Supplemental Data. Linkies et al. (2009). Ethylene interacts with abscisic acid to regulate endosperm rupture during germination; a comparative approach using *Lepidium sativum* (cress) and *Arabidopsis thaliana*.



Supplemental Figure 1. TAGGIT analysis of functional categories in the Lepidium sativum FR1 transcriptome datasets. The seed-specific TAGGIT workflow (Carrera et al. 2007; Holdsworth et al., 2008) was applied to Lepidium transcriptome datasets to provide proportional representations of genes in functional categories. (A, B) Comparison of CON array lists: (A) All transcripts belonging to TAGGIT functional categories (6557 of 22025 transcripts, Supplemental Dataset 3 online) and significantly regulated transcripts belonging to TAGGIT functional categories (487 of 1350 transcripts, Supplemental Dataset 5 online). (B) Transcripts belonging to TAGGIT functional categories that are regulated in a significantly different way in the radicle and endosperm at 8 h and 18 h (CON-array, Supplemental Dataset 5 online). (C) Comparison of ABA array lists: All transcripts belonging to TAGGIT functional categories (6036 of 19794 transcripts, Supplemental Dataset 4 online) and significantly regulated transcripts belonging to TAGGIT functional categories (1243 of 3530 transcripts, Supplemental Dataset 6 online). (D) Key to the colour representation of TAGGIT functional categories (Holdsworth et al., 2008). (A-C) The TAGGIT analysis suggests that ethylene-related transcripts are important for germination and for counteracting the ABA inhibition. Supplemental Datasets 3-6 provide lists of the transcripts from which the subsets belonging to different TAGGIT functional categories can be extracted by sorting for the TAGGIT column. For example, the numbers (percentages) of transcripts in the functional category 'ethylene' are for 'all transcrips' and 'regulated transcripts' in (A) 144 (2.2%) and 16 (3.3%) for CON and in (B) 131 (2.2%) and 32 (2.6%) for ABA.



Supplemental Figure 2. The effect of ACC and ABA on the germination of Arabidopsis thaliana: Wild type (Col) and ethylene-related mutants (aco2, ctr1). (A-E) Time course analyses of testa and endosperm rupture of wild type (WT), the ACO2-deficient mutant aco2 and the loss-of-function signaling mutant ctr1. (A) Testa and (B) endosperm rupture under optimal conditions (CON, no hormone addition to the medium). (C,D) Testa rupture of seeds incubated in the presence of (C) ABA or (D) ABA+ACC. (E) Endosperm rupture of seeds incubated in the presence of ABA or ABA+ACC. Incubation conditions: continuous light, 24°C, no cold-stratification. Medium additions, as indicated: 1 μ M ABA, 1 mM ACC. Mean values ± SE of three x 50 seeds are presented.

ATTGGTTTTGGATCAAAGGAGGAGGAGGAGGAGGAGGAGTGCAGGAGTGGAGGAGGAGTGGAGGAGGAGGAGGAGGAGGAGG	AGCA 150 AAGA AGAG CCAA CCAA CCAA CCAA CCAA CCA
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AtACO1 SA* LesaACO1

----- Cosubstrate-binding motif (ascorbate)

