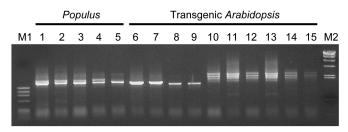
## **Supporting Information**

## Yuan et al. 10.1073/pnas.0906869106

	(1) Call	Culture, MA	Steeding (	Al ON	obio RMS	unding (PA	ato) ang Rwb along Rwb alo	osenstees	19 <sup>1919)</sup>	Probe ID PtpAffx.106238.1.A1_at
ICS		1		1	3.3	0.7	0.7	0.8	0.6	PtpAffx.106238.1.A1_at
MenA		1.1		1.4	5.6	0.9	0.8	1	0.9	PtpAffx.32024.1.A1_at
MenB		1.1		1.2	6.6	0.6	0.5	0.7	0.6	Ptp.5897.1.S1_at
APO2a		1		1	2.3	0.9	0.9	0.8	0.5	PtpAffx.1486.1.S1_at
APO2b		1.3		1.2	4.6	0.6	1	0.8	0.9	PtpAffx.99779.1.A1_at
CM1	1.6	0.6	0.6	1.6	1.2	1.8	1.1	0.7	1.3	PtpAffx.46815.2.S1_a_at
CM2	1.3	0.8	0.7	1.1	1	1.3	1.2	1.2	1	PtpAffx.988.1.A1_at
PAL1	1.5	1.4	1.3	1.8	1	1.7	1	1.5	1.5	PtpAffx.1672.3.A1_a_at
PAL2	2.7	0.7	2.5	1.8	0.1	2.8	1.4	1.2	2.4	PtpAffx.128701.1.S1_s_at
PAL3	1.8	0.7	1.4	1.6	1.2	2.6	1.2	1.6	2.4	Ptp.4730.1.A1_s_at
PAL4	0.8	0.9	2	2.5	0.4	1.8	1.7	1.3	1.9	PtpAffx.2272.1.S1_a_at
C4H1	1.3	0.9	1.5	1.1	1.3	1.4	1.9	1.6	2.3	PtpAffx.12443.4.A1_s_at
C4H2	1.3	0.7	1	1.2	0.4	2	1.4	1.5	3.2	Ptp.336.1.S1_at

**Fig. 51.** Heat map showing that *Populus ICS* is coregulated with orthologs of *Arabidopsis* genes involved in PhQ biosynthesis and PSI function, but not with phenylpropanoid pathway genes. Gene expression ratios (treatment over the respective control) were obtained from Affymetrix GeneChip analysis, and visualized using the HeatMapperPlus Tool (http://www.bar.utoronto.ca/ntools/cgi-bin/ntools.heatmapper\_plus.cgi). MenA, 1,4-dihydroxy-2-naphthoate phy-tyltransferase; MenB, 1,4-dihydroxy-2-naphthoyl CoA synthase; APO2, accumulation of photosystem one 2; CM, chorismate synthase; PAL, phenylalanine ammonia-lyase; and C4H, cinnamate 4-hydroxylase (corresponding probe ID shown on the right). Data were extracted from the following GEO series: (1) *Populus tremuloides* (clone L4) cell culture response to 25  $\mu$ M methyl jasmonate (MJ) feeding (GenBank accession no. GSE16786); (2) and (3) *Populus fremontii x angustifolia* (clone RM5) leaf wounding responses: (2) leaves from leaf plastchron index (LPI) 2 and root tips were analyzed 90 h after wounding (accession no. GSE16785), whereas (3) LPI1 and LPI5 were used 1 week after wounding in a separate experiment (GenBank accession no. GSE16783); (4) and (5) *Populus fremontii x angustifolia* response to nitrogen stress: LPI2 and LPI5 from clone 1979 (4) and clone 3200 (5) were analyzed 4 weeks after the start of the nitrogen stress treatment (GenBank accession nos. GSE14515 and GSE14893). Gray color indicates gene expression was below detection in both treatment and control samples.



