

# SUPPLEMENTARY MATERIAL

The activity of Hydroperoxide lyase 1 regulates accumulation of galactolipids containing 12-oxo-phytodienoic acid in *Arabidopsis thaliana*. Anders K Nilsson, Per Fahlberg, Oskar N Johansson, Mats Hamberg, Mats X Andersson and Mats Ellerström

Co1-0 ATGTTGTTGAGAACGATGGCGGCGACTTCCCCGCGGCCACCACCGTCAACATCCCTAACATCTCAGCAGCCACCATCACCCCTCACAGCTTCCCCTCCGTACAATGCCGGGATCGTAC  
C24 ATGTTGTTGAGAACGATGGCGGCGACTTCCCCGCGGCCACCACCGTCAACATCCCTAACATCTCAGCAGCCACCATCACCCCTCACAGCTTCCCCTCCGTACAATGCCGGGATCGTAC  
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Co1-0 GGCTGGCCGTTGGTTGGACCATTATCGGACCGTTTAGA-----TTCCAAGGACCCGATAAGTTTTCCGGACAAGAGCTGAGAAGTATAAGAGCACTGTGTTCCGTACAAATATT  
C24 GGCTGGCCGTTGGTTGGACCATTATCGGACCGTTTAGATTACTTCTGGTTCCAAGGACCCGATAAGTTTTCCGGACAAGAGCTGAGAAGTATAAGAGCACTGTGTTCCGTACAAATATT  
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Co1-0 CCTCCGACGTTTCTTTCTTCGGCAACGTTAACCCCTAACATCGTCGCGGTTCTTGACGTCAGTCTTTTAGCCATCTTTTGGACATGGATCTAGTTGATAAAAAGAGATGTTCTCATCGGA  
C24 CCTCCGACGTTTCTTTCTTCGGCAACGTTAACCCCTAACATCGTCGCGGTTCTTGACGTCAGTCTTTTAGCCATCTTTTGGACATGGATCTAGTTGATAAAAAGAGATGTTCTCATCGGA  
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Co1-0 GACTTCCGGCCTAGCCTTGGGTTCTACGGCGGCGTTCGTGTTGGAGTTTATCTCGACACTACTGAGCCAAAGCAGCCAAAGATAAAAAGTTTCGCTATGGAAACACTAAAACGAAGCTCA  
C24 GACTTCCGGCCTAGCCTTGGGTTCTACGGCGGCGTTCGTGTTGGAGTTTATCTCGACACTACTGAGCCAAAGCAGCCAAAGATAAAAAGTTTCGCTATGGAAACACTAAAACGAAGCTCA  
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Co1-0 AAAGTATGGCTACAAGAGCTTCGTTCAAACCTAAACATTTTCTGGGGAACAATCGAATCCGAAATCTCCAAAACGGTGCCGCTTCATATATCTCCCTCTCCAACGTTGCATCTTCAGT  
C24 AAAGTATGGCTACAAGAGCTTCGTTCAAACCTAAACATTTTCTGGGGAACAATCGAATCCGAAATCTCCAAAACGGTGCCGCTTCATATATCTCCCTCTCCAACGTTGCATCTTCAGT  
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Co1-0 TTCCTCTGCGCCTCTCTCGCCGGCGTTGACGCTTCCGTATCGCCGGACATCGCTGAGAACGGTTGAAAACAATCAATACTTGGCTTGCCTTGAAGTTATTCCTACTGCTAAACTTGGC  
C24 TTCCTCTGCGCCTCTCTCGCCGGCGTTGACGCTTCCGTATCGCCGGACATCGCTGAGAACGGTTGAAAACAATCAATACTTGGCTTGCCTTGAAGTTATTCCTACTGCTAAACTTGGC  
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Co1-0 GTAGTTCCCTCAGCCTCTTGAAGAGATTTTACTTCATACTTGGCCTTATCCTTCTCTTAAATCGCCGAAATTAACAAAAGCTTTACAATTTTCATCGACGAGAACCCGGAGATTGTCTC  
C24 GTAGTTCCCTCAGCCTCTTGAAGAGATTTTACTTCATACTTGGCCTTATCCTTCTCTTAAATCGCCGAAATTAACAAAAGCTTTACAATTTTCATCGACGAGAACCCGGAGATTGTCTC  
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Co1-0 CGGTTAGGTCAAGAAGAATTTCGGGTTGACCCGAGATGAGGCTATTCAAATCTTCTTTGTTTTAGGTTTTAATGCCTACGGGGCTTTCCGTCTTCTTACCTTCTTTGATCGGGAGA  
C24 CGGTTAGGTCAAGAAGAATTTCGGGTTGACCCGAGATGAGGCTATTCAAATCTTCTTTGTTTTAGGTTTTAATGCCTACGGGGCTTTCCGTCTTCTTACCTTCTTTGATCGGGAGA  
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Co1-0 ATAACCGCGGACAATTCGGGTTTACAGGAGAGATTAGAACCGAAGTCAGGAGAGTTGCGGATCCGGGTCGGATCTTAATTTCAAGACGGTTAACGAAATGGAGCTGGTTAAATCCGTG  
C24 ATAACCGCGGACAATTCGGGTTTACAGGAGAGATTAGAACCGAAGTCAGGAGAGTTGCGGATCCGGGTCGGATCTTAATTTCAAGACGGTTAACGAAATGGAGCTGGTTAAATCCGTG  
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Co1-0 GTTTACGAAACGCTGCGTTTTAGTCTCCGGTTCCGCTGCAATTCGCACGTGCGAGGAAAGATTTTTCAGATAAGTTACACGATGCTGTTTTTGGAGTCAAGAAAGGTGAGCTTCTTTGT  
C24 GTTTACGAAACGCTGCGTTTTAGTCTCCGGTTCCGCTGCAATTCGCACGTGCGAGGAAAGATTTTTCAGATAAGTTACACGATGCTGTTTTTGGAGTCAAGAAAGGTGAGCTTCTTTGT