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	GGGCCCA GGGCCCA ⁶¹⁰	CACT	GATGC GATGC 520	TGGAG	G TA TO	ATCT	TG T TC TG T TC 640	TTTC	AAGAC 650	GAC	AAGG	57 TA 660	GTGG GTGG	TCT	CCAG CCAG	CTI	CTT CTT 6	AAGGA 80
	TGGTGAC TGGTGAC ⁶⁹⁰	TGGA TGGA	TTGAT TCGAT 70	GTTCC GTTCC 0	TCCTC TCCTC	TCAA TCAA	CCACI	CTAT CCAT 720	FG TC A FG TC A	TCA TCA 730	АТСІ АТСІ	TGG TGG	TGAC TGAC 740	САА САА	C T T G C T T G	A G G A G G 750	TGA TTA	FAACC FAAC T 76
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	GPTEGTKV	SNYP	PCPKP	EMIKG	180 180	TDAGG		PODDKI	ISGLO	200	DGDV	110V	PPLN	HST	22 22 V T N T	GDC	LEV	230 230
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Brassica	ceae ACC C	Oxidase 4 a	lignments: A	At ACO4 (A	At1g05010) and Les	a <i>ACO4</i> (G	Q221033)	
₁start	10	20	30	40		50	60	70	
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ACGCTTG	TGAAAACTO	GGGCTTCT	TTGAGTGTG	IGAACCAT	GGGATTTC	ACTCGAG	CTTTTGGAC	AAAGTGG	AGA
1	60	170	180	190	200		210	220	
GATGACC	AAGGAACAI	TACAAGAA	GTGCATGGA	GAGAGAT	TCAAGGAA	TCGATTA	AGAACAGAG	GTCTTGA	CTC
230	240	250	260	2	70	280	290	30	0
CTTCGCT	CTGAAGTCA	ACGACGTT	GACTGGGAA	CCACTTT	СТАССТСА	AGCACCT	FCCCGTCTC	ΤΑΑΤΑΤΟ	TCC
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	390	400	410	420		430	440	450	
GAGCTAC GAGTTAC	TGGATCTGC TGGATTTG1	C TG TG CG AGA	A A T C T C G G T 1 A A T C T C G G A 1	TAGAGAA TAGAGAA	G G G T T A T I A G G T T A T C	TAAAAAAA	G G T G T T T T A A G T G T T T T A	CGGGTCG	AAA AAA
460	470	480	4	90	500	510	520		530
GACCGAC GTCCAAC	T T T T G G A A C T T T T G G A A C	CAAAGTCA CAAAGTGA	G C A A T T A T C (G C A A T T A T C (CACCTTGT	СС ТААТСС СС ТАААСС	GGACCTA	G T C A A G G G 1 A T C A A A G G G	C T C C G A G C T T C G A G	CCC CTC
	40	550	560	570	580		590	600	
CACCGAC TACCGAT	GCCGGCGGC GCCGGTGGA	CATCATCCT	CCTCTTCCA GTTATTTCA	AGACGACA AGACGATA	AAGTCAGI AAGTTAGI	GGACTTC	A G C T T C T T A G C T A C T T A	AAGACGG	CGA AGA
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	770	780	790	800		810	820	830	
TCCGGGA.	AGCGACTCI	IGTTATTTT	TCCGGCGCCC	GGAGCTGA	TCGGAAAA	GAAGCAG	AGAAGGAGA	AGAAAGA	GAA
840	850	860	8	70	880	890	900		910
TATCCGA	GATTTGTGI	TTGAAGAT	ТАСАТБААА	CTCTACTC	TGCTGTCA	AGTTTCA	GGCCAAGGA	ACCAAGG	ТТТ
9	20	930	940	950	960		970 974		
AAGCCAT	GAAAGCTAI	IGGAGACAA	CTGTGGCCA	CAATGTT	GGACCATI	GGCCACT	GCGTGA		
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80	90	100	110		20	130	140	15	
RSEVNDVD	WESTFYLKH	HLPVSNISD	VPDLDDDYR	LMKDFAG I	KIEKLSEE KLEKLAEE	LLDLLCE	NLGLEKGYI NLGLEKGYI	KKV FYGS KKV FYGS	K R P K S P
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	240	250	260	270	280		290	300	
/EHRVLSQ /EHRVISQ	TDGEGRMS TDGEGRMS	IAS FYNPGS IAS FYN	DSVIFPAPEI	JIGKEAEK	EKKENYPF	FVFEDYM	KLYSAVK FÇ) A K E P R F E	AMK
310	320 324				_	- Fe ²⁺ -bin	ding motifs (A	CC and O ₂ b	bindin
IETTVANN	VGPLATA *					Cosubstr	ate-binding m	otif (ascorba	te)

D Brassicaceae ACC Oxidase 2 orthologous cDNA alignments: Lineage I (Arabidopsis, Lepidium): At ACO2 (At1g62380) and Lesa ACO2 (GQ221032) Lineage II (Brassica, Sisymbrium): Br ACO1 (AJ309322) and So ACO2 (EU689115)

