Supplemental Materials for

Liang Zhao, Hong Wang and Yong-Bi Fu. 2020. Analysis of stored mRNA degradation in acceleratedly aged seeds of wheat and canola in comparison to Arabidopsis. Plants 20, e

Table S1. Extracted total RNAs from Arabidopsis seeds aged under different AA temperatures.

AA day		8 days						16 days					
AA temperatures		4°C	22°C	30°C	33°C	37°C	40°C	4°C	22°C	30°C	33°C	37°C	40°C
Yield	a	357	420	415	355	346	367	324	344	208	324	387	331
(ng/μl)	b	342	320	408	298	501	389	177	325	277	304	197	325
$A_{280/260}$	a	2.16	2.07	2.11	2.06	2.13	2.05	2.04	2.11	2.09	2.12	2.11	2.12
	b	2.15	2.12	2.10	2.13	2.10	2.13	2.09	2.13	2.10	2.12	2.15	2.09
$A_{280/230}$	a	2.21	2.11	2.20	2.06	2.10	2.19	1.80	2.19	1.96	2.15	2.15	2.12
	b	2.14	2.12	2.12	2.17	2.19	2.06	2.07	2.14	2.00	2.13	2.17	2.08

Note: Total RNAs were isolated from unaged and aged seeds of Arabidopsis under various temperatures as described in the Materials and Methods. The AA day and temperature were indicated for each seed sample. Two biological replicates of RNA extraction were made for each seed sample, labeled as "a" and "b". For each RNA sample, the RNA yield and purity (A260/280 and A260/230) were assessed using a NanoDrop 8000 spectrophotometer.

Table S2. List of genes (or fragments) and primers used to analyze mRNA degradation in wheat seeds.

Gene code	Gene name	Coding Sequence Length	Fragment analyzed (bps)	Forward primer	Reverse primer
W1	TraesCS7A02G070100	1815	950	TCAGGGAGCGGCGACGTTC	GCTACGACAAGCCGGTGGA
W2	TraesCS5D02G425100	1519	950	AAGTTGAACAAGTATGGTCGTC	GTATCTACCGGCTCGAACTC
W3	TraesCS2B02G309900	2785	950	CCTAGTGATGGAGTATTGTCC	CCACTAGAAGAGCTCGAACTC
W4	TraesCS4A02G296000	1767	949	GGGACTCACGGACGCAGAC	GGCGTTGATCTCGAAGCAC
W5	TraesCS2A02G027800	2274	951	CTCAGTTCATGTTCCCTGGT	TCACCACGTTGAGAAATGTCT
W6	TraesCS3A02G471900	2282	951	CGCTCGTCTGGTCCACGT	CGCTTCTTCTCCATGTCGAA
W7	TraesCS2B02G567600	678	500	CCTGCACCACCAGAAGCAC	TCCAGTTCACCACCTTCAG
W8	TraesCS1A02G045700	1479	950	CCATGCTCACCCTCCCGT	CGGCGGGAGGGCTTACGG
W9	TraesCS5B02G267900	2418	950	TCTGCAAAGGAGATTGATGAG	TTCATCTTCTTTGGACTACGTC
W10	TraesCS5B02G106300	2460	955	CTTCAAGAGAGTCGAATTGTGC	CAAGCGGCTCAGGTTCAGAGC
W11	TraesCS2D02G289100	2832	900	GCTGTGAAGAGATCACGCAC	TCAAAGCGTTGGTGTTGGATC
W12	TraesCS4A02G143200	2910	950	ATTATGCATTGAACCGAGGAGC	CTGGTCCTGGGTTGTCCTTC
W13	TraesCS7A02G517700	2070	2000	CCGTTTTCGCGGTCCTGATC	CTAAAGATACTTAAAGAGGATGC
W14	TraesCS2B02G521600	1458	900	GAGTTCAAGATCGTCCTCACC	GGATGCTAATGTAGTCAGACTG
W15	TraesCS7B02G068100	1605	948	GGCCTTATCTTTGACAAGAAGG	TTACCTTACAGTGACTATATCAT
W16	TraesCS4A02G100500	1128	950	GGTACATCTCCGCGGCGC	TGAACGAGCAATCTTCGCTGC
W17	TraesCS3B02G311900	1899	950	GACACTGCTGTAGAAGAGGC	TGCTCCGGTCTCTCGTTCC
W18	TraesCS3D02G321500	741	655	GCCTAGAGCGGCGGAGAAG	GGACAGATCAACGACCGACG
W19	TraesCS3A02G277700	2304	951	GATGGAGATGACCATTAAATAC	CTTGTCCTTGTCATCCGCAC
W2	TraesCS5D02G425100	1519	107	ATCTTGCTCGCGAAGGTAATG	GTATCTACCGGCTCGAACTC
			295	TGTTATACCGGTAGCTTCAGG	
			490	GGTATGCATTTCCGTGTATTAG	
			703	GCATTACTTGAATGCGACTGC	
			933	AAGTTGAACAAGTATGGTCGTC	
			1131	AGCCAATGGATCTGTTATGTAG	

