Article title: **WRINKLED1 and ACYL-COA:DIACYLGLYCEROL ACYLTRANSFERASE1 regulate tocochromanol metabolism in Arabidopsis**

Authors: **Sébastien Pellaud, Alexandre Bory, Valentin Chabert, Joëlle Romanens, Laurie Chaisse-Leal, Anh Vu Doan, Lucas Frey, Andrea Gust, Katharina M. Fromm and Laurent Mène-Saffrané.**

The following Supporting Information is available for this article:

Fig. S1. Next-generation mapping of the *enhanced vitamin e* (*eve*) *1* mutation using whole genome sequencing. The mutation causing the *eve1* seed phenotype was mapped by whole genome sequencing. A DNA sample was prepared from the 49 BC1F2 mapping recombinants exhibiting the *eve1* seed phenotype (blue). The Col-0 parental DNA sample (control) was prepared from 75 Col-0 parental individuals (green). Both DNA samples were sequenced using the Illumina HiSeq2500 sequencer and v3 chemistry. The Col-0 parental DNA yielded 75'378'937 paired-end reads (120-fold genome coverage) while DNA of the BC1F2 *eve1* recombinants yielded 74'801'935 paired-end reads (119-fold genome coverage). Sequencing files were uploaded and analyzed with the SNPtrack bioinformatic pipeline.

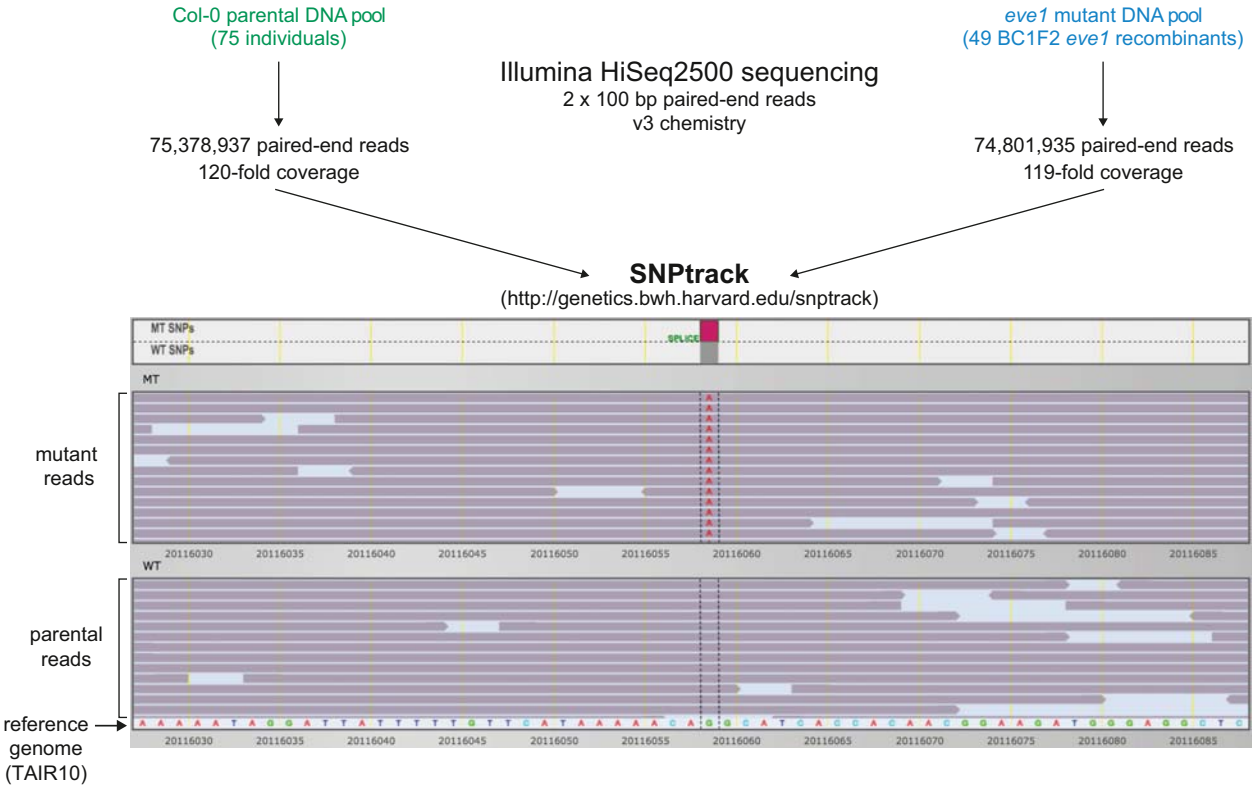

Fig. S2. Molecular characterization of the *eve1* (*wri1-6*) mutation. (**a**) Structure of the Arabidopsis *WRI1* gene (TAIR10) and position of the published *wri1* mutants. Exons are coloured in orange while introns are in blue. Both start and stop codons are highlighted in blue. For insertion mutants, an arrow indicates the orientation of the T-DNA as in the T-DNA Express database (http://signal.salk.edu/cgi-bin/tdnaexpress). (**b**) List of available *wri1* alleles in different Arabidopsis accessions including the type of mutation and the position on chromosome 3 (TAIR10). (**c**) *wri1-6* dCAPS marker assayed on different genomic DNAs. Lane 1, New England Biolabs 100 bp ladder; lane 2, homozygous *WRI1* gDNA; lane 3, homozygous *wri1-6* gDNA; lane 4, heterozygous *WRI1* *wri1-6* gDNA.

(a)

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(b)

Allele	Background	Mutation type	Position	Reference
wri1-1	Col-2	EMS (G-to-A)	20,115,021	Cernac & Benning (2004)
wri1-2	Col-2	EMS (?)	-	Focks & Benning (1998)
wri1-3	Col-0	T-DNA (SALK_085693)	20,115,774	Baud *et al.* (2007)
wri1-4	Col-0	T-DNA (SALK_008559)	20,116,785	Baud *et al.* (2007)
wri1-5	Ws-0	T-DNA (FLAG_158E08)	20,116,043	Baud *et al.* (2007)
wri1-6	Col-0	EMS (G-to-A)	20,116,058	this study
wri1-10	Col-0	T-DNA (GK109D06)	20,117,186	Maeo *et al.* (2009)

(c)

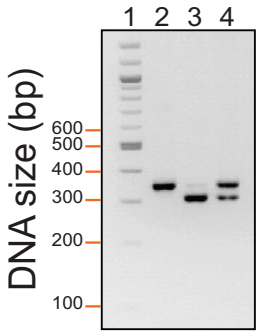


Fig. S3. Representative seed tocochromanol profiles of independent *wri1* mutants. NP-HPLC-FLD chromatograms of lipid extracts prepared from *eve1* (*wri1-6*; **a**), *wri1-3* (**b**), and *wri1-4* (**c**) seeds, respectively (all in red), and their controls (blue). The internal standard tocol is labeled in red. Abbreviations: LU, light unit; PC-8, plastochromanol-8; t1, tocomonoenol; toc, tocopherol.

