The *Arabidopsis sn*-1-specific mitochondrial acylhydrolase AtDLAH is positively correlated with seed viability

Young Sam Seo[1], Eun Yu Kim[1], and Woo Taek Kim*

Department of Biology, College of Life Science and Biotechnology, Yonsei University, Seoul 120-749, Korea

Supplementary materials and methods

Tetrazolium-uptake assay

Seeds were incubated in 1% (w/v) 2,3,5-triphenyltetrazolium chloride (Sigma-Aldrich, St. Louis, MO, USA) for 2 days in the dark at 30°C (Debeaujon and Koornneef, 2000). The tetrazolium salts were reduced to formazon, a red-like colored end product, by NADH-dependent reductases localized to the endoplasmic reticulum (Berridge, 1996). Seed staining was observed under a light microscope (Olympus).

Ruthenium red staining

Dry seeds were incubated with 0.01% (w/v) ruthenium red (Sigma-Aldrich) for 30 min with agitation at room temperature and rinsed by extensive washing with distilled water (Arsovski *et al.*, 2009). Seed staining was observed under a light microscope (Olympus).

Supplementary Table S1. The sequence of primers used for cloning of *AtDLAH* cDNA, construction of MBP fusion, GFP fusion, transgenic plants, genotyping PCR, and RT-PCR in this study.

Gene	Foward Primers (F)	Reverse Primer (R)
Cloning of *AtDLAH* cDNA		
AtDLAH	5'-tcggatccatggagaacgcattggtc-3'	5'-gtaaaacgacggccagtgccaagctttc-3'
Construction of MBP:N terminal lacking *AtDLAH*		
AtDLAH	5'-tcggatcctcagccgacgattttcttg-3'	5'-gtaaaacgacggccagtgccaagctttc-3'
Construction of 35S:*AtDLAH-GFP*		
AtDLAH	5'-caaagagccgtcctgacaag-3'	5'-gactcgagaaaatatccaaaatgtggcgtgag-3'
Construction of 35S:*AtDLAH-HA*		
AtDLAH	5’-gcagatctatggcaggttacccatac-3’	5’-gagcggccgcgagctcagtggacgcctctagaggaacg-3’
Construction of *35S: AtDLAH* transgenic plants		
AtDLAH	5'-gcagatctatggagaacgcattggtcaa-3'	5'-cggagctctcataagctataaataggttttggtc-3'
Genotyping PCR of *atdlah*		
AtDLAH	5'-aacaaaaacctttccaagaggag-3'	5'-ttggaatctttgctccatgtc-3'
RT-PCR		
AtDLAH	5'-gtgcgccgagggtaggtaac-3'	5'-gttttggtccggtcggtaac-3'
AtCYCD3	5'-tcgggtacctcccatcag-3'	5'-gagtggctacgattgccc-3'
AtCYC2b	5'-gagcagcaatggccgg-3'	5'-cattgctaccacaccattgtg-3'
AtPCNA	5'-agggctcgttgttgaag-3'	5'-cttcaatcttaggagcc-3'
AtEXP5	5'-ggtacggcaatctgtatagc-3'	5'-gttcccacacatatattcgc-3'
AtACT8	5'-tactgattacctcatgaagatccttac-3'	5'-aaacgatgtctctttagtttagaagc-3'

Supplementary Figure Legends

Supplementary Fig. S1. Sequence analysis of *Arabidopsis* AtDLAH. (A) Multiple alignments of five DAD1-like acylhydrolases from different plant species. The derived amino acid sequence of AtDLAH is compared with those of poplar protein (*Populus trichocarpa*, XP_002314049.1; 68% identity), caster bean triacylglycerol lipase (*Ricinus communis*, XP_002531054.1; 63% identity), grape protein (*Vitis vinifera*, XP_002272780.1; 59% identity), and rape chloroplast lipase (*Brassica napus*, ACJ76846.1; 45% identity). The lipase consensus sequence (GHSLG) and the catalytic triad (Ser, Asp, and two candidate His residues) are indicated. (B) Phylogenetic relationship of the five DAD1-like acylhydrolase homologs from *Arabidopsis* (AtDLAH), poplar, caster bean, grape, and rape.

