Spatio-temporal Seed Development Analysis Provide Insight into Primary Dormancy Induction and Evolution of the *Lepidium DELAY OF GERMINATION 1* Genes

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Supplemental Data

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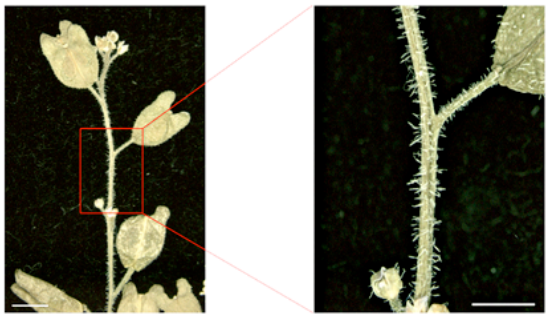
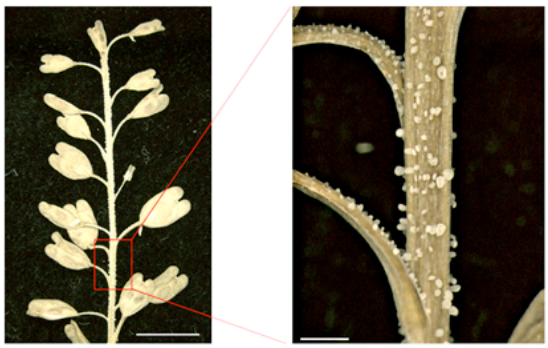
Supplemental Figure S1



Lepidium oxytrichum
Scales: 2 mm (left), 1 mm (right)



Lepidium papillosum
Scales: 5 mm (left), 0.5 mm (right)

Supplemental Figure S1. Comparison of *Lepidium oxytrichum* and *Lepidium papillosum* trichomes. The fruit axis of *L. oxytrichum* is hairy with acicular hairs, whereas that of *L. papillosum* is papillose, with white, clavate or turgig, vescicular hairs (Hewson 1981).

Hewson H (1981) The genus *Lepidium* L. (Brassicaceae) in Australia. Brunonia **4:** 217-308

Supplemental Figure S2

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1 Lepidium oxytrichum_[211]_ITS (Loxy-ITS)
2 Lepidium papillosum_[719]_ITS (Lepa-ITS)
3 Lepidium accession "Mummenhoff 2415" used in this work (Lepa-?)

Loxy-ITS AUTCGATACC TGTTCAAAAC AGAACGACCC GCGAACCAAC TATCATCACT
Lepa-ITS AUTCGATACC TGTTCAAAAC AGAACGACCC GCGAACCAAC TATCATCACT
Lepa-?     tcgatacc tgtccaaaac agaacgaccc gcgaaccaac tatcatcact

Loxy-ITS TGCGGTGGGC CGGTTTCCTA GCAGATCCCG TGTCCTCCGA ATCCTTGGTT
Lepa-ITS TGCGGTGGGC CGGTTTCCTA GCAGATCCCG TGTCCTCCGA ATCCTTGGTT
Lepa-?   tgcggtgggc cggtttccta gcagatcccg tgtcctccga atccttggtt

Loxy-ITS TCGCGTAC-G TTCCGAACGG GAGATCTCTC CCGGACCGGT CCTGCGCGTA
Lepa-ITS TCGCGTACCG TTCCGAACGG GAGATCTCTC CCGGACCGGT CCTGCGCGTA
Lepa-?   tcgcgtacg  ttccgaacgg gagatctctc ccggaccggt cctgcGCGTA

Loxy-ITS GCTGATGGAT ATCACAACAA CACGGCACGA AAAGTGTCAA GGAACATGCA
Lepa-ITS GCTGATGGAT ATCACAACAA CACGGCACGA AAAGTGTCAA GGAACATGCA
Lepa-?   gCTGATGgAT ATCACAaCAA CACGGCAcGA AAAGTGTCAA GGAACATGCA