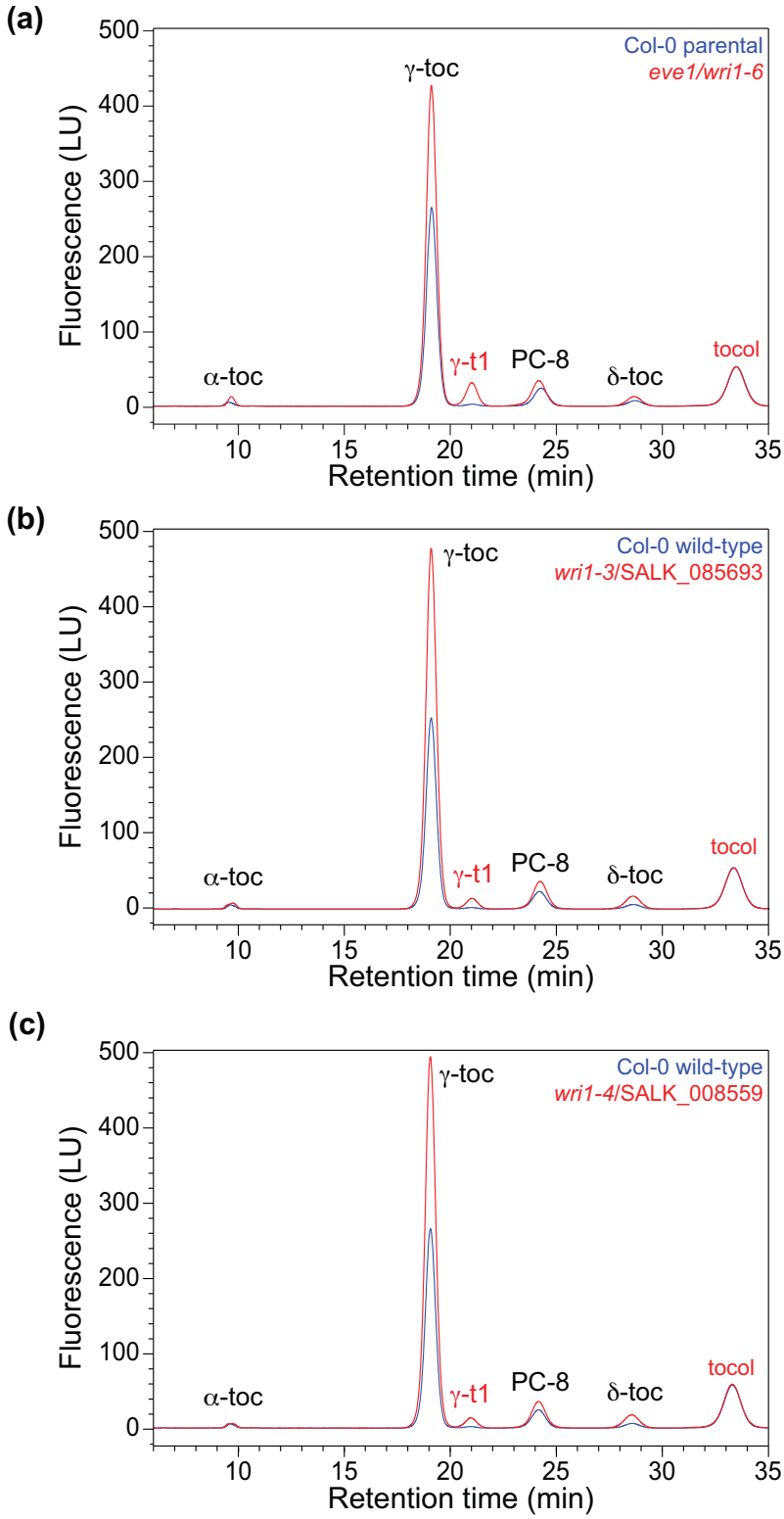


Fig. S4. Representative seed tocochromanol profiles of independent *dgat1* mutants. NP-HPLC-FLD chromatograms of lipid extracts prepared from *dgat1-4* (**a**, **d**), *eve4* (*dgat1-3*; **b**, **e**), and *dgat1-2* (ABX45; **c**, **f**) seeds, respectively (all in red), and their controls (blue). General views (**a**-**c**) and close-up views (**d**-**f**). Extracts prepared for panels **a** to **c** are independent from extracts prepared for panels **d** to **f**. Asterisks in **d** and **f** indicate the presence of a second additional compound that is likely δ-tocomonoenol. Abbreviations: LU, light unit; PC-8, plastochromanol-8; t1, tocomonoenol; toc, tocopherol.

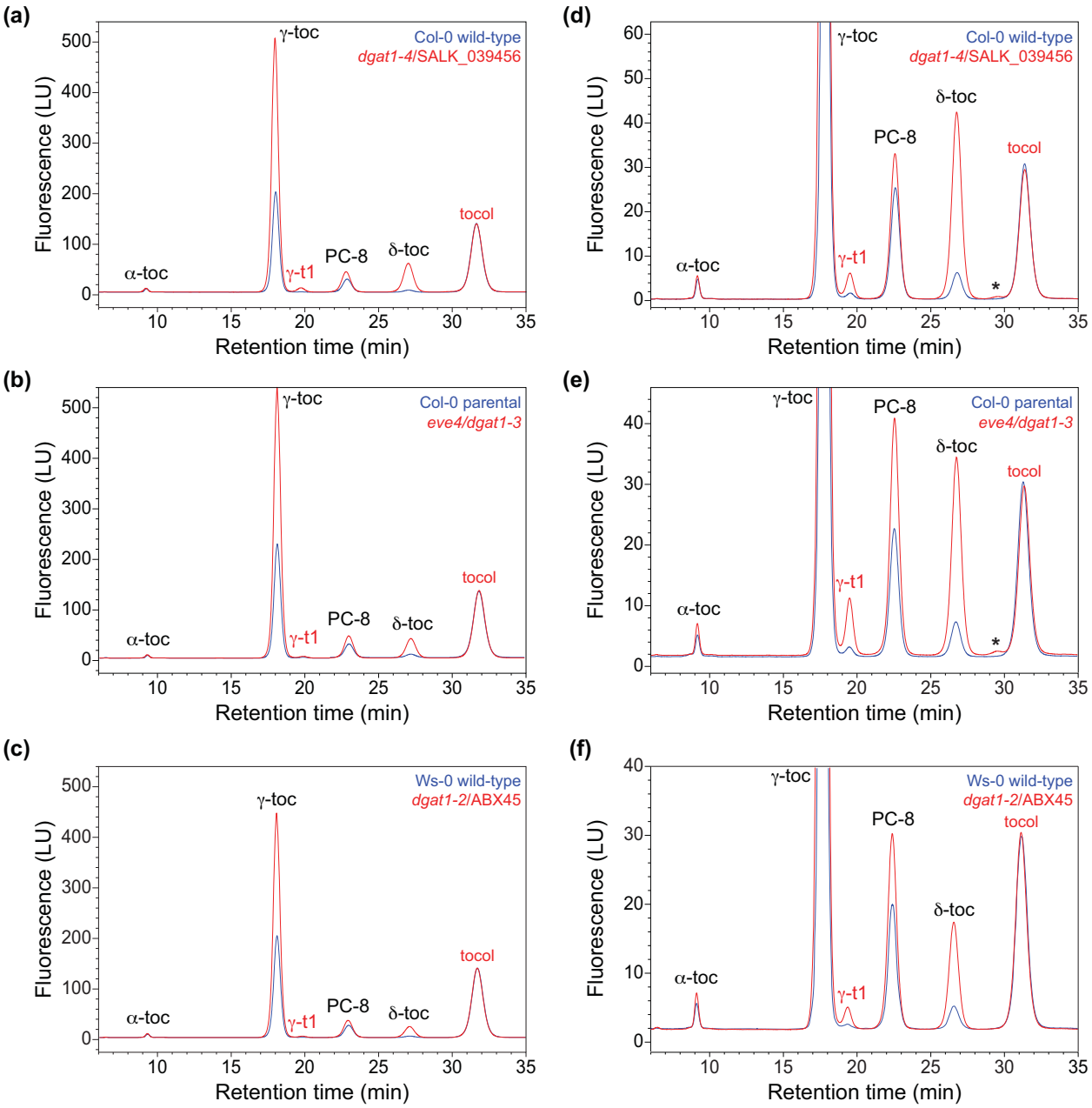
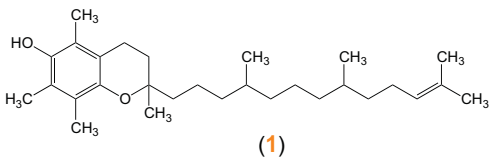


Fig. S5 List of organisms producing tocomonoenols. (**a**) List of species that produce plant- and marine-derived tocomonoenols. This list does not include species that accumulate tocomonoenols originating from their diet such as humans, several fish species, whale and krill. (**b**) Chemical structures of plant-derived α- (1), β- (2), γ- (3), and δ-tocomonoenol (4), respectively, and marine-derived α-tocomonoenol (5).

