Supporting Information (SI Appendix)

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DELAY OF GERMINATION 1 mediates a conserved coat dormancy mechanism for the temperature- and gibberellin-dependent control of seed germination

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Supplementary Methods

Genome Size Analysis by Flow Cytometry and Chromosome Counts

Flow cytometry (FC) was used for the determination of relative DNA amount. Fresh leaf material (ca. 0.5 cm^2) of 202 *L. sativum* accessions, closely related *L. spinescens* and *L. spinosum* plus selected other *Lepidium* species (Supplemental Table 1 online) was chopped with a sharp razor blade into DAPI (4,6-diamino-2-phenylindole) solution and filtered into a sample tube. Subsequent FC analysis was performed on a Partec Ploidy Analyser-I (Partec) using an UV arc lamp. Diploid *Lepidium campestre* (2n=2x=16) was used as an internal standard (FC value = 1.0). DAPI-stained mitotic chromosome metaphase spreads from *L. sativum* flower bud tissue were used for chromosome counts following the protocol of (1). The same methodology was used to prepare meiotic metaphase II spreads.

Seed Germination Assays. For germination analyses of *A. thaliana* and *L. sativum* FR14 seeds were placed onto 1/10 Murashige-Skoog (MS) inorganic salts (Duchefa) solidified in 1% (w/v) agar-agar containing 0.1% plant preservative PPM (Plant Cell Technology) in Petri dishes and incubated in continuous white light (ca. 100 µmol m^{-2} s^{-1}) in a Sanyo Versatile Environmental Test Chamber (MLR-350) at the temperatures indicated. All germination assays were carried out with 3 replicates of 50 seeds each. Testa and endosperm rupture were scored over time with a binocular microscope. Germination assays were partly carried out without (control) or with the following additions as indicated: 10 µM *cis*-S(+)-ABA (Duchefa), 10 µM gibberellin A_{4+7} (GA_{4+7}; Duchefa), 10 µM fluridone, 100 µM paclobutrazol. Cold-stratification was carried out by placing the Petri dishes with the imbibed seeds for 3 days at 4°C. Seed after-ripening was performed by storing fresh mature seeds at 25°C and 50% relative humidity for the time indicated.

Plant Transformation

Plant transformation of *A. thaliana dog1-1* (2) mutant plants was done by floral dip method, as described in (3) with the addition of 0.1% Silwet as a detergent. Briefly, *Agrobacterium tumefaciens* GV3101 harbouring the desired vector were grown for 2 days at 28°C in 500ml YEB medium (5 g/l beef extract, 1 g/l yeast extract, 5 g/l peptone, 5 g/l sucrose, 0.5 g/l $MgCl_2$, pH 7.0). The liquid culture was centrifuged for 20min at 4000 rpm and the pellet was resuspended in 500ml 5% (w/v) sucrose solution, containing 0.1% Silwet. Plants were held upside down into the bacterial solution for approximately 20 s, then the plants were incubated in plastic bags covered from light exposure overnight, then they were placed back into the greenhouse. Plant transformation was repeated twice. For *L. sativum* FR14 plant

transformation slightly differed since we experienced that the use of Silwet as detergent was harmful to *L. sativum* plants which led to strongly impaired seed formation. Therefore bacterial cultures were prepared basically as described as above but without the addition of Silwet. To compensate for the use of Silwet as a detergent vacuum infiltration was used as it has been shown to improve transformation efficiency (3). *Lepidium sativum* plants that just started opening the first flowers were selected for transformation. 9 plants were placed upside down into a custom made device and their flower buds dipped into the *Agrobacterium* solution and vacuum (150 mbar below ambient pressure) was applied to the plants for 5 min. Plants were incubated in plastic bags overnight covered from light exposure before placing them back into the greenhouse. Plants were transformed two times with approximately 10 days in between to allow plant recovery from vacuum treatment. T1 seeds were harvested, stratified on plates and germinated in cont. white light. Ungerminated seeds were treated with GA or scarified to ensure that also potentially dormant seeds produce seedlings which were then exposed to the subsequent selection procedure. Transgenic plants were identified by spraying BASTA on 7-d-old seedlings and repeating the spraying twice more after 4 d and 7 d. Homozygous lines were identified by BASTA selection in subsequent generations. Transgenic lines were propagated for at least 5 generations.

Quantification of the Embryo Growth Potential

For the determination of the embryo growth potential (4) for every data point at least 20 seeds of *L. sativum* wild-type or Lesa-OxAtDOG1-E17 were imbibed in 1/10 MS for 2 h, then the embryos were carefully extracted and transferred to clear microtiter plates containing 0, 10, 15, 20, or 25 mM polyethylene glycol (PEG) 6000 (Carl Roth) in 1/10 MS inorganic salts and incubated for 27 h at 24 °C in continuous white light. For size determination of the radicle the embryos were transferred to a black ceramic plate during the incubation period after 1, 4, 7, 20, 23 and 27 h and photographed using a digital camera (Leica DFC480 with Leica IM1000 software) connected to a Leica MZ125 binocular stereo microscope with 20-fold magnification. Twenty-four-bit RGB images at a resolution of 2560 × 1920 pixel (pixel aspect ratio=1) were saved in TIFF format. The image analysis was done as described in detail in (4) with the modification that in this work only the increase in radicle-hypocotyl axis size was determined as shown in Figure 5 in (4). Individual size increases were calculated relative to the size of each radicle-hypocotyl axis at the 1-h time point. For each sample a linear regression analysis of the size increase over time was performed to determine the growth rate per hour during the 27h measuring interval. Osmolarity of the incubation medium was measured using a OM801 osmometer (Vogel). Osmotic pressure was calculated as described in (5).

RNA Extraction and qRT-PCR Analysis (6)

For each replicate sample *L. sativum* seeds were imbibed at the temperatures and for the times indicated. 10 seeds for each replicate were harvested at the indicated physiological state and time, and either entire seeds or tissues were frozen in liquid nitrogen and subsequent RNA extraction and quality assessment was performed as described (6). RNA was reverse transcribed and qRT-PCR analysis and data normalisation was performed as described (6). Transcript abundance was normalized against the geometric mean of the three validated reference genes LesaG17210 (HQ912755), LesaG04660 (HQ912754) and LesaG20000 (HQ912757) for each sample. Primer sequences for selective amplification of the two *LesaDOG1* paralogs in *L. sativum*: LesaDOG1A: LsDOG1AqPCR-F4 (5'-CTTTGTGTGGCTCCGAAACT-3') and LsDOG1AqPCR-R5 (5'-TTTTGGTCATCTTCTCTTCGTCTTCT-3'), these primers were also used for detecting LesaDOG1A overexpression in transgenic *A. thaliana dog1-1* seeds by semiquantitative PCR; LesaDOG1B: LsDOG1BqPCR-F4 (5'-CTTTGTGTGGCTCTCAAACT-3') and LsDOG1BqPCR-R5 (5'-TCTTGGTCATTTTCTCTTCCTCTTCT-3'). Primers for detecting *L. sativum GID1A*, *GID1B*, *GID1C*, *EXLA1*, *EXPA9*, *XTH19* have been described in (7). Detection of the overexpressed *AtDOG1* transgene in *L. sativum* seeds was performed using general *A. thaliana DOG1* qPCR primers described in (8).

The qRT-PCR primers for transcript detection of genes for which no *L. sativum* sequence was available were designed to match the consensus sequence of 4 closely related Brassicaceae species (*Arabidopsis thaliana*, *Capsella rubella*, *Brassica rapa* and *Thellungiella halophila*) of the respective gene orthologs as determined by the Phytozome database (9) and validated by multiple sequence alignment and phylogenetic analysis. Primer for detecting *EXPA2*: Expa2-F (5'-CAGCCAAGGCTATGGGCTAC-3') and Expa2-R (5'-GCTCACAACAGTCCGACCAT-3'); *KO*: KO-FP1 (5'-ACATGGATAAGAAGCGTTGGGAG-3') and KO-RP1 (5'-GCACCAGCACAAACCCTCT-3'); *KAO1*: KAO1-FP3 (5'-CTTGGGGAGAACAGGCACTATC-3') and KAO1-RP3 (5'-GCCAATGAAAGGCCAACCCA-3'); *KAO2*: KAO2-FP4 (5'-GTCCGGTGATGTTCTTGCCT-3') and KAO2-RP4 (5'-GGCGTCGTTTTGGTGATTCTT-3'); *GA20ox*: GA20ox-gnrl-FP3 (5'-GGTGGTGAACAGCGAGAG-3') and GA20ox-gnrl-RP3 (5'-CGAGAGTGTTCATGTCTGCT-3'); *GA3ox1*: GA3ox1-FP4 (5'-GACATCGTTGAAGAGTACGAGGAA-3') and GA3ox1-RP4 (5'-AGGAGGGTGGAGTCGGTATG-3'). All resulting qRT-PCR amplicons were investigated for specificity by melt-curve analysis and correct amplicon size was determined by gel electrophoresis.

