

Supplementary Material

	225	235	245	255	265	275	285	295	305	315	325
AtSDP1	LSFMHETRHA	FGRTALLLSG	GASLGAFHVG	VVRTLVEHKL	LPRIIAGSSV	GSIIICAVVAS	RSWPELQSFF	ENSLHSLQFF	DQLGGVFSIV	KRVMTQGALH	DIRQLQCMLR
AtSDP1-like	LSFMHETRHA	YGR TALLLSG	GASLGAFHLG	VVKTLVEHKL	LPRIIAGSSV	GSVMCAVVG	RSWPELQSFF	EGSWHALQFF	DQMGGIFTTV	KRVMTQGAHV	EIRHLQWKLR
GmSDP1-1	LSFMHETRHA	FGRTALLLSG	GASLGAFHVG	VVKTLVEHKL	LPRIIAGSSV	GSIMCSIVAT	RSWPELQSFF	EDSLHSLQFF	DQMGGIFTTV	KRVTTYGAVH	EIRQLQMLLR
GmSDP1-2	LSFMHETRHA	FGRTALLLSG	GASLGAFHVG	VVKTLVEHKL	LPRIIAGSSV	GSIMCSIVAT	RSWPELQSFF	EDSLHSLQFF	DQMGGIFTTV	KRVTTYGAVH	EIRQLQMLLR
GmSDP1-3	LAFMHETRHA	FGRTALLLSG	GASLGASHVG	VVKTMVEHKL	MPRIIAGSSV	GSIMCAVVAT	RTWPELQSFF	EDSWHSLQFF	DQMGGIFAVV	KRVTTLGAVH	EIRQLQMLLR
GmSDP1-4	LAFMHETRHA	FGRTALLLSG	GASLGASHVG	VVKTLVEHKL	MPRIIAGSSV	GSIMCAVVAT	RTWPELQSFF	EDSWHSLQFF	DQMGGIFAVV	KRVTTLGAVH	EIRQLQMLLR
	*:*****	:*****	***** *:*	***:*****	:*****	***:***:*	:*****	***:*****	*,* *:****	***:***:*	*** * **:* :*:*** **
	335	345	355	365	375	385	395	405	415	425	435
AtSDP1	NLTSLNLTQFE	AYDMTGRILG	ITVCSPRKHE	PPRCLNYLTS	PHVVIWSAVT	ASCAFPGLFE	AQELMAKDRS	GEIVPYHPPF	NLDPEVGTGS	SSGRRWRDGS	LEVDLPMMQL
AtSDP1-like	NLTSLNLTQFE	AYDITGRILG	ITVCSLRKHE	PPRCLNYLTS	PHVVIWSAVT	ASCAFPGLFE	AQELMAKDRT	GEIVPYHPPF	NLDPEEG--S	ASVRRWRDGS	LEMDLPMIQL
GmSDP1-1	HLTSLNLTQFE	AYDMTGRILG	ITVCSPRKHE	PPRCLNYLTS	PHVVIWSAVT	ASCAFPGLFE	AQELMAKDRS	GEIVPYHPPF	NLGPEKG--S	TSVRRWRDGS	LEMDLPMMQL
GmSDP1-2	NLTSLNLTQFE	AYDMTGRILG	ITVCSPRKHE	PPRCLNYLTS	PHVVIWSAVT	ASCAFPGLFE	AQELMAKDRS	GEIVPYHPPF	NLGPEKG--S	TSVRRWRDGS	LEMDLPMMQL
GmSDP1-3	HLTSLNLTQFE	AYDMTGRILG	ITVCSPRKHE	PPRCLNYLTS	PHVVIWSAVT	ASCAFPGLFE	AQELMAKDRS	GEIIPYHPPF	NLGPEEG--S	TPARRWRDGS	LEIDLPMMLQ
GmSDP1-4	HLTSLNLTQFE	AYDMTGRILG	ITVCSPRKHE	PPRCLNYLTS	PHVVIWSAVT	ASCAFPGLFE	AQELMAKDRS	GEIVPYHPPF	NLGPEEG--S	TPVRRWRDGS	LEIDLPMMLQ
	:**.******	***:*****	***** ****	***** **	***** **	***** **	***** **	***** **	***:*****	**.* ** *	:. ***** ** ****:*
	445	455	465	475	485	495	505	515	525	535	545
AtSDP1	KELFNVNHFI	VSQANPHIAP	LLRLKDLVRA	YGGFFAAKLA	HLVEMEVKHR	CNQVLELGFP	LGGLAKLFAQ	EWEGDVTVM	PATLAQYSKI	IQNP THVELQ	KAANQGRRCT
AtSDP1-like	KELFNVNHFI	VSQANPHIAP	FLRMKEFVRA	CGGRFAAKLA	QLAEMEVKHR	CNQVLELGLP	LREVASLFAQ	EWEGDVTVM	PATFSQYLKI	IQNPSNVEIQ	KAANQGRRCT
GmSDP1-1	KELFNVNHFI	VSQANPHIAP	LLRLKEFVRA	YGGNFAAKLA	HLAEMEVKHR	CNQVLELGFP	LGGLAKLFAQ	DWEGDVTVM	PATLAQYLKI	IQNPSYVELQ	KAANQGRRCT
GmSDP1-2	KELFNVNHFI	VSQANPHIAP	LLRFKEIIRA	YGGNFAAKLA	HLAEMEVKHR	CNQVLELGFP	LGGLAKLFAQ	DWEGDVTVM	PATPAQYLKI	IQNPSYVELQ	KAANQGRRCT
GmSDP1-3	KELFNVNHFI	VSQANPHIAP	LLRLKEFVRT	YGGNFAAKLA	HLVEMEVKHR	CNQILELGFP	LGGLAKLFAQ	DWEGDVTVM	PATLAQYTKI	IQNPSYVELQ	KATNQGRRCT
GmSDP1-4	KELFNVNHFI	VSQANPHIAP	LLRLKEFVRT	YGGNFAAKLA	HLVEMEVKHR	CHQILELGFP	LGGLAKLFAQ	DWEGDVTVM	PATLAQYTKI	IQNPSYVELQ	KAANQGRRCT
	*****	*****	***:***:*	**.******	:*.******	*:***:***:*	* :*.****	:*****:*	*** :** **	****: *	***:*****