**Fig. 52.** RT-PCR amplification of *ICS* from various *Populus* genotypes (lanes 1–5) and transgenic *Arabidopsis* lines (lanes 6–15). Lanes 1–3: three different *P. fremontii*  $\times$  angustifolia hybrid genotypes; lane 4: *P. trichocarpa*; lane 5: *P. tremuloides*; lanes 6 and 7: two independent transgenic *Arabidopsis sid2–2* lines harboring the full-length *Pti/CS* cDNA; lanes 8 and 9: two independent transgenic *Arabidopsis sid2–2* lines harboring *Pti/CS* cDNA without the predicted plastid-targeting presequence; lanes 10–13: independent transgenic *Arabidopsis* lines carrying the 6-kb genomic *Pti/CS* gene under control of the CaMV 35S promoter in the *sid2–2* mutant (10 and 11) or Col-0 background (12 and 13); lanes 14 and 15: two independent transgenic *Arabidopsis sid2–2* lines harboring the 8-kb *Pti/CS* gene in the *sid2–2* mutant background. M1 and M2: molecular markers PhiX174-HaeIII and Lambda-HindIII, respectively.

<

genomic N1 N2 N5 N12	TCAAC <b>TGA</b> TAAGAAGACCAGCAAGAAAGAGCATAAATTCCTGAAGTGTAATTTCTAGACTCCTGAATTGGAGTCATCAGT TCAAC <b>TGA</b> TAAGAAGACCAGCAAGAAAGAGCATAAATTCCTGAAGTGTAATTTCTAGACTCCTGAATTGGAGTCATCAGT TCCAC <b>TGA</b> TAAGAAGACCAGCAAGAAGAGGAGCATAAATTCCTGAAGTGTAATTTCTAGACTCCTGAGTTGGAGTCATCAGT TCAAC <b>TGA</b> TAAGAAGACCAGCAAGAAGGGAGCATAAATTCCTGAAGTGTAATTTCTAGACTCCTGAATTGGAGTCATCAGT TCAAC <b>TGA</b> TAAGAAGACCAGCAAGAAGAGCATAAATTCCTGAAGTGTAATTTCTAGACTCCTGAATTGGAGTCATCAGT ** **********************************
genomic N1 N2 N5 N12	TTGCACCCATTGGTTTCTACGAGGCACTTGTGATGCAAACTAGCTGTAACTTTCACACTATTACCTGGTAGCTTTTGGCC TTGCACCCATTGGTTTCTATGAGGCACTTGTGATGCAAACTAGCTGTTACTTTCACACTATTACCTGGTAGCTTTTGGCC TTGCACCCATTGGTTTCTATGAGGCACTTGTGATGCAAACTAGCTGTTACTTTCACACTATTACCTGGTAGCTTTTGGCC TTGCACCCATTGGTTTTTATGAGGCACTTGTGATGCAAACTAGCTGTTACTTTCACACTATTACCTGGTAGCTTTTGGCC TTGCACCCATTGGTTTCTATGAGGCACTTGTGATGCAAACTAGCTGTTACTTTCACACTATTACCTGGTAGCTTTTGGCC
genomic N1 N2 N5 N12	CGATGGATCTTGGAATCAAGTAAAGCTTAGTTGGCAGGAGAAGTACCAAGAGCTTATAGCATGTTCAGATAGCAGCACTT CGATGGATCTTGGAATCAAGCAAAGCTTAGTTGGCAGGAGAAGTACCAAGAGCTTATAGCATGTTCAGATAGCAGCACTT CGATGGATCTTGGAATCAAGTAAAGCTTAGTTGGCAGGAGAAGTACCAAGAGCTTATAGCATGTTCAGATAGCAGCACTT CGATGGATCTTGGAATCAAGTAAAGCTTAGTTGGCAGGAGAAGTACCAAGAGCTTATAGCATGTTCAGATAGCAGCACCTT CGATGGATCTTGGAATCAAGTAAAGCTTAGTTGGCAGGAGAAGTACCAAGAGCTTATAGCATGTTCAGATAGCAGCACCTT
genomic N1 N2 N5 N12	AAATTGTATCAGTCTGATTTTTTTTCCAGATAGGGAACTGTGTCGGTATTCTTTATTTTTTTT
genomic N1 N2 N5 N12	CTTTTTTACTTTAATTTTTGACCAAGTTGGAGAAGGTGTAATATTCTTTAGCAATTTGATAATGTTTCCCCAACATCTTT CTTTCTTACTTTAATTTTTGACCAAGTCGGAGAAGGTGTAATATTCTTTAGCAATTTGATAATGTTTCCCCAACATCTTG CTTTTTTACTTTAATTTTTGACCAAGTCGGAGAAGGTGTAATATTCTTTAGCAATTTGATAATGTTTCCCCAACATCTTG CTTTTTTACTTTAATTTTTGACCAAGTCGGAGAAGGTGTAATATTCTTTAGCAATTTGATAATGTTTCCCCAACATCTTG CTTTTTTACTTTAATTTTTGACCAAGTCGGAGAAGGTGTAATATTCTTTAGCAATTTGATAATGTTTCCCCAACATCTTG CTTTTTTACTTTAATTTTTGACCAAGTCGGAGAAGGTGTAATATTCTTTAGCAATTTGATAATGTTTCCCCAACATCTTG CTTTTTTACTTTAATTTTTGACCAAGTCGGAGAAGGTGTAATATTCTTTAGCAATTTGATAATGTTTCCCCAACATCTTG CTTTTTTACTTTAATTTTTGACCAAGTCGGAGAAGGTGTAATATTCTTTAGCAATTTGATAATGTTTCCCCAACATCTTG CTTTTTTACTTTAATTTTTGACCAAGTCGGAGAAGTGTGTAATATTCTTTAGCAATTTGATAATGTTTCCCCAACATCTTG
genomic N1 N2 N5 N12	САТТАСТТТТТСАТGCCATGCTTACAGTTTTTATGAGTGAATTTGAATTTTCTGTTCTGCTGGTTCACTGGCTGAAGAGT САТТАСТТТТТСАТGCCATGCTTACAGTTTTTATGAGTGAATTTGAATTTTCTGTTTTACAAAAAAAA

