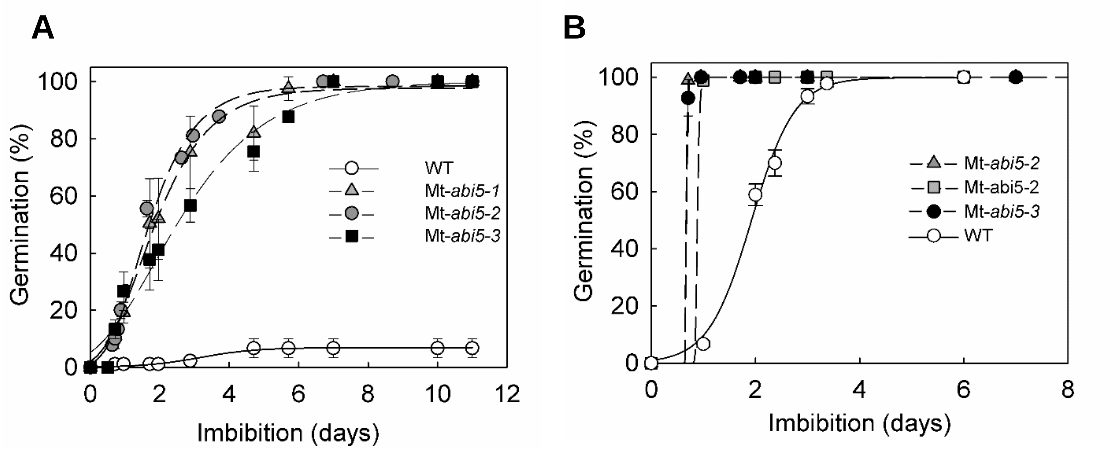


Supplemental Figure 1. Germination of Mt-*abi5* seeds is insensitive to ABA during imbibition in the dark.

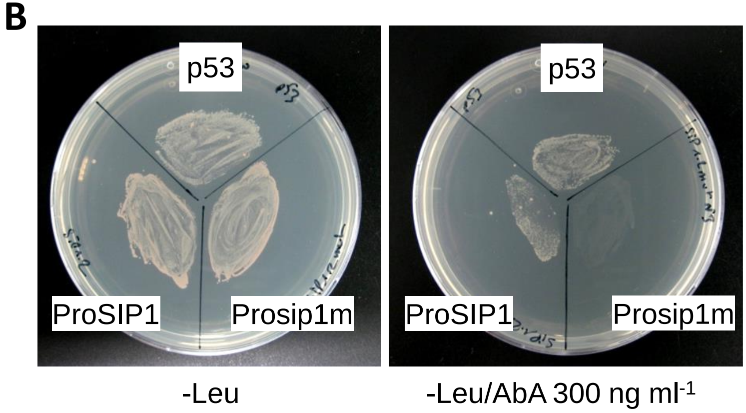
A) Percentages of germination of wild-type (WT) and Mt-*abi5* seeds in the presence of 10 µM ABA and B) in the presence of 0.005% (v:v) methanol (control). Prior to imbibition, seeds were after-ripened for 30 d and scarified. Data represent the mean of 3 biological replicates of 30 seeds pooled from 20 plants (± SE).

