

Genome-wide identification and expression pattern analysis of the ACS gene family during fruit development in peach

Xiaofei Wang, Kang Dong, Jun Cheng, Bin Tan, Xianbo Zheng, Xia Ye, Wei Wang, Langlang Zhang^{#*} and Jiancan Feng^{#*}

College of Horticulture, Henan Agricultural University, 95 Wenhua Road, Zhengzhou 450002, China

[#] These authors contributed equally: Langlang Zhang,

Jiancan Feng

* Corresponding authors, E-mail: llzhang041@henau.edu.cn; jcfeng@henau.edu.cn

Abstract

Ethylene plays an important role in regulating the development and ripening of fruits, and 1-aminocyclopropane carboxylic acid synthase is the key rate-limiting enzyme in ethylene synthesis pathway. In this study, eight *PpACS* genes were identified from the peach genome [*Prunus persica* (L.) Batsch], and their phylogeny, gene structures, promoter motifs and expression patterns were analyzed. The *PpACS* genes could be divided into four types, and the genes with similar structures and motif distribution clustered together. Identification of the cis-elements in the promoters revealed that the *PpACS* genes may respond to various hormones. Furthermore, expression analysis showed that five (*PpACS1*, *PpACS5*, *PpACS6*, *PpACS7* and *PpACS8*) of the eight *PpACS* genes were expressed at different stages during peach fruit development. Among them, *PpACS1* was highly expressed at the ripening stage and induced by ethylene. The expression peaks of *PpACS5*, *PpACS6*, *PpACS7* and *PpACS8* during the transition from first exponential growth to pit hardening (S1 to S2) indicated a potential function of ethylene during this important transition. Taken together, these results provide valuable information for future investigation into the functions of the *PpACS* genes during peach fruit development and ripening.

Citation: Wang X, Dong K, Cheng J, Tan B, Zheng X, et al. 2024. Genome-wide identification and expression pattern analysis of the ACS gene family during fruit development in peach. *Fruit Research* 4: e004 <https://doi.org/10.48130/frures-0023-0040>

Introduction

Ethylene is an important plant hormone that plays a vital role in many aspects of plant growth, including fruit development and ripening^[1–3]. Climacteric fruit like tomato, apple, pear, kiwifruit and peach display enhanced ethylene production and a peak in respiration at the onset of ripening. It is thought that ethylene regulates the initiation of various ripening-related changes like fruit color, cell wall metabolism, sugar content, flavor and aroma compounds in climacteric fruit^[3–5]. In addition, ethylene has been reported to participate in fruit set and early fruit development^[6–8]. The inhibition of ethylene production or ethylene-induced responses by external treatments with chemical inhibitors such as ethylene biosynthesis inhibitor aminoethoxyvinylglycine, perception inhibitor silver thiosulfate and 1-methylcyclopropane (1-MCP), induces fruit set efficiency and the growth of set fruits in pear^[8], and tomato^[6].

Ethylene is synthesized from S-adenosyl-L-methionine (SAM), which is converted to 1-aminocyclopropane carboxylic acid (ACC) under the catalyzation of the enzyme ACC synthase (ACS). Then, ACC is oxidized into ethylene by ACC oxidase (ACO)^[9,10]. In the ethylene synthesis pathway, the formation of ACC is generally thought to be the rate-limiting step and therefore, ACS proteins are rate-limiting enzymes in ethylene synthesis^[11]. ACS belongs to the family of pyridoxal-5'-phosphate dependent aminotransferases, which require vitamin B6 as a co-factor for activity^[9]. According to the presence of particular sequences at the C-terminus, ACS genes can be divided into

three major groups: Type I genes encode target sites for mitogen-activated and calcium-dependent protein kinases (MAPK and CDPK, respectively), Type II genes encode target sites for CDPKs and E3 ligases, and Type III genes encode no signaling target sites^[12].

Early work has discovered several genes in the ACS family in different species and their differential expression during plant development. There are 12 ACS genes (*ACS1* to *ACS12*) in Arabidopsis, and all of them display different expression patterns throughout growth and development^[1,13]. In tomato, nine genes encoding ACS (*LeACS1A*, *LeACS1B*, and *LeACS2–8*) have been reported^[1], with *LeACS1A*, *LeACS2*, *LeACS4*, and *LeACS6* are expressed in different stage of fruit development^[14,15]. Three of the genes, *LeACS1A*, *LeACS2*, and *LeACS4* showed a ripening-related increase in expression, while *LeACS6* transcripts were present in mature green fruit but declined as ripening was initiated^[15]. A total of 19 ACS genes were identified in apple, with six expressed in fruit^[16]. Among them, *MdACS3a* is expressed lower and before fruit ripening, while *MdACS1* is expressed abundantly after fruit ripening^[17]. These results indicated that fruit development relies on the expression of different ACS genes to form ethylene at distinct developmental stages.

There are two systems of ethylene biosynthesis in higher plants: System 1 is auto-inhibitory and responsible for producing basal levels of ethylene in all tissues, including non-ripening fruit; System 2 is auto-stimulatory and produces the burst of ethylene during the ripening of climacteric fruit^[15,18]. The ethylene production in Systems 1 and 2 is regulated by differential

expression of ACS genes. In tomato, System 1 is regulated by the expression of *LeACS1A* and *LeACS6*. As fruit transitions to the ripening stage, the expression of *LeACS1A* and *LeACS4* is induced, dependent on the RIN MADS-box transcription factor, and System-2 ethylene synthesis is subsequently initiated and maintained by *LeACS2*^[15]. In apple, *MdACS1* is well-known to control the climacteric ethylene burst during fruit ripening, while *MdACS6* is responsible for ethylene production in System-1 and *MdACS3a* is a main regulator of ethylene production through the transition from System 1 to System 2^[17,19].

Six *PpACS*s were identified in nectarine, and three of them are expressed during nectarine fruit development and ripening^[20]. Among them, *PpACS1* is expressed abundantly at the ripening stage and plays important roles in regulating fruit softening^[20,21]. However, peach fruit development and ripening consists of several growth phases: the first exponential growth phase (S1), the onset of pit hardening (S2), the second exponential growth phase (S3), and ripening (S4). Aside from the ripening stage, the regulation of *PpACS* genes to control ethylene synthesis during the other development phases especially the transition between different growth phases, remains unclear.

In this study, we identified eight *PpACS* genes in peach and analyzed their gene structures, conserved domains, motifs and promoters. Samples were taken from additional, intervals before and after full bloom to perform detailed examinations of ACS activity and transcript levels of the *PpACS* genes during fruit development and ripening. The *PpACS* transcript expression patterns were also examined under ethylene and 1-MCP treatments. These results provide valuable information for the study of the *PpACS* gene functions during peach fruit development.