Fig. S3. Alignment of representative Pupulus ICS 3' UTR sequences showing alternative polyadenylation patterns. Translational stop codon is shown in boldface. Clone N12 represents the predominant transcript type (6 of 9) among the sequenced clones.

PNAS PNAS

A										
	clone		#							
	ICS v18	2,102	. ,	2 <b>-</b>						
	v10 v19	2,960 2,615	. ,							-
	v19 v20	2,504	• •			F 7		▋_▋┤ ▋_▋┤		
	v20 v21	2,304	. ,				_			
	v22	2,406	• •							
	v23	2,406		22						
	v24	2,390	• •	1 <b>1</b>						-
	v25	2,384		i i i i i i i i i i i i i i i i i i i			_			-
	v26	2,179	(1)			H-H			<u> </u>	H
	v27	2,098	(1)			H-H	-			
	v28	2,095	(1)							-
	v29	2,086	(1)			H-11	-			
	v30	2,081	(1)							H
	v31	2,040	(1)			** **				-
	v32	2,024	(1)			•• ••	-			ł
	v33	1,991	. ,			•••••				-
	v34	1,670	. ,							-
	v35	1,478	• •							H
	v36	811	• •		-					-
	v37	420	(1)							
в	100	4 740	$\langle 0 \rangle$							
	ICS	1,719 1,833	• •							
	a1 a2	1,633	. ,							
	az a3	1,773	• •							-
	a4	2,137	. ,					— H		
	a5	1,732	• •	<u> </u>			_			•
	a6	1,538	. ,	<b>H</b>					<u> </u>	
	a7	1,769	. ,							ĺ
	a8	1,716	(3)						-	ĺ
	a9	1,542	(3)							1
	a10	1,425	(3)							
	a11	2,420	(2)			-	_	_	<u> </u>	
	a12	2,319	(2)			-	-	_	H	
	a13	2,080	. ,			8 -				
	a14	1,522	. ,							
	a15	1,478	. ,			88-88				
	a16	1,421	. ,							1
	a17	2,683	. ,							
	a18	2,667	. ,							
	a19	2,638	• •				_			1
	a20 a21	2,303 2,222	. ,							1
	a21 a22	2,222	. ,							
	azz a23	1,437	• •							 
	a20	1,437	(י)							L

**Fig. S4.** Additional *Populus ICS* splice variants not shown in Fig. 3. (*A*) Splice variants from *Populus* leaf RT-PCR with a single occurrence among the 184 sequenced clones. (*B*) Representative splice variants recovered from transgenic *Arabidopsis* expressing the 6-kb genomic *Pti/CS* under control of the 35S promoter. Clones are arranged in order of abundance (frequencies in parentheses), with the normal *ICS* transcript shown at the top. A total of 64 RT-PCR clones were sequenced. Exons affected by alternative splicing are shown in gray.