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Col-0  GGTATCAGCCGCTTGTGATGAGAGACGCTAATGTTTTGACGAACCGGAGGAATTTAAACCGGACCGGTATGTAGGTGAGACCGGGTCTGAATTGCTGAATTATCTCTACTGGTCTAAC
C24    GGTATCAGCCGCTTGTGATGAGAGACGCTAATGTTTTGACGAACCGGAGGAATTTAAACCGGACCGGTATGTAGGTGAGACCGGGTCTGAATTGCTGAATTATCTCTACTGGTCTAAC
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Col-0  GGTCCACAAACCGGTACCCCGAGCGCGTCGAACAAACAGTGTGCAGCTAAGGACATTGTCACTCTCACGGCTTCCTTGCTCGTTGCCGATTTATTTCTCCGGTATGATACGATTACTGGT
C24    GGTCCACAAACCGGTACCCCGAGCGCGTCGAACAAACAGTGTGCAGCTAAGGACATTGTCACTCTCACGGCTTCCTTGCTCGTTGCCGATTTATTTCTCCGGTATGATACGATTACTGGT
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Col-0  GACTCCGGTTCAATTAAAGCTGTTGTTAAAGCTAAA TAA
C24    GACTCCGGTTCAATTAAAGCTGTTGTTAAAGCTAAA TAA
*****

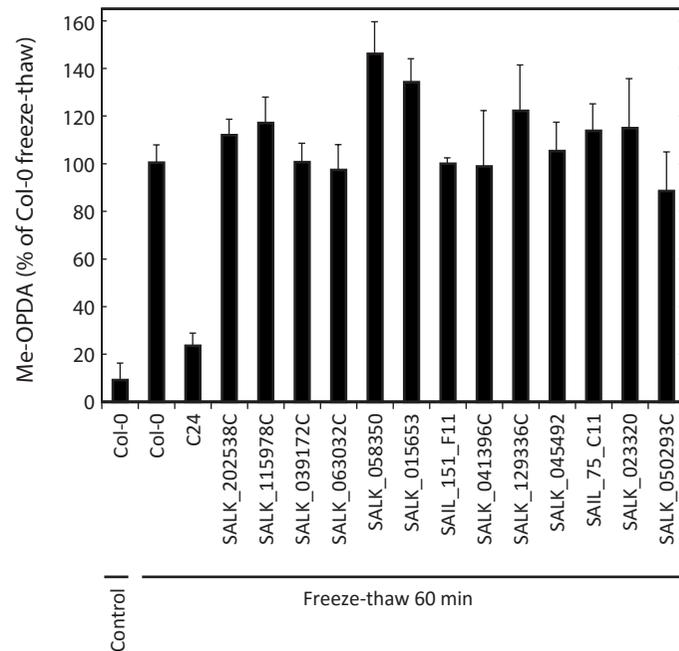
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**Fig. S1.** Alignment of the coding sequences of Col-0 and C24 *HPL1* alleles. Highlighted in green is the transcriptional start codon suggested by Duan, Huang et al. (2005), red stop codon, dashed line indicates the 10 bp deletion found in Col-0. Col-0 genetic sequence was downloaded from TAIR ([www.arabidopsis.org](http://www.arabidopsis.org)) and C24 sequence was determined by sequencing cDNA. Alignment was made using Clustal Omega (<http://www.ebi.ac.uk/Tools/msa/clustalo/>).

A

GOT35								
TAIR ID	Gene	Description	Localization	SNPs	NSS	NSSP	T-DNA line	
AT4G15396	CYP702A6	A member of the cytochrome P450 family	Secreted <sup>1</sup> /Membrane <sup>2</sup>	22	7	3	SALK_202538C	
AT4G15400	ABS1, BIA1	Encodes BIA1, a member of the BAH2 acyltransferase family	Cytoplasm <sup>2</sup>	8	2	1		
AT4G15410	PUX5	Serine/threonine protein phosphatase 2A 55 kDa regulatory subunit B prime gamma	Nucleus <sup>2</sup>	3	1	0	SALK_115978C	
AT4G15415	ATB' GAMMA	B' regulatory subunit of protein phosphatase 2A	Chloroplast <sup>1</sup> /Nucleus <sup>2</sup>	1	1	1	SALK_039172C	
AT4G15417	RTL1	RNAse II-like 1	Cytoplasm <sup>2</sup>	2	1	1		
AT4G15420		Ubiquitin fusion degradation UFD1 family protein	Nucleus <sup>2</sup>	1	1	1	SALK_063032C	
AT4G15430		ERD (early-responsive to dehydration stress) family protein	Secreted <sup>1</sup> /Membrane <sup>2</sup>	18	7	4**	SALK_058350, SALK_015653	
AT4G15440	HPL1	Encodes a hydroperoxide lyase. A member of the CYP74B cytochrome p450 family	Chloroplast <sup>2</sup>	*	-	-		
AT4G15450		Senescence/dehydration-associated protein-related	Mitochondrion <sup>1</sup>	3	2	0		
AT4G15460		Glycine-rich protein	Secreted <sup>1</sup> /Membrane <sup>2</sup>	1	0	0		
AT4G15470		Bax inhibitor-1 family protein	Unknown <sup>1</sup>	2	1	0	SAIL_151_F11	
AT4G15475		F-box/RNI-like superfamily protein	Nucleus	26	5	4	SALK_041396C	
AT4G15480	UGT84A1	Encodes a protein that might have sinapic acid:UDP-glucose glucosyltransferase activity	Unknown <sup>1</sup>	44	13	8	SALK_129336C	
AT4G15490	UGT84A3	Encodes a protein that might have sinapic acid:UDP-glucose glucosyltransferase activity	Unknown <sup>1</sup>	7	5	2	SAIL_75_C11, SALK_045492	
AT4G15500	UGT84A4	Encodes a protein that might have sinapic acid:UDP-glucose glucosyltransferase activity	Unknown <sup>1</sup>	1	0	0		
AT4G15510	PPD1	A nuclear-encoded thylakoid luminal protein that is associated with photosystem I (PSI)	Chloroplast <sup>1,2</sup>	0	0	0		
AT4G15520		tRNA/rRNA methyltransferase (SpoU) family protein	Cytoplasm <sup>2</sup>	0	0	0		
AT4G15530	PPDK	Encodes a dual-targeted protein believed to act as a pyruvate, orthophosphate dikinase	Chloroplast <sup>2</sup>	2	1	0	SALK_023320	
AT4G15540	UMAMIT38	Nodulin MtN21-like transporter family protein	Membrane <sup>2</sup>	3	2	1	SALK_050293C	
AT4G15545			Unknown <sup>1</sup>	1	0	0		
AT4G15550	IAGLU	UDP-glucose:indole-3-acetate beta-D-glucosyltransferase	Chloroplast <sup>1</sup> /Cytoplasm <sup>2</sup>	1	1	1		
GOT6								