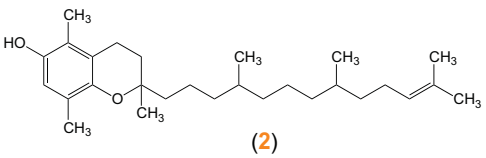
(a)

Tocochromanol	Organism	Species	Tissues	References
11'-12' α-tocomonoenol (1)	palm	*Elaeis guineensis*	seed oil	Puah *et al.*, 2007
	pumpkin	*Cucurbita pepo*	seed oil	Butinar *et al.*, 2011
	rice	*Oriza sativa*	bran oil	Matsumoto *et al.*, 1995
	rapeseed	*Brassica napus*	seed oil	this study
	sunflower	*Helianthus annuus* cv. Sanluca	seed oil	this study
11'-12' β-tocomonoenol (2)	kalanchoe	*Kalanchoe daigremontiana*	leaves	Kruk *et al.*, 2011
11'-12' γ-tocomonoenol (3)	palm	*Elaeis guineensis*	seed oil	Butinar *et al.*, 2011
	pumpkin	*Cucurbita pepo*	seed oil	Butinar *et al.*, 2011
	kalanchoe	*Kalanchoe daigremontiana*	leaves	Kruk *et al.*, 2011
	bean	*Phaseolus coccineus*	etiolated seedlings	Kruk *et al.*, 2011
	Arabidopsis	*Arabidopsis thaliana*	seed oil	this study
	flax	*Linum usitatissimum*	seed oil	this study
	rapeseed	*Brassica napus*	seed oil	this study
11'-12' δ-tocomonoenol (4)	kiwi	*Actinidia chinensis*	fruit pulp & peels	Fiorentino *et al.*, 2009
	kalanchoe	*Kalanchoe daigremontiana*	leaves	Kruk *et al.*, 2011
12'-13' α-tocomonoenol (5)	phytoplankton	not determined	-	Yamamoto *et al.*, 2001

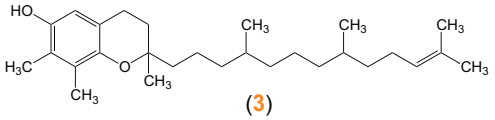
(b)



(1)



(2)



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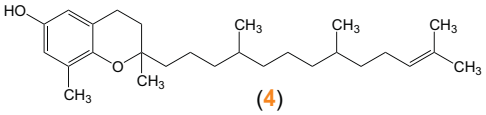
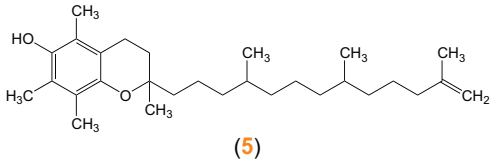
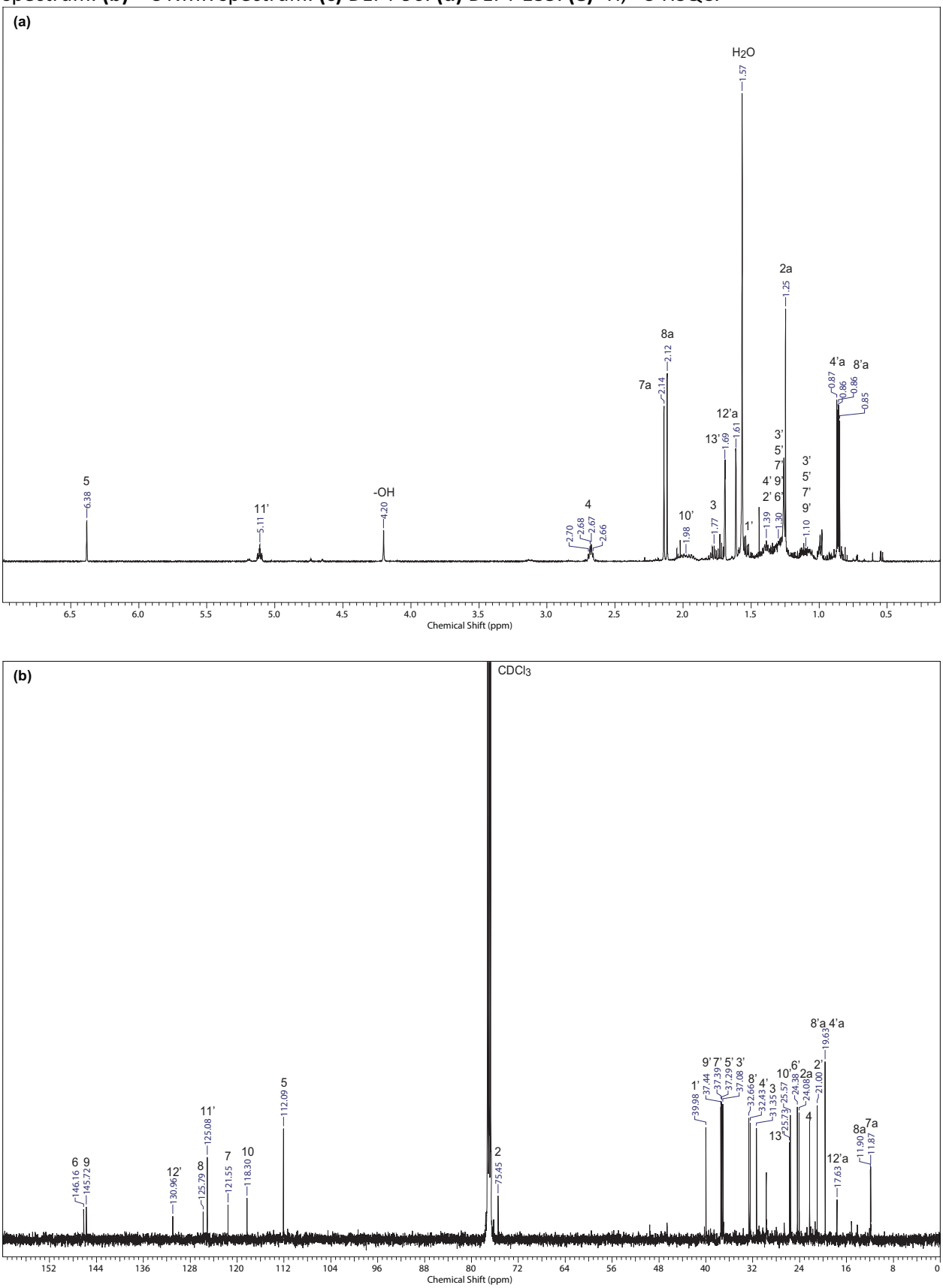