Loxy-ITS ACCGAACGGC [CA-C] GTTCGC CTTCCCGGAG ACGGTGCGAG -CGCGA [-ATT]
Lepa-ITS ACCGAACGGC [CA-C] GTTCGC CTTCCCGGAG ACGGTGCGAG -CGCGA [-AT-]
Lepa-?   ACCGAACGGC [CAGC] GTTCGC CTTCCCGGAG ACGGTGCGAG -CGCGA [-AT-]

Loxy-ITS GCTGTGCTGC GATCTAAAGT CTATCGTCGT CCCACTCA [CG AAATTTT-G] C
Lepa-ITS GCTGTGCTGC GATCTAAAGT CTATCGTCGT CCCACTCA [CG AAATTTT-G] C
Lepa-?   GCTGTGCTGC GATCTAAAGT CTATCGTCGT CCCACTCA [CG AAATTTT-G] C

Loxy-ITS GAGTGCGGGG CGGAA-CTGG TCTCCCGTGT GTTACCGCAC GC [-GG] TTGGC
Lepa-ITS GAGTGCGGGA CGGAAGCTGG TCTCCCGTGT GTTACCGCAC GC [-GG] TTGGC
Lepa-?   GAGTGCGGGA CGGAAGCTGG TCTCCCGTGT GTTACCGCAC GC [-GG] TTGGC

Loxy-ITS CAAAATCTGA GCTGAGGATG CTGGGAGCGT CCCGACATGC GGTGGTGATC
Lepa-ITS CAAAATCTGA GCTGAGGATG CTGGGAGCGT CCCGACATGC GGTGGTGATC
Lepa-?   CAAAATCTGA GCTGAGGATG CTGGGAGCGT CCCGACATGC GGTGGTGATC

Loxy-ITS TAAAGCCTCT TCATATTGCC GGTCGCTCCT GTCCGTAAGC TCTCGTTGAC
Lepa-ITS TAAAGCCTCT TCATATTGCC GGTCGCTCCT GTCCGTAAGC TCTCGTCGAC
Lepa-?   TAAAGCCTCT TCATATTGCC GGTCGCTCCT GTCCGTAAGC TCTCGTCGAC