A

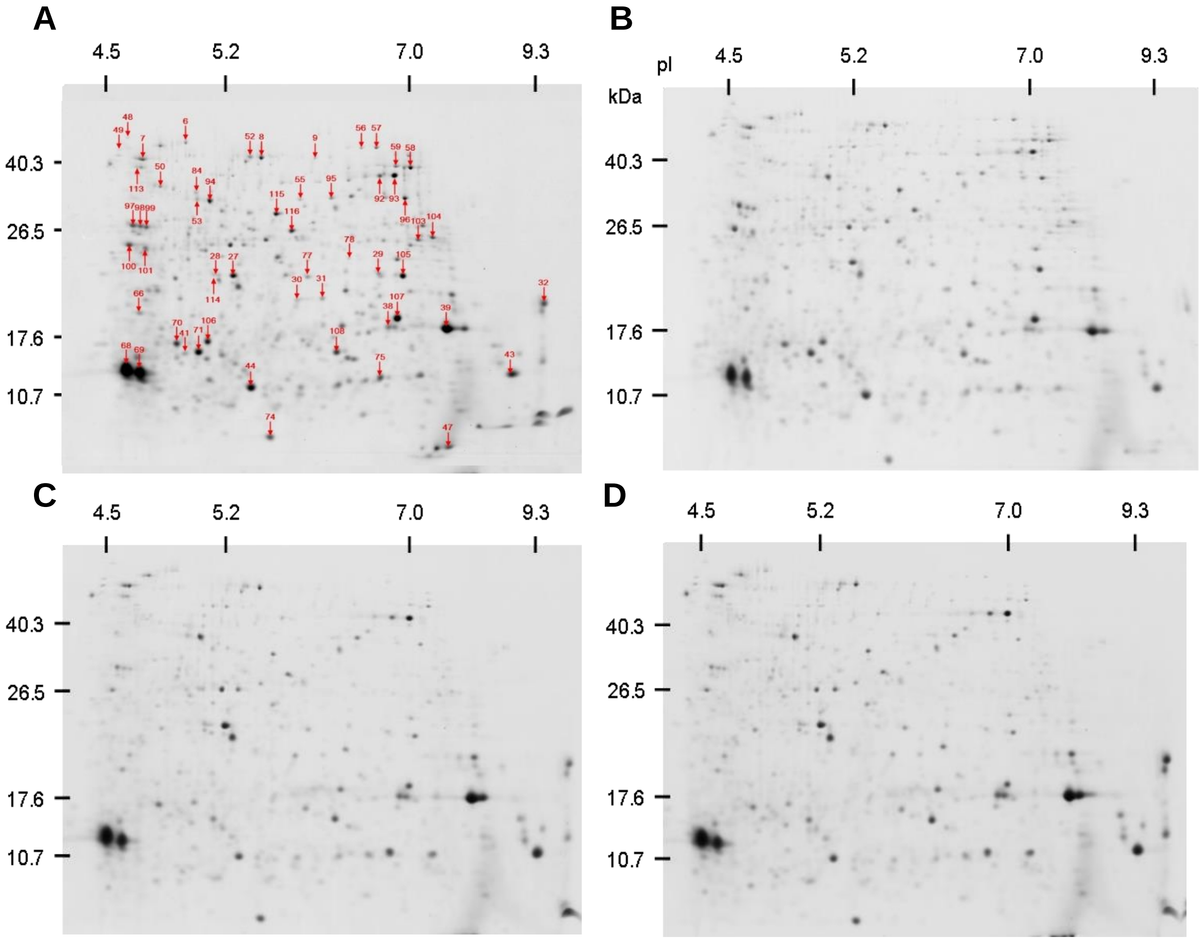
-1326

ProSIP1: ---TGTT**ACGT**GTGT---

Prosip1m: ---TGTT**TTCA**CTGT---

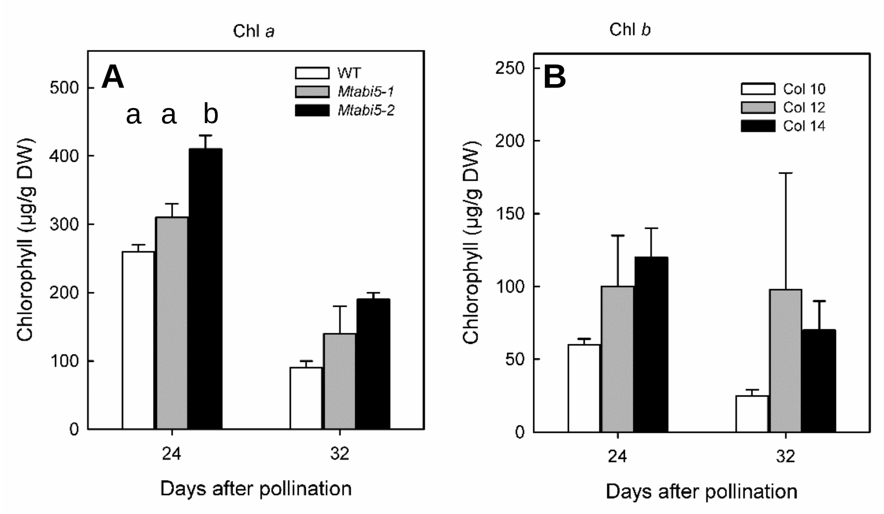


Supplemental Figure 2: Yeast one-hybrid binding assays of ABI5 to the ABRE in the promoter region of Mt-*SIP1*. A) Sequence of the -1326 bp region surrounding the ABRE (bold, underlined) used for the binding assay of the SIP1 promoter (ProSIP1) and its mutated version (Prosip1m, indicated in green). B) Binding assay using indicated baits. p53 sequence containing a p53 binding motif was used as a positive control (p53) whereas the mutated version (Prosip1m) was used as negative control (Prosip1m).