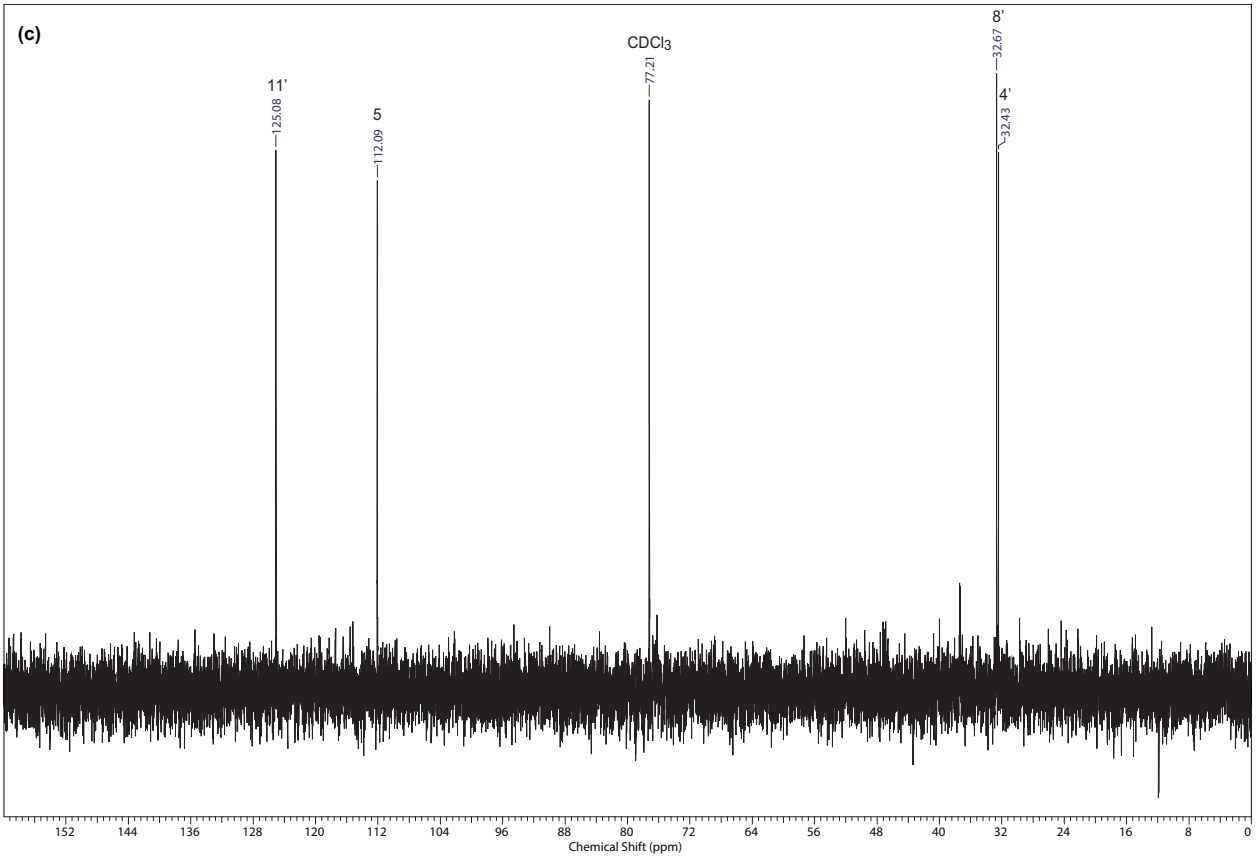
(4)

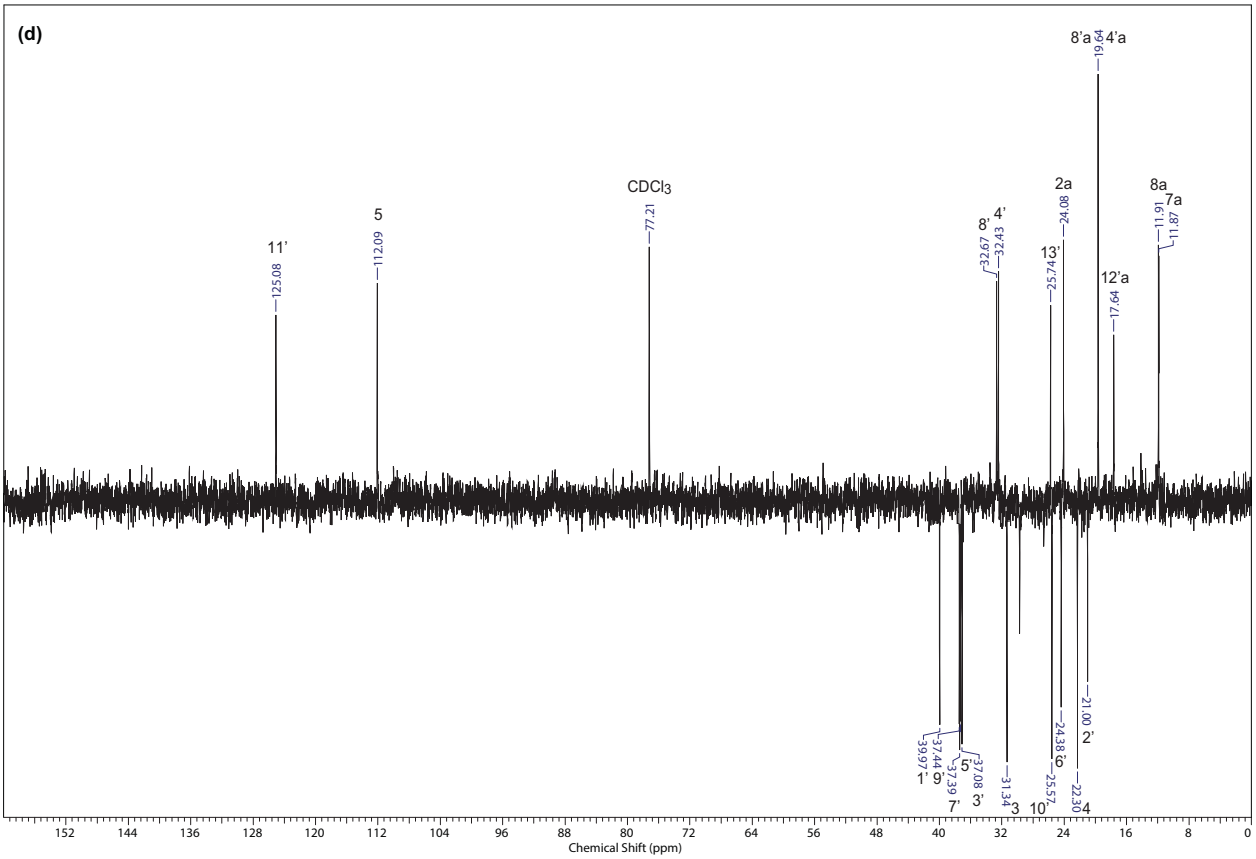


(5)

Fig. S6. NMR spectra for 11'-12' γ-tocomonoenol purified from pumpkin seed oil. (a) ^{1}H NMR spectrum. **(b)** ^{13}C NMR spectrum. **(c)** DEPT 90. **(d)** DEPT 135. **(e)** ^{1}H,^{13}C-HSQC.