Supplementary Fig. S2. Expression levels of cell cycle- and cell elongation-related genes in wild-type, *35S:AtDLAH*, and *atdlah* mutant seedlings. Steady-state mRNA levels of cell cycle- and cell elongation-associated genes were determined by RT-PCR in light-grown 3- or 7-day-old wild-type, *35S:AtDLAH* transgenic (lines #2 and #3), and *atdlah* mutant seedlings. The D-type cyclin *AtCYCD3*, cell cycle-dependent kinase-related gene *AtCDC2b*, and S-phase-specific proliferating cell nuclear antigen (*AtPCNA*) were used as cell cycle-related genes and expansin *AtEXP5* as a cell elongation-related gene. Actin (*AtACT8*) was used as a loading control.

Supplementary Fig. S3. Germination analysis of wild-type, *35S:AtDLAH,* and *atdlah* mutant seeds in response to ABA. Wild-type, *35S:AtDLAH* transgenic (lines #2, #3, #4, #6 and #7), and *atdlah* mutant seeds were germinated in the presence or absence of different concentrations (0.1, 0.5, and 1 μM) of ABA. Enlarged pictures in the lower panels depict the detailed germination patterns of wild-type, *35S:AtDLAH* transgenic (line #2), and *atdlah* mutant plants 3-days after germination.

Supplementary Fig. S4. Vital staining with tetrazolium and mucilage release of seeds. (A) Wild-type, *AtDLAH*-overexpressing (lines #2 and #3), and *atdlah* mutant seeds were stained with tetrazolium under normal (upper panel) or accelerated-aging (lower panel) conditions and observed under a light microscope. Scale bars = 500 μm. (B) Mucilage release of wild-type, *AtDLAH*-overexpressing T4 transgenic, and *atdlah* mutant seeds. Light microscopic examination of the mucilage layers of wild-type, *AtDLAH*-overexpressing, and *atdlah* mutant seeds following incubation with ruthenium red. Scale bars = 500 μm.

Supplementary Fig. S5. Neutral lipid content of wild-type, *AtDLAH*-overexpressing T4 transgenic, and *atdlah* mutant seeds. Total lipids were extracted from wild-type, *AtDLAH*-overexpressing transgenic (lines #2 and #3), and *atdlah* mutant seeds under normal and accelerated-aging conditions. TLC of total neutral lipids (12 μg in left panel and 200 μg in right panel) was developed with petroleum ether/ether/CH_3COOH (70:29:1, v/v/v) and visualized by iodine vapor. Three neutral lipids, TAG, DAG, and MAG, are indicated.

A

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AtDLAH        1 MENALVKTPLR-KLRRRRTKRVWRLKQKLKLAWKSIKIRVKS---HLPGFLSTKKHLFHIKSRKEEQDLSQVAQRIC----KISNDSTKS
caster_bean   1 MEMSPLRQPVA-TKKTMSRKARRIWKLKLTVTWRAIKKAFTS---KHRLRLSCTGNMKQLSTFKLHQELATKTKKNG----DCHP--HKT
grape         1 MELLPSKKVTSGKMRRGKTKRAR----KSGFSWSAIKKALSSAMKKHHLHMTCASSLKQLSGISDGGMVTPLRQLQSEEPKKHVSHGGKS
rape          1 MATIPSHN-------------------LLLPNPTINQSAHS------LSFKPQSTLLNFPARSSPAAVTRAVSR------TDGASISSR
poplar        1 ------------------------------------------------------------------------------------------