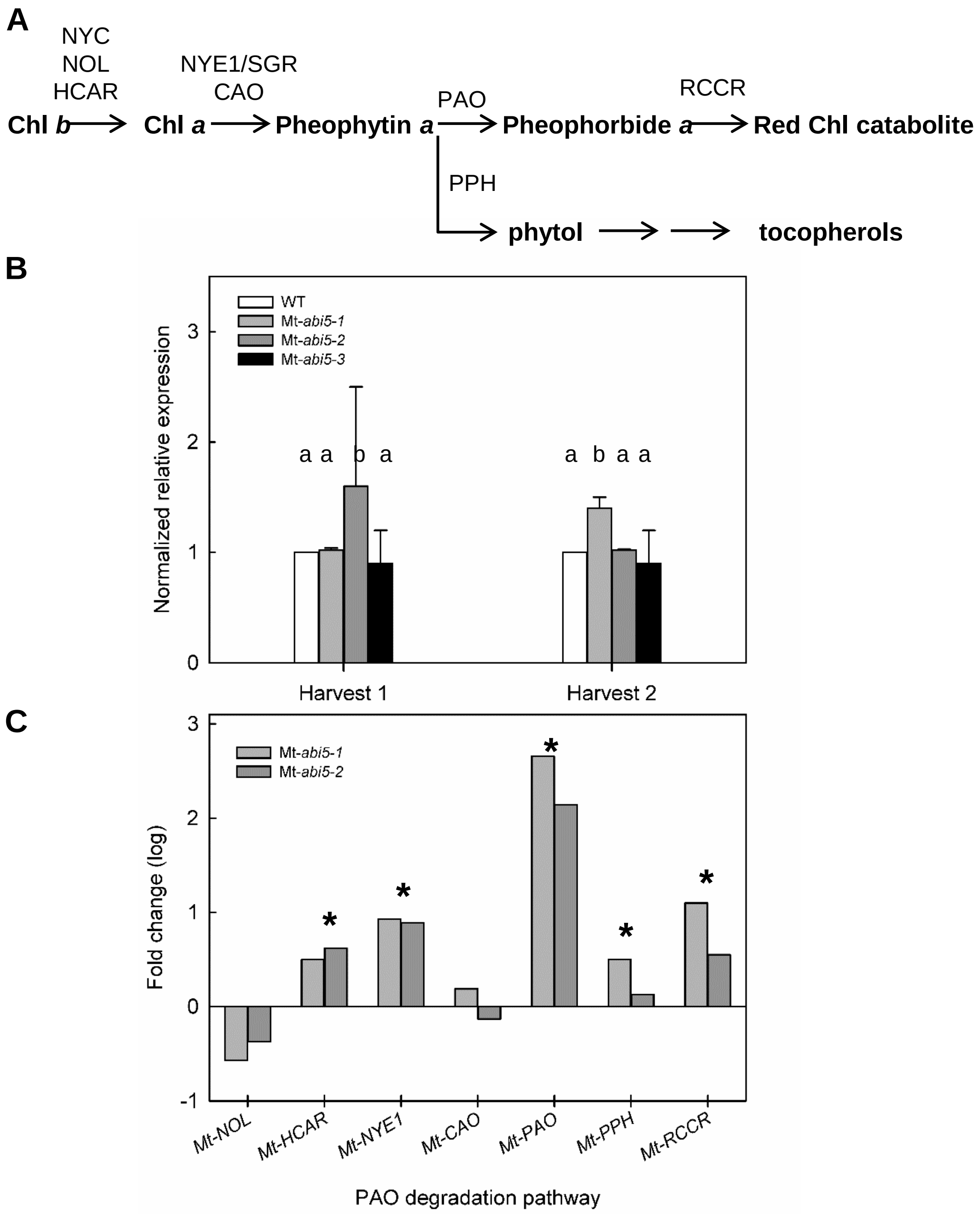
Supplemental Figure 3. **Two dimensional electrophoresis gels of the heat-stable proteome of *M. truncatula* seeds.**

A) Reference map of the heat-stable proteome of mature seeds, genotype R-108. B-D) Representative gels of the heat-stable proteome of mature wild-type (B), Mt-*abi5-1* (C) and Mt-*abi5-2* seeds (D). One hundred milligrams of the heat-stable proteins was separated by SDS-PAGE using 24 cm non linear immobilized pH gradient strips (3-10). pI and molecular mass (in kDa) are indicated. Numbers indicate the spots that were identified (see Supplementary table 1).