Materials and methods

Plant materials

For *PpACS* expression profiles and ACS activity analysis, peach fruit at different developmental stages were collected from the cultivar 'Jinquehongmi' about 7 days' before and after full bloom (DAFB). For ethylene and 1-MCP treatment, the variety 'Huangshuimi' peach fruit at 70 DAFB were collected and treated with 100 g·L⁻¹ of ethephon, 10 μL·L⁻¹ of 1-methylcyclopropene (1-MCP) or distilled water according to Cheng et al^[22]. Fruit samples were collected at 0, 2 and 4 d after treatment. All collected materials were immediately frozen in liquid nitrogen and stored at -80 °C until use. The peach cultivars used in this study were seven-year-old trees and maintained at the Fruit Tree Germplasm Repository of Henan Agricultural University (Henan Province, China).

Identification of the *PpACS* genes in peach

The genome sequences (Version 2.0) used in this study were downloaded from GDR (www.rosaceae.org/gb/gbrowse/prunus_persica_v2.0/). All *PpACS* genes in peach were identified by BlastP using the ACS genes of *Arabidopsis thaliana* as the query sequence^[12] downloaded from the TAIR database (www.arabidopsis.org/). The potential *PpACS* genes were reconfirmed through comparison with the Swiss-Port database in NCBI-BLASTP (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

The exon-intron structures and the conserved motifs of the *PpACS* genes were analyzed and visualized using TBtools^[23]

with the parameters of zero or one repetition and 10 maximum number of motifs.

The 2,000 bp upstream of each *PpACS* coding sequence (CDS) was extracted as the promoter by TBtools^[23]. The promoter cis-acting elements were predicted through the PlantCARE (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>)^[24] and visualized using TBtools^[23].

Multiple sequence alignment, phylogenetic analysis, and protein structure prediction of *PpACS*s

The predicted CDS length, predicted isoelectric point (pI) and molecular weights (MWs) of all confirmed *PpACS*s were predicted by ExPASy-ProtParam (<https://web.expasy.org/prot-param/>), and their subcellular localization was predicted by Plant-mPLo (www.csbio.sjtu.edu.cn/bioinf/plant-multi/).

The multiple sequence alignment was generated by the BioEdit Sequence Alignment Editor^[25]. A total number of 23 ACS proteins from Arabidopsis, tomato and apple were downloaded from the Phytozome database (<https://phytozome-next.jgi.doe.gov/>)^[26] and were used for phylogenetic analysis together with the *PpACS*s. Maximum likelihood and neighbor-joining trees of ACS proteins were generated using MEGA 11.0 with 1,000 replicates (Jukes-Cantor distance matrix).

Quantitative real-time PCR analysis

Total RNA was extracted using the Spin Column Plant total RNA Purification Kit (Sangon Biotech, Shanghai, China), and the first-strand cDNA was synthesized using the PrimeScript™ RT reagent Kit (TaKaRa, Dalian, China). qRT-PCR was conducted in a total reaction volume of 20 μL containing 10 μL of SYBR Green I Master Mix (Takara, Dalian, China), 0.2 μM of each primer, and 100 ng of template cDNA. The amplification was performed on an ABI Prism 7500 FAST Sequence Detection System (Applied Biosystems, Madrid, CA, USA) with the following program: pre-denaturation at 95 °C for 30 s, followed by 40 cycles of 95 °C for 30 s, 60 °C for 34 s, and 72 °C for 1 min. The peach *PpTEF2* (Prupe.4G138900) gene was selected as constitutive controls^[27]. According to the Ct value, the gene expression level was calculated by the 2^{-ΔΔCT} method^[28]. Primers used for qRT-PCR were designed using Primer-BLAST^[29] and are listed in Supplemental Table S1. Three biological replicates were performed for each analysis.

Analysis of ACS activity during fruit development

The peach fruits collected at different developmental stages after full bloom were also used to detect ACS activity. Firstly, fruit samples were ground with liquid nitrogen, and 0.1 g samples were homogenized by hand with 2 mL phosphate buffer (pH 7.4), and then centrifuged at 1,000 g for 20 min. The supernatant was collected to detect ACS activity. The Plant ACC synthase (ACS) ELISA Kit (Ruishou, Shanghai, China) was used to perform a chromogenic reaction according to the instructions. Briefly, a total reaction volume of 50 μL samples or standard, 100 μL of enzyme conjugate were added to a microtiter plate, covered with an adhesive strip and incubated for 60 min at 37 °C, the microtiter plate was washed four times. Then, 50 μL Substrate A and Substrate B were added to each well respectively, gently mixed and incubated for 15 min at 37 °C. Lastly, 50 μL Stop Solution were added and the reaction solution was measured at a wavelength of 450 nm using a microplate reader within 15 min. A standard curve was generated and used to calculate the ACS activity. Three biological replicates were performed.

Results

Phylogenetic analysis and multiple sequence alignment of *PpACS* genes

An early study identified six *PpACS* genes in the nectarine genome^[20]. Here, we have found eight *PpACS* genes in the peach genome, including the six reported *PpACS*s and two other genes named *PpACS7* and *PpACS8* (Supplemental Table S2). According to previous studies, the ACS proteins can be classified into four types based on their C-terminal sequence characteristics^[12,30]. Here, a phylogenetic tree was constructed, and sequence alignment of the C-terminal amino acids of the ACS proteins was performed.

The phylogenetic analysis showed that the eight *PpACS* proteins from peach were classified into four types (Type I, Type II, Type III and AT, or aminotransferase) together with the ACS proteins from Arabidopsis, tomato and apple (Fig. 1). For instance, *PpACS1* and *PpACS5* clustered with Type I ACS proteins, *PpACS4* and *PpACS6* with Type II, and *PpACS2* and *PpACS3* with Type III (Fig. 1). The proteins that cluster in the AT type, namely *PpACS7* and *PpACS8* from peach, *AtACS10* and *AtACS12* from Arabidopsis, and *MdACS6* from apple, are likely aminotransferases (AT) that lack ACS activities (Fig. 1).

Further amino acid sequence alignment of the C-termini of the ACS proteins from Arabidopsis and peach confirmed the

conservative characteristics of the *PpACS* proteins (Fig. 2). The Type I ACS proteins have four conserved serine residues, three of which are phosphorylation sites of MAPKs and one of which is a phosphorylation site of CDPKs (Fig. 2). The Type II ACS proteins contained only one CDPK phosphorylation sites or conserved serine residues at the C-terminus (Fig. 2). Both the Type III and AT-type showed the shortest C-terminals, lacking both types of conserved serine residues (Fig. 2).

