

Supplementary data

- **Manuscript Title:** Transient induction of a subset of ethylene biosynthesis genes is potentially involved in regulation of grapevine bud dormancy release
- **Running Title:** Regulation of ethylene biosynthesis in grape buds
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Table S1: Schematic details of all the treatments in Fig 1.

Figure	Experimental system Sealed vases (SV), Open Vases (OV)	Treatment label	Incubation solution (150 ml, replaced every 48h)	Spray	Replicates/treatment	Monitoring time points	Notes
1A	SV	Control	tap water	Triton X-100 (0.02%)	3 groups of 30 cuttings	6, 24, 48	Jars were sealed 2 h before air sampling
		HC		3% HC + Triton X-100 (0.02%)			
		AZ		1% AZ + Triton X-100 (0.02%)			
1B	OV	Control	tap water	Triton X-100 (0.02%)	15 groups of 10 cuttings	10, 14, 18, 21, 24, 28	HC was applied to buds 48 h after incubation in solution (CoCl ₂ or water). After 10 days in CoCl ₂ solutions, cuttings were transferred to tap water.
		HC	tap water	3% HC + Triton X-100 (0.02%)			
		CoCl ₂ -C	3.6 mM CoCl ₂	Triton X-100 (0.02%)			
		CoCl ₂ -HC	3.6 mM CoCl ₂	3% HC + Triton X-100 (0.02%)			
1C	OV	Control	tap water	Triton X-100 (0.02%)	9 groups of 10 cuttings	10, 14, 18, 21, 24	HC was applied to buds 24 h after incubation in solution (STS or water).
		HC	tap water	3% HC + Triton X-100 (0.02%)			
		STS (0.5%)	STS (0.5%)	Triton X-100 (0.02%)			
		STS (0.5%)-HC	STS (0.5%)	3% HC + Triton X-100 (0.02%)			
		STS (2%)	STS (2%)	Triton X-100 (0.02%)			
		STS (2%)-HC	STS (2%)	3% HC + Triton X-100 (0.02%)			
1D	SV	HC	tap water	3% HC + Triton X-100 (0.02%)	3 groups of 10 cutting	10, 14, 18, 21, 24, 28	*Open tubes with 5 ml NBD were placed in jars of relevant treatment. After incubation in sealed Jars for 48 h, cuttings were transferred to open vases.
		HC+NBD		3% HC + Triton X-100 (0.02%) +NBD*			
1E	SV	AZ	tap water	2% AZ + Triton X-100 (0.02%)	9 groups of 7 cuttings	10, 14, 18, 21, 24, 28	
		AZ+NBD		2% AZ + Triton X-100 (0.02%) + NBD*			
1F	SV	Control	tap water	KMnO ₄ * +Triton X-100 (0.02%)	9 groups of 10 cuttings	12, 14, 18, 21	*Perforated tube with vermiculite saturated with 7.43 g/100 ml KMnO ₄ solution was placed in control jars. **Ethylene was injected to jars. After incubation in sealed Jars for 48 h cuttings were transferred to open vases.
		HC		3% HC + Triton X-100 (0.02%)			
		Ethylene		100 ppm ethylene**+ Triton X-100 (0.02%)			
1G	OV	Control	tap water	Triton X-100 (0.02%)	9 groups of 10 cuttings	24	HC and Ethrel were applied to buds 24 h after incubation in water.
		HC		3% HC + Triton X-100 (0.02%)			
		Etherel		0.7% etherel + Triton X-100 (0.02%)			
1H	OV	Control	tap water	Triton X-100 (0.02%)	4 blocks of 3 vines	42	Vines were pruned to three-node spurs on mid January, and the total number of buds was determined. The pruned vines were treated (1L/vine).
		HC		3% HC + Triton X-100 (0.02%)			
		Etherel		0.8% etherel+ Triton X-100 (0.02%)			