Identity	∔ start	10	20	30	40	50		50	70	80	90
AtACO2 LesaACO2 BrACO1 SoACO2	ATGG AGA ATGG AGA ATGG AGA	AGAACAT AGAACAT AGAACAT	GAAGTTTCC GAAGTTTCC TAAGTTTCC	AG T AG T AG A (AA T TG T TG A (GG T TG T AG A (CTTGTCCAAG CATGTCCAAA CTTGTCCAAG	CT CAATGG CT CAATGG 1 CT CAT TGG 1	GAAGAGAGAG GAAGAGAGAG GAAGAGAGAG	AGACCAAAC AGACCAAAC AGACCAAAC	CATGGCTCTA. CATGGCTTTA. CATGGCTTTG.	ATCAATGAA ATCAATGAT ATCAACGAT AT	GCT TG TGAG A GCT TG TGAG A GCT TG TGAG A GCT TG TG – TA
Identity	100	110	12	0 1	30	140	150	160	170	180	190
AtACO2 LesaACO2 BrACO1 SoACO2	ATTGGGG ATTGGGG ATTGGGG ATTGGGG	CTTCTTT CTTCTTT CTTCTTT CTTCTTT	GAG AT AGTG GAG AT AGTG GAG AT AGTG GAG AT AGTA	AACCATGGAT AATCATGGAT AACCATGGTT AACCATGGGT	TACCACATG TACCACATG TACCACATG TACCACATG	ACTTAATGO ACTTAATGO ATTTGATGO ATTTATTGO 240	ACAAGATCO ACAAGATTO ACAACGTCO ACAACGTCO 250	G AG A AG A TG G AG A AG A TG G AG A AG A	ACAAAGGACC. ACAAAAGGATC. ACAAAGGAAC. ACAAAGGAAC.	ATTACAAGA ATTACAAGA ATTACAAGA ATTACAAGA ATTACAAGA 280	CATGCCAA CTTGTCAA TATCAATG AATCAATG
Identity	200		210	220	230	210	2.70	200	270	200	250
AtACO2 LesaACO2 BrACO1 SoACO2	GAACAAA GAACAGA GAACAAA GAACAAA	AGTTCAA AGTTCAA AGTTCAA AGTTCAA 300	TGACATGCT TGAGACGCT CGACATGCT CGACATGCT 310	CAAGTCCAA TAAGTCCAA CAAATCCAA CAAGTCCAA 320	AGG TT TGG AT AGG TT TGG AT AGG TT TGG AA AGG TT TGG AA 330	AATCTTGAC AATCTTGAC AATCTTGAC AATCTTGAC 340	ACAGAAGT ACAGAAGT CGAGAAGT AGAGAAGT 350	CGAAGATGT TGAAGATGT TGAGGATGT TGAGGATGT 3	CGATTGGGAA. TGATTGGGAA. TGATTGGGAA. CGATTGGGAA. ⁶⁰ 33	AGCACTTTC AGTACTTTC AGCACTTTC AGCACTTTC 7º	TACGTTOGTC TACGTTOGTC TACCTTOGTC TACCTTOGTC 380
Identity	1000000						00.2000.000				
LesaACO2 BrACO1 SoACO2	ACCTCCC ATCTCCC ATCTCCC ATCTCCC 390	TCAATCC TCAATCC TCAATCC TCAATCC 400	AATCTCAAT AACCTCAAC AATCTCTAC AATCTCCAC 410	GACATTTCAC GACATTTCTC GACATTCCTC GACATTCCTC 420	ATGTGTCTG ATATTTCTG ATATGTCTG ATATGTCTG 43	ATGAATACA ATGAATATA ATGAATACO ATGAATACO	IGG ACGG CC/ IGG ACGG CG/ IGG ACGG CC/ IGG ACGG CC/ 440	ATG AAAG AC' ATG AAAG AC' ATG AAAG AT' ATG AAAG AT' 450	TTTGGTAAGA TTTGGGAGGA TTTGGTAAGA TTTGCAAAGA 460	GACTGGAGA GATTGGAGA GATTGGAGA GATTGGAGA 470	ATCTTGCGGA ATCTTGCGGA ATCTTGCTGA ATCTTGCGGA 480
Identity			'		-	_		_			
AtACO2 LesaACO2 BrACO1 SoACO2	GGATTTG GGATTTG GGATTTG GGATTTG	TTGGATC TTGGATC TTGGATC TTGGATC	TACTGTGTG TATTGTGTG TATTGTGTG TATTGTGTG TATTGTGTG	AGAATCTAGO AGAATCTAGO AGAATTTAGO AAAATCTAGO	G TTAG AG AA ATTAG AG AA G TTAG AG AA G TTAG AG AA	AGGG TATT AGGG TACT AGGG TACT AGGG TACT	GAAGAAAG GAAGAAAG GAAGAAAG GAAGAAAG	TGTTTCATGO TGTTTCGTGO TGTTTCATGO TGTTTCATGO	GAACAAAAGG GAACTAAAGG GAACAAAAGG GAACAAAAGG	CCCAACCTT TCCAACCTT TCCAACCTT TCCAACCTT	TGGGACAAAG TGGGACCAAA TGGGACTAAG TGGGACCAAG
Identity	450	_		510	520	550	510	510	-	570	
AtACO2 LesaACO2 BrACO1 SoACO2	G TGAG CA G TGAG TA G TGAG CA G TGAG CA	ATTATCC ATTATCC ACTATCC ATTATCC 590	ACCATGTCC ACCTTGTCC AGCTTGTCC ATCTTGTCC 600	TAAACCAGAG TAAACCAGAG TAAGCCAGAG TAAACCAGAG 610	ATGATCAAA ATGATCAAA ATGATAAAA ATGATCAAA 620	GGTCTTAGC GGTCTTAGC GGTCTTAGC GGTCTTAGC GGTCTTAGC 630	GCCCACAC GCCCACAC GCCCACAC GCCCACAC GCCCACAC	TGATGCAGG TGATGCTGG TGATGCAGG TGATGCAGG 650	AGG CAT CAT C' AGG TAT CAT C' AGG CAT CAT C' AGG CAT CAT C') 660	TTG TTG TTT TTG TTG TTT TTG TTG TTT TTG TTATTT)	CAAGACGACA CAAGACGACA CAAGATGACA CAAGACGACA
Identity			<u> </u>	_	_				<u>'</u>		
AtACO2 LesaACO2 BrACO1 SoACO2	AGGTCAG AGGTTAG AGGTCAG AGGTCAG 680	TGG TCTC TGG TCTC TGG TCTC TGG TCTC 690	CAG CTTCTT CAG CTTCTT CAG CTTCTT CAG CTCCTT 700	AAAGATGG TC AAGGATGG TC AAAGATGG TC AAAGATGG TC 710	ACTGGATTG ACTGGATCG ACTGGATTG ACTGGATCG 720	ATGTTCCT ATGTTCCT ATGTTCCT ATGTTCCT 7	CTCTCAAC CTCTCAAC CACTCAAC CACTCAAC	CACTCTATTO TACTCCATTO CACTCTATTO CACTCCATTO 740	GTCATCAATC' GTCATCAATC' GTCATCAATC' GTCATCAATC' 750	TTGGTGACC TTGGTGACC TTGGTGACC TTGGTGACC 760	AA CTTG AGG T AA CTTG AGG T AA CTTG AGG T AA CTTG AGG T 770
Identity	<u></u>			_		-					
AtACO2 LesaACO2 BrACO1 SoACO2	GATAACC TATAACT GATAACT GATAACC	AACGGGA AATGGCA AACGGCA AACGGCA	AG TATAAG A AG TACAAG A GG TACAAG A AG TACAAG A	GTG TG CTG C <i>I</i> GTG TG ATG C <i>I</i> GTG TG ATG C <i>I</i> GTG TG ATG C <i>I</i>	ACCGTGTGGT ACCGTGTGGT ACCGTGTGGGT ACCGTGTGGGT	GACTCAAC GACTCAAA GACTCAGA GACTCAGA	AG AAGG AA AG AAGG AA AG AAGG AA AG AAGG AA AG AAGG AA	ACAGGATGT ACAGAATGT ACAGAATGT ACAGGATGT	CGG TTG CATCO CCATTG CATC CAATTG CATC CCATTG CCTCO	GTTTTACAA ATTCTACAA TTTCTACAA GTTCTACAA	CCCGGGAAGC TCCAGGAAGC CCCGGGAAGC CCCGGGAAGC
Identity	780	191			810 810	820	830	540	850	800	*/*
AtACO2 LesaACO2 BrACO1 SoACO2	GATGCGG GATGCTG GATGCTG GATGCCG ⁸⁸	AGATC AGATTGC AGATC AGATCGC 0	TCACCAGCT TCCAGCT TCTCCAGCT TCCAGCT 890	ACTTCG CTTC ACATCTCTTC TCATCG CTTC TCATCG CTCC 900	TCGAGAAAG T-TGGAAAG CCTGTAAAG CTGGGAAAG 910	-ATTCCGAC GACTCTGAC AAA-CCGAC AAAGC-GAC 920	TACCCGAG TACCCAAG TACCCGAG TACCCGAG	TTTCGTCTT' TTTCGTCTT' TTTTGTTTT' TTTTGTCTT' 940	TGATGACTAC. TGATGACTAC. TGATGACTAC. TGATGACTAC. 950	ATG AAG CTT ATG AAG CTC ATG AAG CTC ATG AAG CTC 960	TATGCAGGGG TATGCAGGAG TATGCTGGGG TACGCTGGGG 968
Identity	TCAACTT	TCACCCC	AACCACCCA	CCCTTCCCA	CANTCARCA	ATCOTTOT	CACTTACA		COTACACCAC	CCCTACACA	CUTTCTA
LesaACO2 BrACO1	TCAAGTT	TCAGCCT	AAGGAGCCA	AGGTTCGAGO	CAATGAAGA	ATGCTTCTC	CAGTTACA	GAGCTGAAT	CCCACAGCAG	CCGTAGAGA	CTTTCTAA