SANG SAT

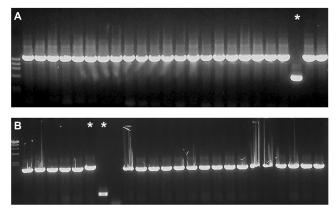
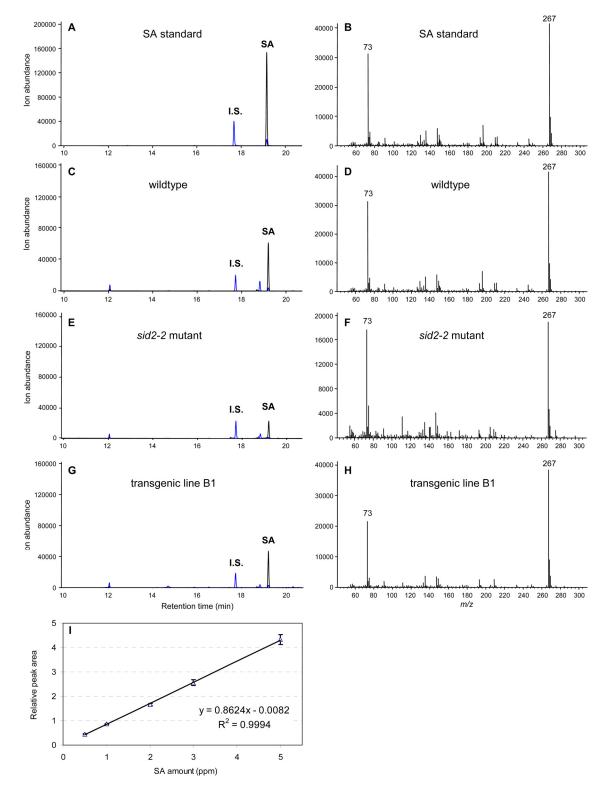


Fig. S5. Colony PCR of At/CS1 and At/CS2 RT-PCR products cloned into the pCRII-TOPO vector. Of the 24 At/CS1 (A) and 23 At/CS2 (B) recombinant clones, one and two were found to be splice variants (denoted by asterisks), respectively. Molecular weight markers used were PhiX174- HaeIII in (A) and Lambda-HindIII in (B).



**Fig. S6.** Quantification of SA in *Arabidopsis* leaves by GC-MS. Representative selected ion chromatograms of silylated SA (m/z 267, black trace) and internal standard (I.S.) *o*-anisic acid (m/z 209, blue trace) are shown on the left (A, C, E, and G), and mass spectra of silylated SA on the right (B, D, F, and H). (A and B) Authentic SA standard; (C and D) wild-type; (E and F) *sid2–2* mutant; and (G and H) transgenic line B1 harboring the full-length *Pti/CS* cDNA under control of the 35S promoter. (I) Calibration curve for SA. Each symbol represents means  $\pm$  SD of three independent measurements.

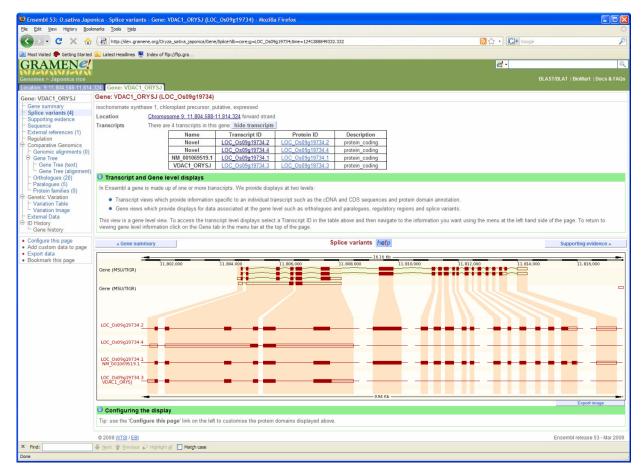
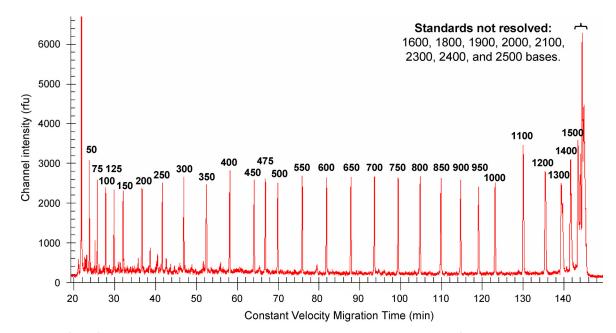