Supplementary Table S2

Table S2: Primers used in quantitative real-time PCR analysis

Gene name	Gene accession	Forward primer (5'-3')	Reverse primer (5'-3')
<i>VvACS1</i>	VIT_02s0025g00360 ^a	GCGAATTCAGGGATGTTGCT	GATCAGCCAAGCAGAAGGTG
<i>VvACS2</i>	VIT_02s0025g04980 ^a	TTGGGCTGAAGAGGGTTTCA	ATTTGCAATCCCGCCCATAC
<i>VvACS4</i>	VIT_16s0022g02010 ^a	GTGGAATCGCGACGTATCAG	CCAAGCAGAAGCAGAGTGTC
<i>VvACS6</i>	VIT_15s0046g02220 ^a	GGCTTCCAGGCTTCAGGGTT	CCAGCCTCCTTGAGCTCTCC
<i>VvACS9</i>	VIT_00s1764g00010 ^a	ATGTTCTGGCTGCTGCTAAG	GTTTCAACCCTGCCACGAAT
<i>VvACO1</i>	VIT_00s2086g00010 ^a	AAGGATGGCCAGTGGATTGA	AATGCTGGTGCTGGGTAGAT
<i>VvACO2</i>	VIT_12s0059g01380 ^a	GGTCGATGTTCCCTCCAATGC	CATCCTGTTGCCGTCTGTTT
<i>VvACO4</i>	VIT_11s0016g02380 ^a	TTCCTTTGGCATCGACCAAC	AGCCCTTCTCCAAACCAAGA
<i>VvTST</i>	VIT_04s0023g03600 ^a	TCAGCAACAGAGGTCGTCAC	GCATGGCCTTTCTTGA ACTC
<i>VvActin</i>	EC969944 ^b	CTTGCATCCCTCAGCACCTT	TCCTGTGGACAATGGATGGA
<i>VvGAPDH</i>	EC958777 ^b	TTCTCGTTGAGGGCTATTCCA	CCACAGACTTCATCGGTGACA

^a Accessions from Ensemblplants: <http://plants.ensembl.org/index.html>

^b Accessions from NCBI

Supplementary Table S3

Table S3. Fold change values in Figure 2

Name	HC vs Control				AZ vs Control			
	12 h	24 h	48 h	96 h	12 h	24 h	48 h	96 h
<i>VvACS1</i>	5.9	14.7	1.6	0.5	8.3	9.8	5.6	0.6
<i>VvACS2</i>	0.9	0.4	0.5	0.5	0.6	0.6	0.6	0.6
<i>VvACS6</i>	28.5	381.5	35.8	15.4	14.6	89.8	177.9	2.7
<i>VvACS9</i>	1.2	0.5	0.5	0.6	0.6	0.6	0.8	0.7
<i>VvACS4</i>	0.9	0.9	0.9	1.3	0.6	0.8	1.0	1.9
<i>VvACO1</i>	1.8	1.6	1.4	2.7	1.5	1.3	1.5	1.6
<i>VvACO2</i>	16.4	18.5	5.8	1.8	27.4	37.1	34.9	1.0
<i>VvACO4</i>	5.2	20.8	12.8	8.8	1.3	3.1	6.0	2.4