B



**Fig. S2. Fine mapping of the QTL determining arabidopside accumulation.** **A**, The QTL accountable for the difference in arabidopside accumulation between Col-0 and C24 could be isolated to a 64 kpb region on chromosome four flanked by the genetic markers GOT35 and GOT6. SNPs, number of single nucleotide polymorphisms; NSS, number of non-synonymous amino acids substitutions; NSSP, number of non-synonymous amino acids substitutions with altered chemical properties. Sub-cellular localization of gene products was retrieved from 1 TAIR (Lamesch et al. 2012) or predicted by 2 TargetP (Emanuelsson et al. 2000). \* denotes a frameshift mutation in Col-0 and \*\* a premature stop codon in C24. **B**, A total lipid extract derived from leaf discs from Col-0, C24, or homozygous T-DNA mutant plants in Col-0 genetic background were transmethylated and analyzed for glycerolipid bound OPDA by GC-MS. Data was normalized to the level of Me-OPDA in Col-0 after 60 minutes of freeze-thaw treatment. Error bars represent standard deviation where n=3-6.

**Table S1.** Quantification of galactolipid species before and 60 minutes after freeze-thaw in Arabidopsis accessions. Letter after lipid species indicate standard used for quantification (see material and methods), a) MGDG, b) DGDG, c) tri18:3-acyl-MGDG, d) Arabidopside A, e) Arabidopside E.

