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Characterization and expression profiles of *MaACS* and *MaACO* genes from mulberry (*Morus alba* L.)*

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Abstract: 1-Aminocyclopropane-1-carboxylic acid synthase (ACS) and 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) are encoded by multigene families and are involved in fruit ripening by catalyzing the production of ethylene throughout the development of fruit. However, there are no reports on *ACS* or *ACO* genes in mulberry, partly because of the limited molecular research background. In this study, we have obtained five *ACS* gene sequences and two *ACO* gene sequences from Morus Genome Database. Sequence alignment and phylogenetic analysis of *MaACO1* and *MaACO2* showed that their amino acids are conserved compared with ACO proteins from other species. *MaACS1* and *MaACS2* are type I, *MaACS3* and *MaACS4* are type II, and *MaACS5* is type III, with different C-terminal sequences. Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) expression analysis showed that the transcripts of *MaACS* genes were strongly expressed in fruit, and more weakly in other tissues. The expression of *MaACO1* and *MaACO2* showed different patterns in various mulberry tissues. *MaACS* and *MaACO* genes demonstrated two patterns throughout the development of mulberry fruit, and both of them were strongly up-regulated by abscisic acid (ABA) and ethephon.

Key words: Mulberry fruit, *MaACS*, *MaACO*, Gene expression, Abscisic acid, Ethephon
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1 Introduction

The fruit is the reproductive organ for higher plants with a complex process of development and senescence, which are regulated by plant hormones (Gillaspy *et al*., 1993; Ozga and Reinecke, 2003). Among these hormones, ethylene, the simplest olefin, is involved in many aspects of the plant life cycle, including seed germination, seedling development, leaf expansion, blossoming, senescence, fruit ripening, and responses to biotic and abiotic stresses (Wang *et al*., 2002; Lin *et al*., 2009). Ethylene is a necessary factor for the initiation of fruit ripening and senescence and promotes the transcription and translation of responsive genes involved in cell-wall metabolism, membrane metabolism, and fruit softening by activating ethylene signaling transduction (Wang *et al*., 2002; Alonso and Stepanova, 2004).

The biosynthesis of ethylene begins with the production of *S*-adenosylmethionine (SAM), which is catalyzed by SAM synthetase (EC 2.5.1.6) from methionine. SAM is converted to 1-aminocyclopropane-1-carboxylic acid (ACC) and 5-methylthioadenosine (MTA) by ACC synthase (ACS; EC 4.4.1.14). ACC is subsequently oxidized by ACC oxidase (ACO; EC 1.14.17.4) to yield ethylene and CO_2 (Yang and Hoffmann, 1984; Kende, 1993). ACS and ACO are the key enzymes producing ethylene, are encoded by

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a multigene family, and have been isolated from numerous plant species. *ACS* and *ACO* genes show different expression profiles in plant tissues, and respond to different stimuli, including low oxygen, mechanical damage, high temperature, and hormones (Arteca and Arteca, 1999; Rieu *et al*., 2005; Choudhury *et al*., 2008).

Ethylene is one of the most important factors regulating softening, senescence, and abscission of fruit, and the concentration of ethylene increases gradually during the development of climacteric fruit. ACS and ACO act as accelerators in fruit ripening by catalyzing the production of ethylene at the late fruit ripening stage, leading to irreversible senescence (Chen *et al*., 2003; Tatsuki *et al*., 2006; Cara and Giovannoni, 2008; Varanasi *et al*., 2011). It has been shown that once the expression levels of *ACS* and *ACO* genes were suppressed or mutated, the production of ethylene was down-regulated and the shelf life of fruit was also prolonged. Oeller *et al*. (1991) transformed the antisense messenger RNA (mRNA) of the *ACS* gene in tomato and inhibited fruit ripening. Ayub *et al*. (1996) generated transgenic melons by introducing an antisense *ACO* gene, and ethylene production of transgenic fruit was down-regulated compared with untransformed fruit. The silencing of *LeACS2* by vacuum-infiltration and the tobacco rattle virus (TRV)-based virus-induced gene silencing (VIGS) method also leads to a significant delay in the post-harvest ripening and senescence of tomato fruit (Xie *et al*., 2006). Moreover, null mutations of the *MdACS1* and *MdACS3* genes have shown no or very low expression levels of ripening-related genes and maintained firmness in apple fruit (*Malus domestica*) (Sunako *et al*., 1999; Wang *et al*., 2009).

Mulberry (*Morus alba* L.) is a deciduous tree and an economically important food crop for the domesticated silkworm (*Bombyx mori* L.). Mulberry has multiple uses in ecology, pharmaceuticals, and traditional Chinese medicine (He *et al*., 2013). Its bark is also used in paper production. In addition, mulberry fruit is one of the most popular fruits worldwide and is especially appreciated for its unique flavor in China. However, the commercialization of mulberry fruit is limited by its short maturity stages and shelf-life. The effective strategy to make use of mulberry fruit is to strengthen deep processing or to utilize ethylene inhibitors, such as silver thiosulfate, 1-methylcyclopropene (1-MCP), aminoethoxyvinylglycine, and 2,5-norbornadiene, which delay ripening and senescence (Blankenship and Dole, 2003). Furthermore, a potential tool to delay the ripening of mulberry fruit and to prolong its shelf-life is the use of transgenic approaches to down-regulate the expression of ethylene biosynthesis-related genes.

The ethylene biosynthesis pathway has been detected in some plant species, like *Arabidopsis thaliana*, tomato (*Lycopersicon esculentum*), and apple. However, there is little knowledge on ethylene biosynthesis in mulberry. In this study, we report the isolation and characterization of five *ACS* genes and two *ACO* genes from mulberry, and demonstrate their expression levels in different organs and fruit with different development stages following treatment with abscisic acid (ABA) and ethephon. We hope this work will provide insights into the developmental functions of these genes and lay a foundation for further understanding the mechanism of mulberry fruit development and ripening.

2 Materials and methods

2.1 Plant materials and treatments

Different tissues (e.g., root, stem, stem epidermis, petiole, leaf, and fruit) were collected from the mulberry cultivar "Changjiang 1", which is grown in the mulberry garden of Southwest University, Chongqing, China. The mulberry fruit were sampled after full-bloom stages of 10, 20, 29, 36, and 40 d. All plant materials were frozen in liquid nitrogen and stored at −80 °C.

The immature fruit on 20 d after full-bloom (DAF) with a partial red color were collected from trees treated with 264 mg/L ABA (containing 0.1% Tween-20) and 100 mg/L ethephon (containing 0.1% Tween-20) for 5 min at 25 °C; the control fruit were dipped in double-distilled water (ddH_2O) (Ren and Leng, 2010). After dipping, the fruit were kept at 25 °C for 5 d and were immediately frozen in liquid nitrogen and stored at −80 °C.

