene structure analysis.** To classify the predicted PpPMEs and PpPMEIs on the basis of amino acid sequence similarity and inferred evolutionary relationships, their amino acid sequences were aligned with predicted PMEs and PMEIs from arabidopsis. Following alignment, NJ phylogenetic trees for PMEs (Fig. 2A) and PMEIs (Fig. 2B) were constructed. Four major monophyletic groups of PMEs were defined based on the arabidopsis PME groups. Group 1 included 24 PMEs, including two Type-2 PMEs. There were 11 and 3 PMEs in groups 2 and 3, respectively, and they were all Type-1 PMEs. Group 4 was composed of 33 Type-2 PMEs. In the PMEI phylogenetic tree (Fig. 2B), groups were distinguished by very low bootstrap values in the base nodes, making subclassification of PMEIs ambiguous. Furthermore, we did not find any common sequence features that distinguished subgroups of PMEIs from each other. PMEs contained one to five exons, and PMEI genes mostly contained only one exon (Fig. 3).

**Expression of the PME and PMEI family genes during fruit ripening in MF and SH peaches.** To elucidate the possible functions of the 71 PME and 30 PMEI genes, their expression profiles were investigated by qRT-PCR during fruit ripening in MF and SH peaches. Based on our transcript data, 11 PME and 15 PMEI genes showed a high level of expression (FPKM >1) in the two types of peach fruit during ripening (Fig. 4A and B). Among the 11 PME and 15 PMEI genes, three PME genes (including Prupe.7G190300, Prupe.7G190400, and Prupe.7G192800) showed obvious differences in expression (log2[Fold Change] >1) between MF peaches (‘CN13’ and ‘Goldhoney 3’) and SH peaches (‘CN16’ and ‘Yumyeong’) during fruit ripening, and five PMEI genes (including Prupe.1G113800, Prupe.1G114500, Prupe.1G330900, Prupe.2G279700, and Prupe.2G279800) showed obvious differences in expression (log2[Fold Change] >1).

To validate the gene expression changes determined by transcript data, quantitative PCR analysis was performed on a selection of differentially expressed PME and PMEI genes. Overall, the quantification of three PME genes and five PMEI genes by qRT-PCR exhibited close agreement with transcript data.
data (Fig. 5). One of the three PME genes (Prupe.7G192800) was upregulated during fruit development, and its expression peaked at S4 III in MF peaches (‘CN13’ and ‘Goldhoney 3’), whereas its expression level was low in SH peaches (‘CN16’ and ‘Yumyeong’). Similarly, two of the five PMEI genes (Prupe.1G114500 and Prupe.2G279800) were upregulated during fruit development, and their expression peaked at S4 III in MF peaches (‘CN13’ and ‘Goldhoney 3’), whereas their expression was almost undetectable in SH peaches (‘CN16’ and ‘Yumyeong’). One PME (Prupe.7G190400) was upregulated in ‘Goldhoney 3’ during fruit development, but not in ‘CN13’. One PMEI gene (Prupe.2G279700) was upregulated in ‘CN13’, but not in ‘Goldhoney 3’.

**FRUIT FIRMNESS AND ETHYLENE PRODUCTION CHANGES IN RESPONSE TO AUXIN AND ETHYLENE TREATMENT.**

The effects of auxin and ethylene on peach fruit softening were determined by evaluating ethylene production and flesh firmness. In this study, the SH type ‘CN16’ was used to explore the effect of NAA and ethylene treatment. Ethylene was not detected without treatment, and ethylene treatment did not stimulate the production of endogenous ethylene; however, NAA treatment resulted in the production of abundant endogenous ethylene in SH peaches, with a rapid increase in ethylene production beginning at 36 h that peaked at 96 h [8.34 μL·g⁻¹·h⁻¹ (Fig. 6A)]. Fruit firmness decreased moderately in ‘CN16’ without treatment or with NAA and ethylene treatment; however, fruit firmness decreased to a greater extent with NAA than with ethylene treatment at the same time point, and to a greater extent with ethylene than without treatment at the same time point. ‘CN16’ fruit hardly softened, and flesh firmness was maintained over 20 N/cm² without treatment or with NAA and ethylene treatment (Fig. 6B).