Lipid species	M[NH4] <sup>+</sup> (m/z)	Neutral loss	Product ion	Interpreted fatty acid composition	Trivial name	Treatment	C 24	Ws-0	No-0	Uk-4	Kas-1	Ler	Po-0	Sei-0	Li-0	Nd-0	Ts-U	Per-1	Mo-2	Col-0	
34:3-MG <sup>a</sup>	770.7	MG	591.6	18:3, 16:0		Control	28.2 ± 1.4	26.7 ± 1.4	34.1 ± 2.0	28.4 ± 3.7	26.4 ± 2.6	29.8 ± 2.4	29.2 ± 2.7	30.5 ± 1.3	19.7 ± 2.6	32.1 ± 3.2	28.4 ± 1.0	34.6 ± 1.9	30.8 ± 1.8	31.0 ± 3.0	
						Freeze-thaw	3.7 ± 0.7	2.8 ± 0.3	3.6 ± 0.9	4.3 ± 0.8	1.6 ± 0.1	2.9 ± 0.5	2.4 ± 0.2	1.3 ± 0.1	2.0 ± 0.7	2.8 ± 0.6	2.8 ± 0.8	2.8 ± 0.4	1.7 ± 0.0	2.1 ± 0.4	
34:4-MG <sup>a</sup>	768.7	MG	589.6	18:3, 16:1		Control	70.6 ± 3.4	57.8 ± 2.5	75.9 ± 1.7	56.6 ± 16.7	60.6 ± 3.9	59.1 ± 2.2	68.0 ± 1.0	76.7 ± 2.1	55.1 ± 4.8	71.8 ± 8.1	67.7 ± 4.2	86.1 ± 5.8	70.8 ± 5.6	80.5 ± 11.1	
						Freeze-thaw	6.8 ± 1.3	4.1 ± 0.3	6.1 ± 1.3	6.5 ± 1.0	2.3 ± 0.3	3.9 ± 0.8	3.7 ± 0.3	1.7 ± 0.2	3.0 ± 1.1	3.6 ± 0.9	3.9 ± 1.3	3.0 ± 0.7	2.6 ± 0.2	2.9 ± 0.8	
34:5-MG <sup>a</sup>	766.6	MG	587.5	18:3, 16:2		Control	94.4 ± 2.7	99.9 ± 2.5	134.3 ± 7.4	94.3 ± 5.9	99.4 ± 4.3	95.1 ± 10.6	110.9 ± 2.9	128.0 ± 3.6	68.8 ± 2.4	112.2 ± 17.4	96.5 ± 3.6	97.9 ± 8.6	103.5 ± 10.8	137.8 ± 23.1	
						Freeze-thaw	4.1 ± 0.5	2.8 ± 0.2	3.2 ± 0.8	3.3 ± 0.5	1.3 ± 0.1	2.5 ± 0.6	1.8 ± 0.3	0.9 ± 0.0	1.3 ± 0.4	1.8 ± 0.6	1.7 ± 0.6	1.1 ± 0.6	1.1 ± 0.6	1.6 ± 0.2	1.4 ± 0.5
34:6-MG <sup>a</sup>	764.6	MG	585.5	18:3, 16:3		Control	825.3 ± 3.6	827.7 ± 2.7	800.1 ± 13.1	874.3 ± 38.1	781.9 ± 12.6	817.8 ± 46.6	783.9 ± 2.2	801.5 ± 8.0	737.2 ± 3.3	783.7 ± 45.0	793.9 ± 9.8	821.2 ± 6.8	800.9 ± 33.4	886.1 ± 35.9	
						Freeze-thaw	58.8 ± 11.8	27.1 ± 1.2	20.9 ± 5.5	48.8 ± 7.4	15.5 ± 1.4	32.7 ± 6.9	17.2 ± 2.8	8.7 ± 0.2	18.1 ± 4.5	19.4 ± 3.5	20.2 ± 6.6	15.1 ± 3.3	19.9 ± 1.3	11.5 ± 3.0	
36:4-MG <sup>a</sup>	796.7	MG	617.6	18:3, 18:1		Control	37.7 ± 4.8	29.3 ± 1.2	34.8 ± 3.1	20.4 ± 14.1	22.5 ± 0.8	28.5 ± 2.0	31.9 ± 1.0	32.7 ± 1.0	17.4 ± 2.8	31.1 ± 2.1	34.9 ± 1.8	45.7 ± 4.0	37.1 ± 1.2	38.9 ± 3.4	
						Freeze-thaw	3.1 ± 1.6	4.3 ± 0.5	4.1 ± 0.8	4.6 ± 0.8	2.2 ± 0.2	3.4 ± 0.6	3.4 ± 0.3	1.5 ± 0.7	2.5 ± 0.6	3.6 ± 0.6	3.1 ± 1.8	4.0 ± 0.5	2.8 ± 0.0	2.9 ± 0.4	
36:5-MG <sup>a</sup>	794.7	MG	615.6	18:3, 18:2		Control	47.4 ± 5.4	50.9 ± 3.2	66.1 ± 4.2	35.7 ± 9.9	35.1 ± 0.3	45.3 ± 2.5	68.0 ± 7.9	61.5 ± 3.8	27.6 ± 2.5	53.2 ± 4.3	48.0 ± 2.5	53.6 ± 1.6	46.9 ± 2.1	58.9 ± 8.4	
						Freeze-thaw	4.0 ± 0.7	3.5 ± 0.2	4.1 ± 0.9	4.1 ± 0.6	1.7 ± 0.1	3.4 ± 0.8	3.0 ± 0.4	1.8 ± 0.1	2.2 ± 0.4	3.1 ± 0.5	2.7 ± 0.6	2.3 ± 0.5	2.3 ± 0.0	2.4 ± 0.5	
36:6-MG <sup>a</sup>	792.7	MG	613.6	18:3, 18:3		Control	1238.1 ± 15.4	1307.6 ± 35.3	1368.7 ± 41.0	1294.6 ± 67.4	1231.4 ± 31.5	1369.0 ± 65.3	1308.4 ± 46.7	1278.0 ± 34.9	928.3 ± 34.6	1259.0 ± 134.7	1332.6 ± 43.3	1389.6 ± 44.7	1276.1 ± 22.8	1230.7 ± 143.0	
						Freeze-thaw	69.0 ± 9.8	49.8 ± 2.0	44.4 ± 10.7	81.0 ± 12.5	32.7 ± 1.6	60.6 ± 12.2	34.7 ± 2.7	22.6 ± 0.8	35.7 ± 9.1	49.4 ± 6.8	43.1 ± 10.5	37.3 ± 8.0	40.6 ± 9.0	27.8 ± 4.5	
34:3-DG <sup>b</sup>	932.8	DG	591.3	18:3, 16:0		Control	72.4 ± 2.0	100.2 ± 4.6	86.3 ± 3.6	105.5 ± 7.2	83.8 ± 5.0	93.5 ± 6.8	86.3 ± 8.8	85.3 ± 4.5	65.0 ± 6.1	81.8 ± 5.8	90.3 ± 5.5	98.2 ± 3.8	88.8 ± 0.4	70.9 ± 6.1	
						Freeze-thaw	17.6 ± 2.8	19.3 ± 1.3	13.8 ± 3.8	21.0 ± 3.9	8.0 ± 0.5	15.4 ± 1.3	9.0 ± 1.3	4.8 ± 0.3	7.5 ± 2.0	9.1 ± 1.7	11.0 ± 2.8	8.7 ± 1.1	8.6 ± 0.5	6.5 ± 1.5	
34:4-DG <sup>b</sup>	930.8	DG	589.3	18:3, 16:1		Control	3.8 ± 0.0	4.1 ± 0.1	3.9 ± 0.4	4.2 ± 0.5	2.4 ± 1.9	4.1 ± 0.4	3.7 ± 0.1	1.7 ± 2.4	2.9 ± 0.2	3.9 ± 0.3	4.1 ± 0.2	4.8 ± 0.5	3.2 ± 2.5	4.0 ± 0.5	
						Freeze-thaw	0.5 ± 0.4	0.5 ± 0.4	0.5 ± 0.1	0.4 ± 0.3	0.3 ± 0.0	0.6 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	0.4 ± 0.1	0.2 ± 0.2	0.3 ± 0.1	0.4 ± 0.0	0.3 ± 0.1	
34:5-DG <sup>b</sup>	928.8	DG	587.3	18:3, 16:2		Control	4.8 ± 4.8	4.9 ± 4.9	4.9 ± 4.9	4.0 ± 4.0	3.3 ± 3.3	4.3 ± 4.3	3.9 ± 3.9	5.6 ± 5.6	3.2 ± 3.2	4.6 ± 4.6	4.2 ± 4.2	3.6 ± 3.6	4.4 ± 4.4	5.9 ± 5.9	
						Freeze-thaw	0.9 ± 0.0	0.7 ± 0.0	0.6 ± 0.1	0.5 ± 0.1	0.3 ± 0.0	0.6 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.4 ± 0.0	0.4 ± 0.1	
34:6-DG <sup>b</sup>	926.8	DG	585.3	18:3, 16:3		Control	36.5 ± 1.6	41.6 ± 1.4	30.5 ± 1.1	42.3 ± 5.1	28.9 ± 0.7	37.6 ± 6.3	28.9 ± 0.2	36.1 ± 0.1	29.4 ± 1.3	33.1 ± 2.5	35.4 ± 2.2	35.2 ± 2.3	35.2 ± 2.1	38.9 ± 2.1	