2.2 Isolation of RNA and synthesis of the first strands of complementary DNA (cDNA)

Total RNA was extracted from root, stem, stem epidermis, petiole, and leaf using the RNA Extraction

Kit (TaKaRa, Japan) as described in the manufacturer's instructions. Total RNA of fruit was extracted using the RNA Extraction Kit Transzol Plant (TransGen Biotech, China). To remove genomic DNA, the RNA samples were digested by DNase I (TaKaRa, Japan). The cDNA was synthesized from 3 μg of DNA-free RNA with the reverse transcriptional Moloney murine leukaemia virus (M-MLV; Promega) following the manufacturer's protocol. Ten-fold diluted cDNA was used in quantitative reverse transcriptase polymerase chain reaction (qRT-PCR).

2.3 Identification and isolation of *ACS* and *ACO*

ACS and *ACO* genes from other plant species were downloaded from GenBank (http://www.ncbi.nlm.nih.gov/genbank/), and were used as queries in BLAST searches against Morus Genome Database (http://morus.swu.edu.cn/morusdb/). This yielded non-redundant full mRNA sequences of *ACS* and *ACO* genes. The candidate genes were identified using BLASTN and SMART (http://smart.embl-heidelberg.de/). Five *ACS* genes and two *ACO* genes were finally selected for further research.

2.4 Sequence alignment and phylogenetic analysis

The similarities of *ACS* and *ACO* genes were analyzed by BLASTN. About 64 ACS and 46 ACO amino acid sequences were downloaded from the National Center of Biotechnology Information (NCBI) and used for alignment with putative protein sequences of mulberry *ACS* and *ACO* genes given by MUSCLE 3.6, and a phylogenetic tree was constructed by MEGA 5.0 software.

2.5 qRT-PCR expression analysis of mulberry *ACS* and *ACO* genes

The primers used for qRT-PCR were designed by the online tool of the GeneScript Company of China (http://www.genscript.com.cn/index.html) (Table 1). For the analyses of *ACS* and *ACO* gene expression, 20 μl total reaction volume composed of 10 μl of 2× SYBR® Premix Ex Taq II (TaKaRa, Japan), 0.8 μl of each primer (10 μmol/L), 0.4 μl of 50× ROX Reference Dye II (TaKaRa, Japan), 2 μl of cDNA, and 6 μl of ddH_2O. The thermal parameters for qRT-PCR were: 95 °C for 30 s, followed by 40 cycles of 94 °C for 5 s and 60 °C for 30 s, with a final extension at 72 °C for 10 min. The *MaACTIN3* (HQ163775.1) gene was used as an internal control. Relative expression levels were calculated using the fruit after full-bloom stage of 10 d set at 1.

Table 1 Primers for qRT-PCR

Gene	Primer (5′ to 3′)
MaACS1	Forward: GCAACACTGACCTCATCCAC
	Reverse: TGCGAGGATACTAATCCGAA
MaACS2	Forward: GTCAACGAGGTCAAGCTCAA
	Reverse: GTTTACGGTCTCCTCCTCCA
MaACS3	Forward: TGGCTTCCAAATCACTGAAG
	Reverse: GGTTGTGTTCGTTCGATGTC
MaACS4	Forward: CTCAAACGGCTTCAGAATCA
	Reverse: AGCTCGTCAGGAGTCAAGGT
MaACS5	Forward: CAGCTTCTGTTGAGCTCTCG
	Reverse: CATCATAAGCTTTCCAGCCA
MaACO1	Forward: AAGGTGATGAGGGAATTTGC
	Reverse: GGTCCCTTTGAGCCATAGAA
MaACO2	Forward: TCTTGGACTGGAGAAAGGGT
	Reverse: CCCTTGATCAGGTCTGGTTT
MaACTIN3	Forward: GCATGAAGATCAAGGTGGTG
	Reverse: CATCTGCTGGAAGGTGCTAA

3 Results

3.1 Identification and isolation of *MaACS* and *MaACO* genes

ACS and *ACO* gene sequences from other plants were used to search the mulberry genome sequences and the obtained mRNA sequences were screened by the BLASTN program; their encoded amino acids were analyzed by the SMART program. Five *ACS* genes and two *ACO* genes were finally screened, which were named as *MaACS1*, *MaACS2*, *MaACS3*, *MaACS4*, *MaACS5*, *MaACO1*, and *MaACO2*. *MaACO1* had previously been submitted to the NCBI (GenBank Accession No. KC709482). *MaACO1* may be associated with tissue aging or senescence and respond to stress in mulberry (Pan and Lou, 2008).

The full-length genomic sequences of *MaACS* genes ranged from 1660 bp (*MaACS5*) to 2732 bp (*MaACS2*) and the open reading frame (ORF) length ranged from 1341 bp (*MaACS5*) to 1491 bp (*MaACS2*), encoding polypeptides ranging from 446 to 496 amino acids with predicted molecular masses ranging from 50.16 to 55.57 kDa. The full-length genomic sequences of *MaACO* genes were 1666 bp (*MaACO1*) and 1526 bp (*MaACO2*) and the ORF lengths were 960 and 969 bp, encoding polypeptides of 319 and 322 amino acids with predicted molecular masses of 36.38 and 36.48 kDa, respectively (Table 2).

Table 2 Gene analyses of *MaACS* and *MaACO*

Gene	Genomic full-length (bp)	ORF length (bp)	Amino acids	MW (kDa)	pI	Gene ID
MaACS1	2196	1461	486	54.68	6.47	Morus024218
MaACS2	2732	1491	496	55.57	6.77	Morus012919
MaACS3	1782	1416	471	53.42	8.82	Morus007775
MaACS4	2044	1404	467	52.50	5.93	Morus007092
MaACS5	1660	1341	446	50.16	5.55	Morus027243
MaACO1	1666	960	319	36.38	5.35	Morus004820
MaACO2	1526	969	322	36.48	5.22	Morus014137

MW: molecular weight of the MaACS and MaACO proteins; pI: isoelectric point of the MaACS and MaACO proteins; ID: IDs of *MaACS* and *MaACO* in the Morus Genome Database

3.2 Structure of *MaACS* and *MaACO* genes

To reveal the intron and exon organizations of *MaACS* and *MaACO* genes, we compared all obtained ORF sequences with the corresponding genomic DNA sequences (Fig. 1). The *MaACS* sequences could be divided into two groups. The first group included *MaACS1*, *MaACS2*, *MaACS3*, and *MaACS4*, which have a 4-exon/3-intron structure, and *MaACS5* has a 3-exon/2-intron structure. Both *MaACO1* and *MaACO2* have a 4-exon/3-intron structure.

