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.

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 Ca^{2+} , 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 Ca^{2+} 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; Deytieu-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 (Jolie 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;

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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 on 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) (Zeng

et al., 2015). For ‘Goldhoney 3’ (MF) and ‘Yumyeong’ (SH), four sampling points covering the climacteric stage at 110, 115, 122, and 127 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 $\mu\text{L}\cdot\text{L}^{-1}$ of surfactant (Silwet L-77; Real-Times Biotechnology, Beijing, China) as a surfactant. For ethylene treatment, fruit were dipped in 1000 $\mu\text{L}\cdot\text{L}^{-1}$ ethephon [an ethylene releaser (Solarbio, Beijing, 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), PME1 (PF04043), or both domains were identified from the peach genome (Verde et al., 2013) (accession no. 2.1) using default parameters in hmmsearch/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

Table 1. Primers of five *pectin* methylesterase inhibitor (*PMEI*) and three *pectin* methylesterase (*PME*) genes used for real-time quantitative reverse transcription polymerase chain reaction (RT-PCR) analysis.

Genes ²	Primers (forward/reverse)
Prupe.1G113800	5'-TGCTCTTCTTCTGTAACCTTTTGTC-3' 5'-TGATGAAGCCCATAAAAGAACA-3'
Prupe.1G114500	5'-GTTGTACCCTACTGTCTTTC-3' 5'-CATAGTTCGGTGTCTGTTT-3'
Prupe.1G330900	5'-GTTGGTTTGGACCGTTGGAAT-3' 5'-GATGAAAGACGGAGAGAATGGA-3'
Prupe.2G279700	5'-CCCCGACAAGATTTTTC-3' 5'-TGGAGGCAGTGGAGGGAGT-3'
Prupe.2G279800	5'-CACAACCACATACCCACT-3' 5'-TGAAACTACGGAAGAGGC-3'
Prupe.7G190300	5'-GCTAACCTCCCTTCTCAAAT-3' 5'-AGGCCTACAATAAACTTGAAGTG-3'
Prupe.7G190400	5'-GCTTTGGAACCTTTCAACTGG-3' 5'-GTTGCATGGATTGCTTTAGTT-3'
Prupe.7G192800	5'-GTTTCATAGGGTTTTCAGGAC-3' 5'-TTGTTCAAGTAGAGGGATT-3'
Actin	5'-GATTCCGGTGCCCAAGT-3' 5'-CCAGCAGCTTCCATTCCAA-3'