* Statistically significant values are indicated by bold letters

Supplementary Figure S1

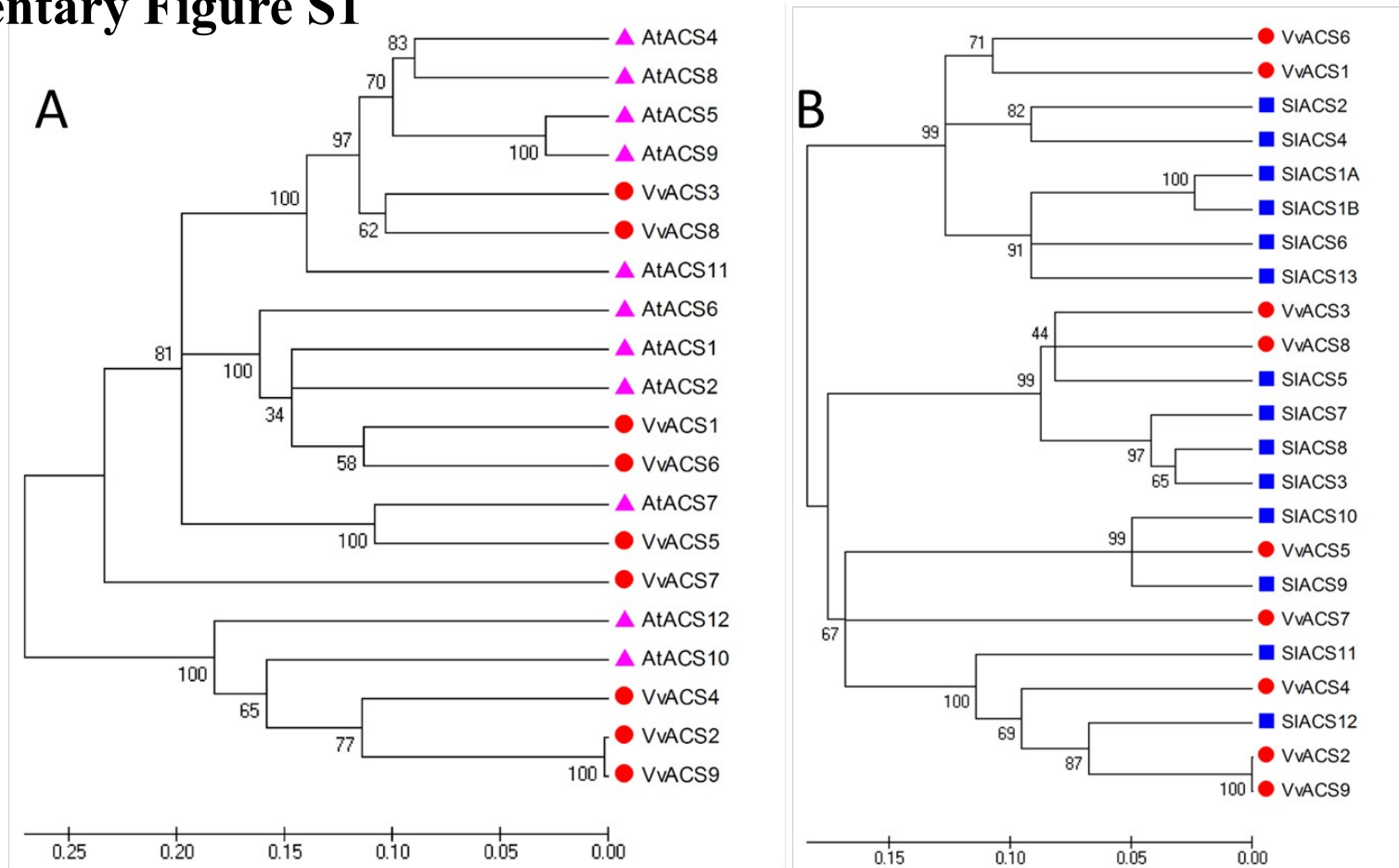
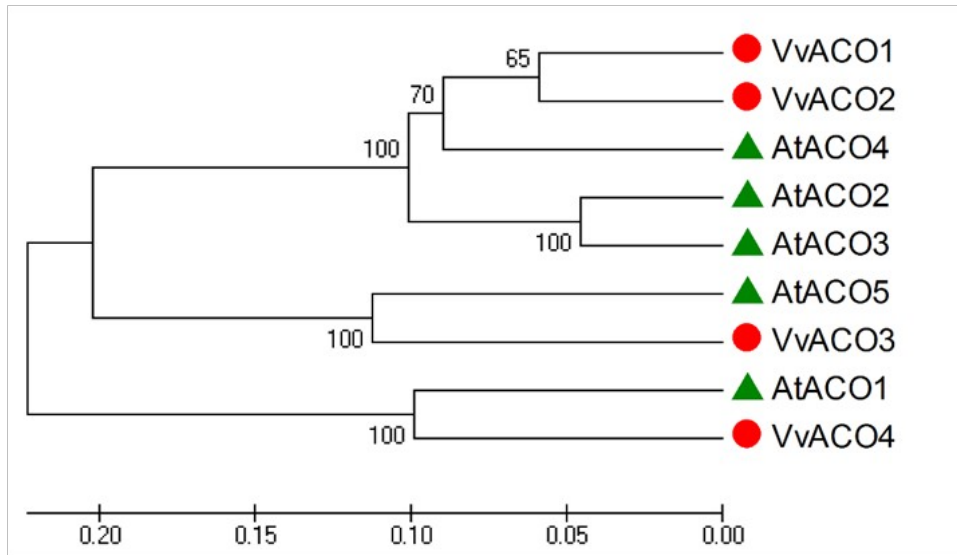