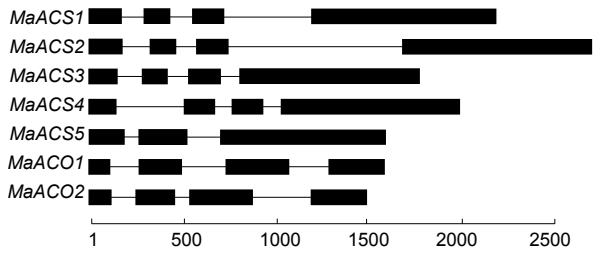


Fig. 1 Schematic diagrams of *MaACS* and *MaACO* genes with exons (black boxes) and introns (lines between exons)

3.3 Multiple-sequence alignments and phylogenetic analysis of *MaACS* genes

Sequence similarity matrix analysis of *MaACS* amino acids has revealed that the sequence identity ranged from 50% to 69% (Table 3). The sequence identities were higher between *MaACS1* and *MaACS2* (69%) and between *MaACS3* and *MaACS4* (68%).

The amino acids encoded by *MaACS* genes were aligned by the Clustal W program and have the pyridoxal phosphate (PLP)-dependent enzyme domain, which is a co-factor associated with ACS catalyzing the conversion of *S*-adenosyl-L-methionine (AdoMet) to ACC, predicted by the SMART program. It was shown that ACS proteins could be divided into three

Table 3 Sequence similarity matrix of *MaACS*

Gene	Sequence identity (%)				
	MaACS1	*MaACS2*	*MaACS3*	*MaACS4*	*MaACS5*
MaACS1	100				
MaACS2	69	100			
MaACS3	53	56	100		
MaACS4	50	54	68	100	
MaACS5	57	60	60	58	100

main types differing in the C-terminal sequences. Type I proteins have an extended C-terminus containing three conserved serine residues that are targets for phosphorylation by mitogen-activated protein kinase 6 (MPK6) and a conserved serine residue that is a phosphorylation site for calcium-dependent protein kinase (CDPK) (Tatsuki and Mori, 2001; Chae *et al*., 2003; Liu and Zhang, 2004; Sebastià *et al*., 2004). Type II proteins have a shorter C-terminus that has only the CDPK phosphorylation site. Type III proteins have a very short C-terminal extension that lacks both phosphorylation sites. The result of multiple-sequence alignment against MaACS proteins showed that MaACS1 and MaACS2 were type I, MaACS3 and MaACS4 were type II, and MaACS5 was type III (Fig. 2).

The amino acids of MaACS were aligned by the Muscle 3.6 program and a phylogenetic tree was constructed by MEGA 5.0. The resulting tree showed that MaACS1 and MaACS2, MaACS3 and MaACS4, and MaACS5 were three branches, respectively (Fig. 3a). A phylogenetic tree was also constructed for ACS from mulberry with other plants via the neighbor-joining method (Fig. 3b). The tree was divided into three groups: I, II, and III; MaACS1 and MaACS2 were group I, MaACS3 and MaACS4 were group II, and MaACS5 was group III. This result is consistent with the classification based on the difference in the C-terminal sequences of MaACS.

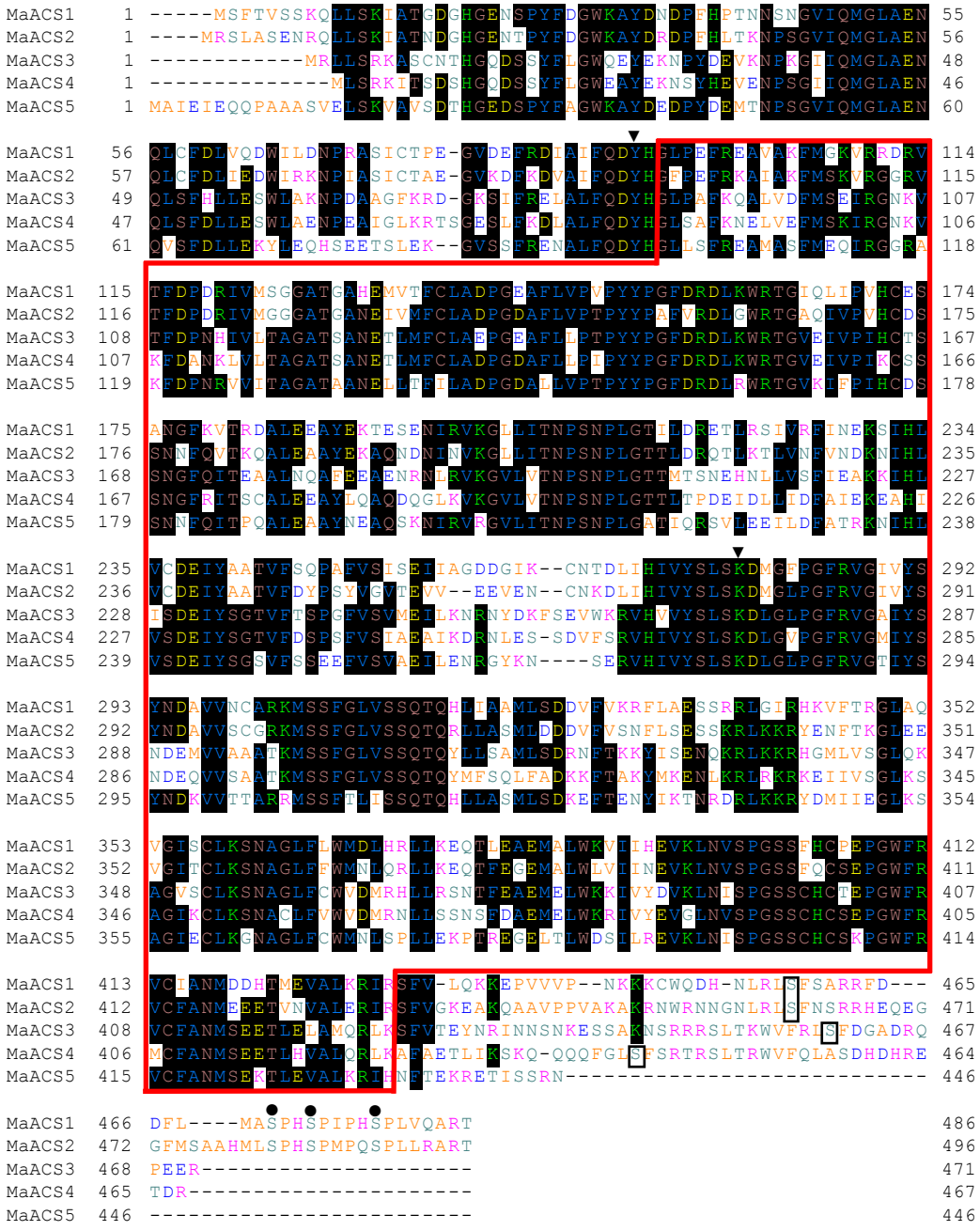


Fig. 2 Amino acid alignment of the inferred *MaACS* gene products

The sequence in the red box indicates the PLP-dependent enzyme domain. Black boxes and black circles (●) indicate CDPK and MPK6 active sites, respectively. ▼ indicates the active sites associated with heterodimeric interactions (Note: for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article)

ACS proteins can act as homodynamic or heterodimeric proteins to form active heterodimers enhancing the isozyme diversity of the *ACS* gene family and providing physiological multifunctionality by operating in different SAM concentrations in various cells and tissues during plant growth and development (Tsuchisaka and Theologis, 2004). In this study, Y92 and K278 residues of MaACS proteins are predicted as the active sites associated with heterodimeric interactions (Fig. 2).