²Five *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 ²	Accession no. 2.1	PFAM ^y	Type ^y	ORF (aa)	Chromosome	pI	M
1	ppa020682	Prupe.1G006800	PF01095	2	346	1	7.13	38,657.37
2	ppa003528	Prupe.1G105400	PF04043 PF01095	1	568	1	9.04	62,478.95
3	ppa017141	Prupe.1G116600	PF01095	2	345	1	9.03	38,034.05
4	ppa003441	Prupe.1G123500	PF04043 PF01095	1	574	1	8.16	63,182.14
5	ppa025631	Prupe.1G123700	PF04043 PF01095	1	574	1	8.58	63,475.56
6	ppa026560	Prupe.1G123800	PF04043 PF01095	1	575	1	8.74	62,895.86
7	ppa020185	Prupe.1G131900	PF04043 PF01095	1	570	1	8.82	62,139.08
8	ppa003569	Prupe.1G132000	PF04043 PF01095	1	564	1	8.91	62,038.41
9	ppa024011	Prupe.1G132300	PF04043 PF01095	1	574	1	8.16	63,114.04
10	ppa003433	Prupe.1G178400	PF04043 PF01095	1	592	1	8.30	64,123.99
11	ppa005208	Prupe.1G330800	PF04043 PF01095	1	542	1	8.57	59,483.88
12	ppa015184	Prupe.1G377100	PF01095	2	480	1	7.67	51,478.01
13	ppa022374	Prupe.1G377500	PF01095	2	335	1	8.36	36,545.32
14	ppb020245	Prupe.1G377600	PF01095	2	356	1	7.04	39,034.98
15	ppa003581	Prupe.1G529400	PF04043 PF01095	1	564	1	6.82	60,907.62
16	ppa003578	Prupe.1G529500	PF04043 PF01095	1	564	1	7.14	61,476.24
17	ppa015611	Prupe.1G537900	PF01095	2	374	1	7.55	40,618.10
18	ppa007766	Prupe.1G538100	PF01095	2	374	1	7.55	40,618.10
19	ppa019857	Prupe.2G003200	PF01095	2	166	2	7.57	18,666.93
20	ppa003474	Prupe.2G058200	PF04043 PF01095	1	572	2	8.62	63,364.74
21	ppa024156	Prupe.2G058300	PF04043 PF01095	1	574	2	9.41	62,785.23
22	ppa003915	Prupe.2G099900	PF04043 PF01095	1	583	2	8.81	64,063.47
23	ppa021056	Prupe.2G100200	PF04043 PF01095	1	539	2	6.67	58,988.46
24	ppa024386	Prupe.2G100500	PF04043 PF01095	1	539	2	6.28	59,142.46
25	ppa004413	Prupe.2G141000	PF04043 PF01095	1	511	2	9.15	56,279.3
26	ppa004770	Prupe.2G141200	PF04043 PF01095	1	492	2	8.72	54,792.18
27	ppa014833	Prupe.2G210700	PF04043 PF01095	1	489	2	6.84	54,199.2
28	ppa018146	Prupe.2G210900	PF01095	2	158	2	6.90	17,260.38
29	ppb018062	Prupe.2G216300	PF01095	2	299	2	9.17	33,224.55
30	ppa017319	Prupe.2G268200	PF01095	2	291	2	6.96	31,874.16
31	ppa024066	Prupe.2G268300	PF01095	2	330	2	8.95	36,354.40
32	ppb015196	Prupe.2G268400	PF01095	2	179	2	8.78	20,103.34
33	ppa007533	Prupe.2G268500	PF01095	2	364	2	9.08	39,542.78
34	ppa021446	Prupe.2G278200	PF04043 PF01095	1	517	2	9.23	56,781.59
35	ppa022308	Prupe.2G278300	PF04043 PF01095	1	526	2	9.63	58,842.08
36	ppa003047	Prupe.2G310600	PF04043 PF01095	1	608	2	9.10	66,088.83
37	ppa003369	Prupe.3G003700	PF04043 PF01095	1	580	3	9.07	63,648.6
38		Prupe.3G031000	PF04043 PF01095	1	1177	3	8.83	12,7824.09
39	ppa007761	Prupe.3G147000	PF01095	2	371	3	5.99	40,261.50
40	ppa018812	Prupe.3G149100	PF01095	2	375	3	7.65	41,168.62
41	ppa006779	Prupe.3G185700	PF01095	2	402	3	8.26	44,362.18
42	ppa008873	Prupe.3G263600	PF01095	2	316	3	7.53	35,317.0
43	ppa003697	Prupe.4G025200	PF04043 PF01095	1	555	4	8.39	60,429.78
44	ppa023424	Prupe.4G239700	PF01095	2	362	4	5.79	40,143.08
45	ppa024139	Prupe.5G069600	PF01095	2	381	5	8.67	41,669.8
46	ppa027083	Prupe.6G128000	PF01095	2	371	6	8.38	40,555.00
47	ppa021246	Prupe.6G128100	PF01095	2	371	6	8.38	40,555.00
48	ppa026550	Prupe.6G128200	PF01095	2	371	6	8.38	40,524.98
49	ppa025119	Prupe.6G128300	PF01095	2	371	6	8.38	40,583.06
50		Prupe.6G215500	PF01095	2	324	6	6.09	35,878.60
51	ppa019999	Prupe.6G215600	PF01095	2	260	6	8.26	28,839.8
52	ppa024542	Prupe.6G215800	PF01095	2	297	6	6.53	33,536.8
53	ppa019869	Prupe.6G312200	PF01095	2	299	6	4.93	32,781.8
54	ppa004437	Prupe.6G318500	PF04043 PF01095	1	560	6	9.03	62,405.87
55	ppa023319	Prupe.6G318600	PF04043 PF01095	1	607	6	8.59	67,655.92
56	ppa015202	Prupe.6G344600	PF04043 PF01095	1	490	6	7.18	54,158.49
57	ppa019696	Prupe.7G011200	PF01095	2	368	7	8.96	40,381.90