AtDLAH       83 LAFLLQLPKYSADDFLDRGDLMTPAASPREKISKMWRELHGSNNWENLLDPLHPWLRREVTKYGEFVESVYDSLDFDPLSEFCGSSRYNR
caster_bean  81 LAHLLVP--RTALDFIDRGDQMTPTLSPKENISSRWQEIHGSRNWENLLDPLHPWLRREVVKYGEFVEATYDAFDFDPLSEYCGSCLYNR
grape        87 LESLMR-VAYTAGDFIDRGNHMTPTRSPREHISAKWREIHGQFNWESLLDPLHPWLRREIVKYGEFSQATYDAFDYDSFSDFCGSCRYNR
rape         59 LEPVEKYEITAAGDVRRRDREAKETKSLRD----TWRKIQGEDDWAGLMDPMDPVLRSELIRYGEMAQACYDAFDFDPFSRYCGSCRFTR
poplar        1 ---------------------MTPTLSPKQSISAVWKEIHGSSNWETLLDPFHPSLRREILKYGEFAQGTYDAFDFDPLSDFCGSCRYNR

AtDLAH      173 NKLFEELGLTRHGYKVTKYIYAMSRVDVPQWFLSSALGETWSKDSNWMGFVAVSGDRESL--RIGRRDIVVAWRGTVTPTEWFMDLRTSM
caster_bean 169 HKIFEELGLTKHGYRATKYIYAMSHVDVPEWFARTHT--TWSKDSNWMGFVAVSNDQESQ--RIGRRDIMVAWRGTVAPTEWYNDLRTDL
grape       176 HKLFDELHLTKHGYKVTKYIYAMTNIDVPSWFERPNTGETWSKDSNWMGYVAVSSDNESQ--RIGRRDIVVAWRGTVAPSEWFLDMKASL
rape        145 KKLFDSLGIFDSGYEAARYLYATSNINLPNFFSKSRWSKVWSKNANWMGYVAVSDDSEATRHRLGRRDIAIAWRGTVTQLEWIADLKDFL
poplar       70 RKFFETLGLTKHGYKVKKYIYALSHVDVPEWLKRSYA--TWSKDSNWMGYVAVSRREESQ--RIGRRDIMVAWRGTVSPSEWFKDLTTSL

AtDLAH      261 EPFD-CEGKHGKTVVKVQSGFLSIYNSKSELTRYNKESASEQTMDEVKRLVNFFKDR-GEEVSLTITGHSLGGALALMNAYEAARDVPAL
caster_bean 255 EYFE-EDQDHKKNHVKVQEGFLSIYKSKSEETRYNKLSASEQVMKELKKLVNLYREN-GEEVSLTLTGHSLGGALALLNAYEAATSIPNV
grape       264 EQIG-E------GGVKVESGFHSIYASKSESTRYNKLSASEQVMEAVKRLLEFFKGR-GEEVSLTVTGHSLGGALALLNAYEAASSLPDL
rape        235 KPVSGNGFRCRDPAVKAESGFLDLYTDKDTSCNFSKFSAREQLLTEVKRLVERYGDEEGGDLSITVTGHSLGGALAVLSAYDVAEMGLNR
poplar      156 EHID-N------TNVKVQEGFLSVYKSKDELTRYNKLSASEQVMQEVMRLVNFYRGK-GEEVSLTVTGHSLGGALALLNAYEAATAIPDL

AtDLAH      349 SG-----NISVISFGAPRVGNLAFKEKLNSLGVKVLRVVNKQDIVPKLPGIVFNK-VLNKLNPITSRLNWVYRHVGTQLKLDVFSSPYVK
caster_bean 343 F-------ISVISFGAPRVGNLAFKEKLNELGVKTLRVVIKQDIVPKLPGIIVNK-ILNKLSKITHKLNWVYRHVGTQLKLDMFISPYLK
grape       346 D------HISVISFGAPRVGNIAFRDKMNEMGVKILRVVVKQDIVPKLPGIICNK-ILRQIHALTRRLKWVYRHVGSELKLDMSLSPYLK
rape        325 TKNGKVVPVTVFTYSAPRVGNIRFKERMEELGVKVLRVVNKHDVVPKSPGLFLNEHAPHALKQLAGGLPWCYCHVGEKLALDHQNSPFLK
poplar      238 F-------VSVISFGAPRVGNIAFKEKLNELGVKTLRVVVKQDVVPKLPG-LLNK-MLNKFHGLTGKLNWVYRHVGTQLKLDAFMSPYLK