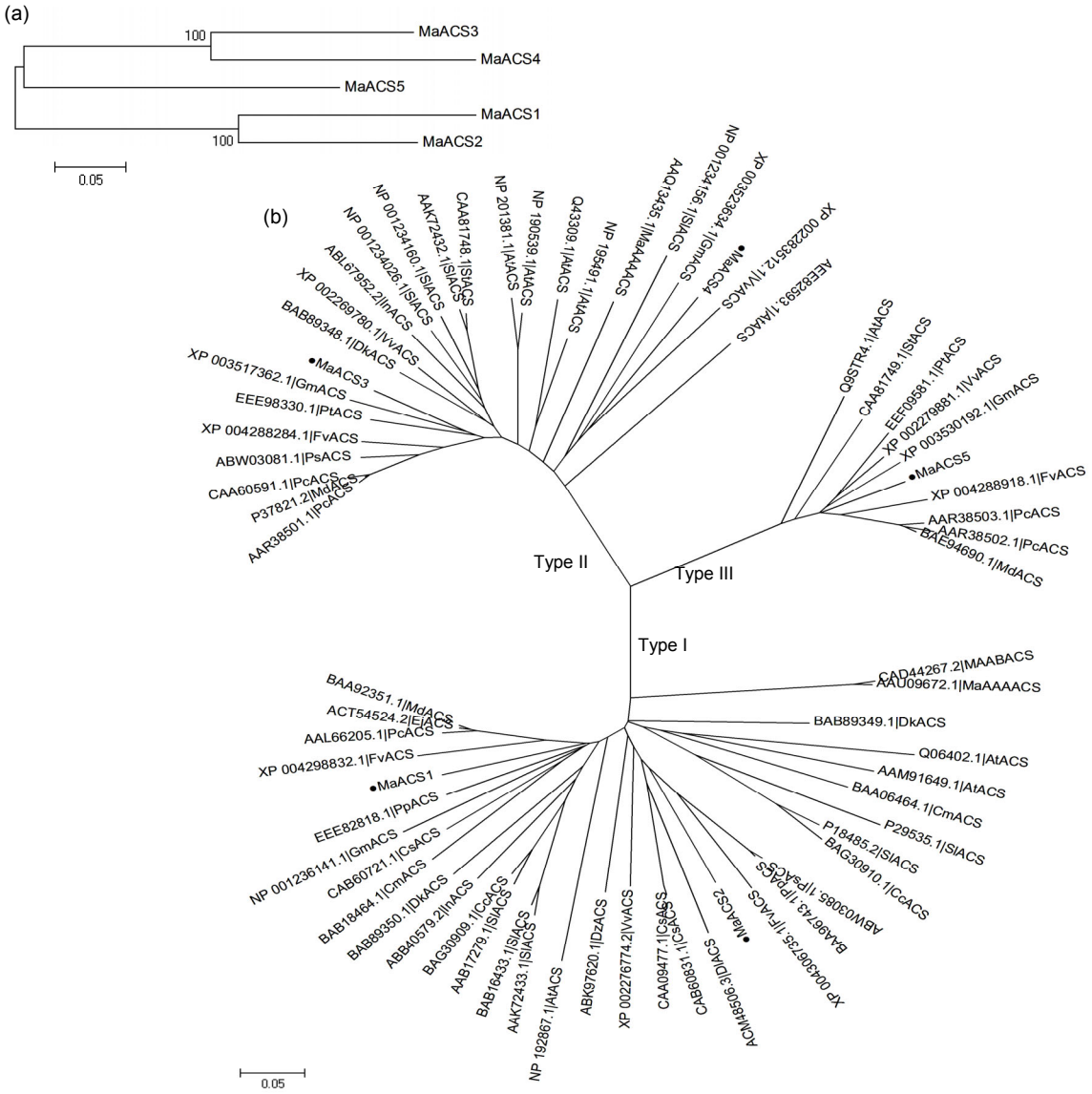


Fig. 3 Phylogenetic tree analysis of MaACS proteins

(a) Phylogenetic relationships between MaACSs; (b) Phylogenetic relationships between MaACS proteins and other ACS proteins from other plant species

3.4 Multiple-sequence alignments and phylogenetic analysis of *MaACO* genes

ACO is a member of a superfamily of non-haem iron-dependent oxygenases, most of which utilize Fe(II) as a co-factor and 2-oxoglutarate (2OG) as a co-substrate (John, 1991; Bidonde *et al*., 1998). The domain of *MaACO* encoding protein sequences was analyzed by the SMART program and NCBI. The results revealed that the sequences of amino acids ranging from 156 to 256 have the 2OG-Fe(II) oxygenase domain (Fig. 4), which indicates that ACO is a member of the ketoglutaric acid dioxygenase family. Crystal structure studies of the *Petunia hybrida* ACO showed that it forms a complex with Fe(II) and Cu(II) and contains a single Fe(II) atom binding site coordinated by three residues (His177, Asp179, and His234), and Arg175 and Arg244 are proposed to be involved in binding bicarbonate; this leads to the activation of the ACO enzyme (Zhang *et al*., 2004). Here, as reported previously, H177, D179, and H234 in MaACO1 and MaACO2 proteins were predicted and identified as Fe(II) binding sites, and R175 and R244 were predicted as 2OG binding sites (Fig. 4).