						Freeze-thaw	3.7 ± 2.5	2.4 ± 0.1	1.5 ± 0.3	2.1 ± 0.3	1.1 ± 0.6	2.3 ± 0.4	1.0 ± 0.2	1.1 ± 0.2	1.2 ± 0.4	0.8 ± 0.2	1.3 ± 0.4	0.8 ± 0.2	1.5 ± 0.2	1.5 ± 0.2	
34:4-DG <sup>b</sup>	958.8	DG	617.3	18:3, 18:1		Control	6.3 ± 0.2	7.0 ± 0.3	6.7 ± 0.3	6.9 ± 1.6	6.0 ± 0.1	6.5 ± 0.4	6.5 ± 0.3	6.3 ± 0.1	4.2 ± 0.3	5.9 ± 0.3	7.1 ± 0.3	9.5 ± 0.7	8.4 ± 0.5	5.2 ± 0.4	
						Freeze-thaw	1.9 ± 0.2	1.9 ± 0.1	1.5 ± 0.3	1.7 ± 0.3	0.9 ± 0.0	1.7 ± 0.1	1.1 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	1.2 ± 0.1	1.4 ± 0.3	1.3 ± 0.1	1.3 ± 0.1	1.0 ± 0.5	
36:5-DG <sup>b</sup>	956.8	DG	615.3	18:3, 18:2		Control	9.5 ± 0.5	10.9 ± 0.4	12.3 ± 0.5	7.3 ± 1.1	7.3 ± 0.2	9.0 ± 0.9	12.8 ± 1.1	13.6 ± 0.5	6.5 ± 0.3	10.7 ± 0.9	9.7 ± 0.2	9.0 ± 0.7	9.8 ± 0.6	11.6 ± 1.1	
						Freeze-thaw	1.8 ± 0.1	2.1 ± 0.1	1.6 ± 0.4	1.1 ± 0.1	0.7 ± 0.0	1.5 ± 0.2	1.0 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	1.1 ± 0.2	0.9 ± 0.2	0.6 ± 0.3	1.0 ± 0.0	1.0 ± 0.2	
36:6-DG <sup>b</sup>	954.8	DG	613.3	18:3, 18:3		Control	493.4 ± 20.2	543.6 ± 15.5	532.2 ± 12.7	533.3 ± 30.4	493.6 ± 11.4	519.8 ± 22.6	539.2 ± 10.4	543.3 ± 8.8	419.7 ± 30.0	516.8 ± 30.5	544.0 ± 15.8	568.6 ± 30.3	500.7 ± 17.6	508.9 ± 19.0	
						Freeze-thaw	87.9 ± 9.2	70.3 ± 1.6	49.5 ± 12.9	63.6 ± 10.7	34.1 ± 2.3	69.3 ± 13.8	35.6 ± 4.6	23.5 ± 1.3	29.7 ± 4.8	40.3 ± 7.1	42.8 ± 9.2	33.6 ± 5.9	44.2 ± 1.8	30.3 ± 6.3	
34:6-MG-16:0 <sup>c</sup>	1002.9	MG-16:0	585.4	18:3, 16:3, 16:0		Control	0.3 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.0 ± 0.0		
						Freeze-thaw	19.0 ± 3.3	9.1 ± 1.1	5.0 ± 0.8	2.3 ± 0.2	6.5 ± 0.8	10.2 ± 1.4	2.3 ± 0.8	2.9 ± 1.7	2.7 ± 0.4	3.6 ± 0.8	2.1 ± 1.0	0.9 ± 0.2	1.1 ± 2.5	2.7 ± 1.2	
34:6-MG-16:3 <sup>c</sup>	996.8	MG-16:3	585.4	18:3, 16:3, 16:3		Control	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0		
						Freeze-thaw	15.0 ± 4.6	4.8 ± 1.5	2.5 ± 0.2	1.6 ± 0.1	5.0 ± 0.6	7.8 ± 1.6	1.4 ± 0.7	2.0 ± 1.4	2.0 ± 0.6	2.2 ± 0.6	1.2 ± 0.8	0.3 ± 0.2	1.16 ± 3.5	1.6 ± 0.7	
34:6-MG-18:3 <sup>c</sup>	1024.8	MG-18:3	585.4	18:3, 16:3, 18:3		Control	4.0 ± 3.6	0.6 ± 0.1	0.6 ± 0.2	0.6 ± 0.4	0.9 ± 0.5	1.8 ± 0.6	0.4 ± 0.0	1.2 ± 0.3	0.6 ± 0.2	2.5 ± 2.4	0.8 ± 0.1	1.1 ± 0.5	2.9 ± 1.0	0.6 ± 0.1	
						Freeze-thaw	98.5 ± 21.5	46.0 ± 8.7	25.4 ± 2.9	16.2 ± 1.7	41.8 ± 6.2	53.8 ± 1.8	63.2 ± 11.0	21.3 ± 11.0	18.3 ± 4.4	21.5 ± 5.0	14.7 ± 7.6	5.6 ± 0.9	79.1 ± 22.6	18.7 ± 8.8	
36:6-MG-18:3 <sup>c</sup>	1052.9	MG-18:3	613.5	18:3, 18:3, 18:3		Control	3.7 ± 2.5	0.6 ± 0.1	0.5 ± 0.5	0.4 ± 0.7	1.1 ± 0.6	2.3 ± 0.4	0.4 ± 0.1	1.1 ± 0.3	0.5 ± 0.2	2.9 ± 2.7	0.8 ± 0.2	1.3 ± 0.6	2.6 ± 0.9	0.4 ± 0.4	
						Freeze-thaw	91.6 ± 21.2	57.4 ± 4.8	41.0 ± 4.6	21.1 ± 1.8	60.2 ± 9.8	86.2 ± 11.1	23.1 ± 6.4	31.7 ± 14.6	26.9 ± 1.9	39.4 ± 5.1	28.8 ± 10.7	17.3 ± 2.6	99.0 ± 14.9	27.3 ± 8.2	
34:4-O-MG <sup>d</sup>	784.7	MG	605.5	OPDA, 16:0		Control	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.2	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.1		
						Freeze-thaw	0.3 ± 0.1	0.4 ± 0.2	0.7 ± 0.1	1.8 ± 0.5	0.6 ± 0.1	0.9 ± 0.1	0.9 ± 0.3	0.4 ± 0.0	1.1 ± 0.3	1.4 ± 0.3	1.4 ± 0.1	2.5 ± 0.7	0.7 ± 0.2	0.8 ± 0.1	
34:5-O-MG <sup>d</sup>	782.6	MG	603.5	OPDA, 16:1		Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0		
						Freeze-thaw	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.0	0.8 ± 0.2	0.5 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.0	1.2 ± 0.1	1.3 ± 0.2	1.4 ± 0.2	3.2 ± 1.1	0.8 ± 0.2	1.3 ± 0.2	
34:6-O-MG <sup>d</sup>	780.6	MG	601.5	OPDA, 16:2		Control	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.2	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0		
						Freeze-thaw	0.4 ± 0.3	0.4 ± 0.3	0.5 ± 0.4	0.1 ± 0.1	0.3 ± 0.2	0.4 ± 0.3	0.1 ± 0.0	0.0 ± 0.0	0.3 ± 0.3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.4 ± 0.2	0.3 ± 0.4	
34:7-O-MG <sup>d</sup>	778.6	MG	599.5	OPDA, 16:3	MGDG-O	Control	3.5 ± 2.6	0.6 ± 0.2	0.8 ± 0.4	2.1 ± 5.0	0.8 ± 0.2	4.0 ± 4.7	0.5 ± 0.3	1.6 ± 0.5	4.7 ± 4.6	12.7 ± 19.0	2.4 ± 1.1	7.8 ± 6.2	9.9 ± 8.6	1.3 ± 0.9	
						Freeze-thaw	8.8 ± 1.4	6.9 ± 0.3	5.9 ± 1.6	1.1 ± 0.2	3.8 ± 0.5	7.2 ± 0.9	3.1 ± 2.3	2.7 ± 0.1	2.7 ± 1.7	3.7 ± 1.0	0.6 ± 0.1	1.4 ± 1.5	4.4 ± 8.1	2.7 ± 2.2	
36:6-O-MG <sup>d</sup>	808.7	MG	629.5	OPDA, 18:2		Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0					