GA and ABA Metabolite Quantification. Seeds of *L. sativum* FR14 and Lesa-OxAtDOG1 lines imbibed at the temperatures and for the times indicated were sampled (17 seeds without testa rupture per replicate), frozen and ground in liquid nitrogen and freeze-dried. This resulted in approximately 30 mg whole seed powder per sample which was used for the quantitation of GA (5 replicates) and ABA (3-4 replicates) metabolites as described (4, 10).

Supplementary References

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4. Voegele A et al. (2012) Embryo growth, testa permeability, and endosperm weakening are major targets for the environmentally regulated inhibition of Lepidium sativum seed germination by myrigalone A. *J Exp Bot*. 63: 5337-5350

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8. Chiang GCK et al. (2011) *DOG1* expression is predicted by the seed-maturation environment and contributes to geographical variation in germination in *Arabidopsis thaliana*. *Mol Ecol* 20:3336–3349.

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Supplementary Figures

Figure S1

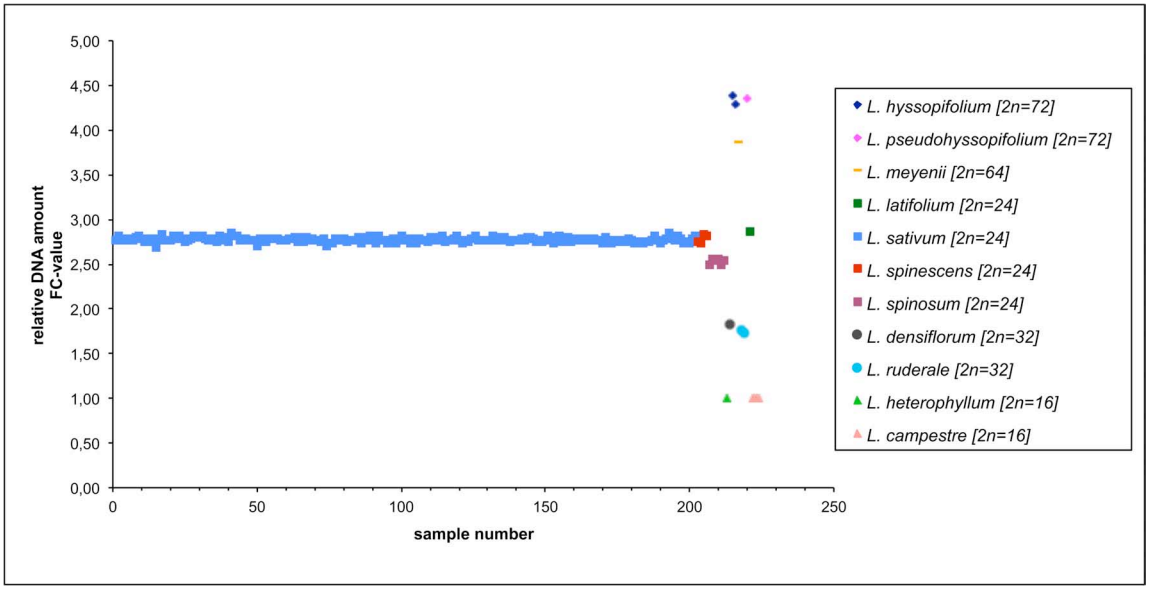

Figure S1. Flow cytometry (FC) analysis to quantify relative genome sizes of *Lepidium* spp. accessions (see Supplemental Table 1 online). Presented are relative DNA amounts (FC values) of *L. sativum, L. spinescens* and *L. spinosum* accessions as well as reference species representing different chromosome numbers and ploidy levels. The diploid *L. campestre* (2n=2x=16) was used as an internal standard (FC =1.0) and the relative FC values of *L. sativum* accessions varied between 2.71 and 2.85 (FC = 2.77±0.08, relative DNA amounts compared to *L. campestre*). Sample numbers are those of Supplemental Table 1 online which also contains the individual FC values and determined chromosome numbers. Based on these results *L. sativum* is a diploid species with 2n=2x=24, a normal mitosis and meiosis, and a base chromosome number of n=12.

Figure S2

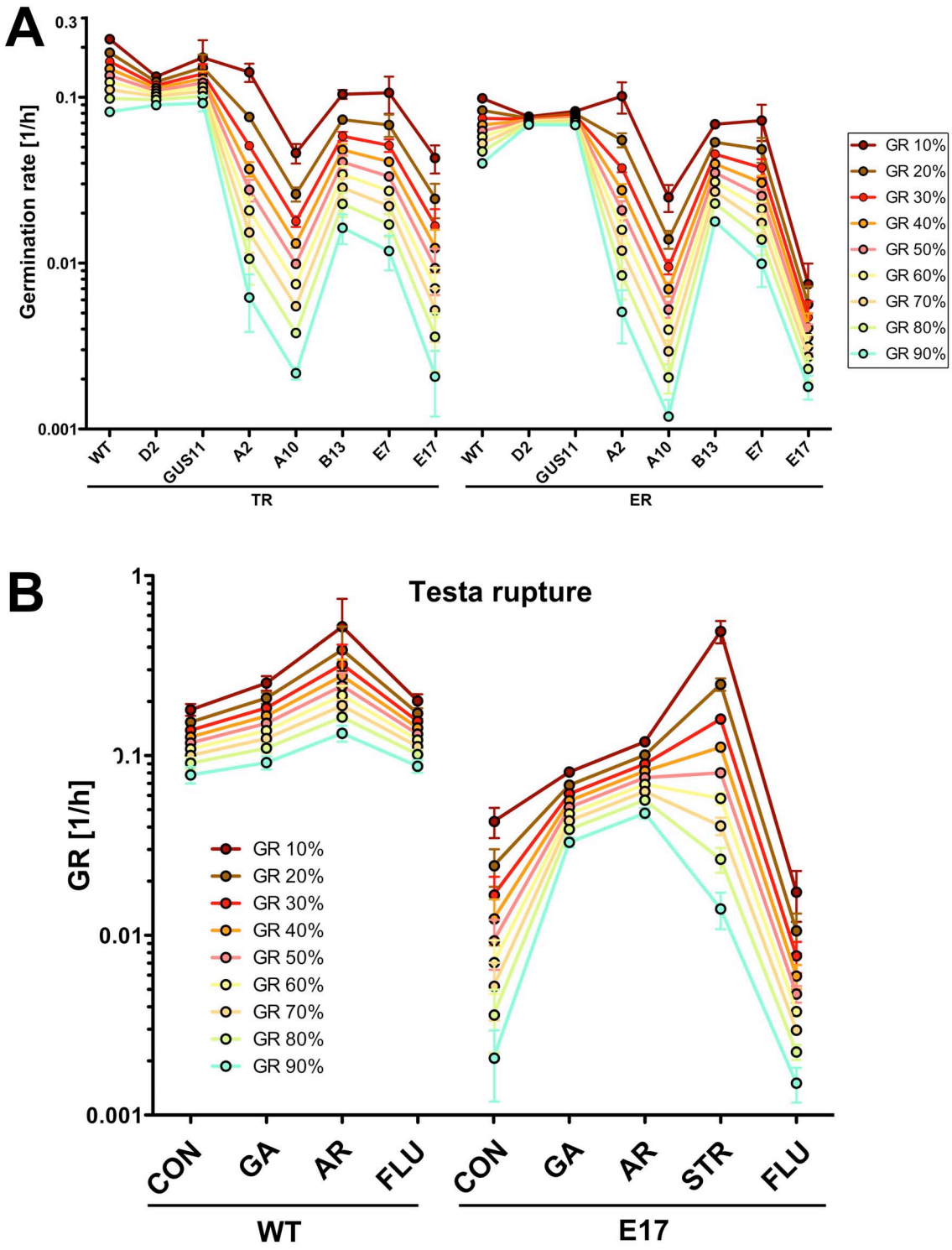
A
Germination rate [1/h]
0.3
0.1
0.01
0.001
WT
D2
GUS11
A2
A10
B13
E7
E17
TR
ER
GR 10%
GR 20%
GR 30%
GR 40%
GR 50%
GR 60%
GR 70%
GR 80%
GR 90%
B
Testa rupture
GR [1/h]
1
0.1
0.01
0.001
CON
GA
AR
FLU
STR
WT
E17