(e)

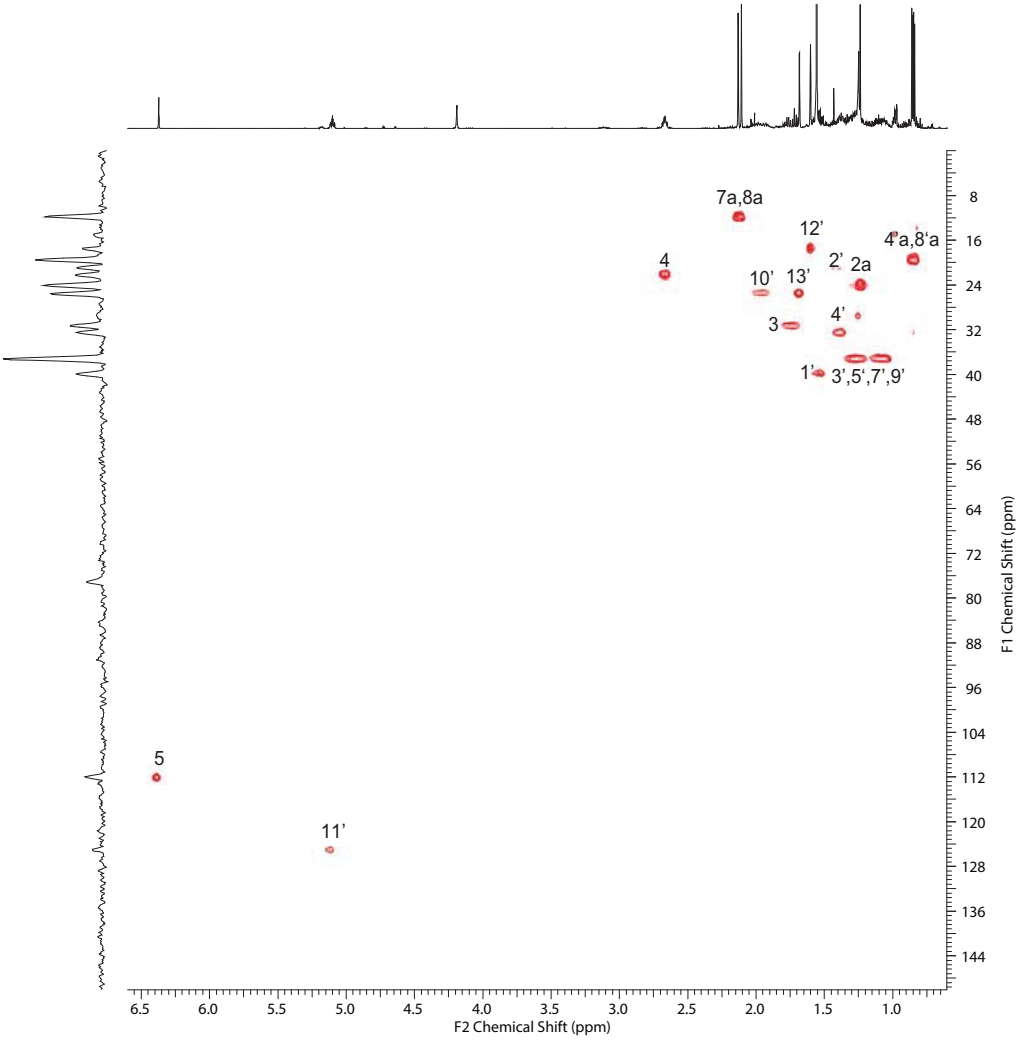


Fig. S7. Determination of the unsaturation position in g-tocomonoenol. Ozonolysis of acetylated 11'-12' **(a)** and acetylated 12'-13' γ-tocomonoenol isomers **(b)** yields [2,7,8-trimethyl-2-(4,8-dimethyl-11-undecanal)-3,4-dihydroxychromen-6-yl] acetate (MW: 430.31) and [2,7,8-trimethyl-2-(4,8,12-trimethyl-12-dodecanal)-3,4-dihydroxychromen-6-yl] acetate (MW: 458.34), respectively. Mass spectrum of the ozonolysis products of γ-tocomonoenol purified from pumpkin seed oil **(c)** and *eve4* seeds **(d)**.

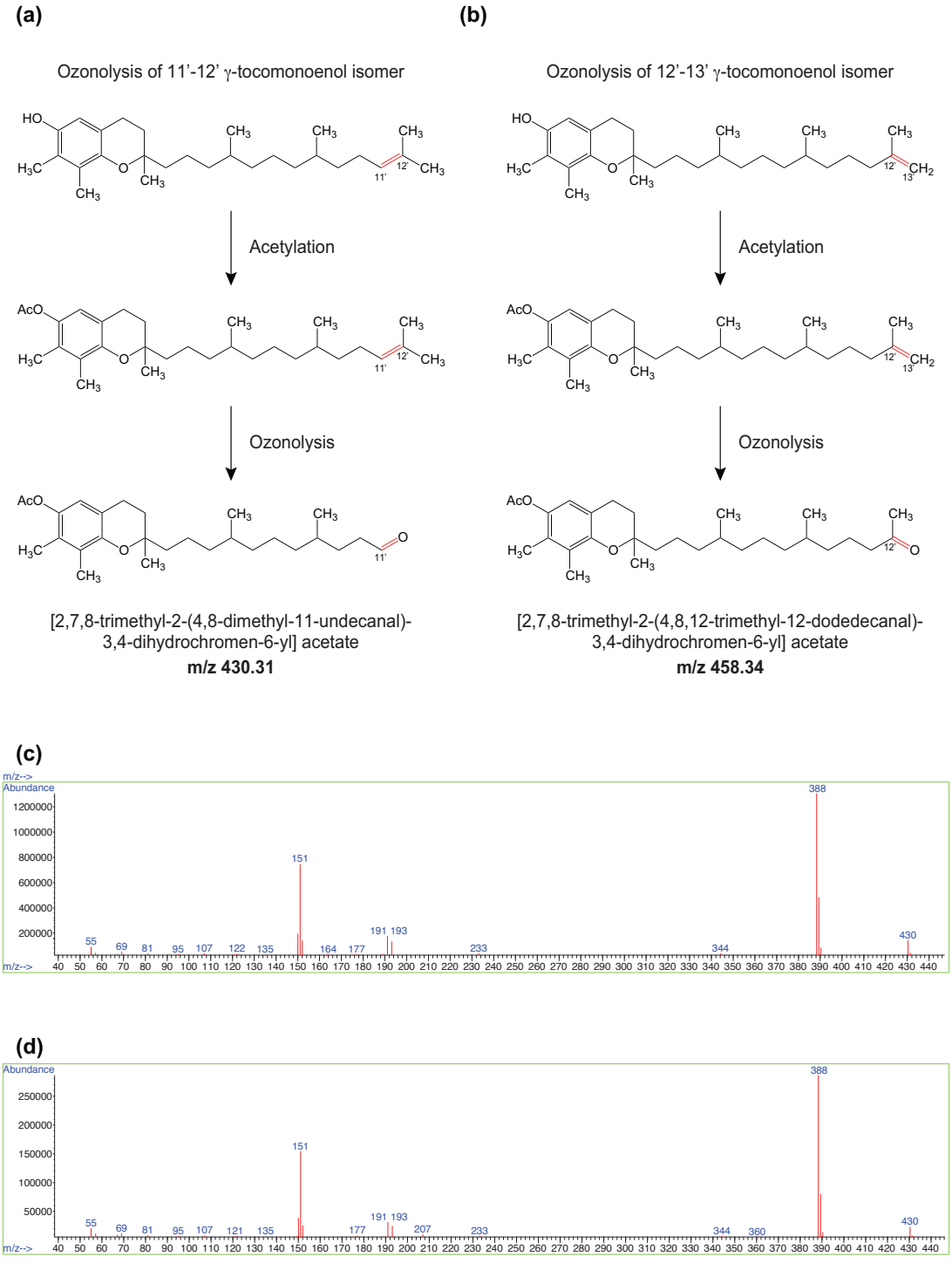


Fig. S8. Mass spectrum of g-tocomonoenol. 70 eV EI mass spectrum of the TMS ether of γ-tocomonoenol purified by HPLC from wild-type Col-0 seeds.