Gene structure and conserved motif composition of *PpACS*s

The analysis of exon-intron structure is essential to understanding the evolutionary history within gene families^[32]. The exons and introns of the ACS genes in peach were analyzed by TBtools. The *PpACS* members in the same subfamily showed roughly similar exon/intron distribution patterns in terms of the exon length and the number of introns (Fig. 3b). The Type I enzymes *PpACS1* and *PpACS5*, *PpACS4* and *PpACS6* (Type II), *PpACS7* and *PpACS8* (AT type) all contained four exons and three introns, while *PpACS2* and *PpACS3* (Type III) contained three exons and two introns (Fig. 3a, b). The conserved motifs of the *PpACS* genes were analyzed, and the results showed that all members contained nine motifs (Fig. 3c). Motif 1–8 were common and distributed among all members, while motif 9 was included in members of Type I, Type II and Type III, but not in the AT Type. Only the AT-type genes contained motif 10,

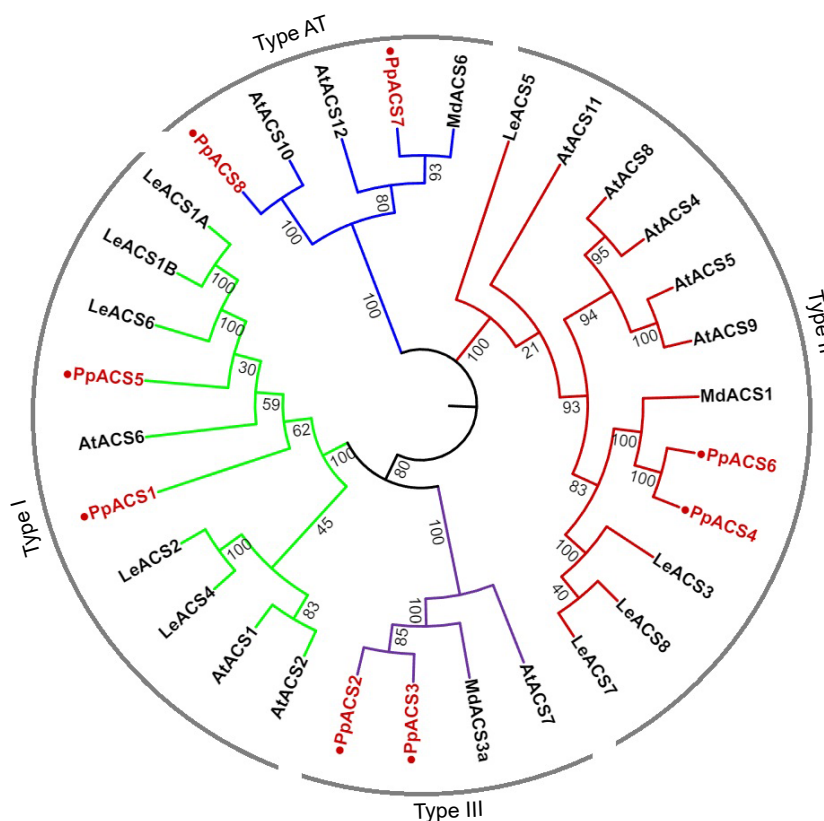


Fig. 1 Phylogenetic analysis of ACS proteins from Arabidopsis, tomato, apple, and peach. A neighbor-joining tree of ACS proteins was generated using the MEGA11.0 with 1000 replicates (Jukes-Cantor distance matrix). ACS proteins of peach are marked with red color. Deduced amino acid sequences from Arabidopsis [AtACS1 (At3g61510), AtACS2 (At1g01480), AtACS4 (At2g22810), AtACS5 (At5g65800), AtACS6 (At4g11280), AtACS7 (At4g26200), AtACS8 (At4g37770), AtACS9 (At3g49700), AtACS10 (At1g62960), AtACS11 (At4g08040), AtACS12 (At5g51690)], apple [MdACS1 (U89156), MdACS3a (AB243060), MdACS6 (MDP0000133334)] and tomato [LeACS1A (U18056), LeACS1B (U18057), LeACS2 (X59139), LeACS3 (U17972), LeACS4 (X59146), LeACS5 (AF167425), LeACS6 (AF167428), LeACS7 (AF043122), LeACS8 (AF167427)] were used. AT type ACS proteins are more like aminotransferases.

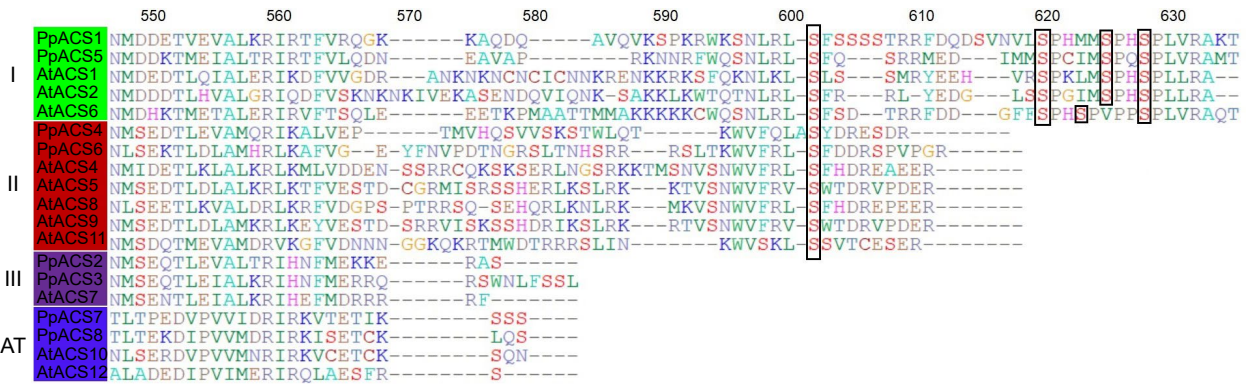


Fig. 2 Amino acid alignment of the C-termini of ACS proteins from Arabidopsis and peach. The conserved serine (S) residues of the ACS proteins are marked with black boxes according to previous research^[31].