**PROMOTER ANALYSIS OF THE PMES AND PMEI5 RELATED TO PLANT HORMONE RESPONSES DURING FRUIT RIPENING.**

Identification and analysis of the regulatory motifs in the promoters of genes expressed during fruit development and ripening is beneficial to improve our understanding of the molecular regulation of these complex developmental processes mediated by numerous transcription factors and various plant hormones. Here, the cis elements in the promoter sequence involved in plant hormone (especially NAA and ethylene) response, were surveyed using PlantCARE, PLACE, and a manual search to understand the transcriptional regulation and potential functions of the fruit ripening–related PME and PMEI genes. Seven types of cis elements were involved in plant hormone response, and one fruit-specific cis element was present in the 1.5 kb upstream sequences of the eight PpPME and PpPMEI genes, representing their predicted promoter regions (Supplemental Table 3, Fig. 7). All members of these cis elements in the promoter regions of PpPME and PpPMEI
genes are shown in Supplemental Table 4 and Fig. 7. The fruit-specific cis elements, TCCAAAAA-motif and TGTCACA-motif, were detected in the promoter regions of two PpPME genes (Prupe.1G114500 and Prupe.2G279800) and two PpPME genes (Prupe.7G190400 and Prupe.7G192800). The ethylene-responsive elements (EREs) (C-repeat/drought-responsive element, ERE, and GCC-box), which play important roles in the regulation of many ethylene-related genes, were detected in the upstream promoter regions of Prupe.1G113800, Prupe.1G330900, Prupe.2G279800, and Prupe.7G190300. The auxin-responsive elements (TGA-element, TGA-box, and S000270), which play important roles in the regulation of auxin-related genes, were detected in the upstream promoter regions of Prupe.1G113800, Prupe.1G114500, Prupe.1G330900, and Prupe.7G192800. Eight promoters contained at least one cis element for plant hormone responses.

**Expression of the eight PME and PMEI family genes in SH fruit (‘CN16’) in response to NAA and ethylene.** To further analyze the cis elements involved in auxin and ethylene response in the promoter regions of the eight PME and PMEI family genes identified, their expression in response to NAA and ethylene treatment was investigated in ‘CN16’ fruit (Fig. 8). NAA induced the expression of one PME gene (Prupe.7G192800) and four PMEI genes (Prupe.1G113800, Prupe.1G114500, Prupe.1G330900, and Prupe.2G279800) in ‘CN16’ fruit, whereas ethylene induced the expression of one PME gene (Prupe.7G192800) and two PMEI genes (Prupe.1G113800 and Prupe.1G114500). Prupe.1G114500 showed the highest upregulation in response to ethylene treatment, whereas Prupe.7G192800 showed the highest upregulation in response to NAA. Other genes were downregulated by NAA or ethylene treatment.

**Discussion**

PME is the first enzyme acting on pectin, a major component of the plant cell wall. PME produces pectin with different structural and functional properties and therefore plays an important role in plant physiology (Giovane et al., 2004). Recent data indicate that several PME isoforms detected in the cell walls are encoded by a multigene family (Pinzón-Latorre and Deyholos, 2013; Wang et al., 2013). In the present study, 71 PMEs were identified in the peach genome, of which 36 were distributed in 15 clusters on peach chromosomes, suggesting that whole-genome duplication and tandem duplication contribute to the expansion of the large family of PME genes (Wang et al., 2013). The 71 PMEs were divided into 36 Type-1 PMEs and 35 Type-2 PMEs, and both types of PME genes function in demethylesterification. Further study should be aimed at clarifying the relationship between gene family expansion and the function of the two types of PME genes. PME activity is controlled at the posttranscriptional level by PMEIs, and the PMEI family appeared later than Type-1 PME and Type-2 PME genes (Wang et al., 2013). Only 30 PMEIs were identified in the peach genome. Phylogenetic analysis with arabidopsis PMEIs showed no common sequence features that distinguished subgroups of PMEIs from each other. This may be because peach has not undergone recent whole-genome duplication (Verde et al., 2013), and the expansion of subclasses is lower than that of other different plants.

Peach fruit are classified into three groups according to the character of textural changes during ripening: MF, NMF, and SH types (Haji et al., 2001). The MF and NMF characters were expressed in response to ethylene treatment in SH fruit, indicating that the SH trait was epistatic to the MF/NMF trait (Haji et al., 2005). In the present study, ‘CN16’ fruit treated
with ethylene and NAA did not soften considerably during fruit storage, suggesting that ‘CN16’ fruit belong to SH NMF type.