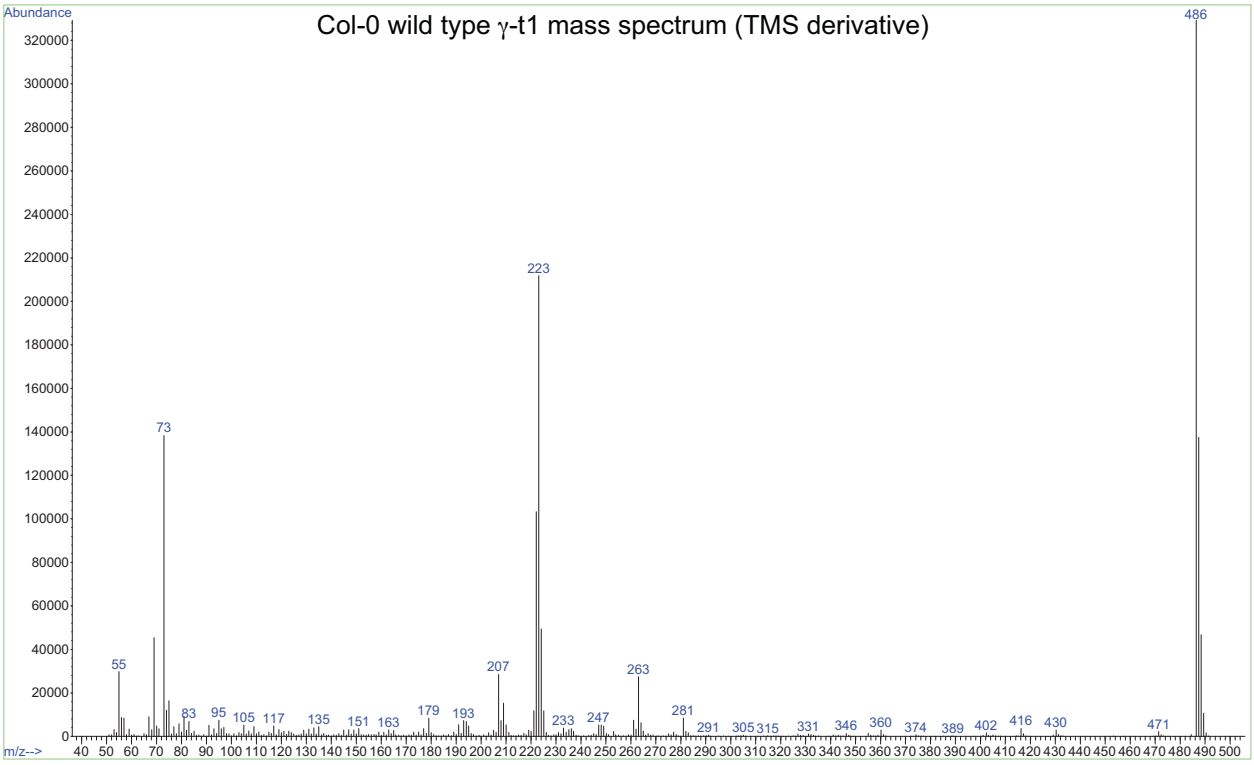
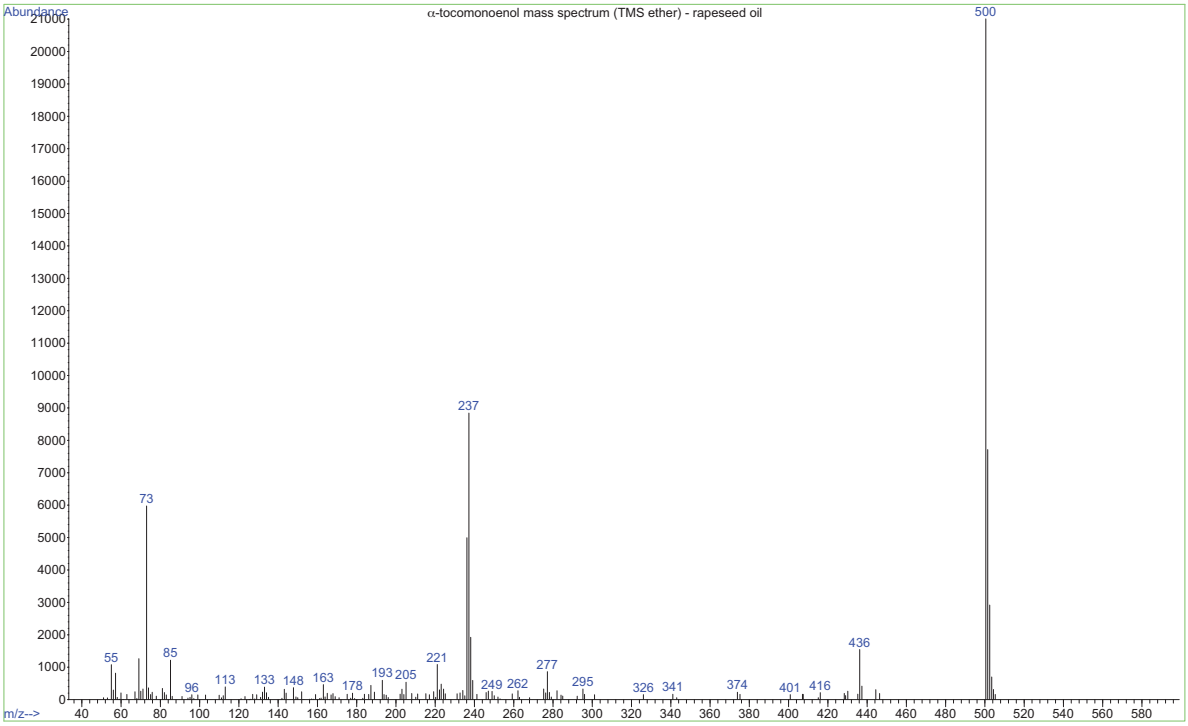
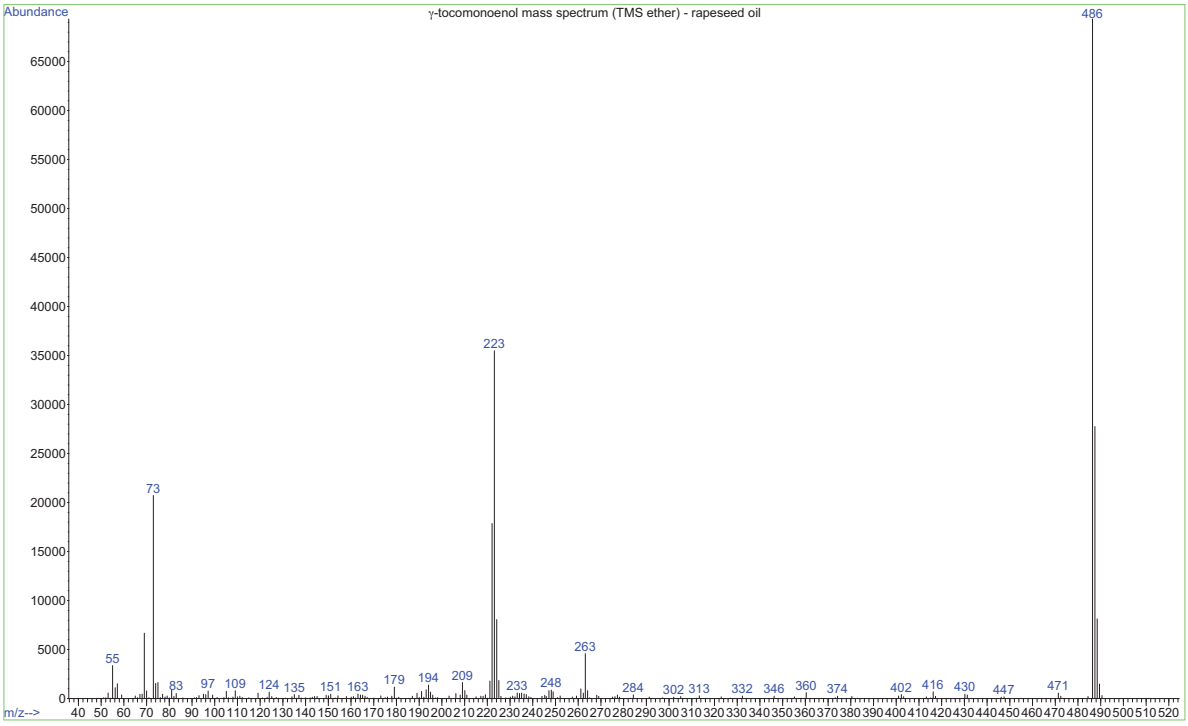


Fig. S9. Mass spectra of tocomonoenols purified from common seed oils. 70 eV EI mass spectrum of the TMS ether of α-tocomonoenol **(a)** and γ-tocomonoenol **(b)** purified from rapeseed oil, of γ-tocomonoenol purified from linseed oil **(c)**, and of α-tocomonoenol purified from sunflower oil **(d)**.