AtDLAH      433 RDSDLGRAHNLEVYLHVLDGFHRKKSGFRVNARRDVASVNKSTDMLLDHLRIPEFWYQVAHKGLILNKQTGRWVKPVRA-PEDIPSPLPT
caster_bean 425 QESDMSGSHNLEVYLHLLDGFLGKKLNYRWNARRDLALVNKSTNMLIEELKIPEFWYQLPHKGLVLN-KYGRWVKPSRV-AEDIPSPFSS
grape       429 REFDLLGFHNLEIYLHLTDGYVGKRLKFRWNARRDLALVNKSSDMLIEELRIPECWYQVPNKGLVFN-SHGRWVKPCRD-QQDIPSPFGE
rape        415 PSVDISTAHNLEALLHLLDGYHGKGQRFVLSSGRDPALVNKASDFLKDHFMVPPYWRQDANKGMVRH-TDGRWIQPDRIRAEDHHAPDIH
poplar      319 PESDLSGSHNLELYLHLIDGFFSKKSKYRWNARRDLALVNKGSDMLIEDLKIPEFWYQFPYKGLVLN-QYGRWVKPGRL-PEDIPSPLSI

AtDLAH      522 GPKPIYSL------------------------
caster_bean 513 EISRLDLSF-----------------------
grape       517 APNNKRINIRKKYSDKASKLINFEDKIGLFSVS