PMEs are involved in peach fruit ripening (Brummell et al., 2004). In the present study, 11 PME genes showed significant expression in two peach texture types during fruit ripening, and three PME genes (including Prupe.7G190300, Prupe.7G190400, and Prupe.7G192800) showed obvious difference in expression between MF peaches and SH peaches during fruit ripening. Previous research showed that PMEI (AB231903) and PME2 (X95991) are upregulated in MF type peaches compared with their expression in SH type peaches, suggesting that these genes are involved in fruit ripening. Although many PME genes were upregulated during peach fruit ripening, the timing and extent of the increase in the PME activity were not necessarily related to ethylene or fruit ripening (Brummell et al., 2004; Hayama et al., 2006). Over-expression of PMEIs in arabidopsis and tobacco (Nicotiana tabacum) results in a lower level of PME activity and a higher degree of pectin esterification (Lionetti et al., 2007, 2012). Therefore, the high expression of PMEIs may lead to decrease of PME activity in peach fruit during ripening or in response to ethylene treatment. Prupe.7G190300 and Prupe.7G190400 are Type-1 PMEs, and Prupe.7G192800 is a Type-2 PME. The proregion of Type-1 PMEs may be involved in the autoinhibition of activity, preventing premature de-esterification during transport in pectin-containing secretory vesicles (Wolf et al., 2009). Because Type-2 PMEs lack the PMEI-like proregion, the specific five PMEIs may be coexpressed during fruit ripening to block activity within secretory vesicles. However, the specific PMEI that regulates PME activity, and the effect of PMEs on pectin during peach fruit ripening remain unclear. Further analysis of the biological functions of these genes (including PME and PMEI) during fruit ripening is necessary.

In addition to PME, PMEI activity is also regulated by hormones. Auxin-induced PME activity increases cell wall extension, and consequently water absorption by the cell (Micheli, 2001). In the present study, NAA induced the expression of one PME and four PMEI genes, whereas ethylene induced the expression of one PME gene and two PMEI genes. The plant hormone response and fruit-specific cis elements in the promoter sequences of the PME and PMEI genes were also investigated. The promoter of a pepper PMEI contains crucial EREs and ethylene-response factors (An et al., 2009). EREs were also identified in the promoter of the E4 gene, which is controlled by an increase in ethylene concentration during tomato fruit ripening (Montgomery et al., 1993), suggesting a common regulatory mechanism during ripening. Most of the promoter regions of PME and PMEI genes contain more than one cis element for fruit-specific and hormone responses, especially to auxin and...
ethylenes, further supporting their participation in peach fruit ripening. Strong evidence makes it imperative to include this new candidate in PME-related studies. Further studies are needed to determine whether and how these cis elements function during fruit ripening.

Conclusion

In conclusion, the peach genome contains 71 PME and 30 PMEI genes that are unevenly distributed on all eight chromosomes. Comprehensive phylogenetic, gene structure, and chromosomal location analyses provided information on this gene family in peach. Analysis of the expression profiles of the PME and PMEI genes during peach fruit ripening in two types of peaches with different textures identified three PME and five PMEI genes with differential expression, and the upstream regulatory cis elements of these genes related to fruit ripening were investigated. One PME (Prupe.7G192800) and two PMEIs (Prupe.1G114500 and Prupe.2G279800) and their promoters were identified as potential targets for future studies on the biochemical metabolism and regulation of fruit ripening. The comprehensive data generated in this study will improve our understanding of the complex PME and PMEI gene families in peach. However, further studies are needed to reveal the detailed biochemical function and regulatory mechanism of PME and PMEI genes during peach fruit ripening.