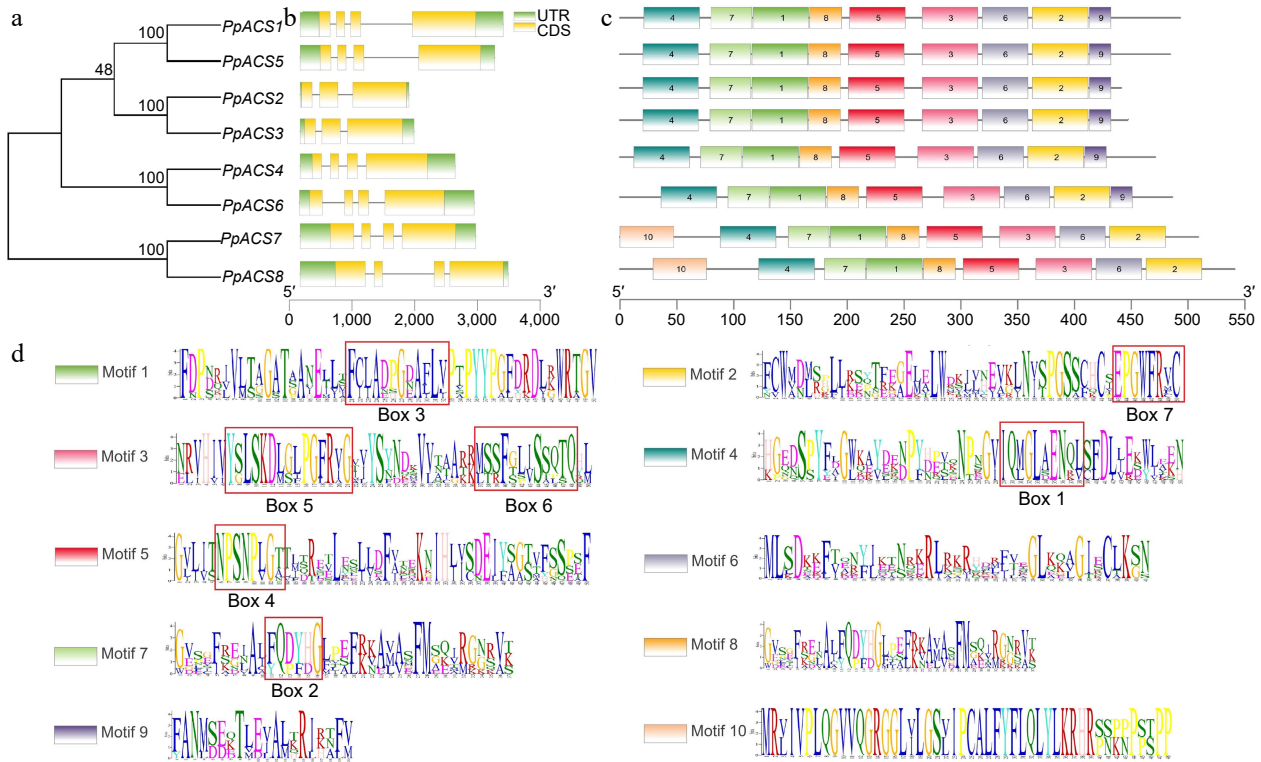


Fig. 3 Gene structure and conserved protein motifs of the *PpACS* family in peach. (a) The phylogenetic tree based on all *PpACS* proteins was constructed using the maximum likelihood method with 1,000 bootstrap. (b) The gene structures of the *PpACS* genes. The green and yellow boxes represent UTRs and CDSs respectively. (c) The conserved motifs of the *PpACS* proteins were identified using the MEME program. (d) The sequence logos of the conserved motifs numbered from 1 to 10. The seven conserved domains of ACS are marked with red boxes and named as box 1–7.

suggesting a highly specific function. Sequence logos of the ten conserved motifs included the seven conserved domains of ACS proteins (Fig. 3d).

Promoter cis-elements analysis of the *PpACS* genes

In order to better understand the potential regulatory function of *PpACS* genes in peach, the possible cis-regulatory elements (CREs) were identified in the 2,000-bp upstream region from each transcription start site using the PlantCARE online web tool. The predicted CREs of the *PpACS* genes contained the core promoter elements, environmental stress-related elements, hormone-responsive elements, light-responsive

elements, site-binding-related elements, and some others binding sites (Supplemental Table S3). The largest number of elements were the core promoter elements that contained the TATA-box, AT-TATA and CAAT-box (Supplemental Table S3). The light-responsive elements were abundant, including Box 4, the G-box, and the TCT-motif (Supplemental Table S3). There were also various hormone-responsive elements, including the TCA-element involved in salicylic acid (SA) responsiveness, the TATC-box and gibberellin (GA)-response element (GARE) involved in GA responsiveness, the abscisic acid (ABA)-responsive element (ABRE), the AuxRR-core and TGA involved in auxin responsiveness, the CGTCA-motif and TGACG-motif involved in MeJA-responsiveness and the ethylene-responsive element

(ERE) (Fig. 4b, c). The most numerous element was the ABRE, which was widely distributed among the *PpACS*s, except for *PpACS3* and *PpACS7*. The second most common element was ERE (Fig. 4b, c). This analysis indicates that the *PpACS* genes are regulated by ABA and ethylene signals.

ACS activity and *PpACS* expression profiles during peach fruit development

To further investigate the potential functions of the *PpACS* genes during peach fruit development and ripening, the ACS activity and *PpACS* expression profiles were determined. Peach fruit samples were numbered negatively as the days before full bloom, while the days after full bloom were divided into four phases: first exponential growth phase (S1), the onset of pit hardening (S2), the second exponential growth phase (S3), and ripening (S4) (Fig. 5a, b).

The total ACS activity fluctuated during fruit development (Fig. 5a). ACS activity was higher during the stage 1 and decreased significantly during the S2 (Fig. 5a). There was a rapid increase in activity within stage-3 at 136 DAFB, and the activity remained high during stage 3 and 4 (Fig. 5a).

The expression profiles of the *PpACS* genes showed that the eight *PpACS* genes were differentially expressed during peach fruit development. Among them, the AT-type genes *PpACS7* and *PpACS8* exhibited higher expression during fruit development (Fig. 6g & h). Transcripts of *PpACS1*, *PpACS5*, and *PpACS6* also accumulated during fruit development and ripening (Fig. 6a–c), while those for *PpACS2*, *PpACS3*, and *PpACS4* exhibited minimal expression in peach fruit (Fig. 6d–f). *PpACS1*, which has been identified as playing important roles in peach fruit ripening^[33,34] showed a burst expression at the S4 stage (Fig. 6a). Interestingly, *PpACS5*, *PpACS6*, *PpACS7*, and *PpACS8* showed clear expression peaks at the S1 to S2 transition (Fig. 6b, c & g). The ACS genes were mainly expressed at the early (S1) and ripening stages (S3–S4) during the fruit development and may function for ethylene synthesis during these stages.