Continued next page

Table 2. Continued.

Number	Accession no. 1.0 ^z	Accession no. 2.1	PFAM ^y	Type ^y	ORF (aa)	Chromosome	pI	M
58	ppb012738	Prupe.7G057300	PF01095	2	170	7	8.58	19,145.00
59	ppa023784	Prupe.7G123900	PF01095	2	423	7	9.11	46,634.31
60	ppa004300	Prupe.7G190300	PF04043 PF01095	1	517	7	6.16	56,807.00
61	ppa003852	Prupe.7G190400	PF04043 PF01095	1	544	7	5.54	59,979.41
62	ppa023802	Prupe.7G190500	PF04043 PF01095	1	596	7	9.45	66,173.96
63	ppa016574	Prupe.7G190700	PF04043 PF01095	1	522	7	8.65	56,986.64
64	ppa019698	Prupe.7G192400	PF04043 PF01095	1	554	7	8.50	62,362.41
65	ppa005976	Prupe.7G192800	PF01095	2	370	7	8.79	41,215.07
66	ppa007112	Prupe.7G218400	PF01095	2	381	7	9.42	42,318.59
67	ppa003639	Prupe.8G263900	PF04043 PF01095	1	560	8	9.18	61,623.43
68	ppa024812	Prupe.8G264000	PF04043 PF01095	1	561	8	6.94	62,078.94
69	ppa003400	Prupe.8G264100	PF04043 PF01095	1	593	8	9.03	65,727.78
70	ppa020130	Prupe.I005000	PF01095	2	349	unknown	6.50	38,286.38
71	ppa026195	Prupe.I005200	PF01095	2	332	unknown	9.12	36,718.55

^zPrupe.3G031000 and Prupe.6G215500 have no peach accession no. 1.0 number.

^yType 1 contain PF04043 and PF01095 domain and Type 2 only contain PF01095 domain.

Table 3. 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 inhibitor (*PMEI*) genes.