Loxy-ITS CCAATGTCCT CAAA
Lepa-ITS CCAATGTCCT CAAA
Lepa-?   CCAATGTCCT CAAA
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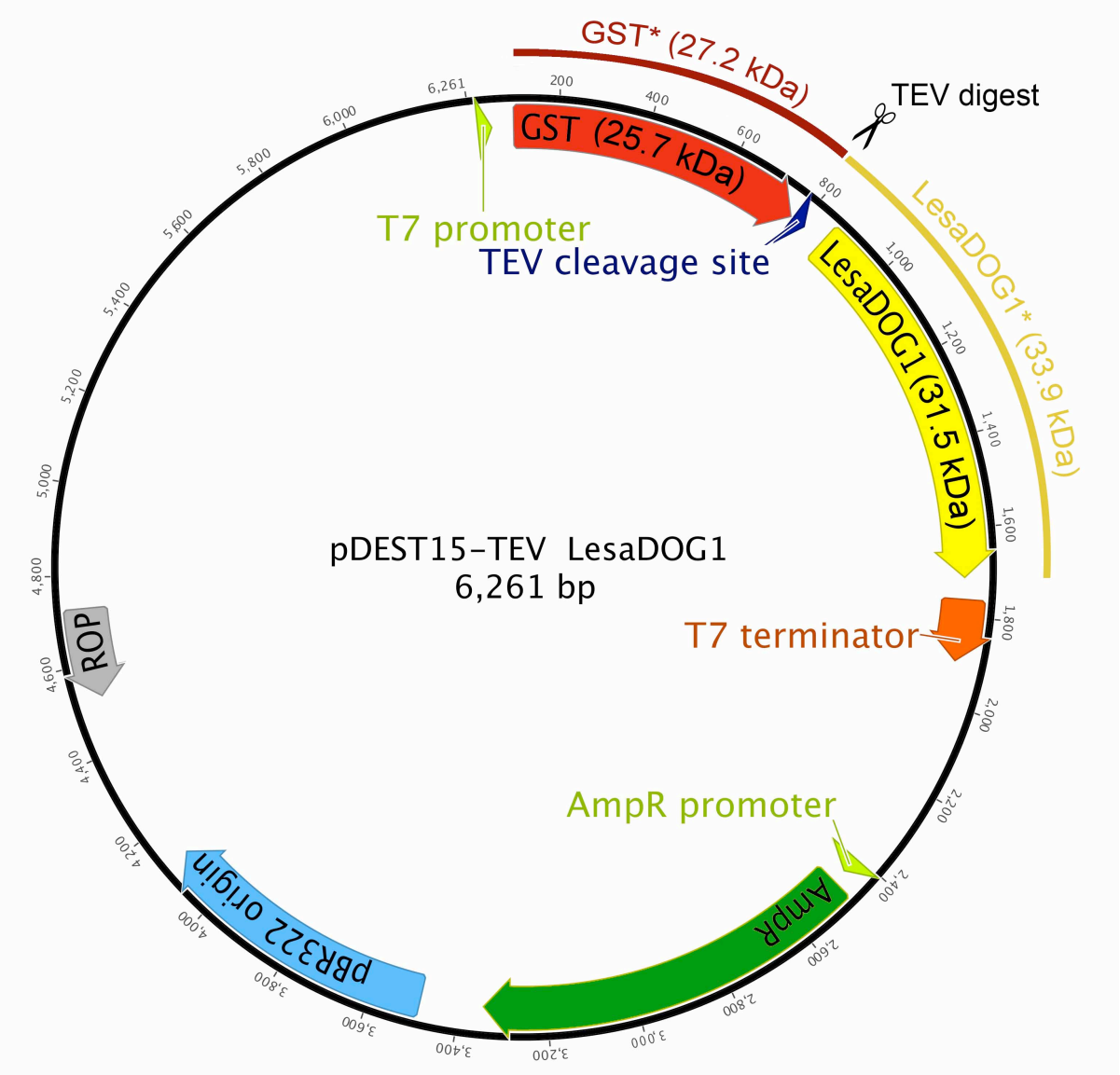
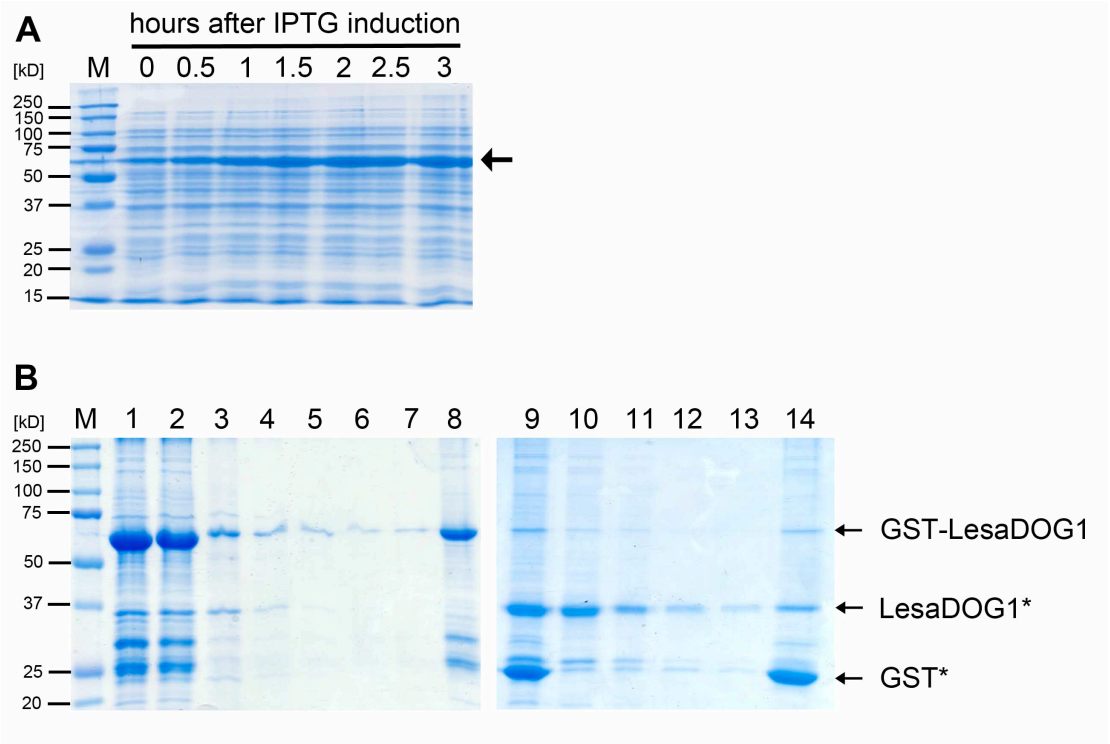
Supplemental Figure S2. ITS sequence comparison between *Lepidium oxytrichum*, *Lepidium papillosum* and the *Lepidium* accession "Mummenhoff 2415" (Lepa-?) used in this work, confirming that this accession is *Lepidium papillosum*. Positions in [ ] indicate ambiguous alignment in the total alignment of the genus *Lepidium*. Small letters mean only one strand was sequenced, capital letters mean both strand were sequenced, positions with sequence differences are in bold, and Lepa-? nucleotide positions identical to the Lepa-ITS are underlined.