Supplemental Figure 3. Brassicaceae ACC oxidase (ACO) sequence comparisons. (A-C) Alignments of Arabidopsis thaliana Col and Lepidium sativum FR14 mRNA (cDNA) and amino acid sequences of the ACO orthologs. The conserved InterPro (www.ebi.ac.uk/interpro/) domain regions of 2OG-Fe(II)-oxygenases are underlined in red. The start and stop codons in the cDNA sequences are underlined and marked as start and stop in black. The conserved active binding site motifs for the substrates ACC and O_2 as well as the cosubstrate binding site for ascorbate (Seo et al. 2004) are underlined in blue in the amino acid sequences and indicate that these proteins are functional ACOs. (A) Arabidopsis ACO1 (At2g19590) and Lepidium ACO1 (GQ221031). (B) Arabidopsis ACO2 (At1g62380) and Lepidium ACO2 (GQ221032). (C) Arabidopsis ACO4 (At1g05010) and Lepidium ACO4 (GQ221033). The corresponding Arabidopsis ACOs and Lepidium ACOs are true orthologs based on the highest BLAST hits and the molecular phylogenetic analysis. (D) Alignment of Brassicaceae ACO2 orthologs which show transcript expression pattern in seeds (Fig. 7F) that are associated with germination responses. Brassicaceae lineage I (Arabidopsis thaliana (At), Lepidium sativum (Lesa)) and lineage II (Brassica rapa (Br), Sisymbrium officinale (So)) cDNA sequences are presented. At least three independent cDNA clones were sequenced for each Lepidium ortholog.