Number	Accession no. 1.0 ^z	Accession no. 2.1	PFAM	ORF (aa)	Chromosome	pI	M
1	ppa011560	Prupe.1G113800	PF04043	206	1	5.40	22,113.48
2	ppa012287	Prupe.1G114500	PF04043	176	1	5.78	18,633.39
3	ppa020077	Prupe.1G118800	PF04043	289	1	8.12	30,879.18
4	ppa023970	Prupe.1G249100	PF04043	175	1	9.03	19,312.44
5		Prupe.1G330900	PF04043	215	1	6.28	23,261.30
6	ppb019226	Prupe.2G115600	PF04043	191	2	9.47	21,718.38
7	ppa011381	Prupe.2G141800	PF04043	213	2	9.62	24,081.01
8	ppa011607	Prupe.2G279700	PF04043	203	2	6.41	21,453.30
9	ppa011478	Prupe.2G279800	PF04043	208	2	8.73	22,255.47
10	ppa011831	Prupe.2G279900	PF04043	194	2	9.37	21,072.15
11	ppa1027160	Prupe.3G007700	PF04043	174	3	5.90	19,286.79
12	ppa020700	Prupe.3G007800	PF04043	171	3	5.23	19,141.99
13	ppa026414	Prupe.3G007900	PF04043	175	3	4.75	19,403.19
14	ppa015672	Prupe.3G008000	PF04043	98	3	8.93	11,057.63
15	ppa022749	Prupe.3G146800	PF04043	181	3	4.93	19,259.24
16	ppa024549	Prupe.4G001200	PF04043	179	4	6.92	19,695.62
17	ppa021843	Prupe.4G137400	PF04043	131	4	7.60	14,374.34
18	ppa023516	Prupe.5G048600	PF04043	199	5	4.78	21,981.36
19	ppa012101	Prupe.5G048900	PF04043	183	5	6.72	20,104.06
20	ppa011699	Prupe.5G076800	PF04043	200	5	6.12	22,170.52
21	ppa015829	Prupe.5G076900	PF04043	208	5	10.02	22,608.89
22	ppa026908	Prupe.5G112600	PF04043	173	5	4.44	18,214.63
23	ppa026135	Prupe.5G189700	PF04043	235	5	5.24	25,380.96
24	ppa021753	Prupe.6G197400	PF04043	181	6	7.64	19,722.46
25	ppa024038	Prupe.6G309200	PF04043	195	6	5.94	21,659.99
26	ppa018153	Prupe.6G309300	PF04043	174	6	5.01	19,800.45
27	ppa012444	Prupe.7G193700	PF04043	202	7	8.72	22,361.37
28	ppa019284	Prupe.8G038100	PF04043	180	8	4.73	19,277.07
29	ppa016389	Prupe.8G261300	PF04043	200	8	9.32	21,498.63
30	ppa014770	Prupe.8G267500	PF04043	451	8	9.47	49,975.54

^zPrupe.1G330900 have no peach accession no. 1.0 number.

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 $2^{-\Delta\Delta C_t}$ 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 TCCAAA 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 *PME*s and 30 putative *PMEI*s (Tables 2 and 3). Independent alignment of 66 arabidopsis *PME* and 71 *PMEI* protein

Fig. 1. Genomic distribution of pectin methylesterase (*PME*) and pectin methylesterase inhibitor (*PMEI*) genes on chromosomes of peach. *PMEI* genes were shown by triangle, others were *PME* genes. The chromosome numbers are demonstrated at the top of each chromosome.

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 PME1 (PF04043) and PME (PF01095) domains] and 35 were Type-2 (i.e., encoding a PME domain, but no PME1 domain). Only one of the genes (Prupe.3G031000) contained two PME and PME1 domains. Detailed information on the PpPME and PpPMEI genes is shown 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 arabidopsis. (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.

B

Fig. 2. (Continued)

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 $\times$ 6; Chr 1 and Chr 2 $\times$ 18; Chr 7 $\times$ 10; and Chr 6 $\times$ 11 genes. Prupe.I005000 and Prupe.I005200 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 Prupe.6G128000, Prupe.6G128100, Prupe.6G128200, and Prupe.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 ($\log_2|\text{Fold Change}| > 1$) between MF peaches ('CN13' and 'Goldhoney 3') and SH peaches ('CN16' and 'Yumyeong') during fruit ripening, and five *PMEI* genes (including

Fig. 3. Gene structure of pectin methylesterase (*PME*) (A) and pectin methylesterase inhibitor (*PMEI*) (B) genes in peach. Exons and introns are indicated by filled boxes and single lines, respectively. Untranslated regions (UTRs) are indicated by thick lines at both ends of the diagrams. CDS = coding sequence.

Prupe.1G113800, Prupe.1G114500, Prupe.1G330900, Prupe.2G279700, and Prupe.2G279800) showed obvious differences in expression ($\log_2|\text{Fold Change}| > 1$). 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

Fig. 3. (Continued)

Fig. 4. Hierarchical cluster analysis of the expression levels of pectin methylesterase (*PME*) and pectin methylesterase inhibitor (*PMEI*) genes in melting flesh (MF) and stony hard (SH) peaches during fruit ripening. (A) 11 *PME* genes and 15 *PMEI* genes in 'Goldhoney 3' and 'Yumyeong'. (B) 11 *PME* genes and 15 *PMEI* genes in 'Zhongyoutao 13' ('CN13') and 'Zhongyoutao 16' ('CN16'). The log₂ ratios and scale bars are shown in the figure, which were obtained using Java TreeView (Saldanha, 2004).