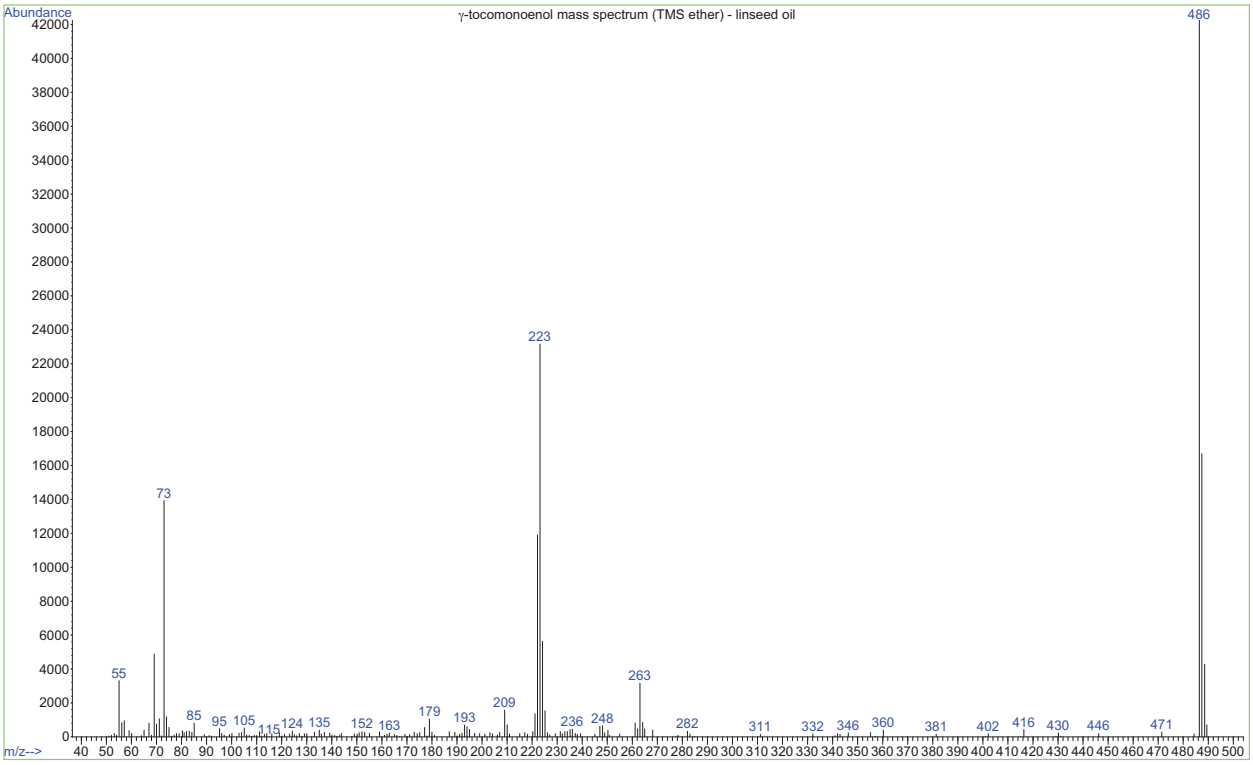
(a)



(b)



(c)



(d)

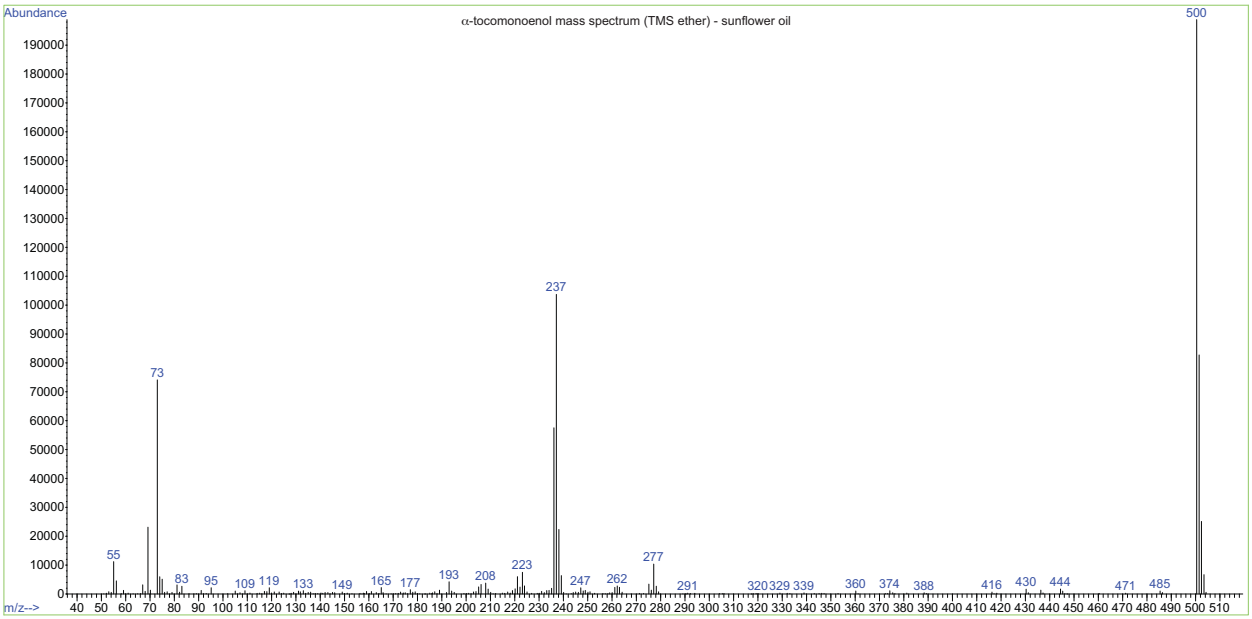
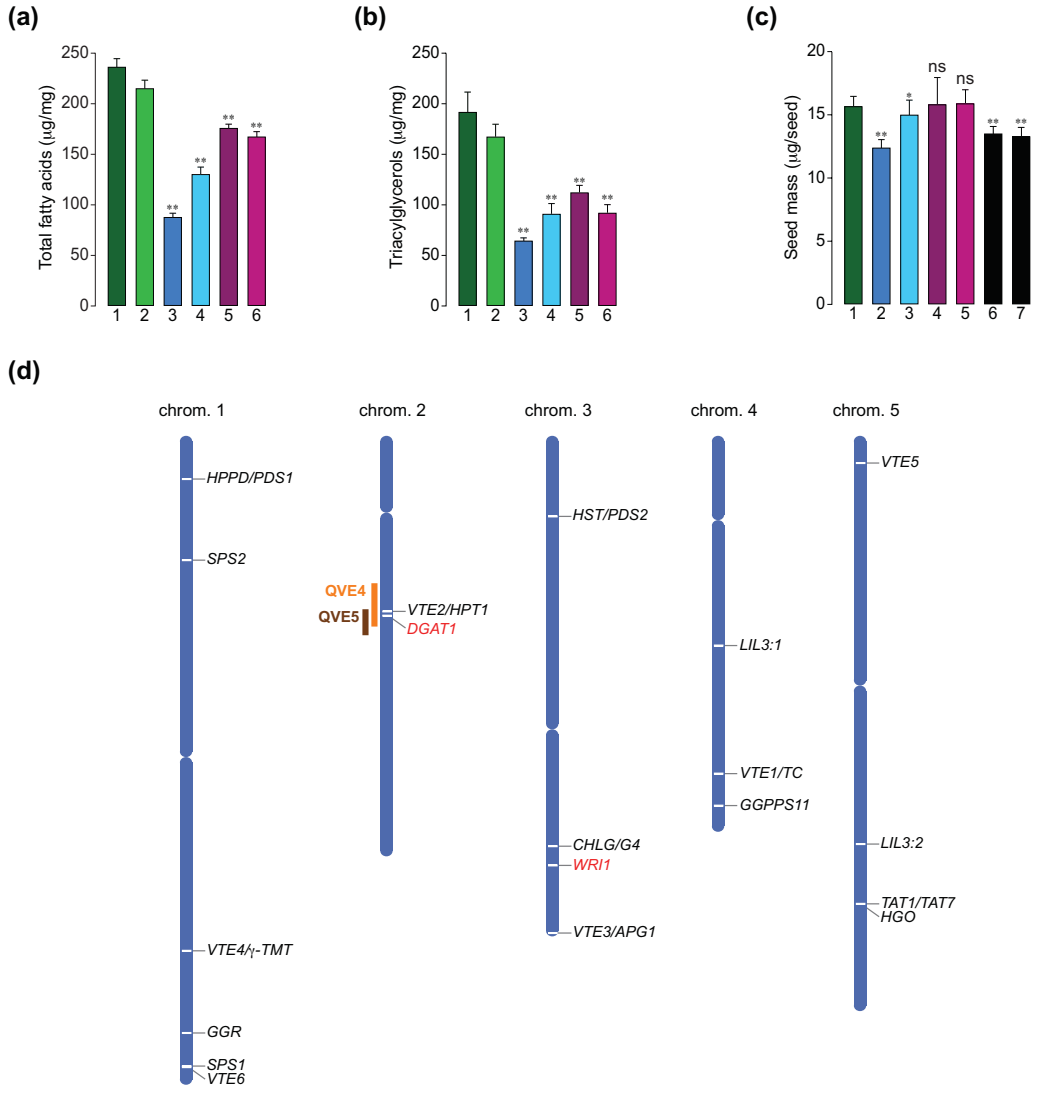