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Fig. 8. Effects of 1-naphthaleneacetic acid (NAA) and ethylene (Eth) treatment on expression of eight pectin methylesterase (PME) and pectin methylesterase inhibitor (PMEI) genes in 'Zhongyoutao 16' ('CN16') peach fruit. Expression levels were normalized to the amount of input RNA, and values at 0 h were set to 1. Data are means ± SD of at least three individual experiments. CK = control.
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Supplemental Table 1. Fragments per kilobase of exon per million fragments mapped (FPKM) values for pectin methylesterase (PME) genes during ‘Zhongyoutao 13’ (‘CN13’) and ‘Zhongyoutao 16’ (‘CN16’) peach fruit ripening.

| Accession no. 1.0 | Accession no. 2.1 | CN16 S3^c | CN16 S4 I^c | CN16 S4 II^c | CN16 S4 III^c | CN13 S3^c | CN13 S4 I^c | CN13 S4 II^c | CN13 S4 III^c |
|------------------|-------------------|-----------|-------------|-------------|-------------|-----------|-------------|-------------|-------------|
| ppa020682 Prupe.1G006800 | 0 | 0.07 | 0 | 0 | 0 | 0 | 0 | 0.05 |
| ppa003528 Prupe.1G105400 | 0 | 0 | 0.03 | 0 | 0 | 0 | 0 | 0 |
| ppa017141 Prupe.1G116600 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa003441 Prupe.1G123500 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa025631 Prupe.1G123700 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa026560 Prupe.1G123800 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa020185 Prupe.1G131900 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa003569 Prupe.1G132000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa024011 Prupe.1G132300 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa003433 Prupe.1G178400 | 0.86 | 1.76 | 0.6 | 0.56 | 0.33 | 0.2 | 0.74 | 0.03 |
| ppa005208 Prupe.1G330800 | 0 | 0 | 0 | 0 | 0 | 0.03 | 0 | 0.03 |
| ppa015184 Prupe.1G377100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa022374 Prupe.1G377500 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppb020245 Prupe.1G377600 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa003581 Prupe.1G529400 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa003578 Prupe.1G529500 | 0.04 | 0.59 | 0.03 | 0 | 0.06 | 0.07 | 0.03 | 0.04 |
| ppa015611 Prupe.1G537900 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa007766 Prupe.1G538100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa019857 Prupe.2G003200 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa003474 Prupe.2G058300 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa007761 Prupe.3G003700 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa017319 Prupe.3G031000 | 25.09 | 30.04 | 75.03 | 75.62 | 37.35 | 59.16 | 28.71 |
| ppa021056 Prupe.3G099900 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa024386 Prupe.3G141200 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa004413 Prupe.3G141000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa004770 Prupe.3G141200 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa014833 Prupe.3G210700 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa018146 Prupe.3G210900 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa018062 Prupe.3G216300 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.1 |
| ppa017319 Prupe.3G268200 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa024066 Prupe.3G268300 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa015196 Prupe.3G268400 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa007533 Prupe.3G268500 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa021446 Prupe.3G268600 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa022308 Prupe.3G278200 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa003047 Prupe.3G310600 | 40.76 | 9.06 | 76.38 | 60.76 | 129.35 | 24.16 | 27.89 | 22.02 |
| ppa003369 Prupe.3G003700 | 32.41 | 24.75 | 54.69 | 59.74 | 45.71 | 40.6 | 22.22 |
| ppa007761 Prupe.3G147000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa018812 Prupe.3G149100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.08 |
| ppa006779 Prupe.3G185700 | 0.66 | 0.18 | 0.72 | 0.4 | 0.17 | 0.77 | 0.26 |
| ppa008873 Prupe.3G263600 | 88.1 | 64.8 | 60.01 | 60.22 | 74.11 | 71.17 | 69.31 | 61.37 |
| ppa003697 Prupe.4G025200 | 2.93 | 0.38 | 0.88 | 0.87 | 2.46 | 2.3 | 2.82 |
| ppa024324 Prupe.4G239700 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa021439 Prupe.4G069600 | 0 | 0 | 0 | 0 | 0.04 | 0 | 0 | 0.05 |
| ppa027083 Prupe.6G128000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa021246 Prupe.6G128100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa026550 Prupe.6G128200 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa025119 Prupe.6G128300 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa019999 Prupe.6G215500 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa024542 Prupe.6G215600 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa019869 Prupe.6G215800 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ppa004437 Prupe.6G318500 | 1.36 | 1.75 | 1.57 | 1.11 | 2.49 | 2.34 | 6.24 | 0.6 |
| ppa023319 Prupe.6G318600 | 0 | 0 | 0 | 0.03 | 0.07 | 0 | 0.04 | 0 |
| ppa015202 Prupe.6G344600 | 0.06 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Continued next page
### Supplemental Table 1. Continued.