**Table S2. Primers used for real time quantitative PCR.** The symbol ~ denotes intron gap.

<b>Gene</b>	<b>Locus</b>	<b>Left primer</b>	<b>Right Primer</b>
<i>HPL1</i>	At4G15440	GCACGCCAAG~ATAAAAGGT	AAGATGCAACGTTGGAGAGG
<i>HPL1</i>	At4G15440	GGCCTAGCCTTGGGTTCTAC	AAGATGCAACGTTGGAGAGG
<i>ACT8</i>	At1G49240	GCTGGATTCGCTGGAGATGA	AGGTCTCCATCTCTTGCTCG
<i>UBQE</i>	At5G25760	CAGTCTGTGTGTAGAGCTATCATAGCAT	AGAAGATTCC~CTGAGTCGCAGTT
<i>LOX2</i>	At3G45140	CTATGGAATCTTGCTAAGACTCATG	CGGCTGAACTTAGCTCTAATGCATA
<i>AOS</i>	At5G42650	CTTACCGGCGCATTGTTTAT	AAGTCAAAGCCGGTCAAATG
<i>AOC1</i>	At3G25760	AATACCGAAAACCCAGACC	TTCGATCACAACGCAGAGAC
<i>AOC2</i>	At3G25770	GCCAAGAAGAACCTCACTGC	TCCGAGACCGAACATTAAGC
<i>AOC3</i>	At3G25780	GACCGAAAACCCAGACCAA	TTCAGGAACGTGTTGGATCA
<i>AOC4</i>	At1G13280	TTCAGAACCATTTCAGCAG	CGGTGATTCCATGCGTTTT

**Table S3. PCR markers used for mapping.** SSLP, Simple Sequence Length Polymorphisms; SNP, Single-Nucleotide Polymorphism; TP-ARMS, Tetra-Primer Amplification Refractory Mutation System (Ye, Dhillon et al. 2001).