+1 +++++1 40 90 100

Time [h]

Supplemental Figure 4

 40 90 100

Time [h]





 40 90 100



Supplemental Figure 4. Analysis of transcript expression by gRT-PCR and microarray analysis in specific seed tissues of Lepidium sativum FR1 during germination. Time course transcript expression data are presented for endosperm caps (Cap) and radicles (Rad) dissected from seeds incubated at 24°C in continuous light in medium without (CON) or with 10 μ M ABA added. For qRT-PCR relative $\Delta\Delta C_t$ expression values based on the comparison with validated constitutive transcripts are presented. Lepidium transcripts named with the prefix 'Lesa' were analysed by gRT-PCR primers designed on the basis of their cloned cDNA sequences. Lepidium transcripts without this prefix in their name were analysed with a gRT-PCR primer design based on Arabidopsis cDNA sequences. Primer sequences for the qRT-PCR are presented in Supplemental Table 1 online. (A) ABA 8'-hydroxylases: Four CYP707A genes are known in Arabidopsis and all provided expression results in the Lepidium seed arrays. Two Lepidium cDNAs were cloned (Lepidium CYP707A2 and CYP707A3) and on the basis of their sequence analyses, represent putative Lepidium orthologs of Arabidopsis CYP707A2 and CYP707A3, respectively. (B) Ethylene receptors. (C) Ethylene signaling components. (D) Ethylene signaling repressor CTR1 (Constitutive Triple Response1); the cDNA of the putative Lepidium ortholog CTR1 was cloned. (E) CTR1-like serine/threonine protein kinase. (F) GPCRtype G protein ABA receptors GTG1 and GTG2 and their interacting G protein α subunit GPA1. (G) RAV1, an AP2/EREBP-type transcription factor with an ABI3/VP1-like domain. (H) PL1, Pectate lyase1. (I) ARL, Argos-like, putative cell expansion gene. (J) EF-1-alpha, Transcription elongation factor 1-alpha. Mean values +SE of four independent biological RNA samples obtained from 1000 endosperm caps or 100 radicles from seeds with ruptured testa, but intact endosperm are presented for the qRT-PCR results. Normalized microarray differences are presented for comparison.