| Accession no. | Accession no. 2.1 | CN16 S3 | CN16 S4 I | CN16 S4 II | CN16 S4 III | CN13 S3 | CN13 S4 I | CN13 S4 II | CN13 S4 III |
|---------------|-------------------|---------|-----------|-----------|-------------|---------|-----------|-----------|-------------|
| ppa019696     | Prupe.7G011200    | 0       | 0         | 0         | 0           | 0       | 0         | 0         | 0           |
| ppo013738     | Prupe.7G057300    | 0       | 0         | 0         | 0           | 0       | 0         | 0         | 0           |
| ppa023784     | Prupe.7G123900    | 0       | 0.05      | 0.07      | 0.14        | 0       | 0.12      | 0         | 0           |
| ppa004300     | Prupe.7G190300    | 0       | 0.36      | 0.21      | 0.16        | 0.22    | 0.54      | 0.14      | 0.23        |
| ppa0016574    | Prupe.7G190700    | 0       | 0.04      | 0.08      | 0.06        | 0.09    | 0.03      | 0.07      | 0.03        |
| ppa003852     | Prupe.7G190400    | 0       | 0.12      | 0.37      | 1.03        | 0.55    | 0         | 2.56      | 9.25        |
| ppa003639     | Prupe.8G263900    | 0       | 0.09      | 0.31      | 0.07        | 0.07    | 0.14      | 0.07      | 1.06        |
| ppa024812     | Prupe.8G264000    | 0       | 0.06      | 0.16      | 0.23        | 0.35    | 0.16      | 0.23      | 0.64        |
| ppa004300     | Prupe.8G264100    | 0       | 0.08      | 0.31      | 0.07        | 0.07    | 0.14      | 0.07      | 1.06        |
| ppa003852     | Prupe.7G190400    | 0       | 0.12      | 0.37      | 1.03        | 0.55    | 0         | 2.56      | 9.25        |
| ppa003639     | Prupe.8G263900    | 0       | 0.09      | 0.31      | 0.07        | 0.07    | 0.14      | 0.07      | 1.06        |
| ppa024812     | Prupe.8G264000    | 0       | 0.06      | 0.16      | 0.23        | 0.35    | 0.16      | 0.23      | 0.64        |

The numbers represent FPKM values of \textit{PME} genes in the stages (S3, S4 I, S4 II, and S4 III) of ‘CN13’ and ‘CN16’, respectively, and the stages indicated as S3, S4 I, S4 II, and S4 III represent fruits from the end of stage S3 (second exponential growth phase) to stage S4 (climacteric).
Supplemental Table 2. Fragments per kilobase of exon per million fragments mapped (FPKM) values for pectin methylesterase inhibitor (PMEI) genes during ‘Zhongyoutao 13’ (‘CN13’) and ‘Zhongyoutao 16’ (‘CN16’) peach fruit ripening.