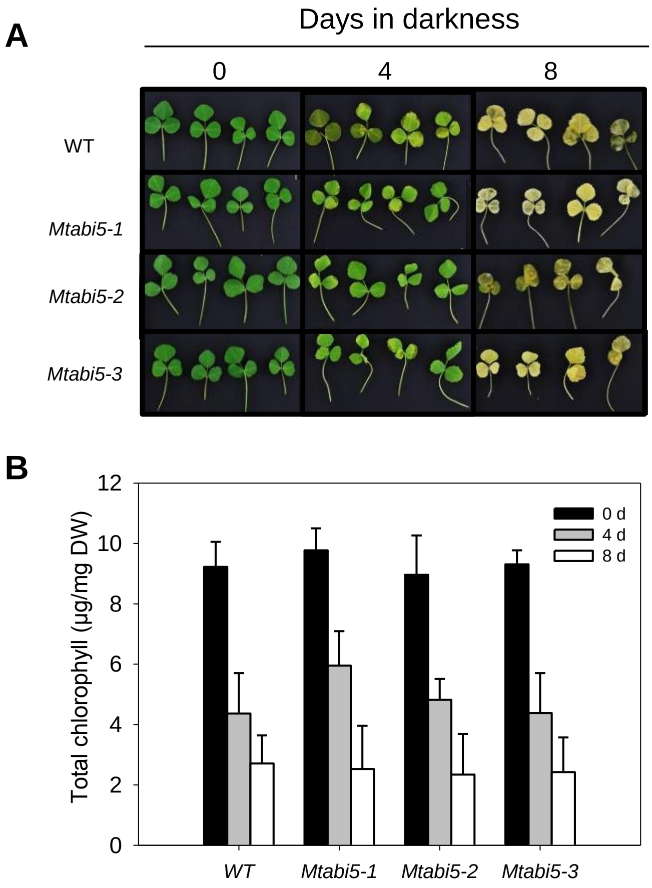


Supplemental Figure 4. **Chlorophyll contents in developing wild-type and Mt-*abi5* seeds**.

Data are the means (± SE) of 3 replicates of 30 seeds pooled from 20 plants. Letters indicate significant differences as assessed by ANOVA (P>0.05, Supplemental Dataset 3) and post-hoc Student-Newman-Keuls test for multiple comparisons.

