Expression Patterns of *ACS* and *ACO* Gene Families and Ethylene Production in Rachis and Berry of Grapes

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Abstract. Ethylene is important during the berry development and in the last stages of rachis development or rachis senescence. Since grapes develop in a cluster that comprises both the fruit berry and the nonfruit rachis, we measured the release of ethylene from both tissues. Detached berries from *Vitis vinifera* ‘Ruby Seedless’ and ‘Thompson Seedless’ showed that ethylene release peaks at the beginning of berry development and at veraison. Ethylene production in the rachis was higher than that in the berry and had an obvious peak before harvest in ‘Thompson Seedless’. In both cultivars, ethephon treatment induced ethylene production in the rachis but not in the berry. Expression of 1-aminocyclopropane-1-carboxylate (ACC) synthase (*ACS*) and ACC oxidase (*ACO*) genes showed diverse temporal and spatial patterns in ‘Thompson Seedless’ and ‘Ruby Seedless’. For most gene family members, the low *ACS* expression levels were observed in berry and rachis. Expression levels of most of the *ACS* and *ACO* genes did not correlate with ethylene released in the same organ. The transcriptional level of *VvACS1* did correlate with ethylene evolution in rachis of ‘Thompson Seedless’ during berry development and storage, which suggested that *VvACS1* may have important roles in rachis senescence. In berries of ‘Thompson Seedless’ and ‘Ruby Seedless’, the transcriptional levels of *VvACO1*, *VvACS2*, and *VvACS6* coincided with ethylene production, indicating possible roles in berry development. Expression of *VvACS2–VvACO9* and *VvACO1–VvACO3* was not consistent with ethylene production during storage or in response to ethephon treatment, which suggests that the expression of *ACS* and *ACO* was affected by other stress factors after harvest.

Grapes (*V. vinifera* L.) are widely cultivated around the world and can be consumed as fresh fruit (table grape) or processed into wine, juice, molasses, or raisins (Mencarelli et al., 2005). Grape berry has a thin skin and a soft, juicy flesh with high sugar content. The berry connects to the main rachis via a short pedicel with a small brush. Table grapes are stored and sold in bunches, and dry, browning rachis and berry drop are unfavorable for storage, transportation, and sale (Balic et al., 2012; Lichter, 2016).

Ethylene plays an important role in pre-harvest ripening in both climacteric and non-climacteric fruit and is involved either directly or indirectly in the regulation of grape development and organ senescence (Chervin et al., 2008; Pech et al., 2008). Previous studies reported that rachis browning were induced

by exogenous abscisic acid (ABA) and ethephon (Hedberg and Goodwin, 1980; Li et al., 2015), and were restrained by ethylene inhibitors, such as 1-methylcyclopropene (1-MCP) (Li et al., 2015), or other plant hormones, such as cytokinin (Balic et al., 2012) and gibberellin (Raban et al., 2013). Although many studies have linked ethylene to grape berry ripening, there is little research on the role of ethylene in rachis development or senescence before and after harvest. Increased understanding of the synthesis and regulation of ethylene production during development and senescence of grape rachis and berry would lay a foundation for prolonging shelf and storage life.

The key enzymes of ethylene biosynthesis in higher plants are *ACS* and *ACO* (Yang and Hoffman, 1984), both of which are encoded by multigene families. For example, eight *ACS* genes and four *ACO* genes have been identified in tomato (Rottmann et al., 1991; Yip et al., 1992). Expression of *ACS* and *ACO* genes are differentially regulated both temporally and spatially, resulting in specific expression patterns. For instance, *EjACS1* and *EjACO1* are specifically expressed in loquat fruit (Jiang et al., 2011), and *LeACO1* is specifically expressed in tomato fruit (Barry et al., 1996). In grapes, there are at least 10 *VvACS* and 3 *VvACO* genes (Munoz-Robredo

et al., 2013; Xu and Wang, 2012). The highest expression of *VvACO1* gene was concomitant with an ethylene peak around veraison (Chervin et al., 2008; Munoz-Robredo et al., 2013; Pech et al., 2008). Moreover, there is little published data about the temporal and spatial expression of *ACS* and *ACO* genes in grapes. To understand how *ACS* and *ACO* genes control ethylene synthesis during development and senescence of both the rachis and the berry, the expression levels of nine *ACS* genes and three *ACO* genes and the production of ethylene were measured in berry and rachis of two seedless table grape cultivars Thompson Seedless and Ruby Seedless.