The effect of ethylene and 1-MCP treatments on *PpACS* gene expression

To further investigate whether *PpACS* gene expression of is under the control of ethylene in peach fruit, the transcript

levels of these *PpACS* genes were examined after 1-MCP and ethylene treatments. The levels of two transcripts, *PpACS1* and *PpACS8* were upregulated under ethylene treatment and repressed by 1-MCP treatment (Fig. 7). The expression of *PpACS3* was repressed by both ethylene and 1-MCP after treatment for 4 d, while *PpACS7* was induced by both ethylene and 1-MCP (Fig. 7). Other *PpACS* genes, including *PpACS2*, *PpACS4*, *PpACS5* and *PpACS6*, were induced by 1-MCP (Fig. 7). All these results indicated that the *PpACS* genes respond differently to ethylene and 1-MCP.

Discussion

Genes in the ACS family have been divided into types I, II, III, and AT^[12]. In Arabidopsis, *AtACS10* and *AtACS12* encode amino-transferases and have ATase activity, which have been reported are not likely to be involved in ethylene biosynthesis^[12]. Here, *PpACS7* and *PpACS8* from peach clustered with the AT types together with *MdACS6* from apple. It has been reported that *MdACS6*, a homolog of *PpACS7*, was expressed continuously during fruit ripening and could regulate ethylene biosynthesis when overexpressed in apple fruit^[35]. These results indicate that the AT type ACS genes may have ethylene biosynthesis function during fruit development and need to be further investigated.

Regulation of ethylene biosynthesis is very important in fruit development, and ACS is the key rate-limiting enzyme in the ethylene synthesis pathway. Here we have conducted a detailed examination of ACS gene expression during peach fruit development and ripening. The results indicate that five ACS genes, *PpACS1*, *PpACS5*, *PpACS6*, *PpACS7* and *PpACS8*, are expressed in peach fruit (Fig. 6). Zeng et al.^[20] found that *PpACS1*, *PpACS4* and *PpACS5* show ripening-related increases in expression during fruit development and ripening in nectarine 'CN13'. *PpACS1* showed extremely high expression at in stage 4 fruit, similar to a previous report. *PpACS4*, which has been reported to show a climacteric pattern from S3-1 to S4-1, exhibited minimal expression in our studies during the entire course of fruit development. Furthermore, the expression of *PpACS5* fluctuated during stages 1 and 3, whereas data presented by

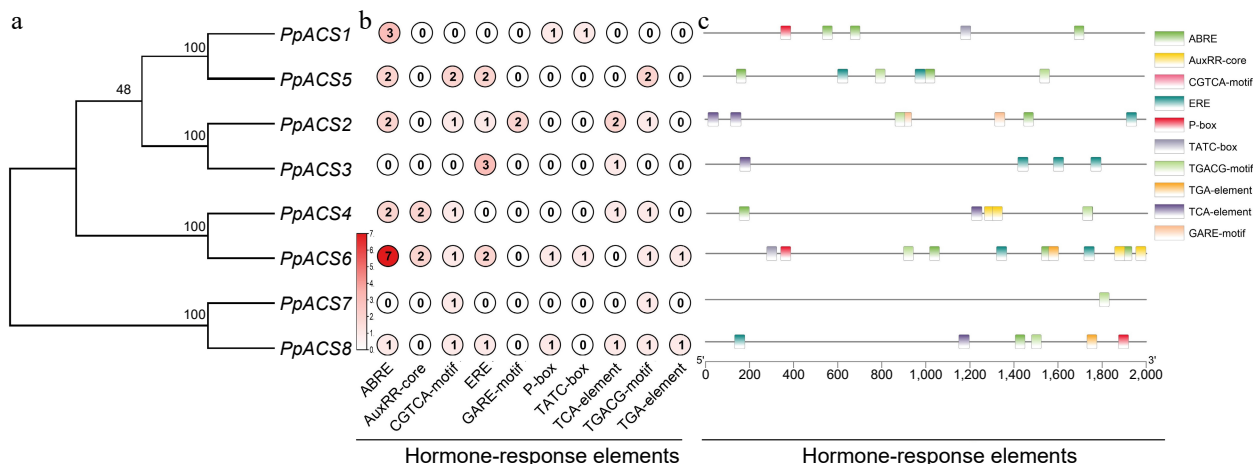


Fig. 4 Analysis of the cis-elements in the promoters of the *PpACS* genes. The cis-elements of the *PpACS* genes were determined by the PlantCARE online web tool and visualized by the TB-tool. (a) Phylogenetic tree of all *PpACS* proteins. (b) Number of hormone-responsive elements detected in each *PpACS* gene promoter. (c) Visualization of hormone-responsive elements in the *PpACS* promoters, with ten different cis-elements displayed by different colored boxes.

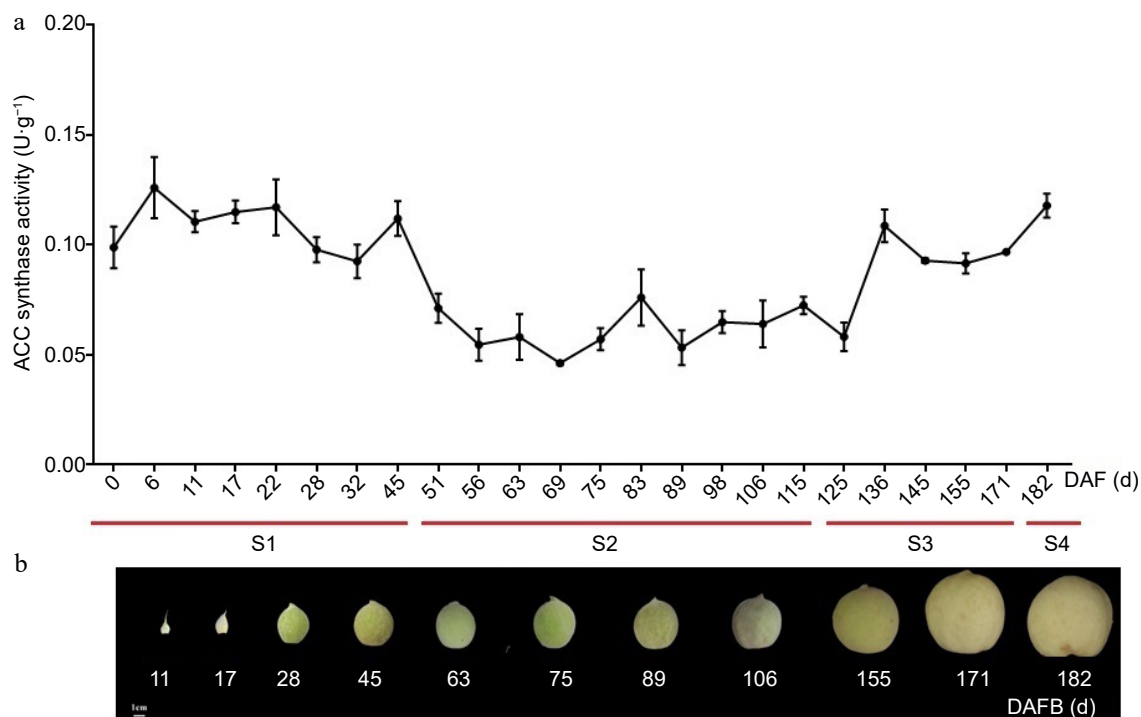


Fig. 5 Total ACC synthase activity at different stages of fruit development in peach. (a) Total ACC synthase activity in different stages of peach fruit development. The values are means \pm SD of three independent biological replicates for ACC synthase activity. (b) Sampling time of peach fruit at different development stages, which was divided into four periods (S1, S2, S3 and S4 identified with red lines) according to the days after full bloom (DAFB).