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 $\mu\text{L}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ (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 PMEIS 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 sequences 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*

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

Fig. 5. Expression of eight pectin methylesterase (*PME*) and pectin methylesterase inhibitor (*PMEI*) genes during peach fruit ripening. (A) Expression of eight *PME* and *PMEI* genes in 'Goldhoney 3' ('GH3') and 'Yumyeong' ('YM'). (B) Expression of eight *PME* and *PMEI* genes in 'Zhongyoutao 13' ('CN13') and 'Zhongyoutao 16' ('CN16'). Expression levels were normalized to input RNA, and the value of 'CN13' and 'GH3' at stage S3 was set to 1. Data are means $\pm$ SD of at least three individual experiments.

genes are shown in Supplemental Table 4 and Fig. 7. The fruit-specific *cis* elements, TCCAAAA-motif and TGTCACA-motif, were detected in the promoter regions of two *PpPMEI* 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

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 *PMEI*s, and the *PMEI* family appeared later than Type-1 *PME* and Type-2 *PME* genes (Wang et al., 2013). Only 30 *PMEI*s were identified in the peach genome. Phylogenetic analysis with arabidopsis *PMEI*s showed no common sequence features that distinguished subgroups of *PMEI*s 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

Fig. 5. (Continued)

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 *PME1* (AB231903) and *PME2* (X95991) are upregulated in MF type peaches compared with SH type peaches (Murayama et al., 2009); the accession no. 2.1 of these two genes are Prupe.7G190300 and

Prupe.7G190400, respectively. *PME1* is present in the aqueous phase of fully ripe fruit, and is probably synthesized as a large precursor that undergoes proteolytic cleavage during the fruit ripening process (Giovane et al., 2004). Of 30 *PMEI* genes identified, 15 were expressed in two types of peach fruit during fruit ripening; five *PMEI* genes were 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 *PMEI*, *PME* 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

Fig. 6. Effects of ethylene (C_2H_4) and 1-naphthaleneacetic acid (NAA) treatment on postharvest 'Zhongyoutao 16' ('CN16') fruit. Values are means $\pm$ SD of three biological replicates. CK = control.

Fig. 7. *Cis* elements in the pectin methylesterase (*PME*) and pectin methylesterase inhibitor (*PMEI*) genes promoter predicted by database analysis. Cylinder represents fruit specific element including TGTCACA-motif and TCCAAAA-motif; parallelogram represents gibberellic acid (GA) response element including TATC-box, gibberellin-responsive element (GARE-motif) and P-box. Triangle represents ethylene response element including ethylene responsive element (ERE), GCC-box and C-repeat/drought-responsive element (CRT/DRE). Rectangle represents auxin response element including TGA-element, TGA-box, S000270; sun represents abscisic acid (ABA) response element including abscisic acid-responsive element (ABRE). Rhombus represents salicylic acid (SA) response element including TCA-element; smiling face represents methyl jasmonate (MeJA) response element including TGACG-motif.