Material and Methods

Plant materials. Grapevines (*V. vinifera*) of two cultivars, namely Thompson Seedless and Ruby Seedless, were grown in the National Vitis Repository of Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences. ‘Ruby Seedless’ is not susceptible to berry drop, and ‘Thompson Seedless’ berry is more susceptible to berry drop (Cooper et al., 1993). Grape berry and rachis of ‘Thompson Seedless’ were sampled from adult vines at 21, 35, 49, 57, 64, and 71 d after full bloom (DAFB), with the final sample collection at harvest [71 DAFB, 21.3% total soluble solids (TSS) and 0.61% tartaric acid]. Grape berry and rachis of *V. vinifera* ‘Ruby Seedless’ were sampled on 35, 49, 57, 64, 81, 91, and 102 DAFB (harvest, 16.9% TSS and 0.68% tartaric acid) (Supplemental Fig. 1; Supplemental Table 1). Samples (the third to eighth node) of leaf, petiole, and tendril, young stem (the first to third node), and shoot tip were collected from the two cultivars when the berry was harvested. All samples were frozen in liquid nitrogen and stored at –80 °C until analysis. For each sample, berries and rachis were collected from three or five clusters with the same maturity. The experiment was conducted in the 2014 growing season. For ethylene analysis and physiological characterization, fresh berry and rachis were taken from ‘Thompson Seedless’ and ‘Ruby Seedless’ on the above date. Fresh samples were transported to the laboratory immediately.

Ethephon treatment. To investigate the expression patterns of *ACO* and *ACS* genes in response to exogenous ethephon, grape clusters of ‘Thompson Seedless’ and ‘Ruby Seedless’ from 12 grapevines were sprayed with 400 mg·L⁻¹ ethephon (Sigma, China) or distilled water on the vine before harvest. Then the grape clusters were harvested and kept in a chamber at 15 °C and 85% to 90% relative humidity. Three days after ethephon treatments berries and rachis were collected every 1 or 2 d during storage. Nine clusters from 12 plants were selected for each treatment, and ethylene production of berry and rachis from nine clusters was measured three times.

Measurement of maturity parameters. To define the stage of berry development, we randomly selected 20 berries on each

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sampling date, measured the level of TSS with a digital refractometer (PAL-1; ATAGO, Japan). Titratable acidity (TA), diameter, and weight parameters were recorded during berry developing stages of ‘Thompson Seedless’. To guarantee berry and rachis with the similar maturity degree in the individual cultivar, different clusters with the similar TSS were collected together in each sampling date (Munoz-Robredo et al., 2013). Three replicates were conducted for each sampling.

GC analysis of ethylene production. Ethylene analysis for each sample was performed using gas chromatography (GC; GC-2010 PLUS, Shimadzu, Japan) with a flame ionization detector as described (Li et al., 2015). At each berry development stage, 200–400 g berries and 5–20 g rachis were sampled from clusters and then sealed in either a 65-mL or a 250-mL bottle or box at 20 °C. Three hours later, 1 mL of the gas from the sample vial was injected into a GC Packed Column (GDX-502, Shimadzu, Japan) for analysis. The temperatures of the injector port, column, and detector were set at 75, 80, and 60 °C, respectively. For each sample, berries and rachis were collected from three or five clusters with the similar maturity. Each experiment was repeated at least three times.

Quantitative real-time RT-PCR analysis and sequencing. Rachis and berries from nine clusters were homogenized in groups of three clusters and RNA extraction was performed on 100–200 mg samples from each replicate using a modified CTAB method (Gambino et al., 2008). RNA quality was

evaluated in a NanoDrop 2000c spectrophotometer (Thermo Scientific, Waltham, MA), selecting RNA samples with an $A_{260/280}$ ratio between 1.8 and 2.0 and an $A_{260/230}$ ratio of more than 2.0 for subsequent analysis. The integrity of RNA samples was assessed by electrophoresis on a 1.5% agarose gel. The primer sequences for *ACS* and *ACO* genes were designed in Primer Express version 3.0 (Applied Biosystems, Madrid, CA), and primer efficiency for each primer pair was calculated (Table 1).