Fig S1: Phylogeny of the ACS homologues from *Arabidopsis*, grape and tomato. The phylogenetic tree was constructed in MEGA5 using the neighbor-joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches. The evolutionary distances were computed using the *p*-distance method and are the number of amino acid substitutions per site. (a) The analysis involved nine *VvACS*s (Red) and eleven *AtACS*s (Vanderstraeten and Van Der Straeten 2017) (Pink) amino acid sequences; (b) The analysis involved nine *VvACS*s (Red) and fourteen *SIACS*s (Liu et al. 2015) (Blue) amino

Supplementary Figure S2

A



B

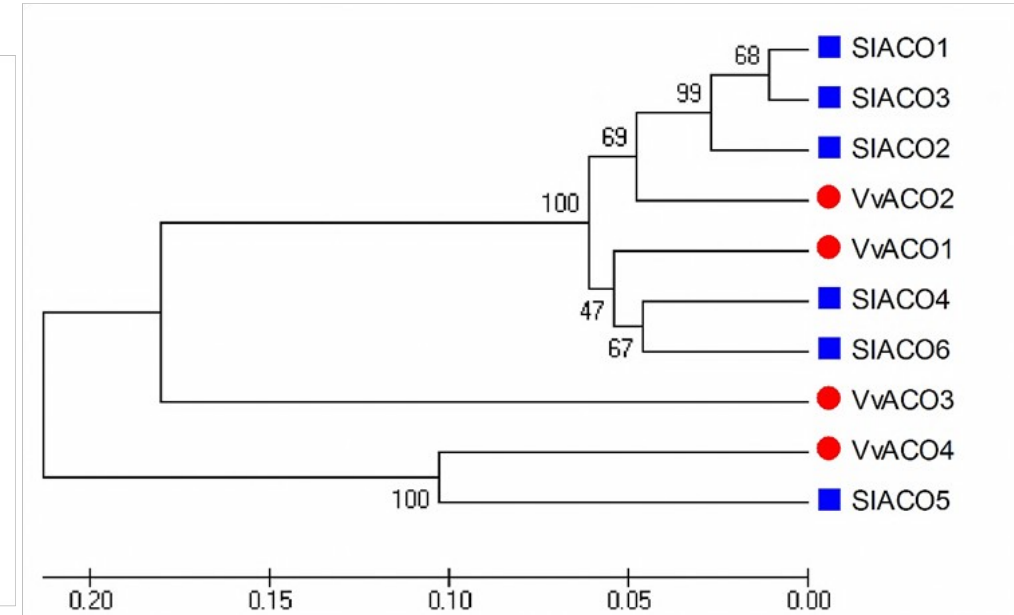


Fig S2: Phylogeny of the ACO homologues from *Arabidopsis*, grape and tomato. The phylogenetic tree was constructed in MEGA5 using the neighbor-joining method. All details are as for Fig S1. (a) The analysis involved four *VvACO*s (Red) and five *AtACO*s (Vanderstraeten and Van Der Straeten 2017) (Green) amino acid sequences; (b) The analysis involved four *VvACO*s (Red) and six *SIACO*s (Liu et al. 2015) (Blue) amino acid sequences.