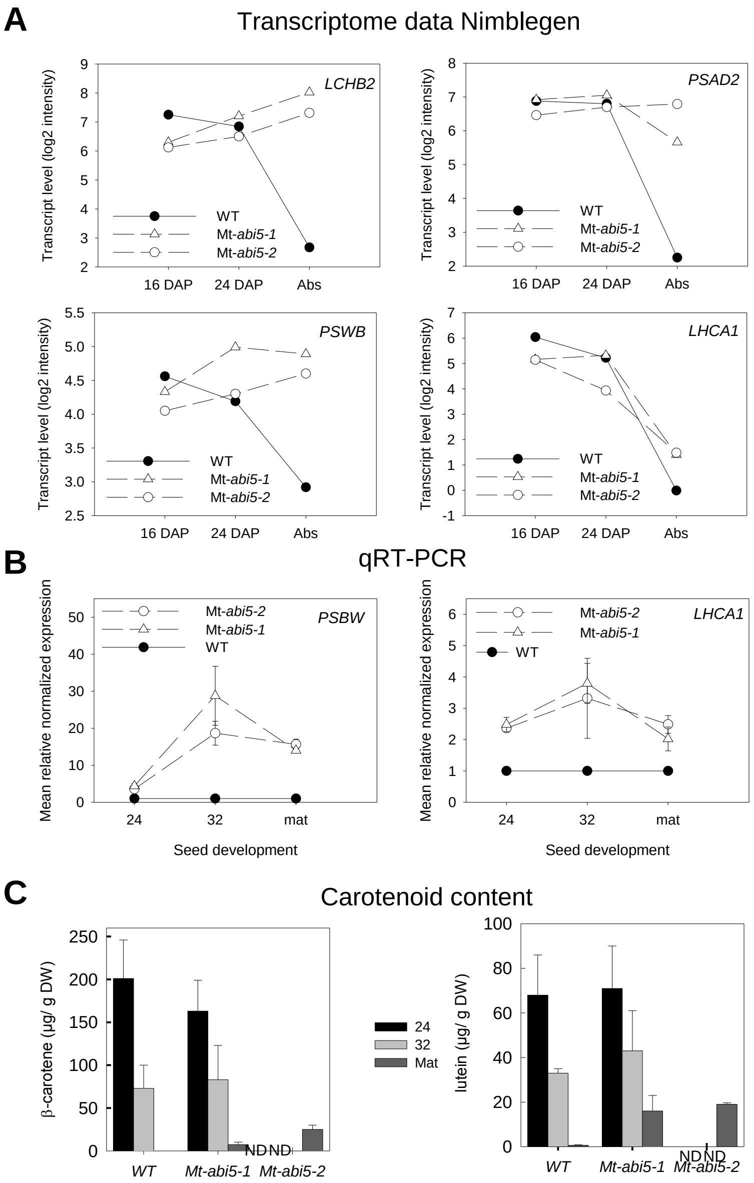


Supplemental Figure 5. Expression of genes involved in the PAO degradation pathway in mature seeds of Mt-*abi5*. A) PAO Chl breakdown pathway at the thylakoid membrane and associated enzymes (*HCAR, 7-hydroxymethyl Chla reductase*, *NYC*, *NON YELLOW COLORING1*, *NOL*, *NYC1-LIKE*), *PAO Pheide a OXYGENASE*, *SGR/NYE1* (*STAYGREEN*, whose role in Chl degradation is not established), *PPH (PHEIDE HYDROLASE)*, *RCCR REDUCTASE (RCC RED CHL CATABOLITE)*, *CAO (CHLOROPHYLIDE a OXYGENASE).* The branching route to tocopherol synthesis is also shown. B) RT-QPCR data obtained for *MtNYC1*. Data are the means (± SE) of 3 biological replicates obtained from 30 seeds of two independent harvests, each from a pool of four plants. Letters indicate significant differences compared to wild-type (ANOVA and Duncan's test for multiple comparisons). C) Expression data obtained from the transcriptome analysis. Asterisks indicate significant differences between wild-type and mutant at $P<0.05$ using BH-corrected values.