First-stand cDNA synthesis from 1 µg of total RNA was conducted with the FastQuant RT Kit (with gDNase I; Tiangen Biotech, Beijing, China). Quantitative real-time reverse transcription polymerase chain reaction (RT-PCR) was performed using the SYBR® Select Master Mix (Applied Biosystems) in an ABI PRISM 7500 Fast Sequence Detection System (Applied Biosystems). Reactions contained 1 µL primer mix (0.5 µM forward and reverse primers), 2 µL template cDNA, 10 µL SYBR® Select Master Mix, and 7 µL RNase-free water to a total volume of 20 µL. Reactions were conducted using the following thermal cycling conditions: 50 °C for 2 min (1 cycle), 95 °C for 2 min (1 cycle), 95 °C for 3 s, and 60 °C for 30 s (40 cycles). The PCR products for each primer pair were sequenced (Shanghai Shengong Company, Shanghai, China) and searched against the NCBI database using BLASTN to ensure data quality and reliability (data not shown). Each experiment was repeated three times with three biological replicates.

Data analysis and statistical analysis. Efficiency of each primer pair was calculated using standard curve method (Larionov et al., 2005). *Actin*, *GAPDH*, and *EF-1α* genes were used as internal controls (Reid et al., 2006), from which the most stably expressed gene was used as a reference gene, according to geNorm analysis (Vandesompele et al., 2002). For each sample, relative expression was calculated by LinRegPCR method (Ruijter et al., 2009). For gene expression in berry development, the value of the sample at harvest was set as “1” for *ACS* and *ACO*. To compare expression in various organs, the value of the shoot tip was set as “1” for *ACS* and *ACO* gene expression.

All statistics were analyzed using SPSS 17.0 with three replications of each experiment. The Duncan’s multiple range test was applied at $P = 0.05$ level to evaluate the significant differences among data.

Results

Ethylene production in berry and rachis during berry development and in storage. To understand ethylene production patterns in ‘Thompson Seedless’ and ‘Ruby Seedless’ fruit, ethylene release rates of rachis and berry were recorded during berry development and after harvest in storage (Fig. 1). There was a general upward trend in ethylene production in the rachis of ‘Thompson Seedless’ during berry development, and a sharp rise of ethylene production was observed at harvest, 71 DAFB (Fig. 1A). In rachis of ‘Ruby Seedless’, two weak ethylene peaks

Table 1. Primer description of *ACS* and *ACO* genes.

Name of genes	Oligonucleotides (5'-3')	GenBank number	Efficiency of primer pairs (%)
<i>VvACO1-F</i>	CGCCCAAACCTGATGGAAACAGAATG	GSVIVT01015220001	111
<i>VvACO1-R</i>	GGGTACACTTCGCTTGTCTCCTTT		
<i>VvACO2-F</i>	GTAGCCCAAACAGACGGCAACA	GSVIVT00025952001	118
<i>VvACO2-R</i>	CGGGTACACTTGATCTTTCTCTGCT		
<i>VvACO3-F</i>	GGCAGACAAGAATGGAAGCAGACT	GSVIVT00016583001	120
<i>VvACO3-R</i>	TGCGAACTTTGTTGTGGCATAAAGC		
<i>VvACS1-F</i>	CGTGCGAGGGTTGAAACAATTAGGA	GSVIVT01019920001	127
<i>VvACS1-R</i>	CCCTTTCTCACTGTAAGAGCGGATT		
<i>VvACS2-F</i>	GACTCCTCAAGGAGCCGACAGT	GSVIVT00019414001	124
<i>VvACS2-R</i>	CGGATCCTTCGTAGGGCCACTT		
<i>VvACS3-F</i>	CTTGAAACGTGAGCCAGGATGC	GSVIVT00004794001	99
<i>VvACS3-R</i>	CTCGACCAGCCGACATGAATGC		
<i>VvACS4-F</i>	AGACGAACCGAGAAAGACTGAGGAA	GSVIVT00015239001	123
<i>VvACS4-R</i>	CAGCTTCACTTCATGCAGGATGGA		
<i>VvACS5-F</i>	CCACCTCTGAAAGAGCCAACAC	GSVIVT00026962001	128
<i>VvACS5-R</i>	GGCCTTTGCCAACAAATGTTCTGAT		
<i>VvACS6-F</i>	GCACCAAGAGCAGTGCTGGTT	GSVIVT00018494001	125
<i>VvACS6-R</i>	GCTGACCCAGGAGTTACATTGATCT		
<i>VvACS7-F</i>	GAGGATTCTACGTGAAGCTGGGTTG	GSVIVT00009284001	125
<i>VvACS7-R</i>	CACCTGCTGGATTCTCTGCCAAG		
<i>VvACS8-F</i>	TCTCTACGACGTCCGCTTAAACATC	GSVIVT00002708001	115
<i>VvACS8-R</i>	GTGGCGGCTTTGGTTGTTGTTG		
<i>VvACS9-F</i>	ACACATTCTGAAGCCGAGATGGAAC	GSVIVT00004571001	111
<i>VvACS9-R</i>	TTGTTGTTGGTGGTGGTTGAGGAG		
<i>EF1-α-F</i>	GAACCTGGGTGCTTGATAGGC	XM_002284888.2	109
<i>EF1-α-R</i>	AACCAAAATATCCGGAGTAAAAAGA		
<i>ACTIN-F</i>	CTTGATCCCTCAGCACCTT	FQ383380.1	108
<i>ACTIN-R</i>	TCCTGTGGACAATGGATGGA		
<i>GAPDH-F</i>	TTCTCGTTGAGGGCTATTCCA	XM_002263109	110
<i>GAPDH-R</i>	CCACAGACTTCATCGGTGACA		