Supplementary Figure S3

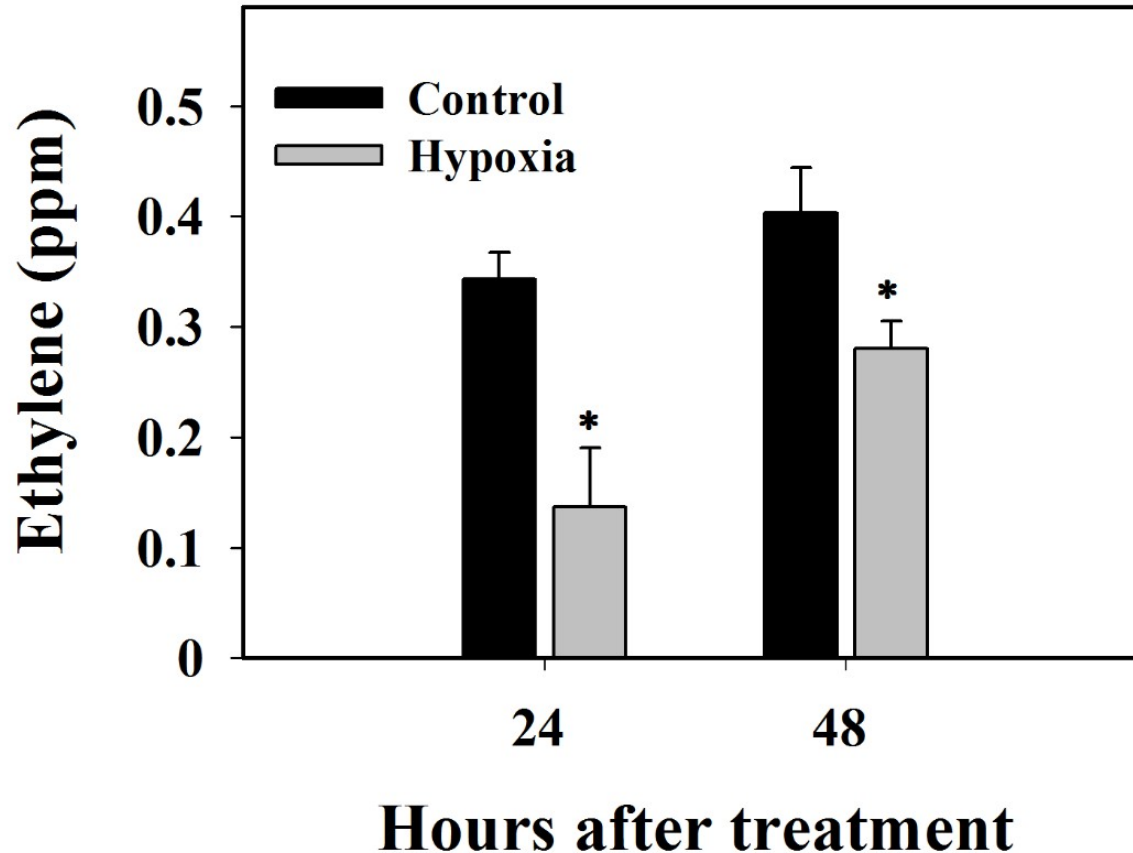


Fig S3: Ethylene production in buds during hypoxia. Cuttings prepared from cv. Early sweet cans collected in the vineyard in Jordan Valley in Dec 3th were exposed to hypoxia as described in Materials and Method, and compared in the SV control without KMnO_4 . Ethylene levels recorded in air samples taken at 24 and 48 h are presented. Three groups of 80 cuttings in 2 L jars were analyzed. Values are the averages \pm SE. Asterisks between treatments indicate significant differences according to Student's t-test (*, $P < 0.05$).