Supplemental Figure S3



Supplemental Figure S3. Plasmid Map of modified pDEST15 vector containing a TEV cleavage site used for recombinant GST-LesaDOG1 fusion protein expression. Predicted molecular masses of native GST and LesaDOG1 proteins are shown on the inside, predicted masses of GST* and LesaDOG1* proteins resulting from TEV cleavage of the fusion protein are shown on the outside of the plasmid map. GST-Linker(TEV)-LesaDOG1 is encoded as a continuous ORF on the plasmid. Numbers on plasmid: bp.

Supplemental Figure S4



Supplemental Figure S4. Recombinant GST-LesaDOG1 fusion protein expression and purification. A, Coomassie stained SDS-PAGE gel showing the IPTG induced expression of the 61 kDa GST-LesaDOG1 fusion protein (arrow) in *E. coli*. Samples of the growing bacterial culture were taken at indicated timepoints, heated in SDS-loading buffer and directly used in SDS-PAGE. B, Coomassie stained SDS-PAGE gel showing successive fractions of second round of protein purification process (see methods for details). 1) eluted protein fraction of first purification round after dialysis; 2) matrix bound proteins after incubation of fraction 1 with Glutathion-Sepharose matrix overnight at 4 °C; 3) unbound protein; 4 -7) wash fractions, unbound protein; 8) matrix bound protein after washes; 9) matrix bound protein after TEV cleavage; 10) unbound protein after TEV cleavage; 11 -13) wash fractions, unbound protein; 14) matrix bound protein after washes. Note: a large amount of GST-LesaDOG1 binds to matrix (2) whilst a small amount is not bound (3) and subsequently washed away (4-7); the matrix bound GST-LesaDOG1 (8) is efficiently proteolytically cleaved at its TEV recognition site resulting in LesaDOG1* appearing at ca. 37 kD and GST* at 25 kD (compare Supplemental Fig. S3) only leaving a small amount of uncut GST-LesaDOG1 (9); relatively pure LesaDOG1* is present in the unbound fractions (10-13) while GST* remains bound to the membrane (14); Fraction 10 was used in western experiments in Figs. 6 and 7 as a control and is referred to as 'DOG1'. Note the remaining presence of GST-LesaDOG1 and free GST* as detected by anti-GST antibody (Fig.6C) in fraction 10, which is hardly visible in the Coomassie stain. M, molecular mass marker.

Supplemental Table S1 Putative *DOG1* orthologs cloned from diverse *Lepidium* species.