There are only three nucleotides acid sequence difference at 3' end of open reading frame between *ACS10* and *ACS8* genes based on grapevine database sequence information (<http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/>) (Xu and Wand, 2012), so the relative expression of *ACS8* was a mix gene in our study.

Fig. 1. Ethylene production rate from berry and rachis during berry development and in storage. Ethylene production rates in (A) rachis of *Vitis vinifera* 'Thompson Seedless' during berry development, (B) rachis of *V. vinifera* 'Thompson Seedless' in storage, (C) rachis of *V. vinifera* 'Ruby Seedless' during berry development, (D) rachis of *V. vinifera* 'Ruby Seedless' in storage, (E) berry of 'Thompson Seedless' during berry development, (F) berry of 'Thompson Seedless' in storage, (G) berry of 'Ruby Seedless' during berry development, and (H) berry of 'Ruby Seedless' in storage. CK, untreated control; ETH, samples that were treated with ethephon at harvest; H, harvest date. The veraison stage of 'Thompson Seedless' was at 49 d after full bloom (DAFB), and 'Ruby Seedless' was at 81 DAFB. Different letters indicated significant differences between transgenic lines and the wild type at $P < 0.05$.

were observed at 49 and 81 DAFB before harvest (Fig. 1C). In 'Thompson Seedless' and 'Ruby Seedless' berry, a general downward trend in ethylene production was displayed as the berry developed (Fig. 1E and G), with the highest ethylene production at 35 DAFB. In general, ethylene production in the rachis ($1\text{--}4.5\ \mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) was higher than

that of the berry ($0.02\text{--}1\ \mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) in both 'Ruby Seedless' and 'Thompson Seedless' (Fig. 1). Comparing the two grapevine cultivars, ethylene production in berry and rachis of 'Thompson Seedless' was generally higher than that of 'Ruby Seedless'. For example, ethylene release rate from berry of 'Thompson Seedless' and 'Ruby Seedless' at

35 DAFB was 1.0 and $0.4\ \mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$, respectively, and release rate from rachis at 71 DAFB was 4.3 and $2.2\ \mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$, respectively (Fig. 1).

Little changes in ethylene production were observed in berry of either of the two cultivars during storage (Fig. 1F and H). At harvest, the berries produced very little

ethylene, and that continued through the 10–15 d of storage. Ethylene production in the rachis of ‘Thompson Seedless’ continued to decrease in storage (Fig. 1B). On the other hand, ethylene production in the rachis of ‘Ruby Seedless’ first increased from 0.75 to 1.5 $\mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ over 7 d, and then began to decrease at 7 d after harvest (DAH) (Fig. 1D).

In response to ethephon treatment, there was little change in ethylene production in berries of either ‘Thompson Seedless’ or ‘Ruby Seedless’ (Fig. 1F and H), whereas ethylene production of the rachis in both cultivars was increased (Fig. 1B and D).

Expression of ACS and ACO genes in various tissues and organs. Expression levels of nine ACS gene family members were analyzed in various tissues and organs at harvest. The results indicated that grapevine ACS (*VvACS*) genes were differentially expressed in ‘Thompson Seedless’ and ‘Ruby Seedless’ (Fig. 2).