Figure S1. GmSDP1 paralogs possess high amino acid sequence identity with Arabidopsis SDP1. The patatin-like domain is underlined and the conserved GX SXG serine esterase motif is outlined with a box. * indicates amino acid identity for all six sequences.

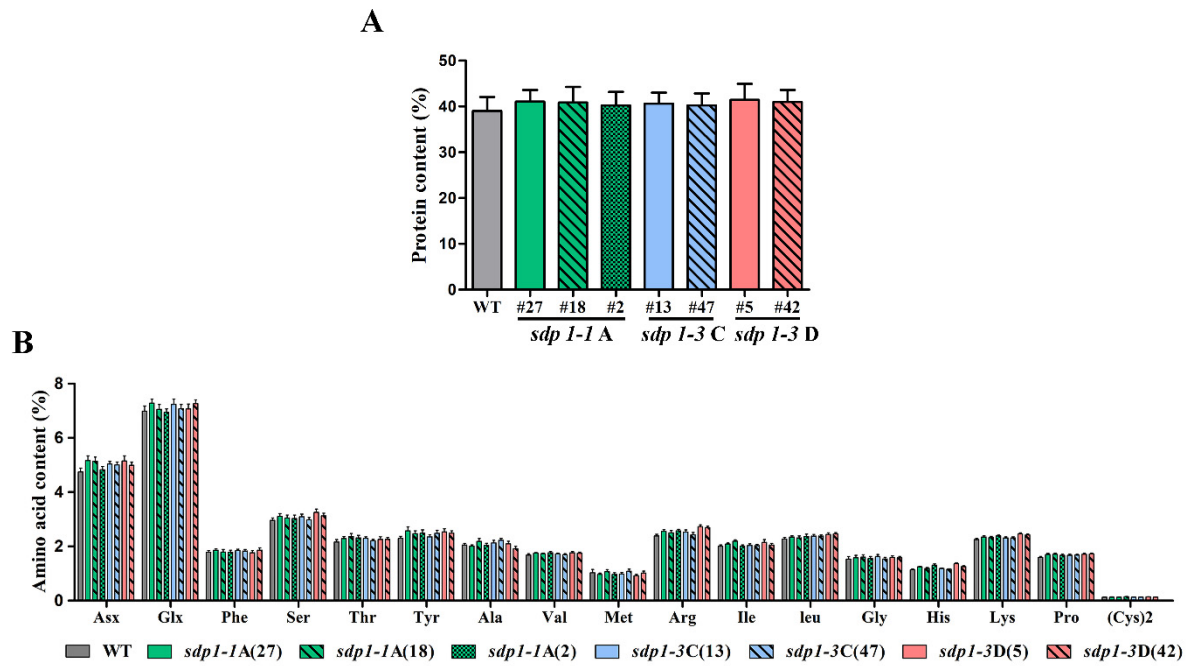


Figure S2. Protein content of mature seed of *GmSDPI*-suppressed transgenic lines. **(A)** Total protein content as a percentage of seed weight was quantified for select transgenic lines and wild-type (WT) plants. **(B)** Amino acid composition of wild-type and *GmSDPI*-suppressed transgenic lines. Values represent the mean $\pm$ SD of six technical replicates each for seed collected from two plants. There was no statistical difference between WT and transgenic lines.

Supplementary Material