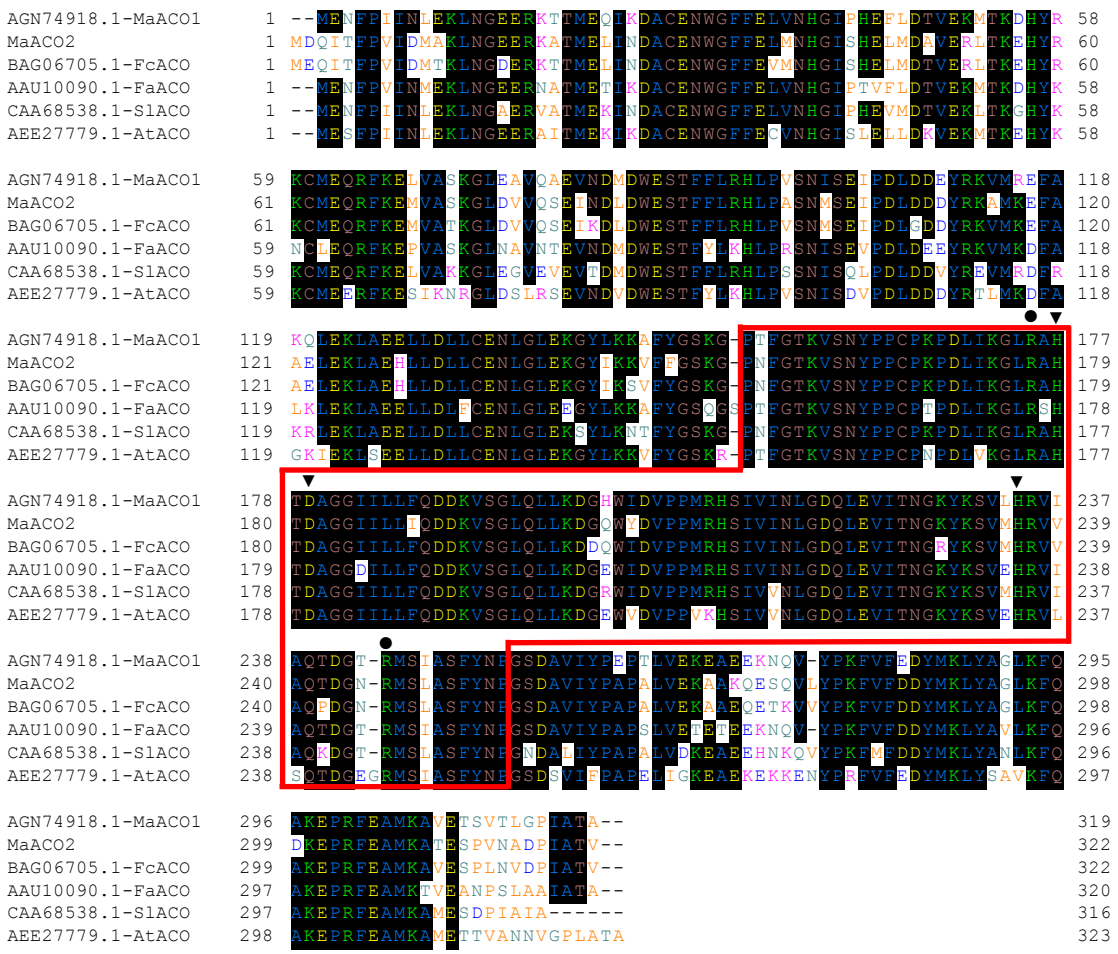


Fig. 4 MaACO protein sequence alignments with other ACO proteins

The sequence in the box indicates the 2OG-Fe(II) oxygenase enzyme domain. ▼ indicates Fe(II) binding sites and ● indicates the 2OG binding sites

Multiple-sequence alignment results showed that ACO amino acids are relatively conserved with high similarities in higher plants. The results of phylogenetic analysis of MaACO suggested that VvACO (XP_002278331.1), AtACO (AAK68810.1), and ZmACO (NP_001151658.1) could be clustered into the same clades, and MaACO1 and MaACO2 could be clustered into a branch with another 43 ACO proteins from NCBI (Fig. 5).

3.5 Tissue specificity in qRT-PCR expression analyses of *MaACS* and *MaACO* genes

Tissue-specific expression analysis revealed that *MaACS* and *MaACO* genes were differentially expressed in various mulberry tissues (Fig. 6). Within the *MaACS* gene family, *MaACS1* and *MaACS4* showed similar expression patterns, with transcripts strongly expressed in fruit and more weakly in roots, stem, stem epidermis, petioles, and leaves. *MaACS2* and *MaACS5* showed somewhat similar expression patterns, and had higher expression in roots, old leaves, and fruit, and lesser levels in others, although *MaACS5* transcripts were also low in roots. *MaACS3* transcripts were more abundant in petiole and fruit tissues, and the expression level was higher in petioles than in fruit.

The expression of *MaACO1* and *MaACO2* showed different patterns. The transcript of *MaACO1* had higher levels in roots and old leaves, and lower levels in other tissues. *MaACO2* had higher levels in stem epidermis, petioles, and immature and old leaves, and lower levels in other tissues.

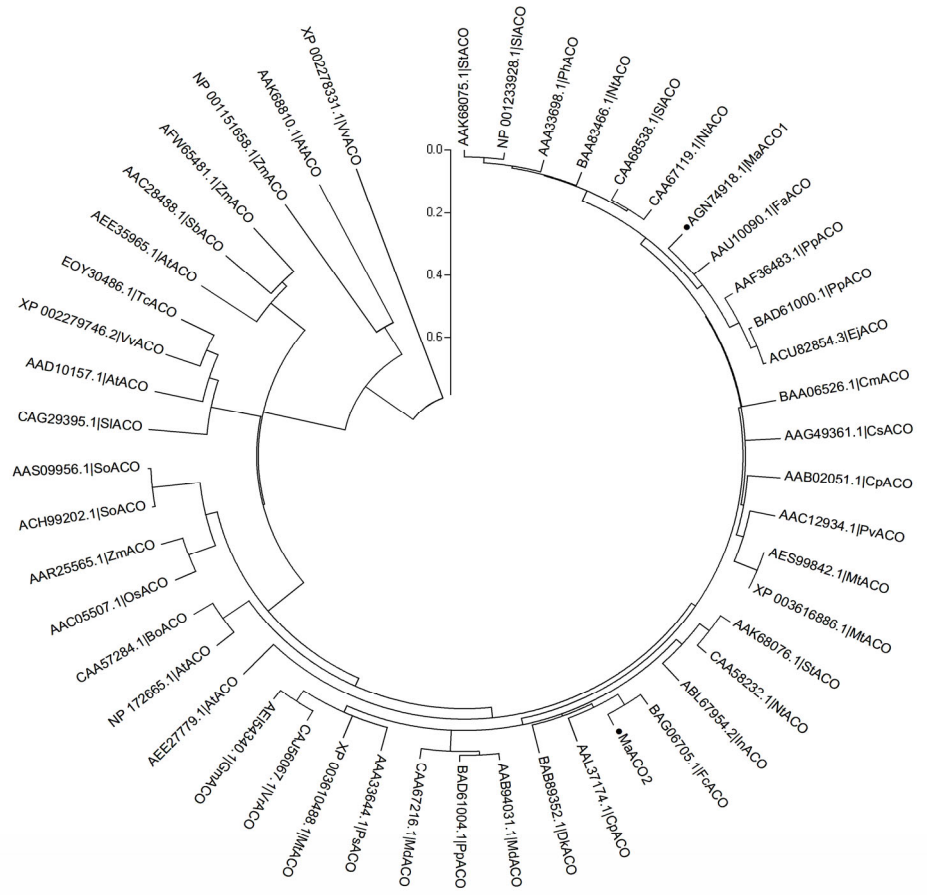


Fig. 5 Phylogenetic relationships between MaACO proteins and other ACO proteins from other species

3.6 qRT-PCR expression analysis of *MaACS* and *MaACO* genes in fruit

The expression of the different *MaACS* and *MaACO* genes showed two patterns during the development of mulberry fruit (Fig. 7). *MaACS1*, *MaACS2*, *MaACS4*, *MaACS5*, and *MaACO1* had strong peaks of expression in early stages of fruit, and decreased throughout development. However, transcript abundance of *MaACS3* and *MaACO2*, unlike other genes, increased rapidly throughout fruit development.