In ‘Thompson Seedless’, expression of *VvACS1* was detected at the highest level in grape rachis, at a level 44 times higher than that in shoot tip or leaves. The highest expression of *VvACS2* was observed in young stem, and lowest in berry and rachis. Transcriptional levels of *VvACS3*, *VvACS7*, *VvACS8*, and *VvACS9* were higher in leaf

and lower or undetectable in grape berry and rachis. Expression of *VvACS4–VvACS6* was highest in the shoot tip and lowest in rachis (Fig. 2A).

In ‘Ruby Seedless’, *VvACS4* and *VvACS7–VvACS9* expression levels were high in young stem, but low in berries and rachises (Fig. 2B). Transcript levels of *VvACS5* were higher in shoot tip, young stem, and berry, but quite lower in rachis. The highest expression of *VvACS3* was in rachis at a level 10 times higher than that in berry (Fig. 2B). The highest expression of *VvACS1* and *VvACS6* was observed in leaves (Fig. 2B).

The expression trends of *ACO1* and *ACO3* genes were similar in ‘Thompson Seedless’ and ‘Ruby Seedless’ (Fig. 2C and D). The highest expression level of *VvACO1* was in the tendril, although the relative levels between the cultivars were quite different. The highest expression level of *VvACO3* was in the berry, and the lowest expression level was in the rachis, especially in ‘Thompson Seedless’ (Fig. 2C and D). Transcript level of *VvACO2* was highest in the rachis of ‘Thompson Seedless’, and quite lower in the rachis of ‘Ruby Seedless’ (Fig. 2C and D).

Expression of ACS and ACO genes in berry and rachis during grape berry development. Expression patterns of the

VvACS genes in berry and rachis were analyzed during grape berry development. The expression levels of these genes were quite different between ‘Thompson Seedless’ and ‘Ruby Seedless’ (Fig. 3). In the rachis of ‘Thompson Seedless’, expression levels of *VvACS2*, *VvACS5*, *VvACS6*, *VvACS8*, and *VvACS9* were highest at 57 DAFB, then lower at 21 DAFB, and the lowest appeared at harvest (Fig. 3A and B). The expression peaks of *VvACS1* and *VvACS3* were the most consistent with ethylene production in rachis, with the highest expression level of these two genes at harvest (Fig. 3A).

In the ‘Thompson Seedless’ berry, expression levels of *VvACS1*, *VvACS2*, *VvACS4*, *VvACS6*, and *VvACS8* were highest at 21 DAFB, then lower at 35 DAFB, and remained at low level until ripening, similar to ethylene production (Fig. 3C and D). The highest expression levels of *VvACS5* and *VvACS9* were observed at harvest (Fig. 3B).

In ‘Ruby Seedless’ rachis (Fig. 3E and F), the highest transcript accumulations of *VvACS1* and *VvACS6* were observed at 35 and 49 DAFB, respectively, and the lowest at 81 DAFB. The highest transcriptional levels of *VvACS2* and *VvACS5* were observed at 91 DAFB, with relatively lower levels at other berry developmental stages. The expression

Fig. 2. Expression patterns of ACS and ACO genes from different organs in *Vitis vinifera* ‘Thompson Seedless’ and ‘Ruby Seedless’. Expressions of ACS genes in (A) *V. vinifera* ‘Thompson Seedless’, (B) *V. vinifera* ‘Ruby Seedless’, (C) *V. vinifera* ‘Thompson Seedless’, and (D) *V. vinifera* ‘Ruby Seedless’. The value of shoot tip was set as “1” for ACS and ACO relative gene expression analysis.

Fig. 2. Continued.

of *VvACS3* gradually decreased from 35 to 57 DAFB, and then slightly increased at harvest. Expression of *VvACS4*, *VvACS7*, and *VvACS8* peaked at 49 DAFB. Little change in rachis expression of *VvACS9* was observed through ‘Ruby Seedless’ berry development.

In ‘Ruby Seedless’ berry (Fig. 3G and H), expression levels of *VvACS2* and *VvACS6* were concurrent with ethylene production, peaking at 35 and 81 DAFB. The highest transcriptional accumulations of *VvACS3* and *VvACS7*, *VvACS8*, and *VvACS9* were at 64 DAFB. The highest expression levels of *VvACS4* and *VvACS5* were detected at harvest, right after its lowest level at 91 DAFB.