Figure S2. Germination rate comparison of Lesa-OxAtDOG1 lines. [A] Transgenic *Lepidium sativum* lines (A2, A10, B13, E7, E17) harboring a chimeric transgene with the CaMV 35S-promoter driving an *A. thaliana* Cvi *DOG1* genomic fragment (Lesa-OxAtDOG1) show a delayed germination phenotype. Shown are germination rates (GR) for testa and endosperm rupture (TR and ER). N=3, mean ±SEM. Note that transgenic lines like Lesa-OxAtDOG1-D2 and GUS11 (a GUS reporter line used as transformation control), which did not accumulate the AtDOG1 protein (maintext Figure 4D) did also not show a delayed-germination phenotype, as evident by GR values similar to wild-type (WT). GRs were determined using the Germinator software (6). [B] Overexpression of *AtDOG1* in *L. sativum* caused delayed germination which can be rescued by dormancy breaking treatments. Testa rupture germination rates (GRs) of WT and Lesa-OxAtDOG1-E17 at 24°C in continuous white light without (CON) or with dormancy breaking treatments: addition of 10 µM GA_{4+7} (GA), seed dry after-ripening storage for 9 month (AR), addition of 10 µM fluridone (FLU) and cold-stratification pre-treatment at 4°C in the dark for 3 days (STR). N=3, mean ±SEM. These TR GRs relate to the endosperm rupture GRs shown in the maintext in Figure 3A.

Figure S3

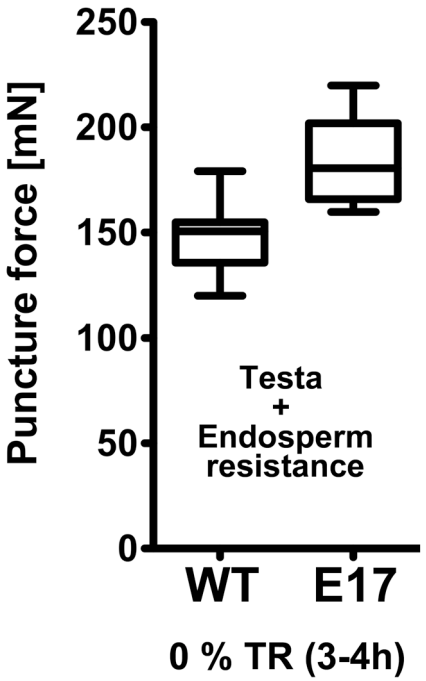

Figure S3. Initial resistance of embryo-encasing tissues of Lesa-OxAtDOG1-E17 and WT. The initial resistance of the combined embryo encasing tissues of the micropylar region (testa + endosperm) at 3-4h of seed imbibition is not significantly different ($p < 0.05$) between *Lepidium sativum* WT and Lesa-OxAtDOG1-E17 as determined by puncture-force measurements. N=8.

Figure S4

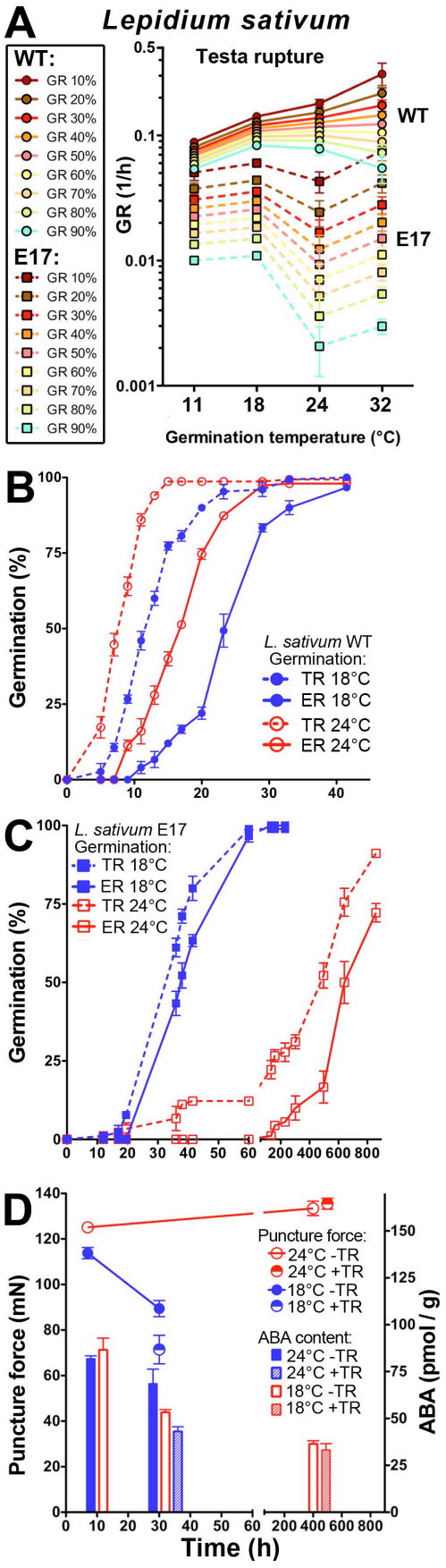

Figure S4. Temperature-dependent germination of Lesa-OxAtDOG1-E17 and WT in association with endosperm resistance and seed ABA levels.

[A] Germination rates for testa rupture at different imbibition temperatures of *Lepidium sativum* WT and transgenic Lesa-OxAtDOG1-E17 seeds overexpressing AtDOG1.

[B] *L. sativum* WT testa rupture (TR) and endosperm rupture (ER) at 18 and 24 °C.

[C] *L. sativum* transgenic Lesa-OxAtDOG1-E17 line TR and ER at 18 and 24 °C. Note that temperature has opposite effects on delaying germination in WT and E17 seeds. Panel B and C are shown for comparative reasons and this data is also presented in Figure 6B.

[D] Endosperm resistance of Lesa-OxAtDOG1-E17 seeds during germination at 18°C and 24°C in relation to seed ABA content. Puncture force measurements of the endosperm are shown (on the left axis) from seeds either with (+TR) or without (-TR) testa rupture. N=20, mean ±SE. ABA levels (on the right axis) during germination at 18 and 24 °C in E17 seeds with (+TR) or without (-TR) testa rupture. N=3, mean ±SE.