Zeng et al.^[20] showed it decreased in nectarine during fruit development. These discrepancies may be caused by the use of different cultivars or may be due to the greater number of fruit samples at closer intervals that we collected. The increased number of fruit samples offered a greater chance to detect any changes in ACS gene expression during fruit development and ripening.

Ethylene plays an important role in regulating the development and ripening of fruits, especially in climacteric fruits like peach. Peach fruit development follows the double sigmoid curve and is divided into four phases^[36]. Fruit ripening in peach was thought to be ethylene dependent. Ethylene production at the ripening stages is mainly regulated by the expression of *PpACS1*^[21,33]. Here, we also found that total ACS activity and the expression of *PpACS1*, *PpACS5* and *PpACS6* were high during S3 and S4 (Fig. 5a & 6). However, it is interesting that there are expression peaks for *PpACS5*, *PpACS6*, *PpACS7* and *PpACS8* at the S1 to S2 transition, with corresponding peaks of total ACS activity. The growth of peach fruit is slowed down at the S1 to S2 transition and is followed by endocarp lignification (onset of pit hardening), which lasts at the end of S2^[37]. The duration of S2 stage is highly dependent on the cultivars, being shorter for early ripening varieties and longer for late ripening varieties^[37]. The increasing expression of these *PpACS* genes at early S2 stage indicated the potential function of ethylene for the peach fruit mature date.

A model for the regulation of *PpACS* gene expression during nectarine fruit development and ripening was proposed^[20]. In immature nectarine fruit, they found that the System 1 ethylene production relies on the expression of *PpACS5*, while expression of both *PpACS1* and *PpACS4* increased with fruit ripening and were responsible for the burst in ethylene

production in System 2. Our data showed that, besides *PpACS5*, other *PpACS* genes, including *PpACS6*, *PpACS7* and *PpACS8*, which were mainly expressed in other tissues beside fruit, were also involved in System 1 ethylene biosynthesis and were responsible for producing a basal level of ethylene during early fruit development (Supplemental Fig. S1). Furthermore, *PpACS4* was not expressed during the whole course of fruit development, suggesting that it may not be involved in System 2 ethylene biosynthesis. These results were also supported by Guo et al.^[34]. Additionally, transcription of *PpACS1* was inhibited after ethylene treatment, suggesting an ethylene-independent (non-autocatalytic) system in nectarine fruit^[20]. In contrast, our data clearly show that the expression of *PpACS1* were significantly induced by ethylene and repressed by 1-MCP, which indicated that *PpACS1* functions in System 2 ethylene biosynthesis via an ethylene-dependent (autocatalytic) way. The result is inconsistent with previous reports, which may be due to the treatment of the fruit at different developmental stages and/or treatment times. The 'CN13' fruits used for treatment by Zeng et al.^[20] were collected at the commercial harvest stage (90 DAFB, S4-1), whereas the fruit used here were harvested at 70 DAFB (S3). Moreover, the increased *PpACS1* transcript levels after 2- and 4-d of ethylene treatment in our study suggests that high ethylene levels required for induction of these genes.

Conclusions

In the present study, we identified eight *PpACS* genes from the peach genome. Phylogenetic analysis showed that the eight proteins could be divided into four types (Type I-III and AT), which was also supported by their exon/intron structures, promoter motifs and protein domains. The ACS activity was