Expression patterns of the *VvACO* genes in berry and rachis were also analyzed during grape berry development. In ‘Thompson Seedless’ rachis (Fig. 4A), expression of *VvACO1* was highest at 57 DAFB. The expression of *VvACO2* peaked at harvest, which was concurrent with the ethylene peak. Little change in expression of *VvACO3* was observed during berry development. In ‘Thompson Seedless’ berry (Fig. 4B), expression levels of *VvACO1* and *VvACO2* were the highest at 21 DAFB and then decreased gradually with berry development, and the patterns were consistent with ethylene production in berry. Slight changes of

VvACO3 expression were observed in ‘Thompson Seedless’ berry (Fig. 4B).

In ‘Ruby Seedless’ rachis (Fig. 4C), expression levels of *VvACO1* were steady throughout grape berry development. The expression levels of *VvACO2* and *VvACO3* peaked at 91 and 81 DAFB, respectively. In ‘Ruby Seedless’ berry (Fig. 4D), the highest expression level of *VvACO1* was observed at 35 DAFB, and the lowest at harvest, an expression pattern consistent with ethylene production. Expression of *VvACO2* had an obvious peak at 81 DAFB, and was relatively lower at other stages. Little expression changes of *VvACO3* were observed in the berry of either ‘Thompson Seedless’ (Fig. 4B) or ‘Ruby Seedless’ (Fig. 4D).

Postharvest expression of ACS and ACO genes in berry and rachis treated with exogenous ethephon during development. Grape clusters were treated with ethephon at 3 d before harvest, and there were no obvious changes in postharvest ethylene production from berries in either cultivar, whether they were treated with exogenous ethephon or not during development (Fig. 1C and D). Therefore, expression of the nine ACS and three ACO genes were analyzed only in rachis during storage. The expression of several ACS and ACO genes in rachis was

modulated by ethephon treatment (Figs. 5 and 6). In ‘Thompson Seedless’ rachis (Fig. 5A–C), expression of *VvACS1* generally decreased during storage, and this trend was almost consistent with ethylene production in rachis. The expression levels of *VvACS2*, *VvACS4*, *VvACS6*, and *VvACS7* increased at the 3rd DAH, and then generally decreased during storage. Expression levels of *VvACS5* and *VvACS9* peaked at 6 DAH. In response to ethephon treatment, expression levels of *VvACS1*, *VvACS2*, *VvACS5*, and *VvACS6* were mostly downregulated during storage, except 4 DAH. Expression levels of *VvACS3*, *VvACS7*, *VvACS8*, and *VvACS9* were downregulated during storage, except 6 DAH. Generally, expression of ACS in rachis was downregulated under ethephon treatment (Fig. 5A–C).

In ‘Ruby Seedless’ rachis during storage (Fig. 5D–F), slight expression changes for *VvACS1* and *VvACS6* were observed, except for dips in expression at 5 DAH. Two expression peaks of ACS2 were observed at 3 and 9 DAH. The expression levels of ACS3–ACS5 and ACS7–ACS9 generally decreased during storage. In response to ethephon treatment, the expression levels of the *VvACS* genes were upregulated or downregulated in rachis of ‘Ruby Seedless’ in

Fig. 3. Expression patterns of *ACS* genes in berry and rachis of 'Thompson Seedless' and 'Ruby Seedless' during berry development. Expressions of *ACS* genes in (A, B) rachis of 'Thompson Seedless', (C, D) berry of 'Thompson Seedless', (E, F) rachis of 'Ruby Seedless', and (G, H) berry of 'Ruby Seedless'. The value of sample at harvest was set as "1" for *ACS* relative gene expression analysis. H = harvest date.

ways that did not seem to correlate with ethylene production.

The response of the three *ACO* genes during storage with or without treatment of ethephon at the 3rd d before harvest was also measured. In 'Thompson Seedless' rachis (Fig. 6A), *VvACO1* expression peaked 3 and 6 DAH. *VvACO2* and *VvACO3* expressions generally increased during storage.

Expression of all three *ACO* genes decreased in response to ethephon treatment, except for *VvACO3* at 6 DAH.

In 'Ruby Seedless' rachis (Fig. 6B), expression of *VvACO1* generally decreased and of *VvACO2* gradually increased during storage, and both were downregulated in response to ethephon treatment. Expression *VvACO3* peaked at 7 DAH and was upregulated in

response to ethephon treatment, which was consistent with ethylene production.