Figure S5

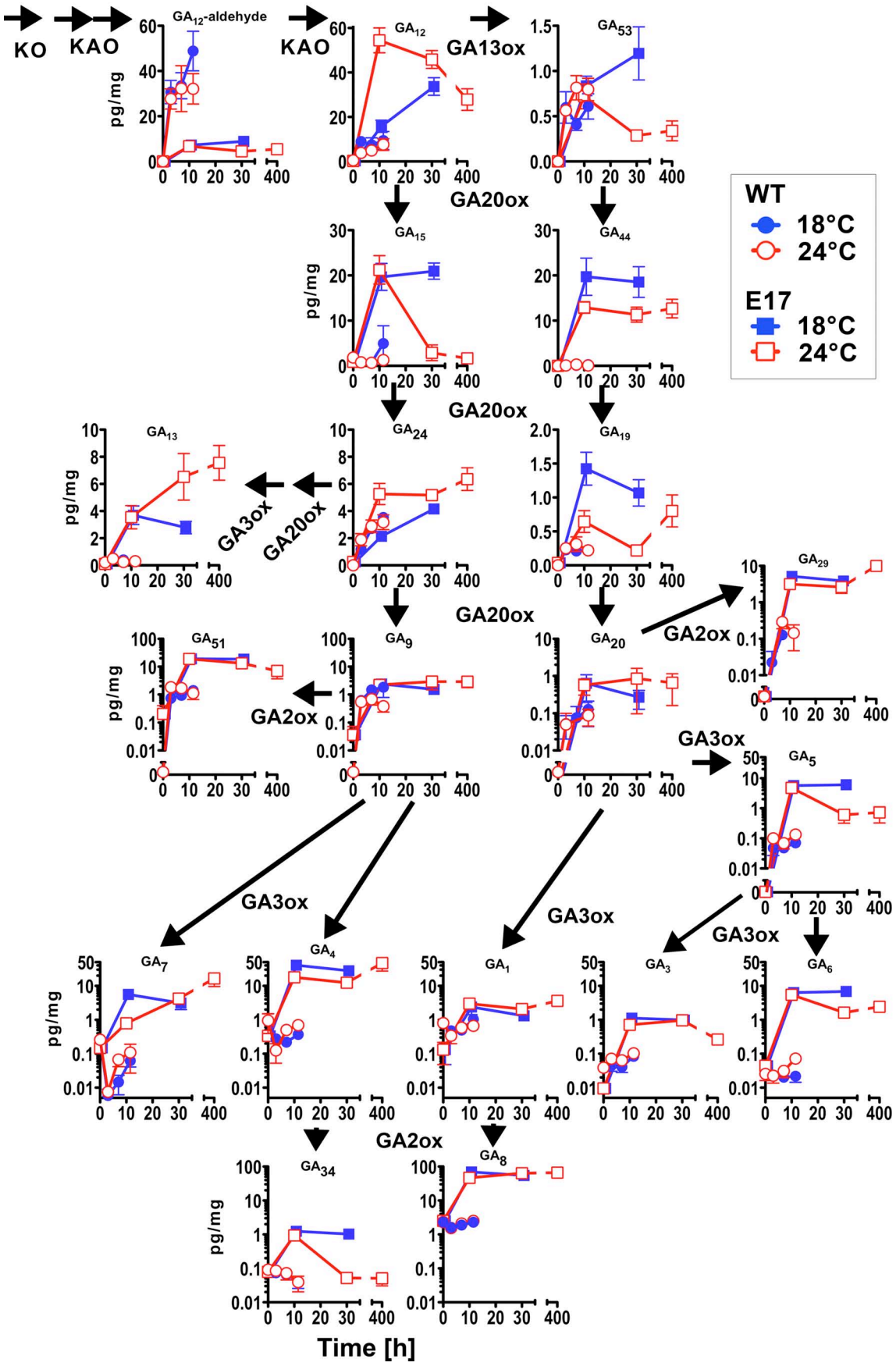
KO
KAO
KAO
GA13ox
GA20ox
GA20ox
GA20ox
GA3ox
GA20ox
GA2ox
GA2ox
GA3ox
GA3ox
GA3ox
GA2ox
GA12-aldehyde
GA12
GA53
GA15
GA44
GA24
GA19
GA13
GA9
GA20
GA29
GA51
GA5
GA7
GA4
GA1
GA3
GA6
GA34
GA8
pg/mg
Time [h]
WT
18°C
24°C
E17
18°C
24°C

Figure S5. DOG1 controls the seed gibberellin metabolism in a temperature-dependent manner. Overexpression of AtDOG1 in seeds of *Lepidium sativum* leads to an increase of gibberellin (GA) metabolites and a strongly altered temperature regulation of GA metabolism during germination. Shown are GA metabolite contents of seed populations of *L. sativum* WT and Lesa-OxAtDOG1-E17 at different times during imbibition at 18°C and 24°C. Samples from WT seeds were taken at 0, 3, 7 and 11h which corresponds to 0, 10, 50 and 90 % TR at 24°C (temperature optimum for WT) and 0, 0, 10 and 50 % TR at 18°C respectively. For Lesa-OxAtDOG1-E17 samples were taken at 0, 10, 30 and 400h corresponding to 0, 0, 10 and 50 % TR at 24 °C and 0, 0, 50 and 100 % TR at 18°C (temperature optimum for E17). All seeds were sampled before testa rupture. Note that no E17 seeds with unruptured testa could be sampled at 400h at 18°C because the whole seed population already completed germination. Shown are all analysed metabolites of the 13-non-hydroxylated (left) and 13-hydroxylated (right) pathway. GA biosynthetic enzymes catalysing resepctive steps are indicated. Results are presented as per amount dry weight. N=5, mean ±SEM. Note that this figure is an alternative presentation of information which is partly shown in Fig. 5B in the maintext. Besides presenting data of all 20 analysed GA metabolites, here GA content changes are visualised on a physical timescale rather than on a physiological timescale as in Fig. 5 in the maintext. For numeric values of GA metabolites see Table S2.

Figure S6

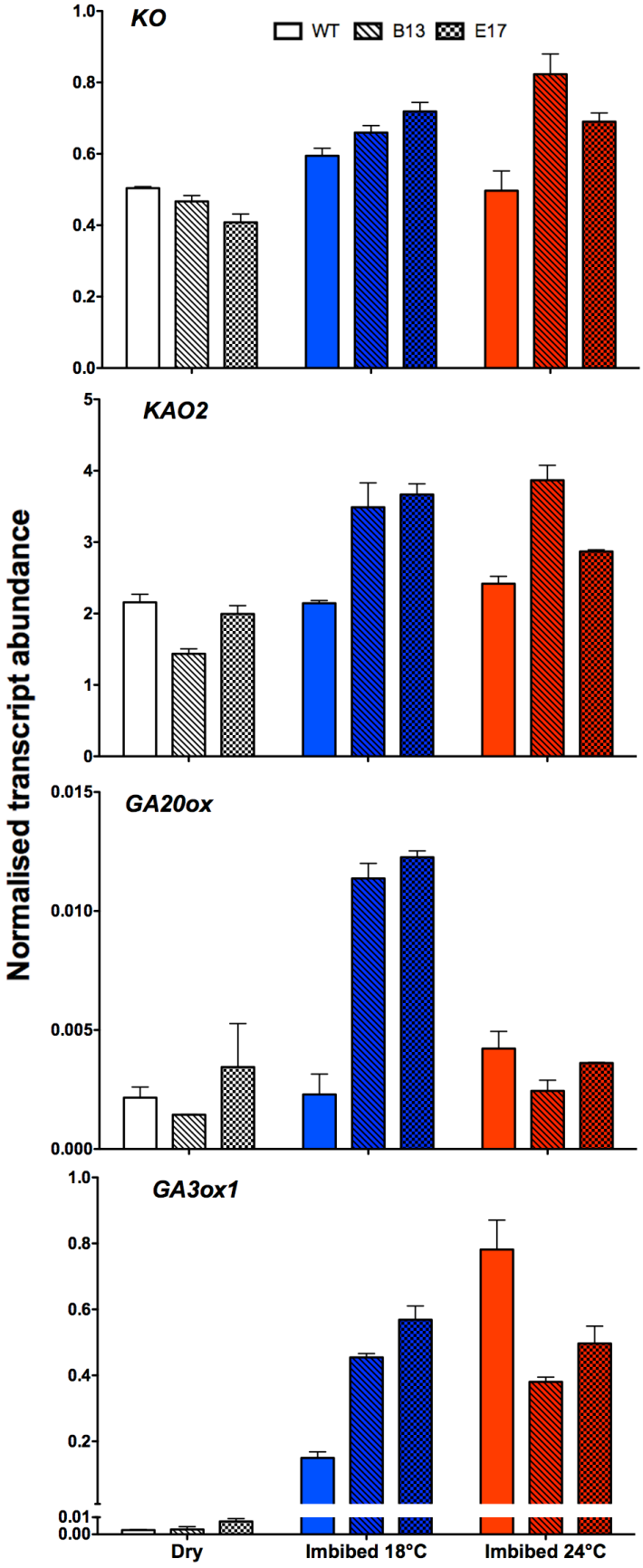

Figure S6. The temperature-dependent effect of DOG1-overexpression on the expression of gibberellin (GA) biosynthesis genes in transgenic *Lepidium sativum* lines. Gene expression (qRT-PCR) profiles of GA biosynthesis gene candidates (*KO*, *KAO1*, *GA20ox*, *GA3ox1*) in dry seeds and during seed germination of Lesa-OxAtDOG1-B13 and -E17 *L. sativum* lines overexpressing AtDOG1 compared to wild type (WT) at 18°C and 24°C. Seeds without testa rupture were sampled during imbibition at physiologically comparable early time points i.e. when the individual seed populations were just about to reach the onset of testa rupture (3h for WT, 10h for B13 and E17). Note that this is an independent experiment conducted with different seed batches than used in Figs. 6 and 2D. The same altered temperature-dependent phenotypic germination responses were obtained for the Lesa-OxAtDOG1 lines as compared to WT; and the same altered GA biosynthesis gene expression patterns were obtained supporting a role for DOG1 in regulating GA metabolism. N=3, mean ±SEM.