Supplemental Figure 5. Analysis of the transcript expression of key ABA metabolism genes, in whole *Lepidium sativum* FR14 seeds, by qRT-PCR following treatment with ACC or NBD. Time course transcript expression data are presented for whole seeds incubated at 24°C in continuous light in absence (CON) or presence of the ethylene precursor ACC (1 mM), the ethylene action inhibitor NBD (100 μ I/I applied via the gas phase), or the combination NBD+ACC. For qRT-PCR, relative $\Delta\Delta C_t$ expression values based on the comparison with validated constitutive transcripts are presented. Primer sequences for the qRT-PCR are presented in Supplemental Table 1 online. (A) qRT-PCR for transcripts of key regulatory genes for ABA biosynthesis: *NCED9* = *NINE-cis-EPOXYCAROTENOID DIOXYGENASE9*, *SDR1* = *SHORT-CHAIN DEHYDROGENASE REDUCTASE1* (also known as *ABA2*), AAO3 = ABSCISIC ALDEHYDE OXIDASE3. (B) qRT-PCR for transcripts of key regulatory genes for ABA degradation: The Lepidium ABA 8'-hydroxylases *CYP707A2* and *CYP707A3* were analysed. Mean values +SE of three independent biological RNA samples are presented.

Supplemental Methods

aRNA labelling and CATMA microarray hybridization

The *Lepidium sativum* FR1 (Freiburg1) aRNA was labelled and the CATMA microarrays were hybridized according to the method described in Lim et al. (2007). Briefly, 5 μ g of aRNA was reverse transcribed using random nonamers (Invitrogen, UK) and SuperScript II (Invitrogen, UK). The Cy3- and Cy5-labelled cDNA probes were prepared, whereby amino allyl-dUTP was incorporated during cDNA synthesis followed by chemical labeling of the amino allyl-modified cDNA using CyDye NHS-esters (Amersham Biosciences, NJ, USA). Reactions were incubated at 42°C for 2.5 h and terminated by the addition of 2 μ L NaOH and an additional incubation at 37°C for 15 min. The labelled cDNA was neutralised by adding 10 μ L 2 M MOPS buffer and purified using the Qiagen PCR Purification Kit (Qiagen, UK). Cy3- and Cy5-labelling efficiency was quantified using a nanodrop ND Spectrophotometer (NanoDrop Technologies, Rockland, DE). An aliquot, containing 40 pmol of Cy dye label, was used for subsequent microarray hybridisations.

The CATMA microarray slides were prehybridised in 60 mL prehybridisation buffer (1% (w/v) BSA, 5 x SSC buffer, 0.1% (w/v) SDS) at 42°C for 2 h. Cy3- and Cy5-labelled cDNAs were freeze-dried and resuspended in 70 μ L of hybridisation buffer (25% (v/v) formamide, 5 x SSC, 0.1% (w/v) SDS, 0.5% (w/v) yeast tRNA (invitrogen, UK). The microarray slides were washed five times in water and twice in isopropanol and dried by centrifugation at 1500 rpm for 2 min. The cDNA probes were denatured by incubation at 95°C for 5 min and applied directly to the microarray slides that were held in a hybridisation chamber (Corning Life Sciences, Netherlands). The microarrays were covered with a coverslip (Sigma Aldrich, UK) and hybridised overnight at 42 °C in a hybridisation oven. The microarrays were then subjected to the following washings: once in Wash Solution 1 (2x SSC, 0.1% (w/v) SDS) pre-warmed to 42°C for 5 min; once with Wash solution 2 (0.1 x SSC, 0.1% (w/v) SDS) at room temperature for 10 min; and five times in Wash solution 3 (0.1 x SSC only) at room temperature for 10 min. The arrays were then transferred to isopropanol for a few seconds and centrifuged at 1500 rpm for 2 min. All washes were performed on an orbital shaker with vigorous shaking. The microarrays were then scanned using an Affymetrix 428 array scanner at 532 nm (Cy3) and 635 nm (Cy5). Scanned data were quantified using Imagene version 4.2 software (BioDiscovery, http://www.biodiscovery.com/).