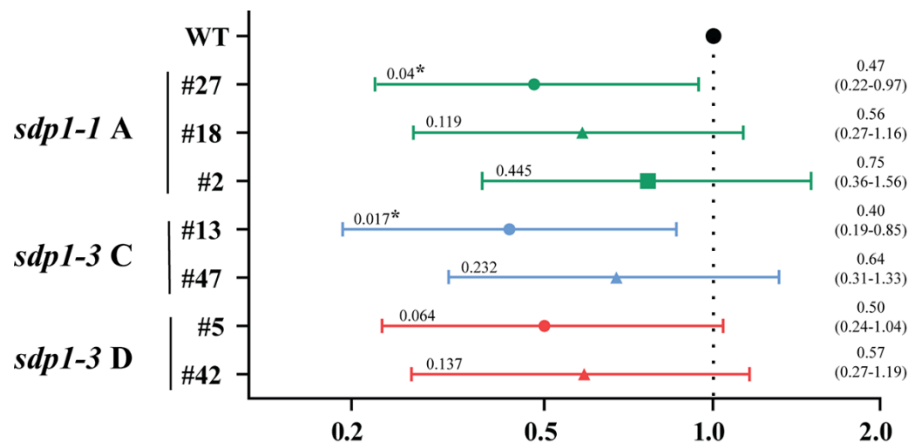


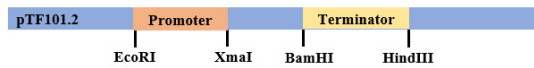
Figure S3. Cox proportional hazard model indicating hazard co-efficient of germination risk. Hazard ratios of estimated error, lower and upper value of estimated difference at 95% confidence interval and *p* values of germination risk of *GmSDPI*-suppressed lines as compared to the wild-type (WT).



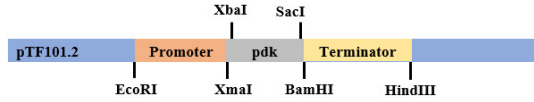
Figure S4. *GmSDPI*-suppressed lines develop similar to wild-type control plants. Representative four weeks old wild-type (WT) and *GmSDPI*-suppressed lines grown under greenhouse conditions.

Supplementary Material

1. Cloning glycine promoter and terminator



2. Cloning pdk intron



3. Cloning sense (S) and antisense (A)

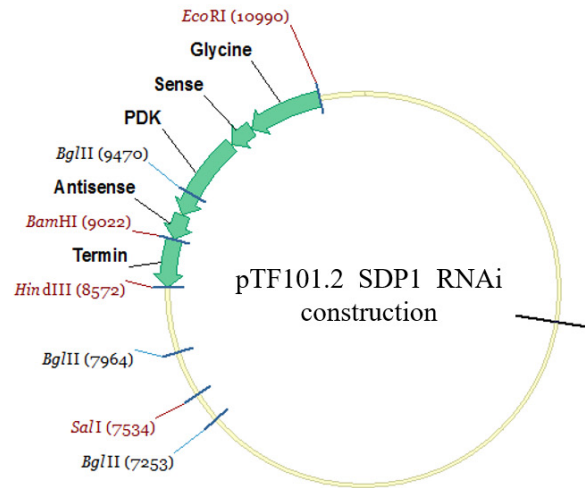
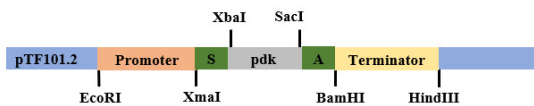