| Accession no. 1.0 | Accession no. 2.1 | CN16 S3 | CN16 S4 I | CN16 S4 II | CN16 S4 III | CN13 S3 | CN13 S4 I | CN13 S4 II | CN13 S4 III |
|------------------|------------------|--------|-----------|-----------|-----------|--------|-----------|-----------|-----------|
| ppa011560        | Prue.1G113800    | 2.98   | 0.39      | 2.18      | 3.21      | 3.89   | 2.06      | 0.55      | 0.86      |
| ppa012287        | Prue.1G114500    | 22.59  | 25.64     | 13.5      | 37.58     | 14.2   | 6.8       | 21.64     | 1944.86    |
| ppa020077        | Prue.1G118800    | 0.17   | 0.18      | 0.14      | 0.25      | 0.6    | 0.65      | 0.23      | 0.57      |
| ppa023970        | Prue.1G249100    | 0      | 0         | 0         | 0         | 0      | 0         | 0         | 0         |
| Prue.1G330900    | 7.66             | 4.24   | 10.72     | 9.93      | 23.76     | 9.22   | 17.76     | 57.16     |
| ppa019226        | Prue.2G115600    | 0.7    | 0.9       | 0.55      | 1.27      | 0.72   | 0.48      | 0.74      | 0.11      |
| ppa011381        | Prue.2G141800    | 0.75   | 0.41      | 0         | 0         | 0      | 0.16      | 0         | 0.15      |
| ppa011607        | Prue.2G279700    | 2.36   | 20.82     | 1.35      | 0.1       | 4.44   | 84.97     | 13.43     | 64.94     |
| ppa011478        | Prue.2G279800    | 9.53   | 75.49     | 9.42      | 15.87     | 2.1    | 48.28     | 207.65    | 2897.7    |
| ppa011831        | Prue.2G279900    | 2448.27| 2836.43   | 2060.34   | 3542.41   | 3051.17| 2142.16   | 2809.55   | 2936.72   |
| ppa1027160       | Prue.3G007700    | 0      | 0         | 0         | 0         | 0      | 0         | 0         | 0         |
| ppa020700        | Prue.3G007800    | 0      | 0         | 0         | 0         | 0      | 0         | 0         | 0         |
| ppa026414        | Prue.3G007900    | 0      | 0         | 0         | 0         | 0      | 0         | 0         | 0         |
| ppa015672        | Prue.3G008000    | 0      | 0         | 0         | 0         | 0      | 0         | 0         | 0         |
| ppa022749        | Prue.3G146800    | 0      | 0         | 0         | 0         | 0      | 0         | 0         | 0         |
| ppa024549        | Prue.4G001200    | 2.61   | 1.43      | 1.19      | 0.7       | 4.65   | 4.89      | 0.38      | 0.2       |
| ppa021843        | Prue.4G137400    | 0      | 0         | 0         | 0         | 0      | 0         | 0         | 0         |
| ppa023516        | Prue.5G048600    | 0      | 0         | 0         | 0.14      | 0      | 0         | 0         | 0         |
| ppa012101        | Prue.5G048900    | 52.88  | 23.86     | 14.02     | 13.04     | 118.63 | 37.2      | 86.47     | 5.1       |
| ppa011699        | Prue.5G076800    | 49.92  | 29.96     | 15.94     | 11.78     | 25.46  | 33.45     | 26.34     | 37.25     |
| ppa015829        | Prue.5G076900    | 5.74   | 5.29      | 6.13      | 6.85      | 15.77  | 7.31      | 8.8       | 6.08      |
| ppa026908        | Prue.5G112600    | 0      | 0         | 0         | 0         | 0      | 0         | 0         | 0         |
| ppa026135        | Prue.5G189700    | 26.52  | 28.45     | 39.43     | 43.5      | 35.83  | 23.46     | 30.82     | 28.78     |
| ppa021753        | Prue.6G197400    | 1.21   | 0.27      | 0.74      | 0.49      | 1.22   | 1.45      | 0.71      | 0         |
| ppa024038        | Prue.6G309200    | 0      | 0         | 0.17      | 0         | 0      | 0         | 0         | 0         |
| ppa018153        | Prue.6G309300    | 0      | 0         | 0         | 0         | 0      | 0         | 0         | 0         |
| ppa012444        | Prue.7G193700    | 0.6    | 0         | 0.9       | 0.25      | 1.47   | 5.42      | 2.33      | 0.07      |
| ppa019284        | Prue.8G038100    | 0      | 0         | 0         | 0         | 0      | 0         | 0         | 0         |
| ppa016389        | Prue.8G261300    | 0.2    | 0         | 0         | 0         | 0      | 0         | 0         | 0         |
| ppa014770        | Prue.8G267500    | 3.17   | 1.8       | 1.71      | 1.27      | 2.22   | 1.49      | 3.1       | 0.55      |

*The numbers represent FPKM values of PMEI genes in the stages (S3, S4 I, S4 II, and S4 III) of ‘CN13’ and ‘CN16’, respectively, and the stages indicated as S3, S4 I, S4 II, and S4 III represent fruits from the end of stage S3 (second exponential growth phase) to stage S4 (climacteric).
Supplemental Table 3. The putative cis-acting regulatory elements of plant hormones response presented in the 5’-upstream region of the pectin methylesterase (PME) and pectin methylesterase inhibitor (PMEI) genes related to fruit development and ripening of peach.