The mulberry fruit on 20 DAF were treated with ABA and ethephon, and fruit turned the full red color (data not shown). Transcript abundance of *MaACS* and *MaACO* genes were up-regulated by ABA and ethephon (Fig. 8). This indicated that ABA and ethephon could regulate the transcription of ethylene biosynthesis-related genes.

4 Discussion

ACS and ACO are the key enzymes producing ethylene, and are typically encoded by multiple *ACS* and *ACO* genes, and some members of these gene families are differentially regulated to respond to different cues in various tissues throughout plant development. In this study, we have isolated and analyzed five *ACS* genes and two *ACO* genes from mulberry genome database. Sequence alignments and phylogenetic analyses of *MaACO1* and *MaACO2* showed that sequence identity is 82% and their amino acids are conserved compared with ACO proteins from other species. Multiple-sequence alignments and phylogenetic analyses of *MaACS* genes show that *ACS* genes have the PLP-dependent enzyme domain and could be divided into three types (I, II, and III) in higher plants based on the difference in the C-terminal sequences. Like *Arabidopsis thaliana*,

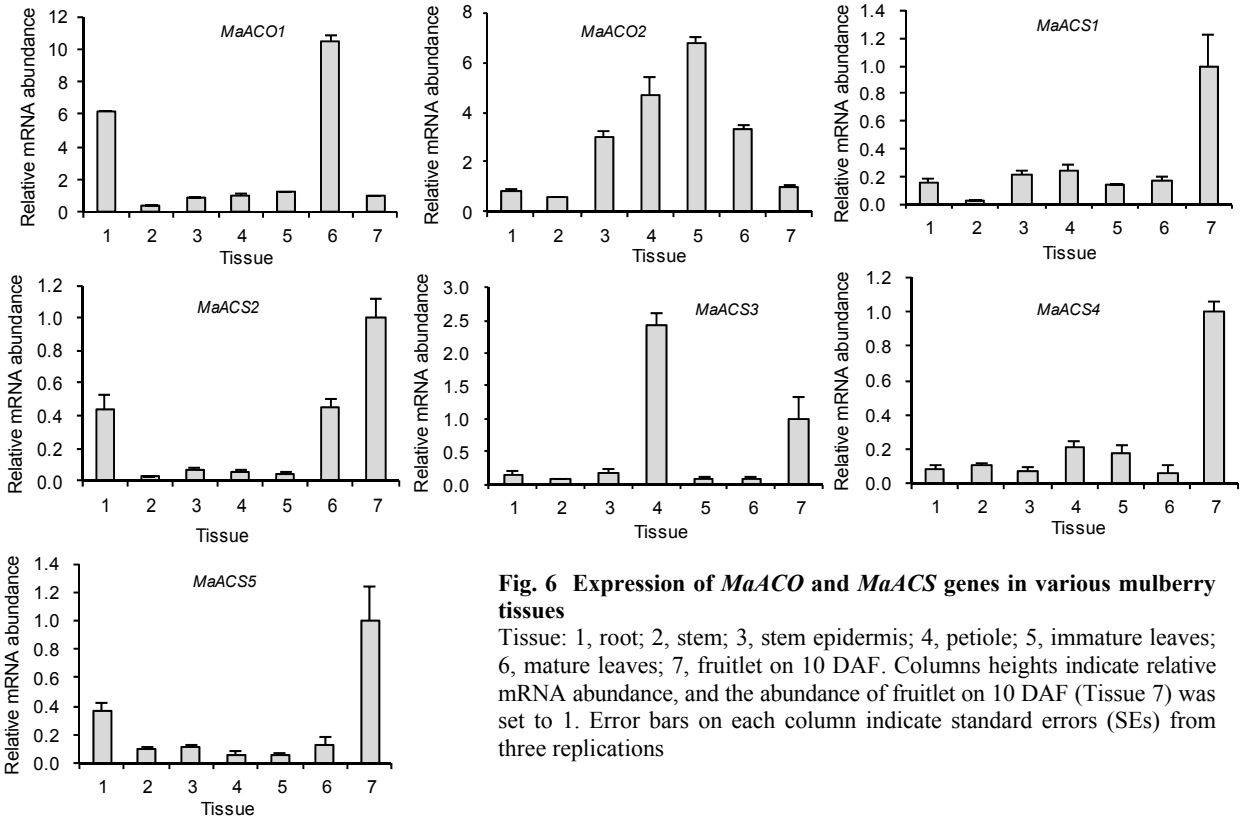


Fig. 6 Expression of *MaACO* and *MaACS* genes in various mulberry tissues

Tissue: 1, root; 2, stem; 3, stem epidermis; 4, petiole; 5, immature leaves; 6, mature leaves; 7, fruitlet on 10 DAF. Columns heights indicate relative mRNA abundance, and the abundance of fruitlet on 10 DAF (Tissue 7) was set to 1. Error bars on each column indicate standard errors (SEs) from three replications