Discussion

A previous study on the grape cultivar Thompson Seedless in Chile showed that the veraison stage at 8–9 weeks after full bloom (WAFB) was accompanied by a peak of

Fig. 4. Expression patterns of *ACO* genes in berry and rachis of 'Thompson Seedless' and 'Ruby Seedless' during berry development. Expression of *ACO* genes in (A) rachis of 'Thompson Seedless', (B) berry of 'Thompson Seedless', (C) rachis of 'Ruby Seedless', and (D) berry of 'Ruby Seedless'. The value of sample at harvest was set as "1" for *ACO* relative gene expression analysis. H = harvest date.

ethylene production of berry at veraison (9 WAFB) during observations from 7 to 12 WAFB (Munoz-Robredo et al., 2013). The highest ethylene production during berry development was also observed at veraison in the grape cultivars Muscat Hamburg (Sun et al., 2010) and Cabernet Sauvignon (Chervin et al., 2004). In nonclimacteric fruits such as strawberry (Perkins-Veazie et al., 1996) and citrus (Katz et al., 2005), the pattern is typified by an ethylene production peak in young fruitlets that then decreases dramatically with fruit development. In our study, the similar ethylene production pattern as strawberry and citrus was observed in 'Ruby Seedless' and 'Thompson Seedless' berries, and the highest ethylene production of berries was observed at the beginning of berry development (3 and 5 WAFB), after which ethylene production sharply decreased before veraison. Munoz-Robredo et al. (2013) and Chervin et al. (2004) started to collect berry samples at 7 and 5 WAFB, respectively, and they may missed the earlier ethylene production peak of berry. The reason(s) behind high ethylene production at the beginning of berry development, whether physiological abscission of fruitlets or wounding stress resulted from sampling, and the mechanisms leading to ethylene changes both require further research.

Although leaves and other vegetative tissues are generally considered as nonclimacteric, ethylene production of detached citrus leaves was demonstrated a climacteric system II behavior during senescence or in response to ethephon treatment (Katz et al., 2004; Li et al., 2015; Morgan et al., 1992). A similar phenomenon was observed in the rachis during berry developing of 'Thompson

Seedless' in our study, and there was a climacteric peak in ethylene production of rachis. In response to ethephon treatment, ethylene production in the rachis of both cultivars was induced, whereas ethylene production of the berry was unaffected, which suggested that rachis of grape may be a climacteric organ in ethylene production. After harvest, ethylene production in berry continuously decreased in 'Ruby Seedless', which is not susceptible to berry drop. However, 'Thompson Seedless' berry, which is more susceptible to berry drop (Cooper et al., 1993), showed a higher ethylene production without an obvious decreasing trend. Similar results were obtained in the rachis of the two cultivars, with higher ethylene production in 'Thompson Seedless' rachis compared with rachis of 'Ruby Seedless'. Li et al. found that the average level of rachis ethylene production of 'Thompson Seedless' was significantly lower than that of 'Mystery' and '3003', and rachis browning levels of 'Thompson Seedless' were much lower than those of the other two cultivars (Li et al., 2015). We also tested other four cultivars, and the results indicated that there were significant differences in ethylene production of rachis among these tested grape cultivars (data not shown). All the above results suggested that ethylene either may have a direct or indirect influence on rachis senescence and berry drop, or may have a reciprocal effect with ABA that results in berry senescence after harvest (Sun et al., 2010).

Organ-specific expression of *ACS* and *ACO* genes was observed in our study. For example, in 'Thompson Seedless', *VvACS1* and *VvACO2* in rachis, *VvACO1* in tendril, and

VvACO3 in berry were highly expressed. In 'Ruby Seedless', similar results were observed, with the highest expression of *VvACS1* and *VvACS3* in rachis, *VvACO1* in tendril, and *VvACO3* in berry. In addition, some organ-specific transcription levels highly correlated with ethylene production. For example, the highest expression levels of *VvACS1* and *VvACO2* in rachis were concurrent with a peak of ethylene production at harvest in 'Thompson Seedless', which may suggest that these organ-specific *VvACS1* and *VvACO2* expressions played important roles in rachis senescence. However, some organ-specific expression did not agree with ethylene production in the same organ. For example, *VvACO3* was highly and specifically expressed in berry, but no obvious changes in its expression were observed in 'Thompson Seedless' or 'Ruby Seedless' berries, when ethylene production decreased during berry development. Although the highest transcriptional levels of *VvACO1* were observed in the tendrils of 'Thompson Seedless' and 'Ruby Seedless', the lower expression pattern of *ACO1* did coincide with ethylene production in berry of both cultivars. Similar results also suggest that *VvACO1* highly correlates with ethylene production in berry development (Munoz-Robredo et al., 2013).