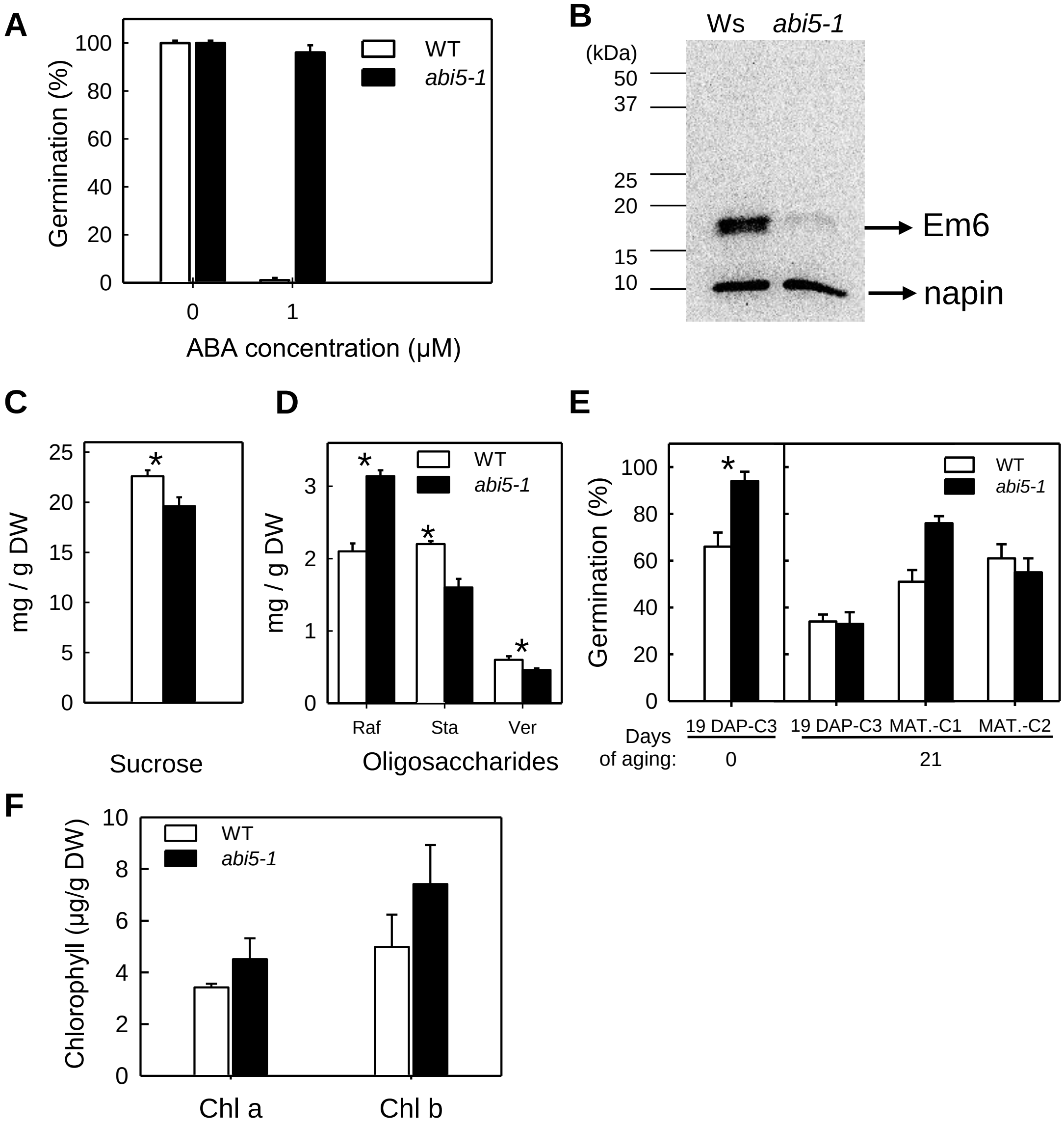


Supplemental Figure 6. Dark-induced senescence in young seedlings of wild-type and *Mtabi5*

Photographs (A) and Chl content using spectrophotometer analysis (B). Data are means (± SE) of 3 replicates of four leaves from two plants. No significant differences (ANOVA P>0.05, Supplemental dataset 3) were observed between wild-type and mutant seeds during senescence.

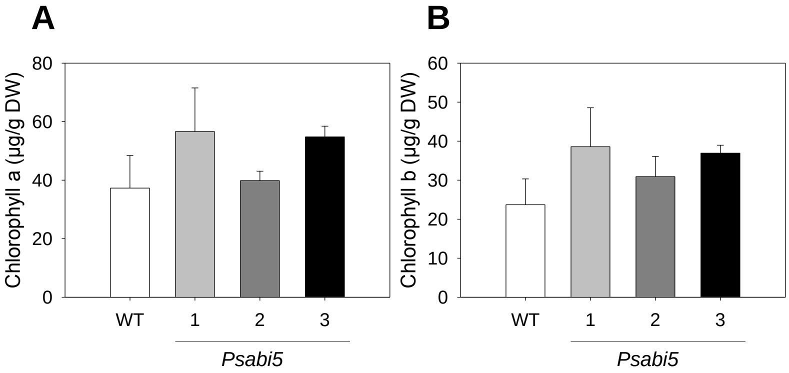


Supplemental Figure 7. Photosynthesis-associated gene expression and carotenoid content during seed development of wild-type and *Mtabi5* seeds. Gene expression (*MtLHCA1,* Medtr6g033320; *MtLHCB.2,* Medtr5g097280; *MtPSAD-2,* Medtr5g006130; *MtPSBW,* Medtr1g070495) was assessed using transcriptome analysis (A) and RT-PCR (B) where data represent the means (±SE) of 3 biological replicates of 25 seeds each pooled from 6 plants. C) β-carotene (left) and lutein contents (right) at indicated time points of days after pollination, (Mat, Mature. Seeds were harvested at the point of abscission). ND, not determined.



Supplemental Figure 8. Longevity of wild-type (Wassilewskija, Ws) and *abi5-1* seeds of Arabidopsis together with sugar content and EM protein levels in dry mature seeds.

A) ABA sensitivity. B) EM6 amount as assessed by immunoblot using an antibody against a synthetic peptide of a consensus amino-acid sequence. C-D) Sucrose and RFO contents of mature *abi5-1* seeds compared to mature wild-type seeds. Data are the means (± SE) of 3 replicates of 15 mg of mature seeds pooled from two plants. E) Seed dormancy and longevity of *abi5-1* mature seeds compared to mature wild-type seeds harvested at 19 DAP and at abscission (MAT). Data are the means (± SE) of 3 replicates of 100-200 mature seeds from 3 independent cultures (indicated as C1, C2, C3) F) Chlorophyll contents measured by spectrometry. Data are the means of (± SE) of 3 replicates of 15-30 mg of mature seeds pooled from four plants. When indicated, stars indicate significant difference (Student t-test, P>0.05) between wild-type and mutant seeds. Raf, raffinose; Sta, stachyose; Ver, verbascose.



Supplemental Figure 9. Chlorophyll contents in seed coats of mature wild-type and *Psabi5* mature seeds.

Chl *a* (A) *and* Chl *b* (B) contents were measured spectrophotometrically. Data are the means (± SE) of two replicates of 3 seeds from a pool of 30 plants.