Lepidium and Arabidopsis Genomic DNA Extraction and Labeling for Hybridisation to CATMA Microarrays

Genomic DNA was extracted from *L. sativum* FR1 and *Arabidopsis thaliana* Cvi leaf tissue using a modified CTAB extraction protocol. Approximately 100 mg of leaf tissue was homogenised in liquid nitrogen and ground fully in 300 μ L of CTAB-Buffer B (100 mM Tris/HCl (pH 8.0), 1.4 M NaCl, 20 mM EDTA and 2% (w/v) hexadecyltrimethyl ammoniumbromide (CTAB)). The homogenate was incubated at 65°C for 15 min and spun in a microcentrifuge at 13000 rpm for 2 min to remove cell debris. The supernatant was extracted twice with an equal volume of chloroform. The genomic DNA was precipitated by adding an equal volume of CTAB-Buffer C (50 mM Tris/HCI (pH 8.0), 10 mM EDTA and 1% (w/v) CTAB) and incubated at room temperature for 2 h. The genomic DNA was pelleted by centrifugation at 13000 rpm for 10 min. The DNA pellets were then resuspended in 400 μ L 1 M CsCl and precipitated once more by the addition of 800 μ L 100% ethanol. The DNA was pelleted by centrifugation at 13000 rpm for 10 mins. The DNA pellets were washed twice with 70% (v/v) ethanol, air dried and re-suspended in TE (10mM Tris/HCI (pH 7.5), 1 mM EDTA) and RNase A (20 μ g/ μ L).

Lepidium and Arabidopsis genomic DNA was fluorescently labelled using the BioPrime Array CGH Genomic Labelling System (Invitrogen, UK). Briefly, 20 µg of genomic DNA was mixed with 2.5x Random Primers Solution (final volume 41 µL) and denatured at 95°C for 5 minutes and immediately placed on ice. For each labelling reaction 5 µL of 10x dCTP mix, 3 µL of Cy3dCTP or Cy5-dCTP CyDye NHS-esters (Amersham Biosciences, www5.amersham biosciences.com) and 1 µL of Exo-Klenow fragment were added. The reactions were incubated at 3 °C for 2 h and the reaction was stopped by adding 5 µL of Stop buffer (0.5 M EDTA). The labelled DNA was purified according to the BioPrime Array CGH Genomic Labelling System. The reaction volumes was adjusted to 100 µL with TE, to which 400 µL Purification Buffer A was added and this mixture was vortexed. They were then loaded on to the column provided with the Kit and centrifuged at 11000 x g for 1 min at room temperature, and the flow-through was discarded. Two-hundred microliters of Purification Buffer B was added to the column and the column was centrifugated at 1000 x g for 1 min at room temperature and the flow-through was discarded. 50 µL dH₂O was then added to the column and the column incubated at room temperature for 1 min. The labelled DNA was recovered by centrifugation at 11000 x g for 1 min at room temperature. Cy3- and Cy5-labelling efficiency was quantified using a NanoDrop ND Spectrophotometer (NanoDrop Technologies, Rockland, DE). Cy3-labelled Arabidopsis genomic DNA and Cy5-labelled Lepidium genomic DNA (reciprocal labelling were also performed) were freeze-dried together for 2 h and re-suspended in hybridisation buffer.

Lepidium and Arabidopsis Genomic DNA hybridisation to CATMA Microarrays

Prehybridisation, hybridisation and washing of the CATMA version 3 microarrays is the same as outlined for the RNA microarrays above. For the genomic DNA microarrays, two independent genomic DNA preps from *A. thaliana* Cvi and *L. sativum* FR1 were labelled with both Cy3- and Cy5-dyes and hybridizing as described above for the aRNA to the CATMA v3 arrays. Thus two biological and two technical replicates were compared. The microarrays were then scanned using an Affymetrix 428 array scanner at 532 nm (Cy3) and 635 nm (Cy5). Scanned data were quantified using Imagene version 4.2 software (BioDiscovery, www.biodiscovery.com/).

Determination of Lepidium genes "present" on the microarray

Spot intensity data from the genomic DNA microarrays determined using Imagene were analysed using the limma package in Bioconductor (Smyth et al. 2005). Background correction

was performed using the 'normexp' method, which is analogous to RMA. Within array normalisation (Smyth et al. 2003) was performed using print tip loess and between array normalisation using quantile normalisation on the 'A' values. For the two species separately, the normalised values for each probe were then compared to those for the 912 empty spots with a one-sided t-test. Probes for which the normalised values were significantly greater than the empty spots (p<0.05) were considered to be "present". The 21527 probes out of the 30343 spotted on the microarray (70.9%) were identified as having significant hybridization for Lepidium and therefore classified as being "present". The equivalent number for Arabidopsis ecotype Cvi was 28146 (93.0%).