Figure S5. Scheme for the creation of the RNAi constructs used to target *GmSDP1* expression. (A) The promoter and terminator are introduced in two steps into the vector via EcoRI/XmaI and BamHI/HindIII respectively. (B) The PDK intron contained within the RNAi hairpin was amplified with specific primers including two restriction sites XmaI/XbaI and SacI/BamHI for each side and introduced in the vector. (C) The sense (S) and antisense (A) sequences were then cloned into the appropriate sites in the vector.

Supplementary Material

Gene	<i>sdp1-1 RNAi A</i>	<i>sdp1-1 RNAi B</i>	<i>sdp1-3 RNAi C</i>	<i>sdp1-3 RNAi D</i>
<i>GmSDP1-1</i> Glyma.02g190000	100	100	90.0	90.0
<i>GmSDP1-2</i> Glyma.10g105200	98.8	97.3	92.0	89.0
<i>GmSDP1-3</i> Glyma.19g132900	89.6	90.6	100	100
<i>GmSDP1-4</i> Glyma.03g130900	91.2	92.0	99.1	98.4

Table S1. Percent homology between each RNAi target sequence and *GmSDP1* paralogs.

Supplementary Material

		C16:0		C18:0		C18:1		C18:2		C18:3	
		MEAN	±SD	MEAN	±SD	MEAN	±SD	MEAN	±SD	MEAN	±SD
WT		13.7	0.41	4.2	0.23	19.6	1.16	54.8	1.35	7.7	0.26
<i>sdpl-1</i> A	2	12.7	0.29	3.7	0.18	21.1	1.35	55.4	0.80	7.1	0.36
	12	13.2	0.33	4.2	0.16	19.3	0.93	55.8	0.63	7.5	0.42
	18	12.3	0.37	3.7	0.16	24.3	2.84	53.0	2.04	6.7	0.64
	27	12.4	0.32	3.8	0.14	23.3	2.22	53.7	1.77	6.9	0.43
	35	12.5	0.48	3.7	0.13	22.4	2.50	54.3	1.93	7.1	0.40
	52	13.1	0.41	4.0	0.23	22.2	1.46	54.0	1.48	6.7	0.26
<i>sdpl-1</i> B	7	12.5	0.37	4.0	0.29	24.5	2.08	52.1	1.92	6.8	0.35
	23	12.7	0.33	3.7	0.13	20.4	2.43	55.7	2.10	7.4	0.72
	27	12.8	0.37	3.9	0.15	22.4	1.74	54.0	1.26	6.8	0.55
<i>sdpl-3</i> C	12	12.7	0.46	3.8	0.20	20.6	1.47	54.9	0.94	8.0	0.50
	13	12.9	0.25	3.8	0.14	20.7	1.83	55.2	1.52	7.4	0.54
	15	12.9	0.32	3.9	0.20	20.9	1.25	55.1	0.74	7.2	0.50
	31	13.3	0.51	3.8	0.19	20.8	0.88	54.6	0.74	7.6	0.40
	47	13.4	0.26	4.0	0.17	20.8	1.19	54.3	0.83	7.4	0.52
	67	13.2	0.26	4.0	0.12	21.8	1.52	53.8	1.41	7.3	0.30
<i>sdpl-3</i> D	5	12.4	0.44	3.8	0.37	23.9	3.12	53.1	2.83	6.8	0.57
	13	12.7	0.42	3.7	0.16	20.7	0.88	55.2	0.72	7.6	0.45
	16	12.5	0.74	3.8	0.31	24.1	1.70	52.6	1.92	7.0	0.45
	42	13.1	0.60	3.9	0.32	23.5	1.39	52.8	1.20	6.7	0.28

Table S2. Mean fatty acid content of T₃ seed harvested from transgenic lines targeting *GmSDPL*. Seeds from two plants were analyzed for each genotype. Shaded data is for lines shown in Figure 4B.