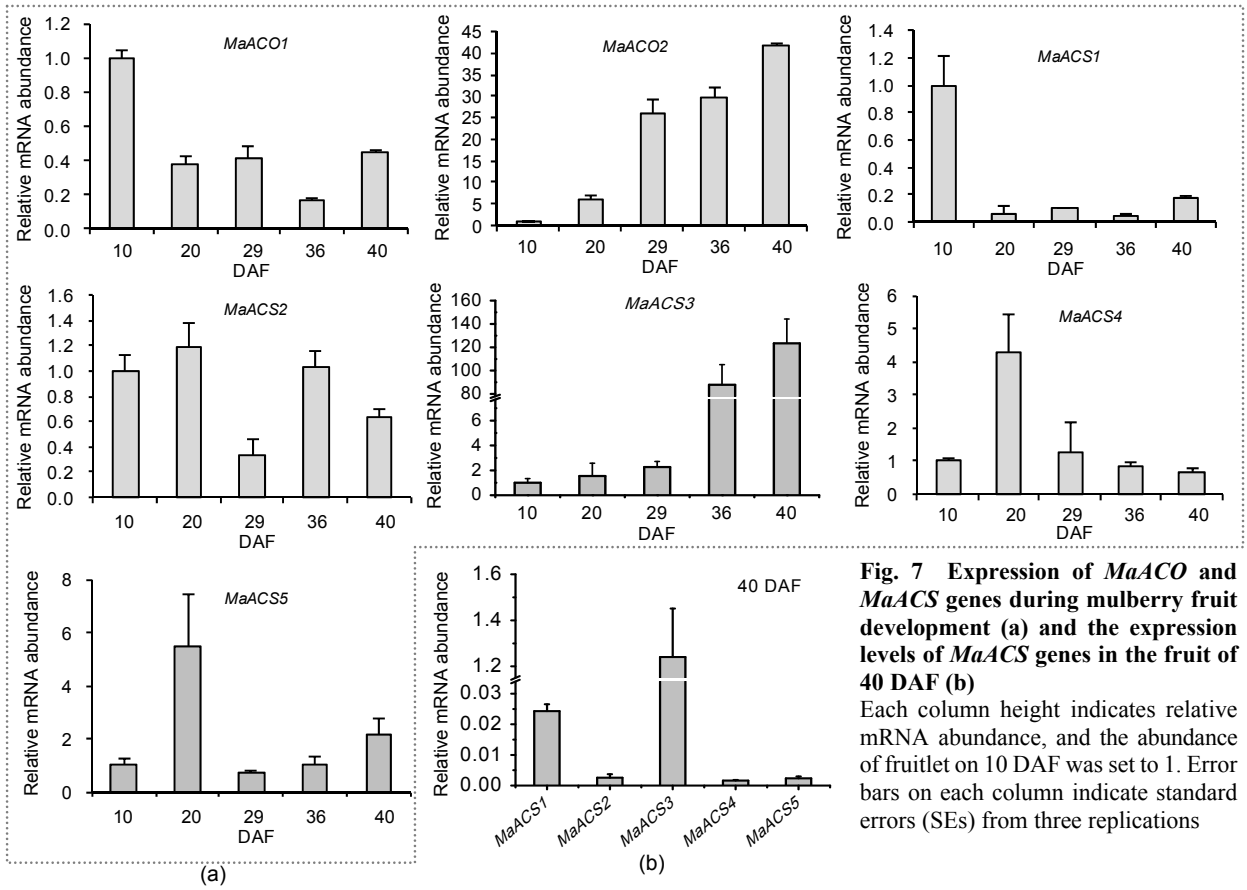


Fig. 7 Expression of *MaACO* and *MaACS* genes during mulberry fruit development (a) and the expression levels of *MaACS* genes in the fruit of 40 DAF (b)

Each column height indicates relative mRNA abundance, and the abundance of fruitlet on 10 DAF was set to 1. Error bars on each column indicate standard errors (SEs) from three replications

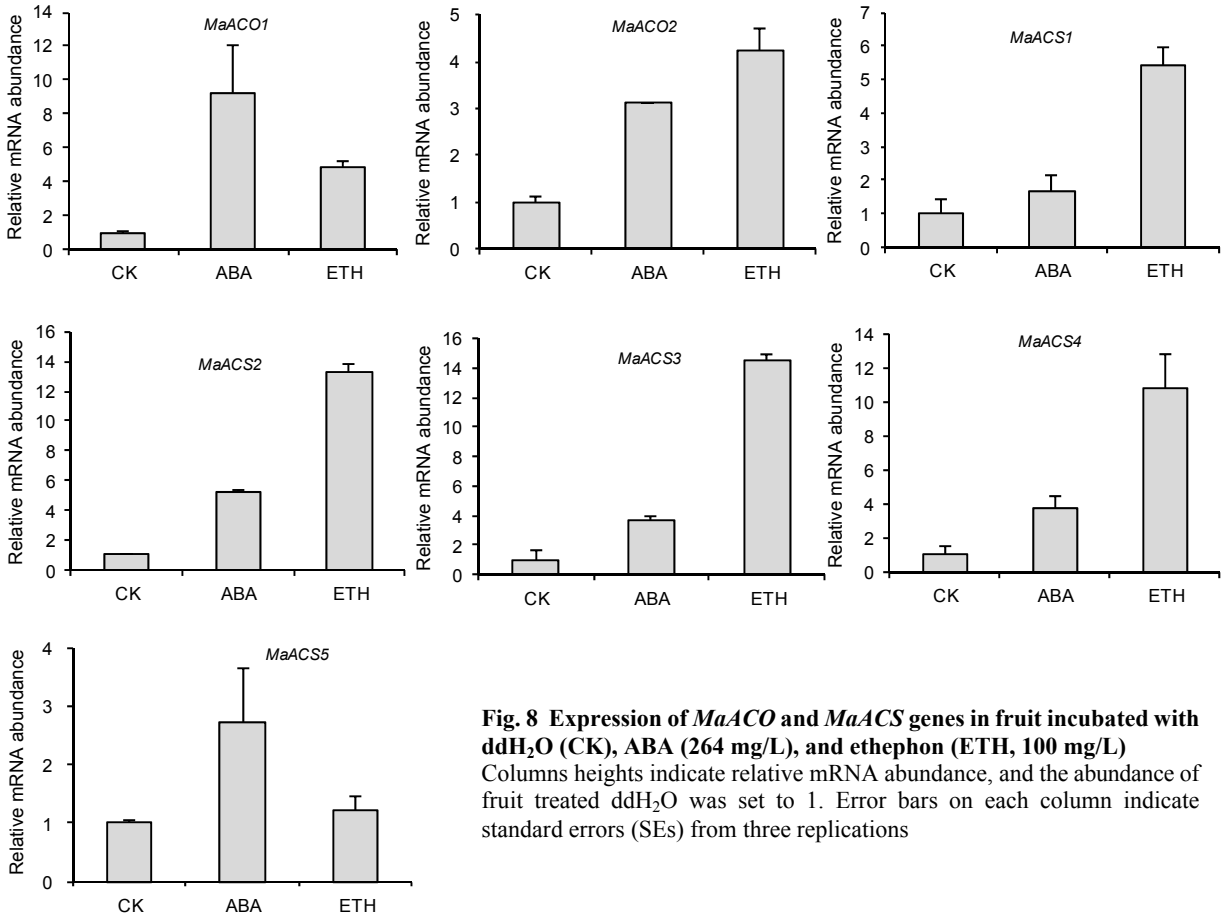


Fig. 8 Expression of *MaACO* and *MaACS* genes in fruit incubated with ddH_2O (CK), ABA (264 mg/L), and ethephon (ETH, 100 mg/L)
Columns heights indicate relative mRNA abundance, and the abundance of fruit treated ddH_2O was set to 1. Error bars on each column indicate standard errors (SEs) from three replications

tomato, apple, and grape (*Vitis vinifera*), *ACS* is classified into three types in mulberry: *MaACS1* and *MaACS2* are type I, *MaACS3* and *MaACS4* are type II, and *MaACS5* is type III.