rape        504 HLLTQLHHPS----------------------
poplar      407 DTPPKHGRQS----------------------
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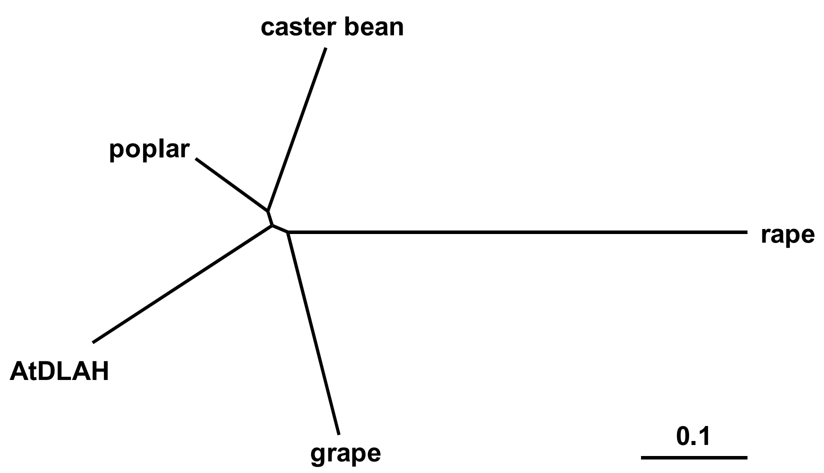
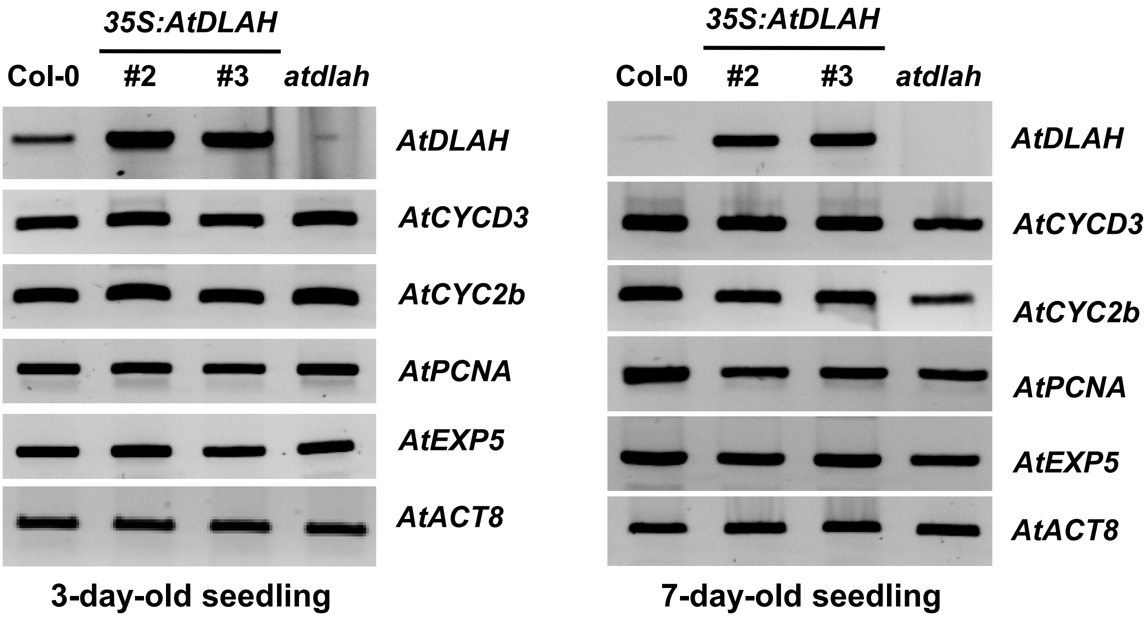
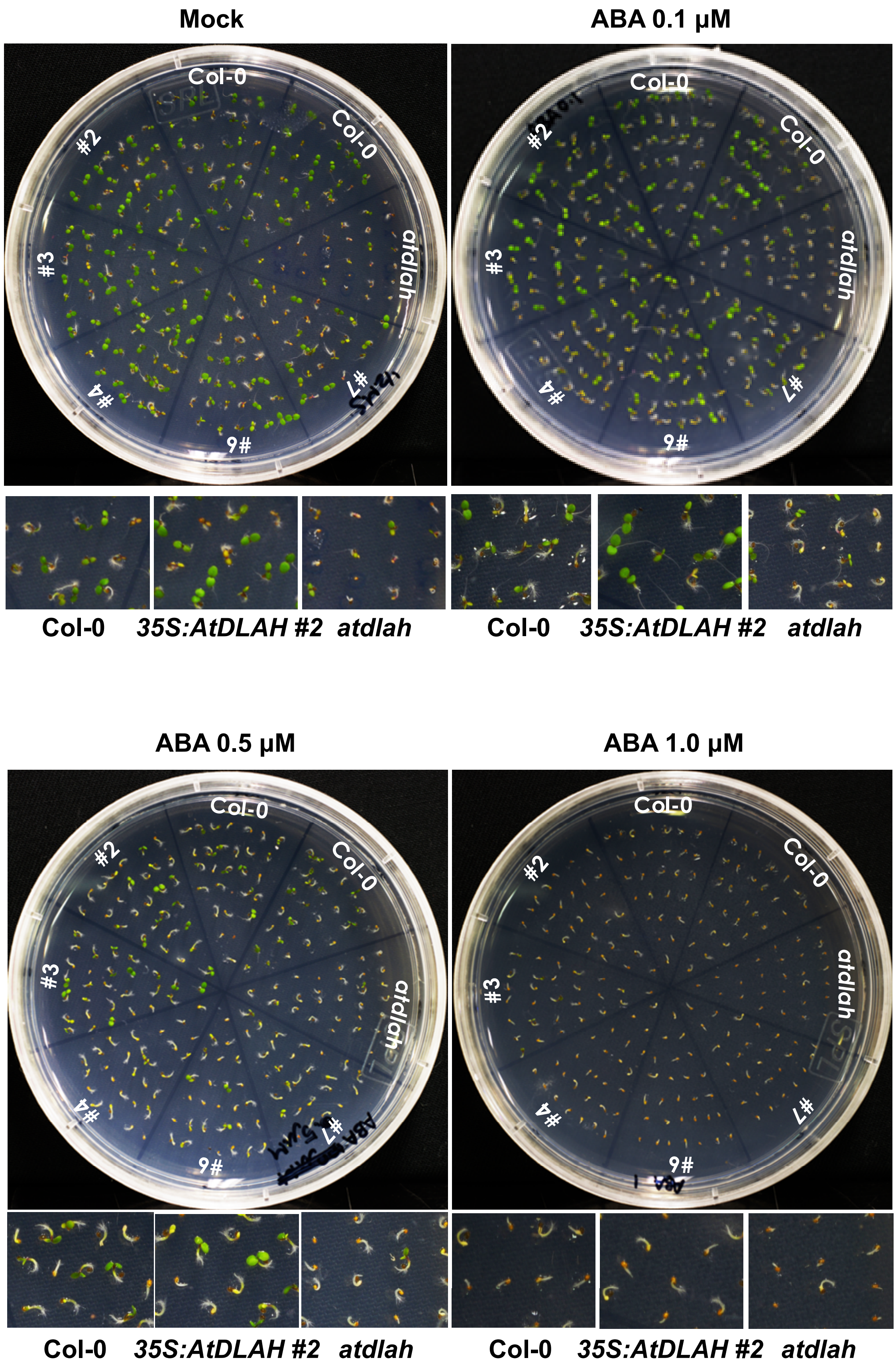
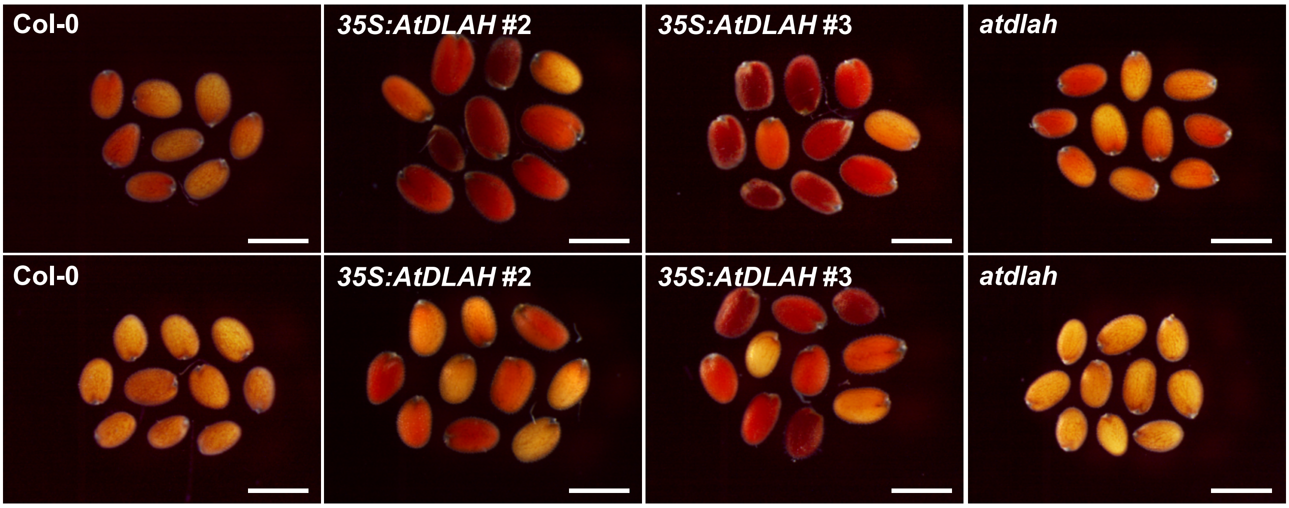
B