ethylene, 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 $\pm$ 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 ^z	CN16 S4 I ^z	CN16 S4 II ^z	CN16 S4 III ^z	CN13 S3 ^z	CN13 S4 I ^z	CN13 S4 II ^z	CN13 S4 III ^z
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.2G058200	0	0	0	0	0	0	0	0
ppa024156	Prupe.2G058300	0	0	0	0	0	0	0	0
ppa003915	Prupe.2G099900	25.09	30.04	75.03	75.62	71.5	37.35	59.16	28.71
ppa021056	Prupe.2G100200	0	0	0	0	0	0	0	0
ppa024386	Prupe.2G100500	0	0	0	0	0	0	0	0
ppa004413	Prupe.2G141000	0	0	0.07	0.03	0	0	0	0
ppa004770	Prupe.2G141200	0	0	0	0	0	0	0	0
ppa014833	Prupe.2G210700	0	0	0	0	0	0	0	0
ppa018146	Prupe.2G210900	0	0	0	0	0	0	0	0
ppb018062	Prupe.2G216300	0	0	0	0	0	0.1	0	0
ppa017319	Prupe.2G268200	0	0	0	0	0	0	0	0
ppa024066	Prupe.2G268300	0	0	0	0	0	0	0	0
ppb015196	Prupe.2G268400	0	0	0	0	0	0	0	0
ppa007533	Prupe.2G268500	0	0	0	0	0	0	0	0
ppa021446	Prupe.2G278200	0	0	0	0	0	0	0	0
ppa022308	Prupe.2G278300	0.13	0.19	0.14	0.19	0	0.04	0.24	0
ppa003047	Prupe.2G310600	40.76	9.06	76.38	60.76	129.3	24.16	27.89	22.02
ppa003369	Prupe.3G003700	0	0	0	0	0	0	0	0
	Prupe.3G031000	32.41	24.75	54.69	59.74	87.2	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	0
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	0.12
ppa023424	Prupe.4G239700	0	0	0	0	0	0	0	0
ppa024139	Prupe.5G069600	0	0	0.04	0	0	0.05	0.13	0
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
	Prupe.6G215500	0	0	0	0	0	0	0	0
ppa019999	Prupe.6G215600	0	0	0	0	0	0	0	0
ppa024542	Prupe.6G215800	0	0	0	0	0	0	0	0
ppa019869	Prupe.6G312200	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

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Supplemental Table 1. Continued.

Accession no. 1.0	Accession no. 2.1	CN16 S3 ^z	CN16 S4 I ^z	CN16 S4 II ^z	CN16 S4 III ^z	CN13 S3 ^z	CN13 S4 I ^z	CN13 S4 II ^z	CN13 S4 III ^z
ppa019696	Prupe.7G011200	0	0	0	0	0	0	0	0
ppb012738	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	197.95	113.98	129.63	80.13	397.96	220.72	285.09	60.5
ppa003852	Prupe.7G190400	17.13	5.71	8.31	4.44	9.68	18.69	15.62	12.98
ppa023802	Prupe.7G190500	0.36	0.21	0.16	0.22	0.54	0.14	0.23	0.19
ppa016574	Prupe.7G190700	0	0	0	0	0	0	0	0
ppa019698	Prupe.7G192400	0.04	0.08	0.06	0.09	0.03	0.07	0.03	0.03
ppa005976	Prupe.7G192800	0.12	0.37	1.03	0.55	0	2.56	9.25	764.21
ppa007112	Prupe.7G218400	0.09	0	0.35	0.06	0.16	0.23	0.64	0
ppa003639	Prupe.8G263900	0	0	0.31	0.07	0.07	0.14	0.07	1.06
ppa024812	Prupe.8G264000	0	0	0	0	0	0	0	0
ppa003400	Prupe.8G264100	1.88	0.32	1.99	1.11	6.96	0.97	1.18	0
ppa020130	Prupe.I005000	0	0	0	0	0	0	0	0
ppa026195	Prupe.I005200	0	0	0	0	0	0	0	0