Figure S7

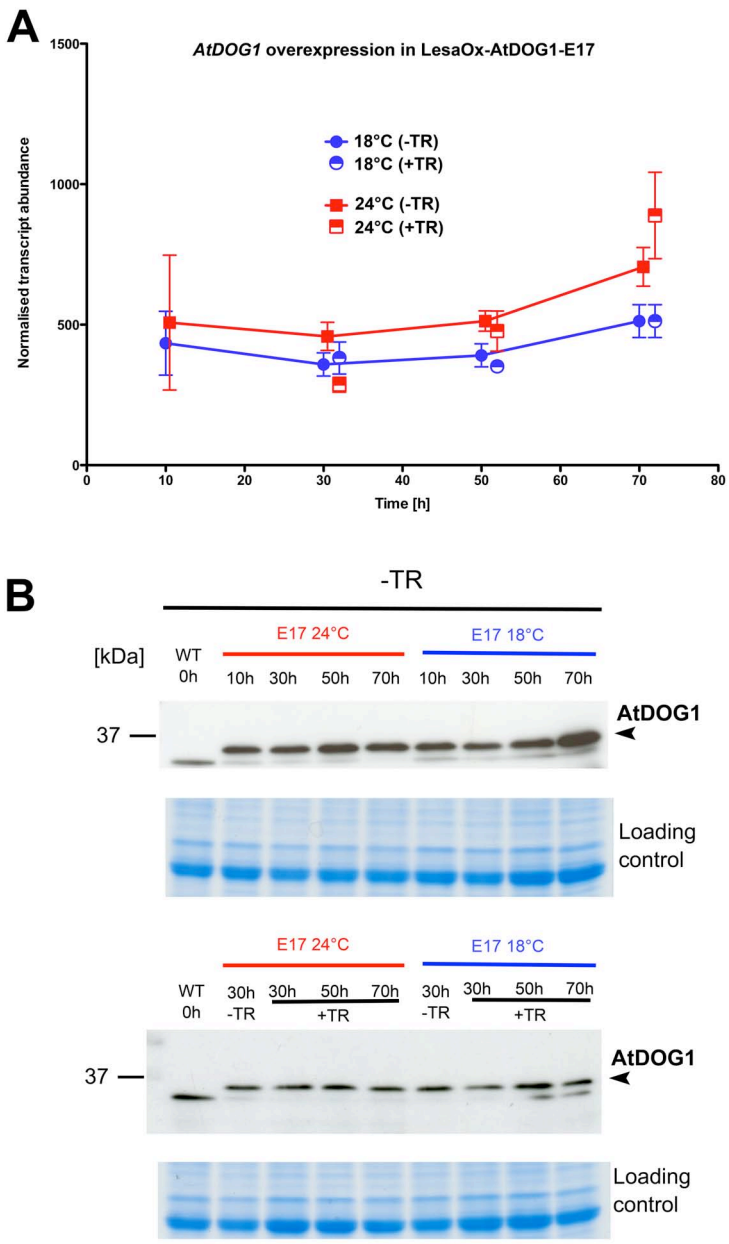

Figure S7. Overexpressed *AtDOG1* transcript and protein in Lesa-OxAtDOG1-E17 seeds during germination is highly abundant and expression level is not appreciably altered by germination temperature or the physiological state of the seed, i.e. having testa rupture (+TR) or not (-TR) as shown by [A] qRT-PCR analysis, N=4, mean ±SEM and [B] Western blot analysis.

Figure S8

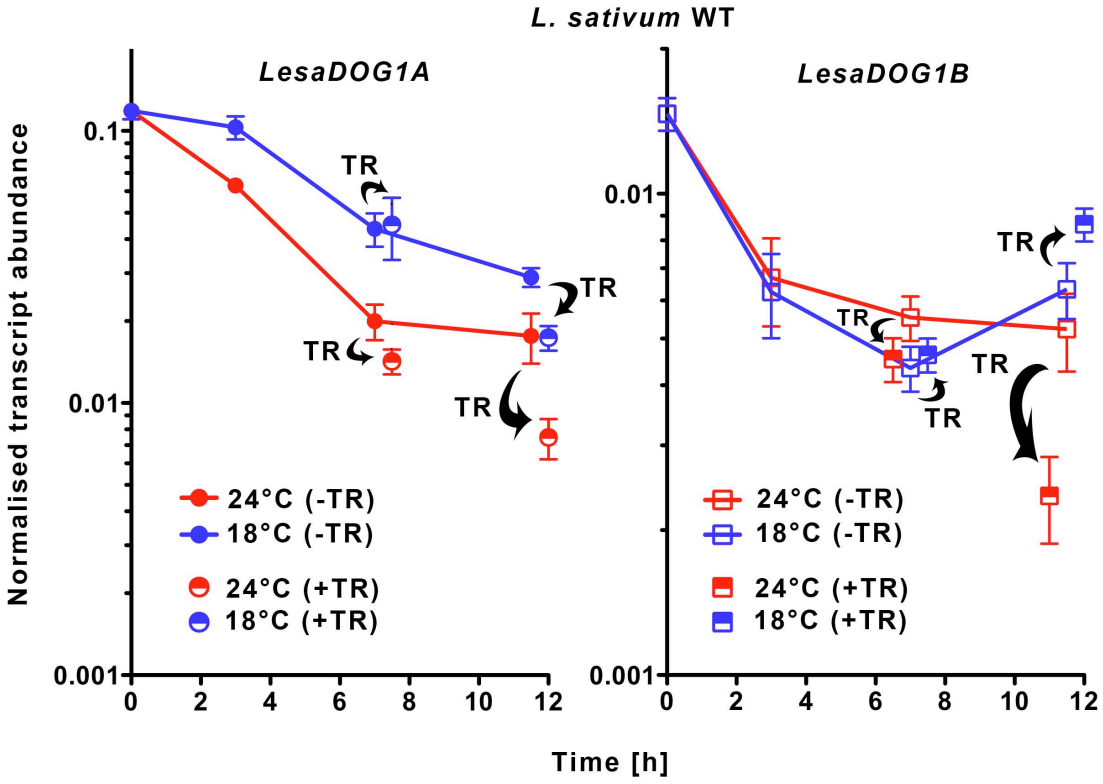

Figure S8. Temperature-dependent expression of endogenous *LesaDOG1A* and *LesaDOG1B* during germination of *L. sativum* WT at 18°C and 24°C. TR = testa rupture. Note that germination temperature differentially affects *LesaDOG1A* and *LesaDOG1B* transcript abundance as determined by qRT-PCR. Note further that TR decreases *LesaDOG1A* transcript abundance at both temperatures whereas temperature has opposing effects on *LesaDOG1B* transcript abundance when undergoing TR late during germination. N=3 ±SEM.

Figure S9

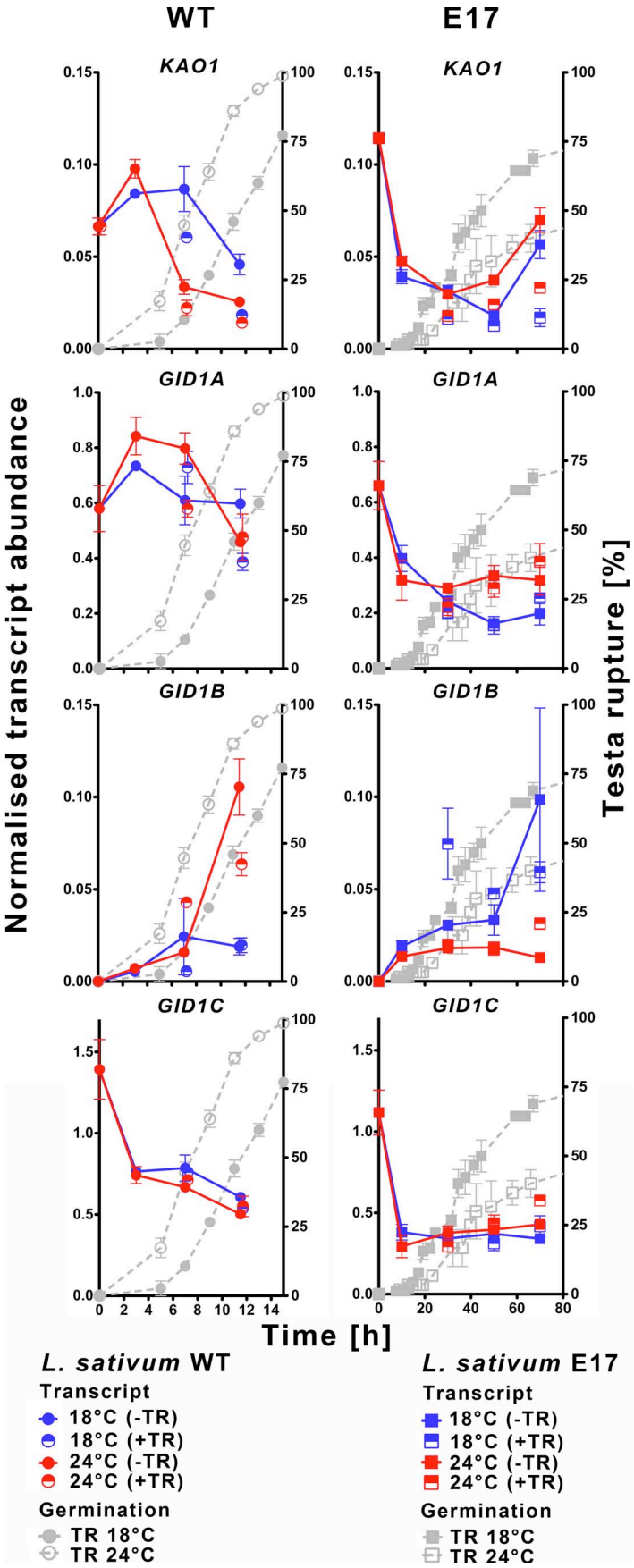
WT
E17
KAO1
GID1A
GID1B
GID1C
Normalised transcript abundance
Testa rupture [%]
Time [h]
L. sativum WT
Transcript
18°C (-TR)
18°C (+TR)
24°C (-TR)
24°C (+TR)
Germination
TR 18°C
TR 24°C
L. sativum E17
Transcript
18°C (-TR)
18°C (+TR)
24°C (-TR)
24°C (+TR)
Germination
TR 18°C
TR 24°C