35S:AtDLAH
Col-0
#2
#3
atdlah
AtDLAH
AtCYCD3
AtCYC2b
AtPCNA
AtEXP5
AtACT8
3-day-old seedling
35S:AtDLAH
Col-0
#2
#3
atdlah
AtDLAH
AtCYCD3
AtCYC2b
AtPCNA
AtEXP5
AtACT8
7-day-old seedling


Mock
ABA 0.1 μM
ABA 0.5 μM
ABA 1.0 μM
Col-0
Col-0
#2
#3
#4
#6
#7
atdlah
Col-0
35S:AtDLAH #2
atdlah

A


Col-0
35S:AtDLAH #2
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atdlah
Col-0
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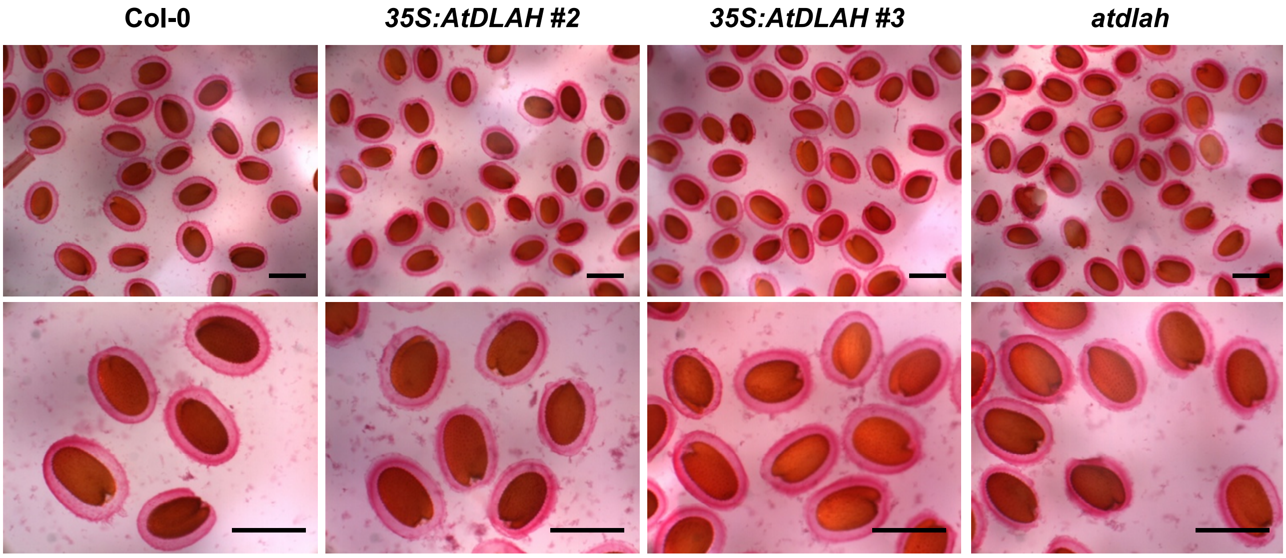
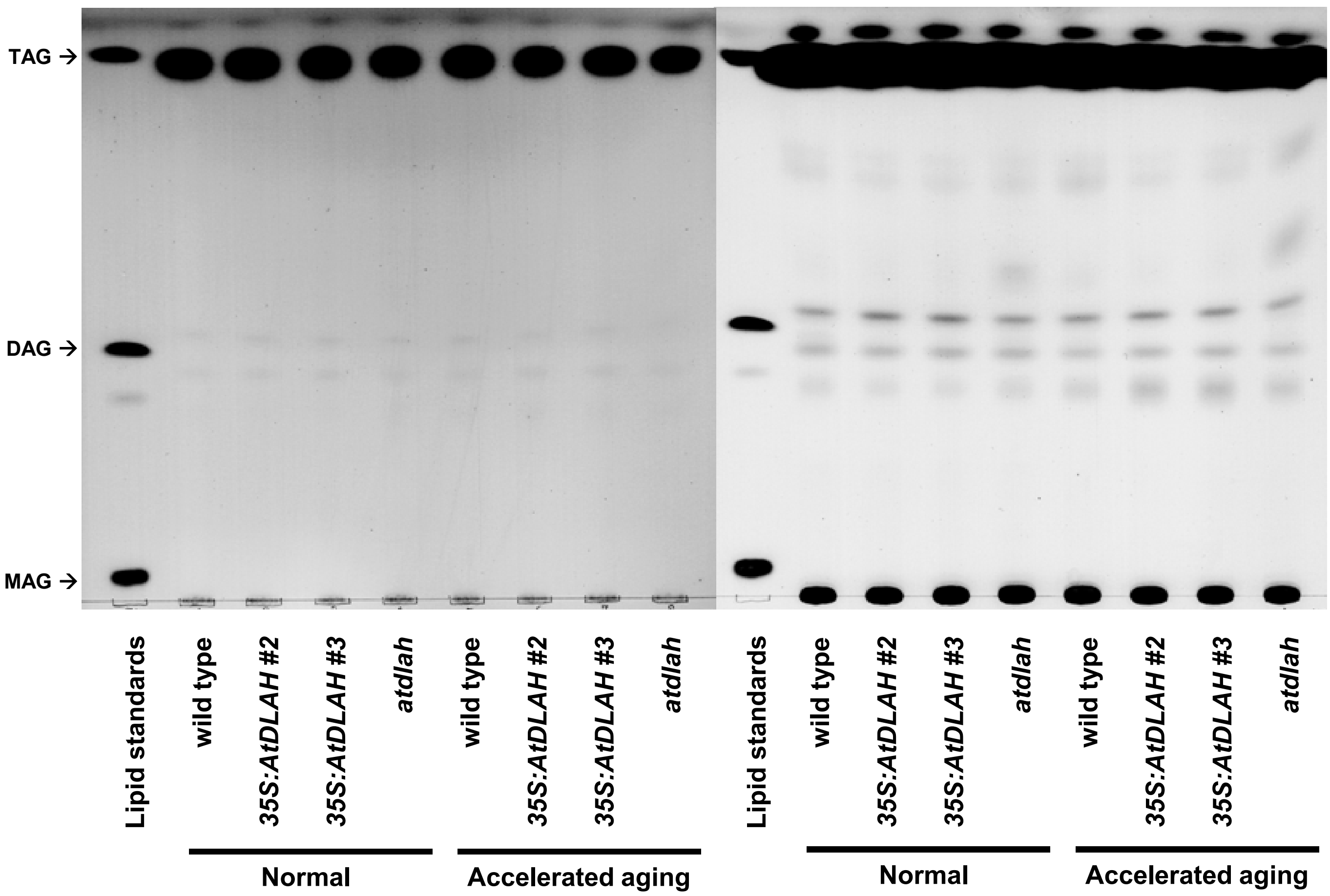
atdlah

B


Col-0
35S:AtDLAH #2
35S:AtDLAH #3
atdlah


TAG →
DAG →
MAG →
Lipid standards
wild type
35S:AtDLAH #2
35S:AtDLAH #3
atdlah
wild type
35S:AtDLAH #2
35S:AtDLAH #3
atdlah
Lipid standards
wild type
35S:AtDLAH #2
35S:AtDLAH #3
atdlah
wild type
35S:AtDLAH #2
35S:AtDLAH #3
atdlah
Normal
Accelerated aging
Normal
Accelerated aging