Supplemental table 1. Detected polymorphisms between Jemalong J-6 and DZA315.16 in the region of ABI5 (Medtr7g104480) on LG7 (Mt4.0)

Position	Ref allele	Alt allele	Alt. Allele Count	Alt. Allele Frequency	Effect	Effect Impact	Nearest Gene	Gene Distance	Codon Change	Amino Acid Change	Exon Rank	Transcript ID
42340607	A	G	10	0.021	Upstream Gene Variant	MODIFIER	Medtr7g104480					Medtr7g104480.1
42340702	A	G	20	0.066	5 Prime UTR Variant	MODIFIER	Medtr7g104480	122			1	Medtr7g104480.1
42340937	C	T	283	0.508	Synonymous Variant	LOW	Medtr7g104480	0	ctC/ctT	L38	1	Medtr7g104480.1
42341240	A	G	167	0.265	Synonymous Variant	LOW	Medtr7g104480	0	caA/caG	Q139	1	Medtr7g104480.1
42341352	A	G	12	0.021	Missense Variant	MODERATE	Medtr7g104480	0	Agg/Ggg	R177G	1	Medtr7g104480.1
42341360	A	G	279	0.479	Synonymous Variant	LOW	Medtr7g104480	0	caA/caG	Q179	1	Medtr7g104480.1
42341399	C	G	11	0.024	Synonymous Variant	LOW	Medtr7g104480	0	acC/acG	T192	1	Medtr7g104480.1
42341519	C	T	14	0.077	Synonymous Variant	LOW	Medtr7g104480	0	aaC/aaT	N232	1	Medtr7g104480.1
42341660	C	T	13	0.028	Synonymous Variant	LOW	Medtr7g104480	0	taC/taT	Y279	1	Medtr7g104480.1
42341946	A	T	13	0.052	Intron Variant	MODIFIER	Medtr7g104480	0			1	Medtr7g104480.1
42341945	T	G	13	0.022	Intron Variant	MODIFIER	Medtr7g104480	0			1	Medtr7g104480.1
42342003	T	C	12	0.023	Intron Variant	MODIFIER	Medtr7g104480	0			1	Medtr7g104480.1
42342082	T	A	72	0.146	Intron Variant	MODIFIER	Medtr7g104480	0			1	Medtr7g104480.1
42342098	A	G	11	0.025	Intron Variant	MODIFIER	Medtr7g104480	0			1	Medtr7g104480.1
42342118	A	G	72	0.145	Intron Variant	MODIFIER	Medtr7g104480	0			1	Medtr7g104480.1
42342462	C	G	21	0.042	Downstream Gene Variant	MODIFIER	Medtr7g104490	951			0	Medtr7g104490.7
42342462	C	G	21	0.042	Intron Variant	MODIFIER	Medtr7g104480					Medtr7g104480.1
42343101	G	A	32	0.069	3 Prime UTR Variant	MODIFIER	Medtr7g104480	475			4	Medtr7g104480.1
42343101	G	A	32	0.069	Downstream Gene Variant	MODIFIER	Medtr7g104490					Medtr7g104490.7

Supplemental table 2: Soluble sugar composition of mature pea cotyledons.
Data (mg/g dry weight) are the means of 4 replicates of 20 cotyledons.

Genotype	**Sucrose**		**Stachyose**		**Raffinose**		**Verbascose**		**RFO/Sucrose**	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Wild-type	19.54	0.84	13.96	0.73	3.92	0.28	33.55	0.63	2.64	0.09
Psabi5-1	29.69	0.55	17.68	0.52	7.21	0.24	22.99	0.63	1.61	0.04
Psabi5-2	27.99	0.98	15.01	0.68	6.05	0.37	23.16	0.63	1.58	0.07
Psabi5-3	35.2	1.1	18.64	0.2	7.82	0.48	18.7	0.38	1.29	0.04

Supplemental table 3. Comparative analysis of affected phenotypes in seeds of the *abi5* mutants of *Medicago truncatula*, *Pisum sativum* and *Arabidopsis thaliana*.

Phenotype	**Mt-*abi5***	**Ps-*abi5***	**At-*abi5-1***
Dormancy	reduced	NA	reduced (at 19 DAP)
Longevity	reduced	reduced	no difference
Chlorophyll retention	yes	yes	no
EM LEA protein/transcript abundance	reduced	reduced	reduced
D-34 LEA protein/transcript abundance	reduced	reduced	reduced[1]
RFO metabolism	affected	affected	affected
PHANG transcripts	increased	ND	ND

DAP, days after pollination. NA, not applicable.ND, not determined.