Supplementary Figure S4

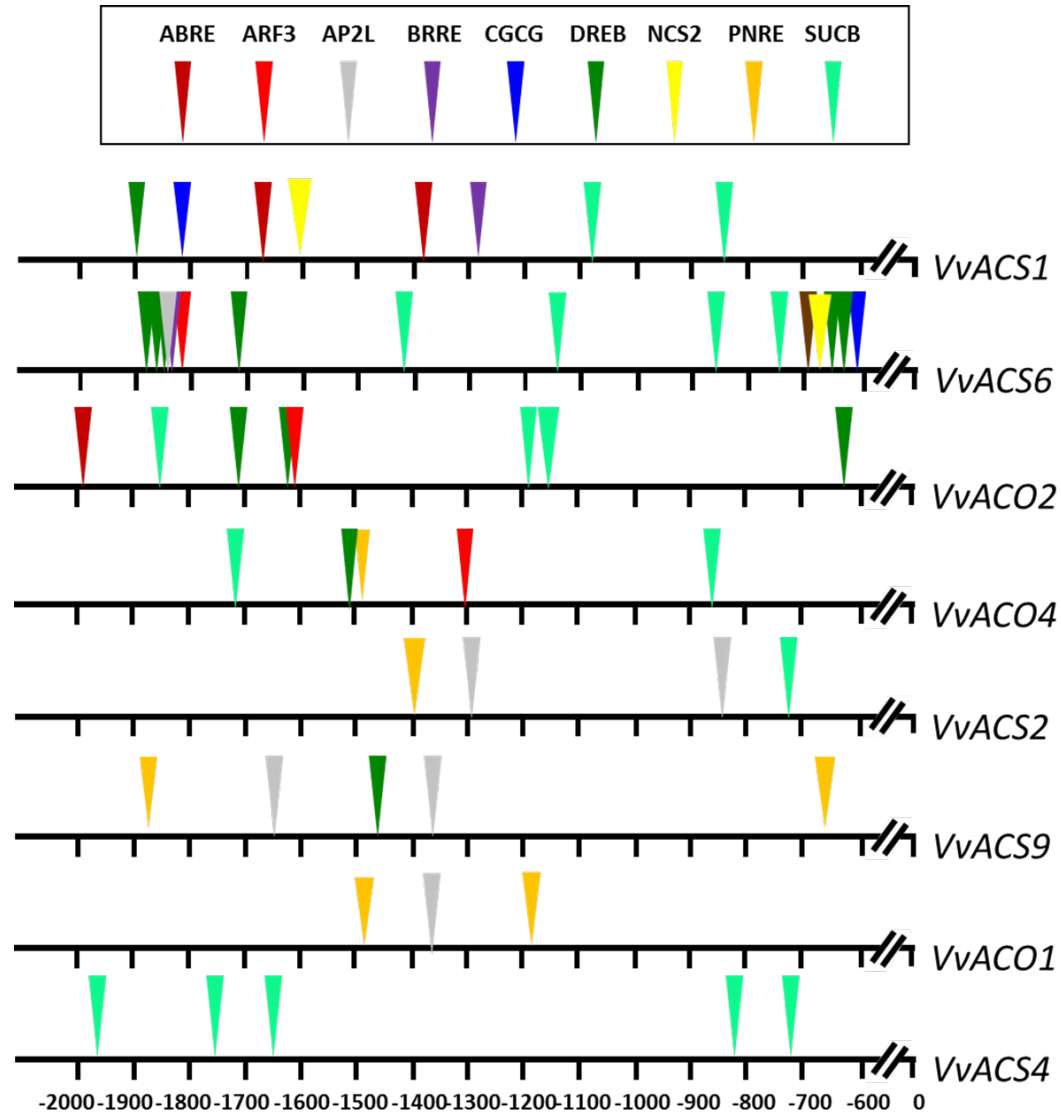


Fig S4: Position of 9 TFBSs in the promoter regions of VvACS and VvACO genes. The sequences used for the analysis are 0.6-2 kbp upstream of the coding sequence. For details see Supplementary Data File1, Table 1 and Methods.