Fig. 57. Evidence of alternative splicing in rice Os/CS based on EST/cDNA sequences. Graph was obtained from the Gramene Rice Genome Browser (www.gramene.org/).

C C C C C C C C C C C C C C C C C C C		rser: chr17:62756786285411 - Mozilla	Firefox				
<pre>vertice for extended for the set of the</pre>	File Edit View History	<u>B</u> ookmarks <u>T</u> ools <u>H</u> elp			0		
Cenoscope Home     Vitis Project     Browser     CMap     Blat-Search     Download     Fetch EMBL     Contact       Showing 9.734 kbp from chr17, positions 6,275,678 to 6,285,411       Instructions       Search Lung a sequence name, gene name, locus, or other landmark. The wildcard character ' is allowed To center on a location, click the ruler. Use the Scroll/Zoom       Examples: chr4 54544.56541, ch6_random, scatbid_1157654.177657, GSVIV70000021001, 052/G4, Harons.       Fride hannel (Bokmark thil) [Link to Image) [High:res Image] [Help] [Reset]       Search     Boronical Decorated FASTA File Configure_ Go       Data Source     Scroll/Zoom: SCroll/Zoom	CD-CX	1 http://www.genoscope.cns.fr/cgi-	bin/ggb/vitis/gbrowse/vitis/	☆ • Google	P		
Showing 3.734 kbp from chr17, positions 6,275,678 to 6,285,411	应 Most Visited 🥐 Getting	Started 🔝 Latest Headlines 💻 Index of ftp://ft	p.gra				
<pre> P Intructions Subject Section Subject Sectin Subject Section Subject Section Subject Section Subject Se</pre>	Genoscope Hom	e    Vitis Project   Browser	CMap   Blat-Search   Blast-Search   Dow	/nload   Fetch EMBL   (	Contact		
Sanch using a sequence name, gene name, locus, or other landmark. The wildcard character " is allowed. To center on a location, click the ruler. Use the Scroll/Zoom butters to charge magnification and position. Examples: chrl 545844.565843, chrl random, scaffold_1157659, GSVIVT00000521001, Q5ZIG4, flavone. [Hide banner] [Bookmark this] [Link to Image] [High: res Image] [High] [Reset] 3 Sanch Data Source Grape Genome Browser Coverview of chrl 7 Coverview of chr	Showing 9.734	kbp from chr17, positions	6,275,678 to 6,285,411				
<pre>Hit banner   Bookmark this   Link to Image  [High: es Image]  Help  [Reet] Bara Landmark or Begion chr7 x50756 26.85811] Search Download Decorated FASTA File Configure. Co Scroll/Zoom: Co Show 9.734 kbp, C P D Configure. Co Scroll/Zoom: Co Show 9.734 kbp, C P D Configure. Co Configure Connect Image (Configure) Configure Configure. Co Scroll/Zoom: Co Co Configure Configure. Co Scroll/Zoom: Co Co Scroll/Zoom: Co Scroll/Co</pre>			ark. The wildcard character * is allowed. To center on a locati	on, click the ruler. Use the Scroll/Z	oom		
<ul> <li>Beach</li> <li>Beach</li> <li>Christer of christer</li> <li>Configure Go</li> <li>Configure Go<!--</td--><td>Examples: chr4:54584</td><td>4565843, chr8_random, scaffold_1:157658</td><td>177657, GSVIVT00000521001, Q5ZJG4, flavone.</td><td></td><td></td></li></ul>	Examples: chr4:54584	4565843, chr8_random, scaffold_1:157658	177657, GSVIVT00000521001, Q5ZJG4, flavone.				
Lundmark or Region:       Reports & Analysis:         Download Decorated FASTA File ♥ Configure Go         Grape Genome Browser         Overview		ark this] [Link to Image] [High-res Image	e] [Help] [Reset]				
<pre>chr12cr25678.6285411 Search Download Decorated FASTA File Configure_ Go Data Source Grape Genoms Browser Configure_ Go Scroll/Zoom: Configure_ Go Configure Configure</pre>			Reports & Analysis:				
Grape Genome Browser     Scroll/Zoom:     Image: Comparison of the factor of th		1 Search					
Yetride	Data Source						
Potential Pot		r		rup			
Details Details Certain the set of th		Overview of chr17					
<pre>     ted:</pre>		M 1M 2M 3M 4M		1 12M 13M			
<pre>Vitis vinifera cDMRs G-Ho_R-Se nodels (all) G-Ho_R-Se nodels (a</pre>							
		<pre>X-Se models (all)</pre>					
lone	× Find:	📕 Next 👚 Previous 🖉 Highlight all 🚺	Match case				
	Done						