Figure S9. The temperature-dependent influence of DOG1 on the expression of gibberellin (GA) biosynthesis and GA receptor genes. QRT-PCR gene expression profiles of the GA biosynthesis gene *KAO1* and the GA receptor genes *LesaGID1A*, *LesaGID1B* and *LesaGID1C* during seed germination of *Lepidium sativum* line E17 overexpressing AtDOG1 compared to wild-type (WT) at 18°C and 24°C. Seeds without (-TR) or with (+TR) testa rupture were sampled at the indicated time points which relate to the TR kinetics indicated by the grey curves. N=3, mean ±SEM.

Supplemental Table 1: Origin, microscopically determined chromosome number (mitotic and meiotic chromosome spreads), quantified relative DNA amount (FC-value) compared to *Lepidium campestre* (FC = 1.0) and assumed ploidy level (if x = 8) of *Lepidium sativum, L. spinescens* and *L. spinosum* accessions and reference species representing different chromosome numbers and ploidy levels.

Taxon	**Sample no.**	**Accession** [X]	**Origin**	**Chromosome number (2n / n)**	**FC-value (relative)** [Y]	**Ploidy level (if x = 8)** [Z]
L. sativum	1	KM 2392/ FR 14	Germany	24 / 12	2.760	3x
	2	KM 1877	Afghanistan	24 /	2.813	3x
	3	KM 1878	Greece	24 /	2.761	3x
	4	KM 1879	unkown		2.764	3x
	5	KM 1880	unkown		2.787	3x
	6	KM 1881	unkown		2.771	3x
	7	KM 1882	unkown		2.759	3x
	8	KM 1883	former UDSSR		2.805	3x
	9	KM 1884	Italy		2.810	3x
	10	KM 1885	Libya		2.781	3x
	11	KM 1886	Iraq		2.752	3x
	12	KM 1887	Iraq	24 /	2.754	3x
	13	KM 1888	Iraq	24 /	2.782	3x
	14	KM 1889	Iraq	24 /	2.791	3x
	15	KM 1890	Iraq	24 /	2.694	3x
	16	KM 1891	Georgia		2.768	3x
	17	KM 1892	Italy		2.825	3x
	18	KM 1893	Georgia		2.757	3x
	19	KM 1894	North Korea		2.765	3x
	20	KM 1897	Georgia		2.756	3x
	21	KM 1898	Former UDSSR		2.806	3x
	22	KM 1899	Germany		2.788	3x
	23	KM 1900	Germany		2.814	3x
	24	KM 1901	Germany		2.779	3x
	25	KM 1902	Germany		2.752	3x
	26	KM 1903	Germany		2.760	3x
	27	KM 1904	Germany		2.783	3x
	28	KM 1905	Germany		2.814	3x

Supplemental Table 1: Continued

Taxon	Sample no.	Accession [X]	Origin	Chromosome number (2n / n)	FC-value (relative) [Y]	Ploidy level (if x = 8) [Z]
L. sativum	29	KM 1906	Yemen	24 / 12	2.798	3x
	30	KM 1907	Germany		2.814	3x
	31	KM 1908	unknown		2.810	3x
	32	KM 1909	unknown		2.787	3x
	33	KM 1910	unknown		2.757	3x
	34	KM 1911	Austria		2.761	3x
	35	KM 1912	former UDSSR		2.792	3x
	36	KM 1913	former UDSSR		2.750	3x
	37	KM 1914	Hindukush	24 /	2.814	3x
	38	KM 1915	unknown		2.761	3x
	39	KM 1916	unknown		2.804	3x
	40	KM 1917	unknown		2.749	3x
	41	KM 1919	Oman		2.852	3x
	42	KM 1920	Oman		2.810	3x
	43	KM 1982	Germany		2.806	3x
	44	KM 1983	Russia	24 /	2.760	3x
	45	KM 1984	Armenia		2.781	3x
	46	KM 1985	Russia	24 /	2.791	3x
	47	KM 1986	India		2.773	3x
	48	KM 1987	Iraq		2.758	3x
	49	KM 1988	Iraq	/ 12	2.757	3x
	50	KM 1989	Iraq		2.713	3x
	51	KM 1990	Nepal		2.788	3x
	52	KM 1991	Armenia	24 /	2.767	3x
	53	KM 1992	Armenia		2.748	3x
	54	KM 1993	Denmark		2.791	3x
	55	KM 1994	Denmark		2.789	3x
	56	KM 1995	Sweden		2.787	3x
	57	KM 1996	former UDSSR		2.783	3x

Supplemental Table 1: Continued

Taxon	Sample no.	Accession [X]	Origin	Chromosome number (2n / n)	FC-value (relative) [Y]	Ploidy level (if x = 8) [Z]
L. sativum	58	KM 1997	CSK		2.768	3x
	59	KM 1998	Russia		2.756	3x
	60	KM 1999	France		2.775	3x
	61	KM 2000	unknown		2.780	3x
	62	KM 2001	Iran		2.788	3x
	63	KM 2002	Germany		2.801	3x
	64	KM 2003	Armenia	24 /	2.749	3x
	65	KM 2004	Afghanistan		2.798	3x
	66	KM 2005	Kazakhstan		2.748	3x
	67	KM 2006	Azerbaijan		2.803	3x
	68	KM 2007	Netherlands		2.766	3x
	69	KM 2008	unkown		2.790	3x
	70	KM 2009	Nepal		2.771	3x
	71	KM 2010	Nepal		2.768	3x
	72	KM 2011	Somalia	24 / 12	2.748	3x
	73	KM 2012	Switzerland		2.776	3x
	74	KM 2013	Iraq		2.714	3x
	75	KM 2014	Iraq		2.735	3x
	76	KM 2015	Iraq		2.727	3x
	77	KM 2016	Iraq		2.782	3x
	78	KM 2017	Netherlands		2.758	3x
	79	KM 2018	Kazakhstan		2.763	3x
	80	KM 2019	Azerbaijan	24 / 12	2.787	3x
	81	KM 2020	Romania		2.750	3x
	82	KM 2021	Syria	24 /	2.764	3x
	83	KM 2022	Denmark		2.768	3x
	84	KM 2023	Azerbaijan		2.759	3x
	85	KM 2024	Netherlands		2.802	3x
	86	KM 2025	Belgium		2.739	3x