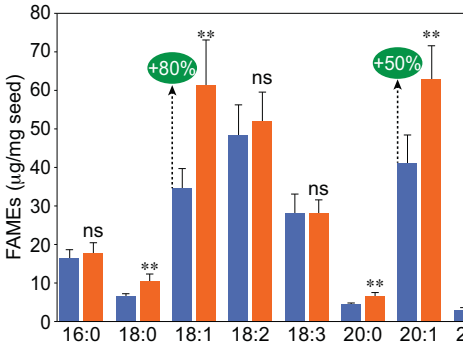
Fig. S10. ***eve1* and *eve4* supplementary data**. Total fatty acids (**a**) and triacylglycerols (**b**) in *wri1* and *dgat1* seeds. Col-0 wild-type (1), Col-0 parental (2), *wri1-4* (3), *eve1* (*wri1-6*; 4), *eve4* (*dgat1-3*; 5), *dgat1-4* (6). Data are the average ± SD of four independent measurements. (**c**) Dry seed masses of *wri1* and *dgat1* mutants. Col-0 wild-type (1), *wri1-4* (2), *eve1* (*wri1-6*; 3), *eve4* (*dgat1-3*; 4), *dgat1-4* (5), *wri1-4 eve4* hybrids (6), and *eve1 dgat1-4* hybrids (7). Data are the average ± SD of three independent measurements. (**d**) Localization of genes involved in tocochromanol metabolism and QVE4 and 5. The two newly identified regulatory genes are coloured in red. Gene positioning was performed with the Arabidopsis Chromosome Map Tool (http://www.arabidopsis.org/jsp/ChromosomeMap/tool.jsp). (**e**) Quantification of seed lipids in Col-0 and L*er* seeds according to three different protocols: direct transmethylation of intact seeds, transmethylation of solvent-extracted lipids, and transmethylation of TLC-purified triacylglycerols. Lipids are the sum of individual fatty acids determined by GC-FID. (**f**) Individual fatty acid amounts in Col-0 (blue bars) and L*er* (orange bars) of TLC-purified TAGs. Fatty acid amounts are the average ± SD of 4 independent measurements for (**e**) and (**f**). Percentages in green circles show the increase of the specific fatty acids in L*er* versus Col-0. Asterisks indicate statistically significant differences using Student's *t* test (**, P<0.01; *, P<0.05) while ns (not significant) indicates P>0.05.



(e)

	Col-0	L*er*	Col/L*er*
Intact seeds	214.9 ± 19.7	258.0 ± 7.8	0.83**
Solvent-extracted lipids	248.2 ± 28.6	286.5 ± 15.6	0.86*
TLC-purified TAGs	187.5 ± 29.0	248.1 ± 36.9	0.75*

(f)



Methods S1. Arabidopsis transgenic *promAt1g51850:dao1* line.

The Arabidopsis EMS-mutagenized plants screened for seed tocochromanol were originally produced to perform a screen on plant defences and carry a T-DNA with the promAt1g51850:*dao1* construct in the Col-0 accession. The cauliflower 35S terminator sequence from pAeq-Hyg was cloned into the XbaI/SacI sites of pGreen0229 (http://www.pgreen.ac.uk/JIT/JIT_fr.htm), yielding plasmid pGreen-T35. The *dao1* sequence (Erikson et al., 2004) was amplified from plasmid pRLM208qcz (BASF Plant Science, Limburgerhof, Germany) using the primers DaoF-EcoRI-F (5'-AATTGAATTCATGCACTCGCAGAAGCGCGTC-3') and DaoR-XbaI-F (5'-AATTTCTAGACTACAACTTCGACTCCCGCGCC-3') introducing restriction sites for *Eco*RI and *Xba*I. The PCR-fragment was cloned into the *Eco*RI/*Xba*I sites of pGreen-T35 to create the vector pGreen-dao1:T35. Subsequently, 853 bp of the promoter region of the PAMP-responsive gene *At1g51850* were amplified using primers At1g51850promF-ClaI (5'-AATTATCGATATGTGATTTTATGGGAAAGCAATCTTGTT-3') and At1g51850promR-E5 (5'-AATTGATATCTGTTCTCCTTACTGTCCACAGGAGAGC-3') and cloned into the *Cla*I and *Hind*III sites of pGreen-dao1:T35, fusing the promoter to the *dao1* coding sequence. The construct together with helper plasmid pSOUP were transformed into *Agrobacterium tumefaciens* GV3101 pMP90 via electroporation. *Arabidopsis thaliana* Columbia-0 plants were transformed using the floral dip method described and transformed seedlings were selected after BASTA selection. Homozygous line 9.3.3 containing a single copy of the *dao1*-cassette was selected based on segregation on BASTA-containing half-strength MS medium and southern blot analysis. For Southern blot analysis, genomic DNA was digested with *Eco*RV and probed with the dao1-fragment obtained via PCR from pRLM208qcz using primers DaoF-EcoRI-F and DaoR-XbaI-F.

References S1. References about tocopherol QTL and GWA studies.

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