Lepidium species	GenBank accession number	Sequence type [Gene name used in text]	Size (bp)	Primer pair[d]	Sequence similarity (%) to *LesaDOG1*[a]	Accession number	Origin
L. campestre	JX512181	genomic	1146	B	72.0	OSBU 96-0070-30-00	Greece, Thrace, Nomos Evrou, Korimvos
L. africanum[c]	JX512177	genomic	1323	B	66.3	OSBU 22265	Australia, Melbourne, Royal Park, south of Park Street; coll. N. Scarlett
L. pseudohyssopifolium[c]	JX512179	genomic	1152	B	66.6	OSBU 22263	Australia Victoria, Mitre Rock, near Mt. Arapiles; 141°50`E 36°44`S; coll. N. H. Scarlett
L. ruderale[c]	JX512180	genomic	1369	B	64.1	OSBU 05-0209-10-00	East Kazakhstan, Zaissan distr., Akzhan; coll. Antonyuk
L. bonariense[c]	JX512178	genomic	1238	B	71.8	OSBU 95-0022-10-00	Australia, NSW, Broken Hill; coll. H. Hurka
L. papillosum	JX512183	genomic [*LepaDOG1a*]	1343	A[e]	85.0	Mummenhoff 2415	Australia, NSW, 10 km west of Broken Hill
L. papillosum	JX512187	mRNA [*LepaDOG1a*]	442	E	100.0	Mummenhoff 2415	dito
L. papillosum	JX512184	genomic [*LepaDOG1b*]	1317	B[e]	73.5	Mummenhoff 2415	dito
L. papillosum	JX512185	genomic [*LepaDOG1c*]	1583	C[e]	65.6	Mummenhoff 2415	dito
L. papillosum	JX512188	mRNA [*LepaDOG1c*]	386	F	90.4	Mummenhoff 2415	dito
L. papillosum	JX512186	genomic [*LepaDOG1d*]	832	D	43.6	Mummenhoff 2415	dito
L. didymum	JX512176	genomic	1216	B	74.0	OSBU 22266	Germany, Osnabrück, near City Hall; coll. K. Sperber
L. leptopetalum[b]	JX512182	genomic	279	C	87.5	OSBU 22264	Australia, Victoria, near Carwarp; 142°06`E 34°38`S, coll. A.H. Brown

[a] Cloned genomic fragments have been aligned against genomic *LesaDOG1* sequence; cloned cDNA sequences have been aligned against *LesaDOG1* cDNA sequence. Similarity is the % pairwise identity within the aligned region. Global alignment was performed using MUSCLE. All genomic fragments cover at least partially exon 1 and exon 2 including the whole intron.
[b] This genomic fragment only covers exon 1.
[c] For these species also another smaller genomic fragment covering only exon 1 was cloned, which is not identical to the listed larger genomic fragment; this indicates the presence of multiple *DOG1* genes in these species.
[d] Primer pair abbreviations refer to supplemental Table S2.
[e] These primer pairs were used for the initial cloning of *L. papillosum DOG1* gDNA fragments. To extend these sequences primers were designed based on an alignment of these gDNA sequences at highly divergent regions to obtain specific genomic fragments of the individual *L. papillosum DOG1* genes. Consensus sequences of these genomic fragments were derived for each *L. papillosum DOG1* gene.

Supplemental Table S2. Primer sequences used for cloning of *Lepidium DOG1* gene sequences and primer pair combinations (indicated by a common letter) as used in Supplemental Table S1.

Primer name	**Primer Sequence (5'-3')**	**Primer pair combination**
1-exFP1-DOG1	ACCAAGAGTGGATGAATTTGC	C
1-exFP2-DOG1	CGCGTACCTGATCTCAAACA	B
2-exRP2-DOG1	CGGCGACGATCTCTCATAGT	A, B, D
viPCR-FP2	ACCGGATTCGACATTATACCA	D
iPCR-RP2	CAAACCATCGATGTCACGAA	C
LoxyDOG1a FP2	TTATGCGCCGTCTTGGAACA	E
LoxyDOG1a RP2	GCAAGAAATTGGCCGCTTCG	E
LoxyDOG1b FP1	TTACGCCGGAAGAAGAGCTG	F
LoxyDOG1b RP1	TGCTTGTCGAGAGCTTGGTC	F
viPCR-FP1	CCAAAACACAAAACACAGCA	A
LesaDOG1a-FP3-wgDNA	TATGTATTTTAGAAAAATGGGATCTTC	
LesaDOG1-FP1-GW	GAAGGAGATAGAACCATGGGATCTTCAAAGAAGAACATCG	
LesaDOG1-RP1-GW	CTATTGCTTCTTCTCCTCCTCTCCTTTGG	
GSP-3	AGCCACACAAAGCATAAACGA	