Supplemental Table 1: Continued

Taxon	Sample no.	Accession [X]	Origin	Chromosome number (2n / n)	FC-value (relative) [Y]	Ploidy level (if x = 8) [Z]
L. sativum	87	KM 2026	Belgium		2.798	3x
	88	KM 2027	Ukraine		2.764	3x
	89	KM 2028	Azerbaijan		2.807	3x
	90	KM 2029	Germany		2.802	3x
	91	KM 2030	unknown		2.728	3x
	92	KM 2031	unknown	24 /	2.809	3x
	93	KM 2032	unknown		2.764	3x
	94	KM 1298	Tadzhikistan		2.760	3x
	95	KM 1833	Afghanistan	24 /	2.728	3x
	96	KM 1834	Afghanistan	24 /	2.756	3x
	97	KM 1835	unknown	24 /	2.780	3x
	98	KM 1836	Russia	24 /	2.744	3x
	99	KM 1837	Georgia		2.761	3x
	100	KM 1838	Germany		2.819	3x
	101	KM 1839	former UDSSR		2.757	3x
	102	KM 1840	Georgia		2.795	3x
	103	KM 1841	Georgia	24 /	2.740	3x
	104	KM 1842	Georgia		2.778	3x
	105	KM 1843	Georgia		2.734	3x
	106	KM 1844	Georgia	24 /	2.784	3x
	107	KM 1846	Georgia		2.771	3x
	108	KM 1847	Georgia		2.766	3x
	109	KM 1848	Georgia		2.746	3x
	110	KM 1849	Georgia		2.799	3x
	111	KM 1851	Georgia		2.757	3x
	112	KM 1852	Georgia		2.796	3x
	113	KM 1853	Ukraine		2.758	3x
	114	KM 1854	Georgia		2.816	3x
	115	KM 1855	Georgia		2.754	3x

Supplemental Table 1: Continued

Taxon	Sample no.	Accession [X]	Origin	Chromosome number (2n / n)	FC-value (relative) [Y]	Ploidy level (if x = 8) [Z]
L. sativum	116	KM 1856	Georgia		2.761	3x
	117	KM 1857	Georgia		2.767	3x
	118	KM 1858	Georgia		2.777	3x
	119	KM 1859	Georgia		2.805	3x
	120	KM 1860	Georgia		2.768	3x
	121	KM 1861	Georgia		2.743	3x
	122	KM 1862	Georgia		2.768	3x
	123	KM 1863	Tadshikistan		2.755	3x
	124	KM 1864	Georgia	24 /	2.781	3x
	125	KM 1865	Georgia		2.778	3x
	126	KM 1866	Aserbaidshan		2.805	3x
	127	KM 1867	Germany		2.765	3x
	128	KM 1868	Turkey		2.755	3x
	129	KM 1869	Georgia		2.771	3x
	130	KM 1870	former UDSSR		2.757	3x
	131	KM 1872	Armenia		2.770	3x
	132	KM 1873	former UDSSR		2.807	3x
	133	KM 1874	former UDSSR		2.762	3x
	134	KM 1875	unknown		2.767	3x
	135	KM 1876	former UDSSR		2.802	3x
	136	KM 1933	Georgia	24 /	2.798	3x
	137	KM 1934	Armenia	24 / 12	2.764	3x
	138	KM 1935	Azerbaijan	24 /	2.749	3x
	139	KM 1936	Georgia	24 /	2.758	3x
	140	KM 1937	Armenia	/ 12	2.767	3x
	141	KM 1938	Armenia		2.773	3x
	142	KM 1939	Armenia		2.783	3x
	143	KM 1940	Georgia		2.771	3x
	144	KM 1941	Georgia	24 /12	2.805	3x

Supplemental Table 1: Continued

Taxon	Sample no.	Accession [X]	Origin	Chromosome number (2n / n)	FC-value (relative) [Y]	Ploidy level (if x = 8) [Z]
L. sativum	145	KM 1942	Azerbaijan	24 /	2.805	3x
	146	KM 1943	Azerbaijan		2.789	3x
	147	KM 1944	Russia		2.747	3x
	148	KM 1945	Iran		2.756	3x
	149	KM 1946	unknown	24 / 12	2.748	3x
	150	KM 1947	Iran		2.760	3x
	151	KM 1948	Iran		2.812	3x
	152	KM 1949	Iran		2.795	3x
	153	KM 1949b	Iran		2.731	3x
	154	KM 1950	Armenia	24 /	2.748	3x
	155	KM 1951	Georgia		2.820	3x
	156	KM 1952	Georgia		2.774	3x
	157	KM 1953	Afghanistan		2.754	3x
	158	KM 1954	Georgia		2.799	3x
	159	KM 1955	Georgia		2.752	3x
	160	KM 1956	Georgia	24 /	2.791	3x
	161	KM 1957	Azerbaijan		2.773	3x
	162	KM 1958	Azerbaijan		2.785	3x
	163	KM 1959	Azerbaijan		2.768	3x
	164	KM 1960	Azerbaijan	24 /	2.777	3x
	165	KM 1961	Azerbaijan	24 /	2.774	3x
	166	KM 1962	Azerbaijan		2.761	3x
	167	KM 1963	Armenia		2.783	3x
	168	KM 1963b	Armenia		2.765	3x
	169	KM 1964	Azerbaijan		2.772	3x
	170	KM 1965	Azerbaijan		2.740	3x
	171	KM 1966	Georgia		2.796	3x
	172	KM 1967	Georgia		2.754	3x
	173	KM 1968	Georgia	24 /	2.733	3x

Supplemental Table 1: Continued

Taxon	Sample no.	Accession [X]	Origin	Chromosome number (2n / n)	FC-value (relative) [Y]	Ploidy level (if x = 8) [Z]
L. sativum	174	KM 1969	Georgia	24 /	2.771	3x
	175	KM 1970	Georgia		2.746	3x
	176	KM 1971	Georgia		2.762	3x
	177	KM 1972	Georgia		2.760	3x
	178	KM 1973	Georgia		2.751	3x
	179	KM 1974	Georgia		2.791	3x
	180	KM 1975	Georgia		2.760	3x
	181	KM 1976	Georgia	/ 12	2.739	3x
	182	KM 1977	Georgia		2.746	3x
	183	KM 1978	Georgia		2.748	3x
	184	KM 1979	Georgia		2.744	3x
	185	KM 1980	Georgia		2.750	3x
	186	KM 1981	unknown		2.746	3x
	187	KM 1826	unknown	24 /	2.775	3x
	188	KM 1827	unknown	24 /	2.814	3x
	189	KM 1828	unknown	24 /	2.759	3x
	190	KM 1829	unknown	24 /	2.731	3x
	191	KM 1830	unknown	24 /	2.775	3x
	192	KM 1831	unknown	24 /12	2.787	3x
	193	KM 1832	unknown		2.843	3x
	194	KM 1924	Canada	24 /	2.777	3x
	195	KM 1925	Canada	24 /	2.770	3x
	196	KM 1926	Canada		2.807	3x
	197	KM 1927	Canada		2.793	3x
	198	KM 1928	Canada	24 /	2.736	3x
	199	KM 1929	Canada	/ 12	2.754	3x
	200	KM 1930	Australia	24 /	2.733	3x
	201	KM 1931	Great Britain	24 / 12	2.790	3x
	202	KM 1932	Botswana	24 /	2.820	3x

Supplemental Table 1: Continued

Taxon	Sample	Accession [X]	Origin	Chromosome number (2n / n)	FC-value (relative) [Y]	Ploidy level (if x = 8) [Z]
L. spinescens	203	KM 1921	Lebanon	24 /	2.752	3x
	204	KM 1923	Lebanon	24 /	2.737	3x
	205	KM 1806	Israel	/ 12	2.826	3x
	206	96-0065-10-00	Israel	24 /	2.806	3x
L. spinosum	207	KM 1922	Lebanon	24 /	2.500	3x
	208	94-0165-10-00	Turkey	24 /	2.557	3x
	209	95-0352-30-00	Spain/Mallorca		2.542	3x
	210	97-0084-30-00	Spain/Mallorca		2.557	3x
	211	KM 1805	Israel	/ 12	2.514	3x
	212	KM 1807	Israel	24 /	2.537	3x
L. heterophyllum	213	KM 2084	Greece	16 /	1.001	2x
L. densiflorum	214	KM 2085	unknown	32 /	1.829	4x
L. hyssopifolium	215	KM 1703	Australia	72 /	4.490	9x
	216	KM 1672	Australia	72 /	4.290	9x
L. meyenii	217	KM 754	Peru	64 /	3.867	8x
L. ruderale	218	KM 2086	unknown	32 /	1.786	4x
	219	KM 2481	Germany	32 /	1.728	4x
L. pseudo-hyssopifolium	220	KM 1666	Australia	72 /	4.357	9x
L. latifolium	221	01-0138-10-00	Germany	24 /	2.869	3x
L. campestre	222	96-0070-30-00	Greece	16 /	1.000	2x

Supplemental Table 1: Continued

Taxon	Sample	Accession [X]	Origin	Chromosome number (2n / n)	FC-value (relative) [Y]	Ploidy level (if x = 8) [Z]
L. campestre	223	KM 2082	Germany	16 /	1.000	2x
L. campestre	224	KM 2083	Germany	16 /	1.000	2x

[X] KM refers to accession numbers of K. Mummenhoff, other numbers refer to the Brassicaceae seed collection of the University of Osnabrück (OSBU).