It is interesting to note that expression level of *VvACS1* gene was about 40 times higher in rachis than that in other tested organs at 71 DAFB, and there is an obvious positive correlation between *VvACS1* gene expression and ethylene production in rachis of 'Thompson Seedless' ($r = 0.796$) during berry development and in storage, which indicate that *VvACS1* is one of the key genes

Fig. 5. Expression of *VvACS* genes in rachis of *Vitis vinifera* 'Thompson Seedless' and 'Ruby Seedless' during storage and in response to ethephon treatment. Expression of *ACS* genes in rachis of (A–C) 'Thompson Seedless' and (D–F) 'Ruby Seedless'. The value for each tissue type at harvest (Day 0) was set as "1" for *ACS* relative gene expression analysis. CK = untreated control; ETH = samples that were treated with ethephon at harvest.

synthesizing ethylene in the grape rachis and playing important roles in rachis senescence (Rosales et al., 2013). In apple, it was also found that *MdACS1* gene was initiated at fruit ripening of apple when climacteric ethylene started to evolve, and the *MdACS1* expression pattern paralleled the changes of ethylene production of fruit tissue in storage (Tan et al., 2013), which suggested that *ACS1* gene seemed to operate in system II ethylene biosynthesis during berry ripening.

Conclusions

In our study on the grape cultivars Ruby Seedless and Thompson Seedless, ethylene

production peaks of detached berries were observed at the beginning of berry development, and berry ethylene production was unaffected by ethephon treatments. Ethylene production of rachis was higher than that of berry and was greatly induced by exogenous ethephon treatment in both cultivars. Among nine *ACS* gene family members, *VvACS1* was the only gene with the highest expression in rachis and showed expression levels coincident with ethylene production in rachis of 'Thompson Seedless' during berry development and in storage. We propose that this organ-specific *VvACS1* expression played important roles in rachis senescence. In berry, the transcriptional level

of *VvACO1*, *ACS2*, and *ACS6* coincided with ethylene production in berry in both 'Thompson Seedless' and 'Ruby Seedless', which implies that *VvACO1* correlates with ethylene production during berry development.

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Fig. 5. Continued.

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Fig. 6. Expression of *VvACO* genes in rachis of *Vitis vinifera* 'Thompson Seedless' and 'Ruby Seedless' during storage and in response to ethephon treatment. Expression of ACO genes in rachis of (A) 'Thompson Seedless' and (B) 'Ruby Seedless'. The value for each tissue type at harvest (Day 0) was set as "1" for ACO relative gene expression analysis. CK = untreated control; ETH = samples that were treated with ethephon at harvest.

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Supplemental Table 1. Quality trait of *Vitis vinifera* 'Thompson Seedless' during berry developing.

WAFB	TSS (% Brix)	Titrateable acidity (%)	Wt (g)	Diam (mm)
21	4.38 ± 0.69 e	3.75 ± 0.12 a	0.21 ± 0.01 f	5.99 ± 0.11 d
35	5.58 ± 0.11 de	3.42 ± 0.37 b	0.43 ± 0.01 e	7.34 ± 0.41 c
49	9.70 ± 0.44 d	3.25 ± 0.49 b	0.5 ± 0.01 e	8.41 ± 0.44 b
57	15.95 ± 0.64 c	1.3 ± 0.22 c	0.85 ± 0.02 d	11.51 ± 0.10 a
64	17.89 ± 0.21 b	1.29 ± 0.21 c	0.94 ± 0.01 c	11.52 ± 0.07 a
71	21.32 ± 0.24 a	0.61 ± 0.00 d	1.07 ± 0.02 b	11.93 ± 0.13 a
79	21.36 ± 0.23 a	0.60 ± 0.00 d	1.24 ± 0.03 a	11.92 ± 0.07 a

WAFB = week after full bloom.

Supplemental Fig. 1. Total soluble solid contents of grape during berry developing. DAFB = days after full bloom.