Supplementary Material

Name	Sequence 5' → 3'
GmSDP1-1For	TTG TCC AGG ACT TAG AAT TTC ATA AAG
GmSDP1-1Rev	TCG TCT CAG ACC ACC TAT TAA C
GmSDP1-2For	AAA TGG CAA CAG GAT GAT TGC C
GmSDP1-2Rev	ATT CCT ATT TGA AGG GGT CAA AAA GTC
GmSDP1-3For	TAG ATG CTG CTA GCT CAG CTT C
GmSDP1-3Rev	TGC TCT GAT CCA TAC CCG AAT G
GmSDP1-4For	TGA TAG CTC TGC ATC TGA ACA C
GmSDP1-4Rev	TGA TCC ATA CCC GAA TCT GTC C
ATP-For	TTT GCT GAA GCC TGT GGA GAT ATG
ATP-Rev	ACA ACC AAA TCA GTT TTC CCT CTG TG
SKIP16-For	AAC ATG GTC GCC GTT TAG AAC AC
SKIP16-Rev	CAT CTT GAA GAT CCA TCA ACT CAG GG
ELF1B-For	GCT TGA CAG TTG AGC CAT GC
ELF1B-Rev	ACA GAC TGA TCA GCC GCT TG

Table S3. Sequence of the oligonucleotides for RT-qPCR.

Supplementary Material

Name	Sequence 5' → 3'
pKMS3gly-F	<u>GAATTCT</u> ACGTAAGTACGTACTCAA
pKMS3gly-R	AAGCTT <u>AAGTCATGAAGA</u> ACCTGATAAGAC
pKMS3glyprom-F	<u>GGATCC</u> AGCCCTTTTGTATGTGCTAC
pKMS3glyprom-R	<u>CCCGGGG</u> GTGATGACTGATGAGTGTTTAAG
Pdk-F	<u>CCCGGGTCTAGACCA</u> ATTGGTAAGGAAATAATTATTTTC
Pdk-R	<u>GGATCCGAGCTCTT</u> TCGAACCCAATTTCCCAACTGTAATC
i_SDP1-1a senseF	<u>CCCGGGG</u> CATTGTTGGGAGGAC
i_SDP1-1a senseR	<u>TCTAGA</u> ACCCTCTTCACAACTGTAAAAATCCC
i_SDP1-1a antisF	<u>GAGCTC</u> ACCCTCTTCACAACTG
i_SDP1-1a antisR	<u>GAGCTC</u> GCATTTGGGAGGACTGCTT
i_SDP1-1b senseF	<u>CCCGGGG</u> AGATGTCTTAACACTTGAC
i_SDP1-1b senseR	<u>TCTAGAT</u> GCATCATAGGTAAATCATTCTCCAAGC
i_SDP1-1b antisF	<u>GAGCTCT</u> GCATCATAGGTAAATCATTCTCC
i_SDP1-1b antisR	<u>GGATCC</u> AGATGTCTTAACACTTGACTTCACC
i_SDP1-3c senseF	<u>CCCGGGG</u> ATTATGTGTGCTGTTGTTG
i_SDP1-3c senseR	<u>TCTAGAC</u> CTGTCATGTCATAAGCTTCTTG
i_SDP1-3c antisF	<u>GGATCCC</u> CTGTCATGTCATAAGCTTC
i_SDP1-3c antisR	<u>GGATCC</u> ATTATGTGTGCTGTTGTTGCC
i_SDP1-3d senseF	<u>CCCGGGG</u> CGCCGTTGGAG
i_SDP1-3d senseR	<u>TCTAGAG</u> CAAGTCCACCTAATGGAAAACC
i_SDP1-3d antisF	<u>GAGCTC</u> GCAAGTCCACCTAATGGAAAACC
i_SDP1-3d antisR	<u>GGATCCC</u> GCCGTTGGAGGGATG

Table S4. Sequence of the oligonucleotides for RNAi construction.