[Y] Flow cytometry was used for the determination of relative DNA amount (FC-value). Diploid *L. campestre* (2n = 2x = 16) was used as an internal standard (FC = 1.0).

[z] Ploidy level is based on x = 8.

Table S2. DOG1 controls the seed gibberellin metabolism in a temperature-dependent manner. Shown are GA metabolite contents of seed populations of *L. sativum* WT and Lesa-OxAtDOG1-E17 at different times during imbibition at 18°C and 24°C. Samples from WT seeds were taken at 0, 3, 7 and 11h which corresponds to 0, 10, 50 and 90 % TR at 24°C (temperature optimum for WT) and 0, 0, 10 and 50 % TR at 18°C respectively. For Lesa-OxAtDOG1-E17 samples were taken at 0, 10, 30 and 400h corresponding to 0, 0, 10 and 50 % TR at 24 °C and 0, 0, 50 and 100 % TR at 18°C (temperature optimum for E17). All seeds were sampled before testa rupture. Results are presented as pg per mg dry weight. N=5, mean ±SEM. These numeric values relate to Figures 5B and 5S in the maintext and supplements.

	Wild-type							**E17**					
	DS	**18°C**			**24°C**			**DS**	**18°C**		**24°C**		
% TR (time) >	0% (0h)	0% (3h)	10% (7h)	50% (11h)	10% (3h)	50% (7h)	90% (11h)	0% (0h)	0% (10h)	50% (30h)	0% (10h)	10% (30h)	50% (400h)
13-Non-hydroxylation pathway													
GA_{12ald}	0.00 ±0.00	30.68 ±5.13	33.48 ±5.73	48.84 ±8.74	27.65 ±4.48	32.19 ±10.13	32.12 ±6.73	0.00 ±0.00	7.26 ±1.73	8.89 ±1.401	6.71 ±1.22	4.50 ±1.43	5.42 ±0.82
GA_{12}	0.50 ±0.21	8.95 ±2.01	7.46 ±3.15	9.41 ±4.05	3.86 ±1.79	4.93 ±0.51	7.60 ±2.61	0.22 ±0.10	15.81 ±2.70	33.70 ±3.95	54.44 ±5.58	45.84 ±3.10	27.84 ±4.88
GA_{15}	1.83 ±0.71	0.93 ±0.24	0.76 ±0.20	4.95 ±3.92	0.77 ±0.18	0.68 ±0.22	1.27 ±0.38	0.91 ±0.08	19.67 ±2.98	20.94 ±1.79	21.23 ±3.16	2.89 ±1.73	1.67 ±0.56
GA_{24}	0.00 ±0.00	1.17 ±0.26	2.71 ±0.24	3.53 ±0.14	1.89 ±0.45	2.88 ±0.46	3.18 ±0.54	0.25 ±0.16	2.14 ±0.28	4.16 ±0.34	5.26 ±0.78	5.17 ±0.22	6.35 ±0.83
GA_{9}	0.00 ±0.00	0.61 ±0.07	1.48 ±0.46	1.82 ±1.04	0.55 ±0.14	0.68 ±0.25	0.38 ±0.14	0.04 ±0.04	2.39 ±0.87	1.53 ±0.26	2.21 ±0.58	2.91 ±1.44	2.89 ±1.11
GA_{51}	0.00 ±0.00	0.74 ±0.26	0.93 ±0.34	1.38 ±0.38	1.82 ±0.33	1.70 ±0.47	1.12 ±0.46	0.20 ±0.20	19.15 ±2.65	18.64 ±3.90	18.92 ±3.92	13.29 ±2.63	7.06 ±3.40
GA_{4}	0.95 ±0.55	0.28 ±0.08	0.22 ±0.06	0.37 ±0.09	0.13 ±0.07	0.50 ±0.14	0.70 ±0.19	0.34 ±0.07	40.72 ±7.24	28.03 ±4.18	17.86 ±0.51	12.43 ±2.26	47.14 ±19.85
GA_{34}	0.09 ±0.03	0.07 ±0.00	0.06 ±0.01	0.04 ±0.02	0.09 ±0.02	0.07 ±0.04	0.04 ±0.02	0.08 ±0.008	1.24 ±0.09	1.03 ±0.07	0.94 ±0.05	0.05 ±0.01	0.05 ±0.02
GA_{13}	0.22 ±0.11	0.45 ±0.046	0.40 ±0.05	0.31 ±0.07	0.49 ±0.03	0.25 ±0.04	0.30 ±0.07	0.107 ±0.008	3.69 ±0.68	2.79 ±0.46	3.54 ±0.86	6.52 ±1.72	7.56 ±1.28

13-Hydroxylation pathway													
GA_{53}	0.00 ±0.00	0.60 ±0.17	0.41 ±0.07	0.61 ±0.14	0.56 ±0.03	0.82 ±0.13	0.80 ±0.12	0.00 ±0.00	0.84 ±0.11	1.19 ±0.29	0.72 ±0.14	0.29 ±0.05	0.34 ±0.11
GA_{44}	0.00 ±0.00	0.08 ±0.08	0.08 ±0.08	0.29 ±0.12	0.10 ±0.06	0.29 ±0.14	0.09 ±0.09	0.00 ±0.00	19.71 ±4.11	18.53 ±3.40	12.90 ±1.26	11.33 ±1.65	12.68 ±2.05
GA_{19}	0.00 ±0.00	0.23 ±0.07	0.21 ±0.04	0.23 ±0.07	0.25 ±0.08	0.31 ±0.11	0.22 ±0.07	0.04 ±0.04	1.42 ±0.24	1.07 ±0.19	0.65 ±0.16	0.22 ±0.04	0.80 ±0.24
GA_{20}	0.00 ±0.00	0.05 ±0.03	0.08 ±0.08	0.13 ±0.08	0.05 ±0.05	0.00 ±0.00	0.09 ±0.04	0.00 ±0.00	0.64 ±0.44	0.27 ±0.14	0.56 ±0.26	0.85 ±0.75	0.66 ±0.49
GA_{29}	0.00 ±0.00	0.02 ±0.02	0.13 ±0.13	0.00 ±0.00	0.00 ±0.00	0.29 ±0.10	0.14 ±0.10	0.00 ±0.00	5.17 ±0.55	3.86 ±0.99	3.16 ±0.58	2.63 ±0.87	9.98 ±1.05
GA_{1}	0.80 ±0.06	0.48 ±0.11	0.50 ±0.09	1.04 ±0.15	0.34 ±0.14	0.58 ±0.08	0.67 ±0.11	0.13 ±0.08	2.39 ±0.97	1.31 ±0.38	2.99 ±0.70	2.08 ±0.59	3.62 ±0.91
GA_{8}	2.32 ±0.10	1.59 ±0.19	1.89 ±0.10	2.29 ±0.13	1.55 ±0.13	2.09 ±0.23	2.49 ±0.45	2.52 ±0.08	69.74 ±6.89	54.68 ±2.73	46.73 ±2.30	64.08 ±11.67	66.52 ±5.72
GA_{5}	0.00 ±0.00	0.0475 ±0.021	0.05 ±0.01	0.07 ±0.02	0.10 ±0.03	0.07 ±0.02	0.13 ±0.04	0.00 ±0.00	5.73 ±0.31	6.06 ±0.39	4.77 ±0.67	0.61 ±0.29	0.72 ±0.40
GA_{6}	0.03 ±0.01	0.02 ±0.00	0.02 ±0.00	0.02 ±0.00	0.02 ±0.01	0.03 ±0.01	0.07 ±0.02	0.04 ±0.00	6.33 ±1.01	6.84 ±1.27	5.46 ±0.82	1.63 ±0.50	2.42 ±0.34
GA_{3}	0.04 ±0.01	0.05 ±0.01	0.04 ±0.01	0.08 ±0.03	0.07 ±0.02	0.06 ±0.01	0.10 ±0.03	0.01 ±0.00	1.12 ±0.11	0.99 ±0.23	0.71 ±0.21	0.97 ±0.19	0.26 ±0.07
GA_{7}	0.26 ±0.08	0.01 ±0.00	0.01 ±0.01	0.06 ±0.02	0.01 ±0.00	0.07 ±0.03	0.11 ±0.08	0.15 ±0.01	5.61 ±1.73	3.13 ±1.13	0.79 ±0.05	4.24 ±0.81	16.55 ±6.93