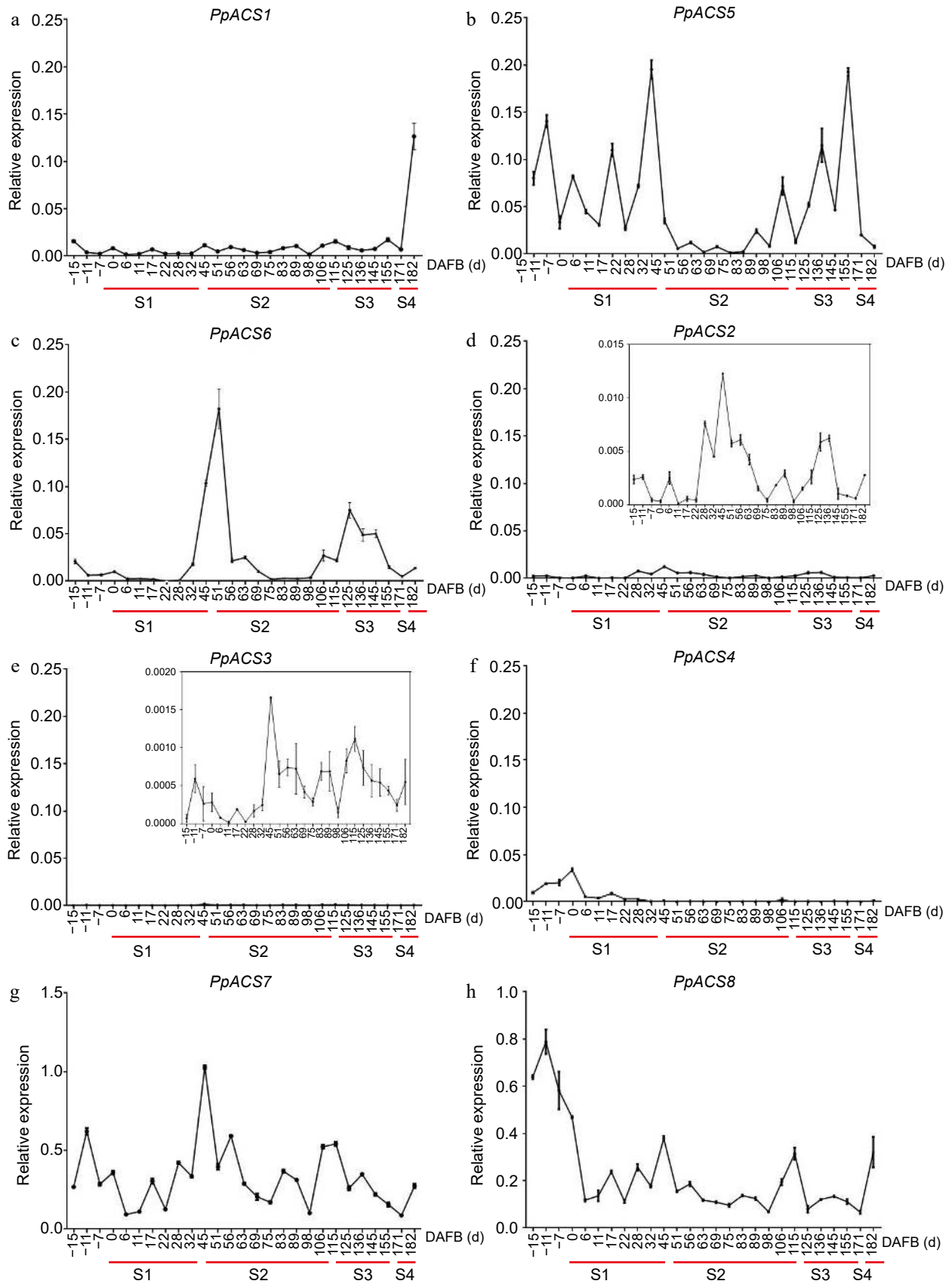


Fig. 6 Expression profiles of *PpACS* genes during fruit development of peach. The expression values are means \pm SD of three independent biological replicates as analyzed by qRT-PCR. The fruit development stage was divided into four periods (S1, S2, S3 and S4; identified with red lines) according to the days after full bloom (DAFB). (a)–(h) show the expression profiles of *PpACS1*–*PpACS8*, respectively.

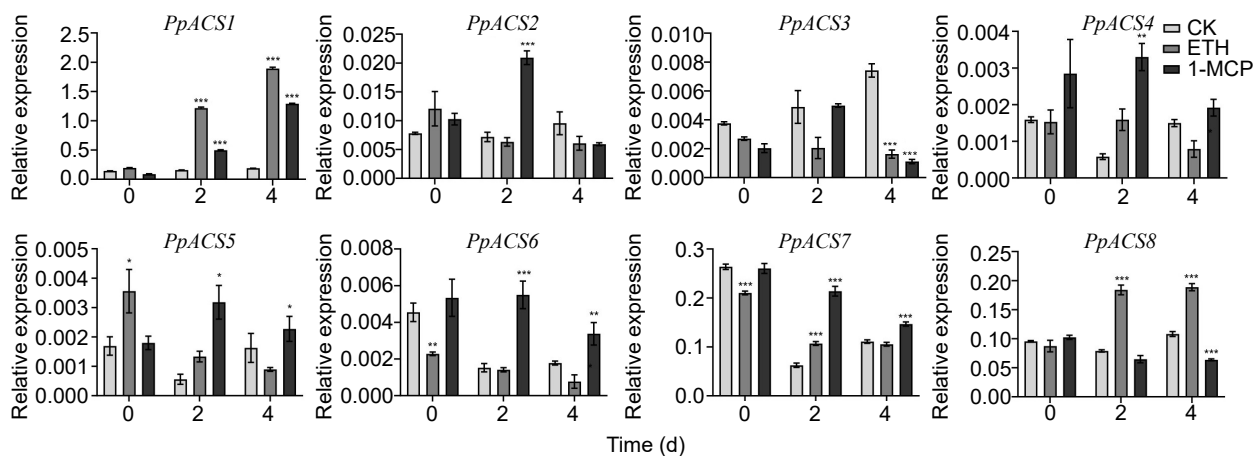


Fig. 7 Effect of ethylene and 1-MCP treatments on the expressions of *PpACS* genes. The expression values are means \pm SD of three independent biological replicates as determined by qRT-PCR. Fruits were treated with ethylene and 1-MCP and then, stored at room temperature until sampling at 2 and 4 d. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Student's t-test).

higher during the early (S1) and ripening (S3 and S4) fruit developmental stages. The *PpACS*s expression profiles during peach fruit development were investigated, and five *PpACS* genes (*PpACS1*, *PpACS5*, *PpACS6*, *PpACS7* and *PpACS8*) were expressed in peach fruit. Among them, *PpACS1* was highly expressed during peach fruit ripening and induced by ethylene treatment, while *PpACS5*, *PpACS6*, *PpACS7* and *PpACS8* were expressed at the S1 to S2 transition, suggesting their different roles in ethylene biosynthesis systems during peach fruit development.

Author contributions

The authors confirm contribution to the paper as follows: study conception and design: Zhang L, Feng J; draft manuscript preparation: Wang X, Zhang L; manuscript revision: Tan B, Zheng X, Feng J; experiments performed: Wang X, Dong K; formal analysis: Ye X, Cheng J, Wang W. All authors reviewed the results and approved the final version of the manuscript.

Data availability

All data generated or analysed during this study are included in the published article and its supplementary information files.

Acknowledgments

The work was conducted at the Henan Provincial Key Laboratory of Fruit and Cucurbit Biology and supported by the National Natural Science Foundation of China (32002014), the National Key Research and Development Program of China (2019YFD1000104), and the Special Fund for Henan Agriculture Research System (S2014-11-G02).

Conflict of interest

The authors declare that they have no conflict of interest.

Supplementary Information accompanies this paper at (<https://www.maxapress.com/article/doi/10.48130/frures-0023-0040>)

Dates

Received 18 August 2023; Accepted 23 October 2023; Published online 5 January 2024