Fruit can be classified into two groups, climacteric and non-climacteric fruit, based on pattern of respiration and ethylene release during maturation and ripening. Further, the ethylene biosynthesis in fruit is controlled by two systems (McMurchie *et al*., 1972): system I is responsible for ethylene production during normal vegetative growth in all tissues including climacteric and non-climacteric fruit; system II only exists in climacteric fruit, and is essential for the burst of ethylene production during the ripening of fruit (Seymour *et al*., 1993). The transition from system I to system II is considered to be an important step in fruit ripening (Lelièvre *et al*., 1997; Cara and Giovannoni, 2008). In climacteric fruit, system I-like *ACS* and *ACO* genes showed lower expression levels in the later stages of fruit, but transcript abundance of system II-like genes increased rapidly throughout fruit development. The expression of the different *MaACS* and *MaACO* genes showed two patterns during the development of mulberry fruit. *MaACS1*, *MaACS2*, *MaACS4*, *MaACS5*, and *MaACO1* had strong peaks of expression in early stages of fruit, and decreased throughout development. However, transcript abundance of *MaACS3* and *MaACO2*, unlike other *MaACS* and *MaACO* genes, increased rapidly throughout fruit development. However, these data could not support the view that *MaACS3* and *MaACO2* are responsible for system II ethylene biosynthesis in ripening fruits and that other genes are responsible for system I, because all *MaACS* and *MaACO* genes were stimulated by ethephon as shown in Fig. 8; more studies are needed to support *MaACS* and *MaACO* gene classification.

Within the *MaACS* genes, the expression pattern of *MaACS3* was different from those of other *MaACS* genes, and its transcript abundance was increased gradually during fruit development, especially increased by 38.8-fold from 29 to 36 DAF of fruit. In

addition, the expression level of *MaACS3* was higher than those of the other four *MaACS* genes in the fruit on 40 DAF. Therefore, the high accumulation of *MaACS3* is closely associated with the production of ethylene in later stages of mulberry fruit. It is possible that mulberry fruit is climacteric on the basis of the results from Luo (2003a; 2003b), and mulberry fruit certainly decays readily. It is probable that using *MaACS3* as the target gene to suppress its expression in the later stages of mulberry fruit development will prolong shelf-life.

Tissue-specific expression analysis revealed that the *MaACS* and *MaACO* genes were differentially expressed in various mulberry tissues. Within the *MaACS* gene family, the transcript abundance in the fruit on 10 DAF was much higher than that in other tissues. However, the expression of *MaACO* genes showed different patterns, the transcriptional levels of *MaACO1* and *MaACO2* in fruit being equal to or lower than those in other tissues. It is indicated that *MaACS* genes, unlike *MaACO* genes, tend to be expressed in fruit compared with other tissues. Promoters are the key factor driving time and space expression of the correspondent gene, and fruit-specific promoters can control gene expression in fruit but not in other tissues. There are some promoters, like tomato E8/E4 promoter, peach (*Prunus persica*) *ACO1* promoter, and apple *ACO1* promoter, being expressed specifically in fruit (Deikman *et al*., 1992; Blume and Grierson, 1997; Moon and Callahan, 2004), and the target genes fused to these promoters could overexpress specifically in fruit. There are no reports on fruit-specific promoters using the promoter of *ACS* genes. Based on the expression analysis of *MaACS* genes in different tissues and fruit at different stages, *MaACS* genes are potential targets to develop fruit-specific promoters. Considering that *MaACS1*, *MaACS2*, *MaACS4*, and *MaACS5* have lower expression levels in the later stages of fruit (Fig. 7b), it is probable that we could use *MaACS3* promoter as a candidate to research the fruit-specific promoter in mulberry.

ABA and ethylene are considered as the key factors regulating fruit maturity and senescence. Some research has confirmed that ABA plays an important role in triggering ethylene biosynthesis and softening during the ripening of fruit (Zhang *et al*., 2009; Zaharah *et al*., 2013). ABA can stimulate the production of ethylene by promoting the activities of ethylene biosynthesis enzymes ACS and ACO, and regulating the expression of its encoded genes, thus starting the ripening process. ABA, like ethephon, can rapidly up-regulate the expression levels of *MaACS* and *MaACO* genes. This indicates that ABA can promote the biosynthesis of ethylene in mulberry fruit by enhancing the expression of *ACS* and *ACO* genes. Sun *et al*. (2010) thought that the productions of ABA and ethylene at the onset of berry ripening and after harvest are affected by each other. Thus, the relationship between these two hormones and how they affect fruit ripening need further studies.

5 Conclusions

We are the first to report *ACS* and *ACO* genes from mulberry and demonstrate their expression profiles. Tissue-specific expression analysis revealed that *MaACS* and *MaACO* genes showed different patterns in various mulberry tissues and two patterns throughout the development of mulberry fruit. *MaACS* genes also tended to be expressed in fruit rather than in other tissues. Further, the transcription levels of *MaACS* and *MaACO* genes could be up-regulated by ABA and ethephon. In future practice, we may be able to use *MaACS3* as the target gene to develop a fruit-specific promoter and to prolong the shelf-life of mulberry fruit by down-regulating its expression.

Compliance with ethics guidelines

Chang-ying LIU, Rui-hua LÜ, Jun LI, Ai-chun ZHAO, Xi-ling WANG, Umuhoza DIANE, Xiao-hong WANG, Chuan-hong WANG, Ya-sheng YU, Shu-mei HAN, Cheng LU, and Mao-de YU declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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中文概要：

本文题目： 桑树 *MaACS* 和 *MaACO* 基因的鉴定和表达模式研究

Characterization and expression profiles of *MaACS* and *MaACO* genes from mulberry (*Morus alba* L.)

研究目的： 分离和鉴定桑树中参与乙烯生物合成的酶的编码基因 *MaACS* 和 *MaACO*，研究其表达模式。

创新要点： 基于最新公布的桑树基因组数据库数据，获得 5 个 *MaACS* 基因和 2 个 *MaACO* 基因，对其进行了生物信息分析，同时鉴定了其在不同桑树组织中、不同发育时期桑椹中和不同激素作用下的表达模式。

研究方法： 通过生物信息学方法筛选和鉴定基因，利用荧光定量逆转录聚合酶链式反应（qRT-PCR）分析基因的表达量。

重要结论： *MaACS* 和 *MaACO* 基因在根、茎、叶等不同组织中呈现出不同的表达模式，在桑椹发育过程中呈现出两种表达模式，其表达量被脱落酸和乙烯利上调。

关键词组： 桑椹；*MaACS*；*MaACO*；脱落酸；乙烯利