Marker name	Chromosome	Nearest locus	Genomic position	Marker type	Left primer(s)	Right primer(s)	PCR product length (bp)
<b>MN1.5</b>	1	AT1G03620	898430	SSLP	TTATTATCAAGATCAAAGATTGTATGGTTT	CTTGTTTTATATCTGTTTGGTTTAATTGT	Col 308; C24 320
<b>CIW12</b>	1	AT1G27660	9621344	SSLP	AGGTTTTATTGCTTTTCACA	CTTCAAAGCACATCACA	Col 128; C24 100
<b>NGA280</b>	1	AT1G55840	20873698	SSLP	GGCTCCATAAAAAGTGCACC	CTGATCTCACGGACAATAGTGC	Col 105; C24 85
<b>ATPASE</b>	1	AT1G76030	28533837	SSLP	GTTACAGAGAGACTCATAAACCA	CTGGGAACGGTTCGATTCGAGC	Col 85; C24 69
<b>NGA692</b>	1	AT1G76820	28836552	SSLP	AGCGTTTAGCTCAACCTTAGG	TTTAGAGAGAGAGAGCGCGG	Col 110; C24 90
<b>GOT10</b>	2	AT2G02540	693817	SSLP	GCGGTGAGTATCTCATTGCAT	AAGCCATTCCCTAAACCAC	Col 214; C24 165
<b>GOT42</b>	2	AT2G21620	9249365	SSLP	ATTCATTTGTCGCATTTGCT	CAACAACACGAGCCACACTC	Col 207; C24 188
<b>GOT11</b>	2	AT2G41905	17493070	SSLP	GGTTTCCATGTTGGCTCAGT	GAACCAACGATTGGGCTAGA	Col 126; C24 147
<b>NGA172</b>	3	AT3G03340	786296	SSLP	CATCCGAATGCCATTGTTC	AGCTGCTTCCTTATAGCGTCC	Col 162; C24 150
<b>CIW11</b>	3	AT3G26610	9775545	SSLP	CCCCGAGTTGAGGTATT	GAAGAAAATTCCTAAAGCATT	Col 180; C24 230
<b>GOT47</b>	3	AT3G44735	16292750	SSLP	AACATGTTTTTGATAATCATCCATC	CATCTAAAAGATCTCCAGATTAAGTGA	Col 218; C24 250
<b>GOT48</b>	3	AT3G44755	16301990	SSLP	TCTCTTGATGGTGGTGTTC	AGCCAGACCAGAACTACG	Col 177; C24 189
<b>GOT39</b>	3	AT3G61550	22776727	SSLP	TCGTCTGGAGGTCTTGAT	ACGGGACAAGAACCATTGAG	Col 244; C24 229
<b>GOT45</b>	4	AT4G00880	367804	SSLP	ATCGAAAAACGAATCCATGC	AATATCCCCACTTAGATATTCCA	Col 139; C24 154
<b>GOT46</b>	4	AT4G00890	372994	SSLP	GCCTCTGTTATTTAGGCTGAAAAT	CAGCTGTTGGTCAAAGTATACA	Col 224; C24 267
<b>MN4.2</b>	4	AT4G07410	4197601	SSLP	TAAGGTCAGACTATATGTTTACGTTTCATT	GTCATCCTCGTTAAAGTTACGATTG	Col 347; C24 357
<b>GOT3</b>	4	AT4G07840	4644916	SSLP	TCCTTAAAGCCCTTTGTTTATG	TTGTTGCTTTATTGGTTGGTTT	Col 175; C24 154
<b>GOT4</b>	4	AT4G09720	6131588	SSLP	GTGGATGCTAAGCGAATGGT	ATCGGGGAAGGGTGATGTAT	Col 209; C24 177
<b>GOT23</b>	4	At4G11750	7077901	SNP	CCATGTTTGGGTTTTGAGATC; CCCATGTTAAGGTTTTGAGATT	TATTCACCAACGGTGCCTTC	Col, C24 206
<b>CIW6</b>	4	AT4G13575	7892624	SSLP	CTCGTAGTGCACCTTCATCA	CACATGGTTAGGGAAACAATA	Col 162; C24 155
<b>GOT22</b>	4	AT4G14400	8297901	SNP	GGTTATATCAGCGATGCCAAC; GGTTATATCAGCGATAGCAAA	GCAAGGCCACATGTAAGGAT	Col, C24 187
<b>GOT31</b>	4	AT4G14590	8375401	SNP	ATCGAGCGAAGCGTTATGC; CACATCGAACGAAGTGTAAGC	TTCACATGAACCGTCTTCTCC	Col, C24 256
<b>GOT25</b>	4	AT4G14760	8484735	SNP	GAGTATTCAGGGACCAATAG; GAGTATTCAGGGACCAATAT	TCTCCAAAAGAGCGCATTGT	Col, C24 243
<b>GOT33</b>	4	AT4G14965	8553090	SNP	TGAAGGTGGAGAGGTAGAACTC; TGAAGGTGGAGAGGTATGAACTT	CTTGCCAAGAGGTTACAGT	Col; C24: 268
<b>GOT29</b>	4	AT4G15100	8625334	SNP	CGAACATATCTATCCGAGACTTG; CGAGCATATCTATCTGAACTCG	GGTGGTGAATGATTCCAGA	Col, C24 322
<b>GOT32</b>	4	AT4G15290	8721450	SSLP	TGACAAAGGAAATATAGCATGTACG	AAGGGTTTGTGAGCATGG	Col 336; C24 302
<b>GOT35</b>	4	AT4G15396	8809580	SSLP	CCCAGATGGTGAGTATCCAA	TGTGATCCTAAGCGACTCATTT	Col 260; C24 278
<b>GOT30</b>	4	AT4G15440	8837250	SSLP	AAAATACAGAAGAACTGTATACAAAA	GATTGATTGTTCCCAAGAA	Col 282; C24 315