| Putative cis-element | Sequence | Probable function |
|----------------------|----------|------------------|
| ERE                  | ATTTCAAA | Ethylene-responsive element |
| GCC-box              | AGCCGCC  | ERF (ethylene response factor) binding site |
| CRT/DRE              | A/GCCGAC | ERF (ethylene response factor) binding site |
| ABRE                 | TACGTG   | Abscisic acid-responsive element |
|                      | GACACGTGGC |                        |
|                      | TACGGTC  |                        |
|                      | CACGTG   |                        |
| TGA-element          | AACGAC   | Auxin-responsive element |
| TGA-box              | TGACGTGGC | Auxin-responsive element |
| S000270              | TGTCTC   | ARF (auxin response factor) binding site |
| TATC-box             | TATCCCCA | Gibberellin-responsive element |
| GARE-motif           | AAACAGA  | Gibberellin-responsive element |
|                      | TCTGTTG  |                        |
| P-box                | CCTTTTG  | Gibberellin-responsive element |
| TCA-element          | GAGAAGAATA | Salicylic acid-responsive element |
|                      | CCATCTTTTT |                        |
| TGACG-motif          | TGACG    | MeJA-responsive element |
| TGTCACA-motif        | TGTCACA  | Potential fruit-specific element |
| TCCAAAA-motif        | TCCAAAA  | Potential fruit-specific element |

*Putative cis-acting regulatory elements in the 5’-upstream region of the PME and PMEI genes were analyzed in the plant cis-acting regulatory DNA elements (PLACE) database and the PlantCARE database. ABRE = abscisic acid-responsive element, ARF = auxin response factor, CRT/DRE = C-repeat/drought-responsive element, ERE = ethylene responsive element, ERF = ethylene response factor, GARE = gibberellin-responsive element, MeJA = methyl jasmonate.*
Supplemental Table 4. The types and number of putative cis-acting regulatory elements of plant hormones response presented in the 5′-upstream region of the pectin methylesterase (PME) and pectin methylesterase inhibitor (PMEI) genes related to fruit ripening of peach.

| Gene       | Plant hormone response elements | No. |
|------------|---------------------------------|-----|
| Prupe.7G190400 | ABRE                            | 1   |
|            | GARE-motif                      | 2   |
|            | TATC-box                        | 1   |
|            | TCCAAA-motif                    | 1   |
| Prupe.7G190300 | ERE                             | 1   |
|            | CRT/DRE                         | 1   |
|            | ABRE                            | 5   |
|            | TCA-element                     | 1   |
|            | TGACG-motif                     | 1   |
| Prupe.1G113800 | S000270                        | 1   |
|            | ERE                             | 2   |
|            | CRT/DRE                         | 1   |
|            | ABRE                            | 1   |
|            | GARE-motif                      | 2   |
|            | TGACG-motif                     | 1   |
|            | TCA-element                     | 1   |
| Prupe.1G330900 | ERE                             | 1   |
|            | TGACG-motif                     | 2   |
|            | TGA-element                     | 1   |
|            | TCA-element                     | 2   |
|            | GARE-motif                      | 1   |
| Prupe.2G279700 | ABRE                            | 3   |
|            | P-box                           | 1   |
|            | TCA-element                     | 2   |
| Prupe.2G279800 | ERE                             | 2   |
|            | GCC-box                         | 1   |
|            | ABRE                            | 4   |
|            | GARE-motif                      | 2   |
|            | TCCAAA-motif                    | 1   |
| Prupe.7G192800 | S000270                        | 1   |
|            | GARE-motif                      | 1   |
|            | TGTCACA-motif                   | 1   |
|            | TCCAAA-motif                    | 1   |
| Prupe.1G114500 | TGA-box                        | 1   |
|            | TGA-element                     | 1   |
|            | ABRE                            | 1   |
|            | TGACG-motif                     | 1   |
|            | TCA-element                     | 1   |
|            | TCCAAA-motif                    | 1   |

*Putative cis-acting regulatory elements in the 5′-upstream region of the PME and PMEI genes were analyzed in the plant cis-acting regulatory DNA elements (PLACE) database and the PlantCARE database. ABRE = abscisic acid-responsive element, CRT/DRE = C-repeat/drought-responsive element, ERE = ethylene responsive element, GARE = gibberellin-responsive element, MeJA = methyl jasmonate.*