- Lim, P.O., Kim, Y., Breeze, E., Koo, J.C., Woo, H.R., Ryu, J.S., Park, D.H., Beynon, J., Tabrett, A., Buchanan-Wollaston, V., and Nam, H.G. (2007). Overexpression of a chromatin architecture-controlling AT-hook protein extends leaf longevity and increases the post-harvest storage life of plants. The Plant Journal 52, 1140-1153.
- Smyth, G.K. (2005). Limma: linear models for microarray data. In: Bioinformatics and Computational Biology Solutions using R and Bioconductor, R. Gentleman, V. Carey, S. Dudoit, R. Irizarry, and W. Huber, eds (New York: Springer), pp. 397-420.
- Smyth, G.K., and Speed, T.P. (2003). Normalization of cDNA microarray data. Methods 31, 265-273.

Supplemental Table 1.	. Primer list for trai	nscript expression	analysis by qRT-PCR.
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Gene name	Description	Primer specificity ^a	Accession Number ^b	Forward Primer: name, sequence (5'→3')	Reverse Primer: name, sequence (5'→3')
LesaACO1	ACC oxidase 1	Lepidium	GQ221031 (At2g19590)	qPAt2g19590-F CGCTTCTGTACTCACATCATA	qPAt2g19590-R CTGAATCAGCAAGATTCTGAC
LesaACO2	ACC oxidase 2	Lepidium	GQ221032 (At1g62380)	Ls-aco2-qP-F3 AGACTTTGGGAAGAGATTGGAG	Ls-aco2-qP-R3 AGGTTGGACCTTTAGTTCCAC
LesaACO4	ACC oxidase 4	Lepidium	GQ221033 (At1g05010)	Ls-aco3-qP-F2 GGTTAAGCATTCAATTGTGGT	Ls-aco3-qP-R1 CATCTGTCTGAGATATCACTCT
LesaCYP707A2	ABA 8'-hydroxylase CYP707A2	Lepidium	GQ221028 (At2g29090)	Lesa-cypA2-F2 AAGAGCTTTCATGCCGGATTC	Lesa-cypA2-R2 GAGATTAGTTCCATCCCATGAA
LesaCYP707A3	ABA 8'-hydroxylase CYP707A3	Lepidium	GQ221029 (At5g45340)	Lesa-cypA3-F ATCAACACCCTCGAACACATG	Lesa-cypA3-R TCAATTTCAGTGGCCTCCTCTT
LesaCTR1	Constitutive Triple Response 1	Lepidium	GQ221030 (At5g03730)	Ls-ctr1-qP-F1 GATCACAGGTTGAATAACCAG	Ls-ctr1-qP-R1 CACTCGATTGTCTCTGCAAC
CTR1-like	Putative CTR1-like serine/ threonine protein kinase	Arabidopsis	At4g24480	At4g24480-F TGGTTGGAGCAGTTGCATTC	At4g24480-R CAGCAAGCTTCCATTAGAGAT
PL1	Pectate lyase 1	Arabidopsis	At1g04680	At1g04680-F CTTCAACCGCAAGTTAACACA	At1g04680-R CAAACAACCAACAACACTTCG
ARL	Agros-like, putative cell expansion gene	Arabidopsis	At2g44080	At2g44080-F TCGGACATTGTCGTCGCAG	At2g44080-R CCAACAAGCACAACCATTGAT
AAO3	Abscisic aldehyde oxidase 3	Arabidopsis	At2g27150	aao3-f1 GTTGGAGCTGCCTTACAAGC	aao3-r1 TGAATGCTCCATGAAGACAG
NCED9	Nine- <i>cis</i> -epoxycarotenoid dioxygenase 9	Arabidopsis	At1g78390	nced9-f1 TCGGTTAGCTACGCTTGTCG	nced9-r1 GTCCGTGAAGCTCTCCAATT
SDR1	Short-chain dehydro- genase reductase 1	Arabidopsis	At1g52340	sdr1-f1 TGAGTGAGTTCGAGATGACC	sdr1-r1 ACCTCCCACACTACATAAGG
EF1a	Translation elongation factor 1-alpha	Arabidopsis	At5g60390	ef1-F TGAGCACGCTCTTCTTGCT	ef1-R GTGGCATCCATCTTGTTACA
ACT7	Actin 7	Arabidopsis	At5g09810	act7-F GGTCGTACAACCGGTATTGT	act7-R GAAGAGCATACCCCTCGTA

Primer were designed within the GST regions of the CATMA array probes for either the Lepidium or the Arabidopsis cDNA sequence. Accession numbers in brackets are the corresponding Arabidopsis orthologs of the Lepidium genes. а b