**Fig. S8.** Evidence of alternative splicing in grape *VvICS* based on G-MO.R-Se assembly and de novo gene model building using RNA-Seq data [Denoeud et al. (2008) *Genome Biol* 9:R175]. Graph was obtained from the Grape Genome Browser (http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/).

PNAS PNAS



**Fig. S9.** Limitation of DNA fragment sizing by an automatic DNA sequencer. A representative chromatogram of DNA size standards, ranging in size from 50 bp to 2,500 bases, analyzed with a Beckman CEQ8000 Genetic Analyzer. Fluorescence (Well Red D1)-labeled DNA size standards were custom synthesized by BioVentures, Inc., and include the following sizes: 50, 75, 100, 125, 150, 200, 250, 300, 350, 400, 450, 475, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1800, 1900, 2000, 2100, 2300, 2400, and 2500 bases. The samples were analyzed using the "Fragment Analysis" module with the run time set at 3 hours, and the voltage set at 3.5 kV. Fragment sizes are indicated above the corresponding peaks, except for those that could not be resolved (>1,500 bases, marked by a bracket).

## Table S1. List of primers used in various PCR and RT-PCR amplifications

PNAS PNAS

Primer name	Sequence (5' to 3')	Application
PtilCSF1	ATGGCAACCGCTACTCTAGC	RT-PCR for PtilCS, PCR for 6-kb gene construct
PtiICSR3	TCAGTTGATGATTCCTGAGTTCTC	RT-PCR for <i>PtilCS</i> , PCR for 6-kb gene construct
PtilCSF1	Same as above	RT-PCR for PtilCS used in clone sequencing
PtilCSexon16R	GGGGAAACATTATCAAATTGC	RT-PCR for PtilCS used in clone sequencing
PtilCSF2noplstid	ATGGCTAATGGCTGCCAAGGAAAC	RT-PCR for mature <i>PtiICS</i>
PtiICSR3	Same as above	RT-PCR for mature PtiICS
PtilCSgF	ACGTCGACTAATGTTAAGAAAGAATTTGGCTTG	PCR for 8-kb gene construct
PtilCSgR	ATGGTACCTAATTCAGACAGATCAGCAACAAA	PCR for 8-kb gene construct
PtilCSF1noplastidNcol	CCATGGCTAATGGCTGCCAAGGAAAC	E. coli complementation
PtiICSR3	Same as above	E. coli complementation
AtICS1F1	ATGGCAACTGCTGTTTTATCTCCGGCAGC	RT-PCR for At/CS1
AtICS1R2new	TCAATTAATCGCCTGTAGAGATG	RT-PCR for At/CS1
AtICS1F1noplastidNcol	CCATGGCTTCTATGAATGGTTGTGATGGA	E. coli complementation
AtICS1R2new	Same as above	E. coli complementation
AtICS2F1	ATGGCAAACGGATGTGAGGCTGACCA	RT-PCR for AtICS2
AtICS2R2	TTAGTTGATTGGTTGCAAAGC	RT-PCR for AtICS2
EntCFNcol	CCATGGCAAATCCGTCCCCTCGCCTTTG	E. coli complementation
EntCRstop	TTAATGCAATCCAAAAACGTTCAACA	E. coli complementation