^zThe numbers represent FPKM values of *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 ^z	CN16 S4 I ^z	CN16 S4 II ^z	CN16 S4 III ^z	CN13 S3 ^z	CN13 S4 I ^z	CN13 S4 II ^z	CN13 S4 III ^z
ppa011560	Prupe.1G113800	2.98	0.39	2.18	3.21	3.89	2.06	0.55	0.86
ppa012287	Prupe.1G114500	22.59	25.64	13.5	37.58	14.2	6.8	21.64	1944.86
ppa020077	Prupe.1G118800	0.17	0.18	0.14	0.25	0.6	0.65	0.23	0.57
ppa023970	Prupe.1G249100	0	0	0	0	0	0	0	0
ppb019226	Prupe.1G330900	7.66	4.24	10.72	9.93	23.76	9.22	17.76	57.16
ppa011381	Prupe.2G115600	0.7	0.9	0.55	1.27	0.72	0.48	0.74	0.11
ppa011607	Prupe.2G141800	0.75	0.41	0	0	0	0.16	0	0.15
ppa011478	Prupe.2G279700	2.36	20.82	1.35	0.1	4.44	84.97	13.43	64.94
ppa011831	Prupe.2G279800	9.53	75.49	9.42	15.87	2.1	48.28	207.65	2897.7
ppa1027160	Prupe.2G279900	2448.27	2836.43	2060.34	3542.41	3051.17	2142.16	2809.55	2936.72
ppa020700	Prupe.3G007700	0	0	0	0	0	0	0	0
ppa020700	Prupe.3G007800	0	0	0	0	0	0	0	0
ppa026414	Prupe.3G007900	0	0	0	0	0	0	0	0
ppa015672	Prupe.3G008000	0	0	0	0	0	0	0	0
ppa022749	Prupe.3G146800	0	0	0	0	0	0	0	0
ppa024549	Prupe.4G001200	2.61	1.43	1.19	0.7	4.65	4.89	0.38	0.2
ppa021843	Prupe.4G137400	0	0	0	0	0	0	0	0
ppa023516	Prupe.5G048600	0	0	0	0.14	0	0	0	0
ppa012101	Prupe.5G048900	52.88	23.86	14.02	13.04	118.63	37.2	86.47	5.1
ppa011699	Prupe.5G076800	49.92	29.96	15.94	11.78	25.46	33.45	26.34	37.25
ppa015829	Prupe.5G076900	5.74	5.29	6.13	6.85	15.77	7.31	8.8	6.08
ppa026908	Prupe.5G112600	0	0	0	0	0	0	0	0
ppa026135	Prupe.5G189700	26.52	28.45	39.43	43.5	35.83	23.46	30.82	28.78
ppa021753	Prupe.6G197400	1.21	0.27	0.74	0.49	1.22	1.45	0.71	0
ppa024038	Prupe.6G309200	0	0	0.17	0	0	0	0	0
ppa018153	Prupe.6G309300	0	0	0	0	0	0	0	0
ppa012444	Prupe.7G193700	0.6	0	0.9	0.25	1.47	5.42	2.33	0.07
ppa019284	Prupe.8G038100	0	0	0	0	0	0	0	0
ppa016389	Prupe.8G261300	0.2	0	0	0	0	0	0	0
ppa014770	Prupe.8G267500	3.17	1.8	1.71	1.27	2.22	1.49	3.1	0.55

^zThe 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 <i>cis</i> -element ^z	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	TATCCCA	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

^zPutative *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 ^z elements	No.
Prupe.7G190400	ABRE	1
	GARE-motif	2
	TATC-box	1
	TCCAAAA-motif	1
Prupe.7G190300	ERE	1
	CRT/DRE	1
	ABRE	5
	TCA-element	1
Prupe.1G113800	TGACG-motif	1
	S000270	1
	ERE	2
	CRT/DRE	1
Prupe.1G330900	ABRE	1
	GARE-motif	2
	TGACG-motif	1
	TCA-element	1
Prupe.2G279700	ERE	1
	TGACG-motif	2
	TGA-element	1
	TCA-element	2
Prupe.2G279800	GARE-motif	1
	ABRE	3
	P-box	1
	TCA-element	2
Prupe.7G192800	ERE	2
	GCC-box	1
	ABRE	4
	GARE-motif	2
Prupe.1G114500	TCCAAAA-motif	1
	S000270	1
	GARE-motif	1
	TGTCACA-motif	1
Prupe.1G114500	TCCAAAA-motif	1
	TGA-box	1
	TGA-element	1
	ABRE	1
Prupe.1G114500	TGACG-motif	1
	TCA-element	1
	TCCAAAA-motif	1
	TCCAAAA-motif	1

^zPutative *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.