References

- Lin Z, Zhong S, Grierson D. 2009. Recent advances in ethylene research. *Journal of Experimental Botany* 60:3311–36
- Barry CS, Giovannoni JJ. 2007. Ethylene and fruit ripening. *Journal of Plant Growth Regulation* 26:143–59
- Kumar R, Khurana A, Sharma AK. 2014. Role of plant hormones and their interplay in development and ripening of fleshy fruits. *Journal of Experimental Botany* 65:4561–75
- Alexander L, Grierson D. 2002. Ethylene biosynthesis and action in tomato: a model for climacteric fruit ripening. *Journal of Experimental Botany* 53:2039–55
- Klee HJ, Giovannoni JJ. 2011. Genetics and control of tomato fruit ripening and quality attributes. *Annual Review of Genetics* 45:41–59
- Shinozaki Y, Hao S, Kojima M, Sakakibara H, Ozeki-Iida Y, et al. 2015. Ethylene suppresses tomato (*Solanum lycopersicum*) fruit set through modification of gibberellin metabolism. *The Plant Journal* 83:237–51
- Martínez C, Manzano S, Megías Z, Garrido D, Picó B, et al. 2013. Involvement of ethylene biosynthesis and signalling in fruit set and early fruit development in zucchini squash (*Cucurbita pepo* L.). *BMC Plant Biology* 13:139
- Dussi MC, Sosa D, Calvo GS. 2002. Effects of Retain™ on fruit maturity and fruit set of pear cultivars Williams and Packham's Triumph. *Acta Horticulturae* 596:767–71
- Adams DO, Yang SF. 1979. Ethylene biosynthesis: Identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. *Proceedings of the National Academy of Sciences of the United States of America* 76:170–74
- Boller T, Herner RC, Kende H. 1979. Assay for and enzymatic formation of an ethylene precursor, 1-aminocyclopropane-1-carboxylic acid. *Planta* 145:293–303
- Kende H. 1993. Ethylene biosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* 44:283–307
- Yamagami T, Tsuchisaka A, Yamada K, Haddon WF, Harden LA, et al. 2003. Biochemical diversity among the 1-amino-cyclopropane-1-carboxylate synthase isozymes encoded by the *Arabidopsis* gene family. *Journal of Biological Chemistry* 278:49102–12

Expression pattern of ACS genes in peach fruit

13. Tsuchisaka A, Theologis A. 2004. Heterodimeric interactions among the 1-amino-cyclopropane-1-carboxylate synthase polypeptides encoded by the *Arabidopsis* gene family. *Proceedings of the National Academy of Sciences of the United States of America* 101:2275–80
14. Nakatsuka A, Murachi S, Okunishi H, Shiomi S, Nakano R, et al. 1998. Differential expression and internal feedback regulation of 1-aminocyclopropane-1-carboxylate synthase, 1-aminocyclopropane-1-carboxylate oxidase, and ethylene receptor genes in tomato fruit during development and ripening. *Plant Physiology* 118:1295–305
15. Barry CS, Llop-Tous MI, Grierson D. 2000. The regulation of 1-aminocyclopropane-1-carboxylic acid synthase gene expression during the transition from system-1 to system-2 ethylene synthesis in tomato. *Plant Physiology* 123:979–86
16. Li T, Tan D, Yang X, Wang A. 2013. Exploring the apple genome reveals six ACC synthase genes expressed during fruit ripening. *Scientia Horticulturae* 157:119–23
17. Tan D, Li T, Wang A. 2013. Apple 1-aminocyclopropane-1-carboxylic acid synthase genes, *MdACS1* and *MdACS3a*, are expressed in different systems of ethylene biosynthesis. *Plant Molecular Biology Reporter* 31:204–9
18. Lelièvre JM, Latchè A, Jones B, Bouzayan M, Pech JC. 1997. Ethylene and fruit ripening. *Physiologia Plantarum* 101:727–39
19. Ji Y, Wang A. 2021. Recent advances in phytohormone regulation of apple fruit ripening. *Plants* 10:2061
20. Zeng W, Pan L, Liu H, Niu L, Lu Z, et al. 2015. Characterization of 1-aminocyclopropane-1-carboxylic acid synthase (ACS) genes during nectarine fruit development and ripening. *Tree Genetics & Genomes* 11:18
21. Tatsuki M, Haji T, Yamaguchi M. 2006. The involvement of 1-aminocyclopropane-1-carboxylic acid synthase isogene, *Pp-ACS1*, in peach fruit softening. *Journal of Experimental Botany* 57:1281–89
22. Cheng X, Cui Z, Jiang Y, Chen Y, Tan B, et al. 2023. *PpeERF115* regulates peach fruit ripening by increasing polyamine turnover through up-regulation of genes involved in polyamine synthesis and catabolism. *Postharvest Biology and Technology* 204:112432
23. Chen C, Chen H, Zhang Y, Thomas HR, Frank MH, et al. 2020. TBtools: an integrative toolkit developed for interactive analyses of big biological data. *Molecular Plant* 13:1194–202
24. Lescot M, Déhais P, Thijs G, Marchal K, Moreau Y, et al. 2002. PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for *in silico* analysis of promoter sequences. *Nucleic Acids Research* 30:325–27
25. Hall T. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41:95–98
26. Goodstein DM, Shu S, Howson R, Neupane R, Hayes RD, et al. 2012. Phytozome: a comparative platform for green plant genomics. *Nucleic Acids Research* 40:D1178–D1186
27. Tong Z, Gao Z, Wang F, Zhou J, Zhang Z. 2009. Selection of reliable reference genes for gene expression studies in peach using real-time PCR. *BMC Molecular Biology* 10:71
28. Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 25:402–8
29. Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S, et al. 2012. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC Bioinformatics* 13:134
30. Chae HS, Kieber JJ. 2005. *Eto Brute?* Role of ACS turnover in regulating ethylene biosynthesis *Trends in Plant Science* 10:291–96
31. Yoshida H, Wang KLC, Chang CM, Mori K, Uchida E, et al. 2006. The ACC synthase TOE sequence is required for interaction with ETO1 family proteins and destabilization of target proteins. *Plant Molecular Biology* 62:427–37
32. Wei C, Zhang R, Yang X, Zhu C, Li H, et al. 2019. Comparative analysis of calcium-dependent protein kinase in Cucurbitaceae and expression studies in watermelon. *International Journal of Molecular Sciences* 20:2527
33. Lü P, Yu S, Zhu N, Chen Y, Zhou B, et al. 2018. Genome encode analyses reveal the basis of convergent evolution of fleshy fruit ripening. *Nature Plants* 4:784–91
34. Guo Z, Zhang Y, Yao J, Xie Z, Zhang Y, et al. 2021. The NAM/ATAF1/2/CUC2 transcription factor PpNAC. A59 enhances *PpERF. A16* expression to promote ethylene biosynthesis during peach fruit ripening. *Horticulture Research* 8:209
35. Li T, Tan D, Liu Z, Jiang Z, Wei Y, et al. 2015. Apple *MdACS6* regulates ethylene biosynthesis during fruit development involving ethylene-responsive factor. *Plant and Cell Physiology* 56:1909–17
36. Tonutti P, Casson P, Ramina A. 1991. Ethylene biosynthesis during peach fruit development. *Journal of the American Society for Horticultural Science* 116:274–79
37. Bonghi C, Trainotti L, Botton A, Tadiello A, Rasori A, et al. 2011. A microarray approach to identify genes involved in seed-pericarp cross-talk and development in peach. *BMC Plant Biology* 11:107



Copyright: © 2024 by the author(s). Published by Maximum Academic Press, Fayetteville, GA. This article is an open access article distributed under Creative Commons Attribution License (CC BY 4.0), visit <https://creativecommons.org/licenses/by/4.0/>.