[1] *Bies-Ethève N, Gaubier-Comella P, Debures A, Lasserre E, Jobet E, Raynal M, Cooke R, Delseny M. 2008. Inventory, evolution and expression profiling diversity of the LEA (late embryogenesis abundant) protein gene family in Arabidopsis thaliana. Plant Molecular Biology 67, 107–124.*

Supplemental table 4 - List of primer combinations used for mutant selection, gene expression validation by qRT-PCR and yeast one-hybrid analysis

Mapping of MtABI5

gene	detection method	Primer F 5'3'	Primer R 5'3'	restriction enzyme	before digestion size fragment	after digestion size fragment
ABI5 (AC147	CAPS	CATGCGTTGACAAAGGCATCT	CGAAATTCTTGCCGCTATCACAG	Hpy188I	497	419 + 78

Mutant selection

species	Genotype	Primer F 5'3'	Primer R 5'3'
M. truncatula	*Mtabi5-1*	ATGGTGGTAAGAGAAGGTGAGAT	AGCAGCAAGATCTAGAGCCAGA
	Mtabi5-2	ATGGTGGTAAGAGAAGGTGAGAT	AGCAGCAAGATCTAGAGCCAGA
	Mtabi5-3	GGTGGAGCAGTTGGAGAACC	TCCTCTGAACACTGCATGAGAT
Arabidopsis	WS/abi5.1	GCGCAAGCGAGACATAATGG3	TCTGAAGACACCGGGCTTAA3

P. sativum

line	detection method	polymorphism	Primer F 5'3'	Primer R 5'3'	restriction enzyme	before digestion size fragment	after digestion size fragment
1805/3434	dCAPS	C310T	TTGTCTGTGATTCAGAAGGGGATTAGTTAA	TACTATTCCCAGGATACCCCGAACC	Hpa I	481	451
3584	CAPS	G384A	GAAGAAGCGATCAGGAACGATGTTA	CTGAGCCATTGTAGCGGTGTTATTC	Nde II	573	307 + 255

qRT-PCR

Gene name	**seq_id_Mt3_5**	**Forward Primer (5'-3')**	**Reverse Primer (5'-3')**	**PCR size (pb)**	**PCR efficiency**	**R²**
MSC27	TC85211	GTTGAAGTAGACATTGGTGCTAAC	AGCTGAGTCATCAACACCCTCAT	100	74.2	0.994
Actin 11	Medtr2g008050	GCTGACCGTATGAGCAAGGA	TGCCAAGATAGACCCACCAA	114	100.4	0.998
LHCA1	Medtr6g033320	TGGCATTTGTTGGAATCGTTGT	ATGTTGTTGTGCCATGGATCAG	100	93.9	0.991
PSBW	Medtr1g070495	TGGTGGATGAAAGAATGAGTACTGA	AATAGAGAGCCCAGATAAAACCGAA	111	100.1	0.995
NYC1	Medtr6g088500	TCTGACAGCCGTCTATGGTTC	CGGGAGATGCAGTATGGACA	107	102.3	0.999
LHCB2	Medtr5g097280	ACCATGCGTCGTACTGTGAA	TGTCCCATCCATAATCACCA	137	107.6	0.999
PSAD-2	Medtr5g006130	GGAAGCACTGGTGGATTGTT	CACCAGTTGGCATCTCAAAG	100	89.8	0.992
SIP1	Medtr3g077280	AACTCTTGTTTTGCCTGATGGT	AAAACACCCGTGTATTTGTTGAGA	141	81	0.990
EM1	Medtr7g069250	TGGGAACAAAAGGAGGGCAG	AGCACGTTGTTCACCAGACT	113	105.7	0.996

Yeast One-Hybrid Assay

Bait	SIP1.2_F	**AGCTT**AAAAAAAAATAATAATCTTTGT**TACGTG**TGTTAATTTAGTAAGATAAGTA**C**
	SIP1.2_R	**TCGAG**TACTTATCTTACTAAATTAACACACGTAACAAAGATTATTATTTTTTTTT**A**
mutated form	SIP1.2 mut_F	**AGCTT**AAAAAAAAATAATAATCTTTGT**T*TTCAC*** TGTTAATTTAGTAAGATAAGTA**C**
	SIP1.2 mut_R	**TCGAG**TACTTATCTTACTAAATTAACAGTGAAAACAAAGATTATTATTTTTTTTT**A**
	Bold red, adapters for HindIII and XhoI; bold underlined, ABRE element; italics, mutated nucleotides	
Prey	MtABI5PREY_F	GCGCCATATG**ATG**GTGGTAAGAGAAGGTGAGAT
	MtABI5PREY_R:	TATACTCGAG**TCA**CAGATGACAACTTGTATTCC
	Bold: start and stop codon of Mt-ABI5	