<b>GOT37</b>	4	AT4G15490	8852180	SSLP	ACGGTGTGTGCAATAACCAA	CCTCAGGCATCATCTCTCGT	Col 432; C24 369
<b>GOT6</b>	4	AT4G15545	8876513	SSLP	GCTGCAACTGACAACGAAAA	GCCGTGGCGTAGTAGTTTGT	Col 167; C24 179
<b>GOT27</b>	4	AT4G15890	9018101	SNP	TGGGAAGATTCTGGGAGGAG; GTGGGAAGATTTTAGGTGGAG	AAACTCTGATTGGCCCTCAA	Col, C24 310
<b>GOT9</b>	4	AT4G16444	9276263	SNP	TAAAGAAACGGCCATTAA; TGGGCTTCGTTGAAATTCTT	TCCTATTCATTCTAAATTTGGATACTT	Col 157; C24 173
<b>GOT17</b>	4	AT4G16770	9434101	SNP	GGTGGAAACCTGAGGCAGG; GGTGGAAACCTGAGGCAGC	CAGACCCAACTGCGTTGTA	Col, C24 226
<b>GOT15</b>	4	AT4G17030	9584800	TP- ARMS	GAAGAAAATGTGAGTTTCTTCTTCTTCT; CGAAAGACATAGTTTGTAGAATCGAAGC	CGTGAGTTTCTTCATACCCAAAGAAG; TTCTTTGGGAAGACTCTAAGAGAATGC	Col 104+166; C24 117+166
<b>GOT18</b>	4	AT4G17140	9630919	SNP	GCTGCTCTTCTCGAGTCCA; GCTGCTCTTCTCGAGTCCG	GCGGCGGTATTTATGCTTAG	Col, C24 180
<b>GOT19</b>	4	AT4g17565	9782371	SNP	TGGTCGATGAATCCCTTATG	CATGACTTCAAGGTGTTATG; CATGACTTCAAGGTGTTATA	Col, C24 165
<b>GOT13</b>	4	AT4G18425	10180210	SNP	CTGGAGTCAAGTCCCAGTTC	ATAATTGCAACAACCTAAAAAGA; ATAATTGCAACAACCTAAAACAA	Col, C24 159
<b>GOT16</b>	4	AT4G19185	10482401	TP- ARMS	GGTTATGCCTAACGTGTTTTCCGGAGTC; TACATCCGTAACGGTCCAAATCCACAGT	TACCGGTGTAACGGCGTCAAGTAGCTC; CTGATTTACCTACGCCGTCGATTAAC	Col 199+365; C24 220+365
<b>GOT7</b>	4	AT4G20330	10977375	SSLP	TCTTGAGCAAGGTGCAAAAA	TAACTTCCAGGCGGCCTTAT	Col 144; C24 135
<b>GOT8</b>	4	AT4G33970	16272270	SSLP	TCGAGAGAGGTGAGAAGGTCA	CACATCTCTCATTGGATGATGAA	Col 138; C24 150
<b>T22D6-1ME</b>	5	AT5G08090	2584790	SSLP	ACGCTTCCAAAAATCCACAA	CTCGGGAGACTCGCACGAT	Col 175; C24 230
<b>NGA139</b>	5	AT5G24620	8428133	SSLP	GGTTTCGTTTCACTATCCAGG	AGAGCTACCAGATCCGATGG	Col 174; C24 132
<b>GOT44</b>	5	AT5G24680	8453593	SSLP	TCACGCTTCTCTCTCCCTA	GGTGATCGGTTTCGATTGT	Col 197; C24 181
<b>GOT38</b>	5	AT5G38840	15551620	SSLP	CTCCGTGTACCAATTTTCAAG	TGAAACCGAGAAATCGAAGG	Col 245; C24 230
<b>CER456385</b>	5	AT5G59160	23882280	SSLP	CACCGCCACCATACGAGCAT	TTCTGGCGGAGGATACTTCTTGAA	Col 210; C24 160

Ye, S., S. Dhillon, et al. (2001). "An efficient procedure for genotyping single nucleotide polymorphisms." *Nucleic Acids Research* **29**(17): e88.

Duan, H., M. Y. Huang, K. Palacio and M. A. Schuler (2005). "Variations in CYP74B2 (hydroperoxide lyase) gene expression differentially affect hexenal signaling in the Columbia and Landsberg erecta ecotypes of Arabidopsis." *Plant Physiology* **139**(3): 1529-1544.

Emanuelsson, O., H. Nielsen, S. Brunak and G. von Heijne (2000). "Predicting subcellular localization of proteins based on their N-terminal amino acid sequence." *Journal of Molecular Biology* **300**(4): 1005-1016.

Lamesch, P., T. Z. Berardini, D. Li, D. Swarbreck, C. Wilks, R. Sasidharan, R. Muller, K. Dreher, D. L. Alexander, M. Garcia-Hernandez, A. S. Karthikeyan, C. H. Lee, W. D. Nelson, L. Ploetz, S. Singh, A. Wensel and E. Huala (2012). "The Arabidopsis Information Resource (TAIR): improved gene annotation and new tools." *Nucleic Acids Research* **40**(Database issue): D1202-1210.