Note: A total of 19 wheat genes were tested, and each was coded with W and numbering. W2 was used to analyze the correlation between the ΔCt value and fragment size, with the following fragments of $W2_{107bp}$, $W2_{295bp}$, $W2_{490bp}$, $W2_{703bp}$, $W2_{933bp}$, and $W2_{1131bp}$. Four genes with similar fragment lengths $W2_{950bp}$, $W3_{950bp}$, $W10_{955bp}$, and $W12_{950bp}$ were assayed.

Table S3. List of canola genes (or fragments) and primers used to study stored mRNA degradation during seed aging.

Gene code	Gene name	Coding sequence length (bps)	Fragment analyzed (bps)	Forward primer	Reverse primer
Bn1	BnaA01g34230D	1659	1001	GGTCATAATAAGGTTTACAAGTC	TAGGTATCATATGAACAAGCTTG
Bn2	BnaC04g38000D	1080	1001	GGTCATAATAAGGTTTACAAGTC	TAGGTATCATATGAACAAGCTTG
Bn3	BnaC06g14690D	1062	1000	CTGTAACTGGATAACTAGTACTG	AGATGGAGCCTCAACAGCAG
Bn4	BnaC05g37990D	1470	1004	CGATCTGCACTATGATCCTTAC	GATTACGTGGTACAATTGCATC
Bn5	BnaA08g15530D	2301	1000	GAGTGCTTCTGCTTGATGGC	CAGTTGGTTAATATCTCAGCTC
Bn6	BnaC07g44190D	3145	1000	GTGCTGCAGAAGCGGAGAG	CATTCCAGAACCTCAAAGTCTC
Bn7	BnaC08g42010D	1493	1000	GAGATGATCCTTCCCATTCAC	CTTCAGCCATAATCCTCCTTG
Bn8	BnaC09g16410D	1443	928	GGTATAAGTTTATATGGTTCGAAC	TGGATTCGACCTTGTCACTTG
Bn9	BnaCnng08950D	1453	995	TATTCGTGGGTGGGAAAGGAG	TCAACTCTTGATCCGTTCCAG
Bn10	BnaA02g02620D	1965	1938	GGTGTATGGAGAATCTGCTGG	CAGCTGCTTATGCAACTCTTC
Bn11	BnaA06g10730D	1490	1490	ATGACTCTCAGAGACAGGCC	CAACCTCTTCTATCTTCGGAC
Bn12	BnaA09g55830D	2925	1851	AGCAGTACTGAAGGAAGTCAC	TCGATAGGAGCATTGTTCTCG
Bn13	BnaAnng00830D	1681	1486	CGTTACAACTCTGGTGCTGTC	AGAGCCTTGACGCATTGATTG
Bn14	BnaC01g16200D	2139	1500	ACCAAGGCTGTTATCACTGTG	GTCGATCACATCATCTCCATC
Bn15	BnaC01g39690D	2415	1920	CTATGCAGACAAGGAGAATGG	TCCACTTCAACTGGTTCAACG
Bn16	BnaC03g03860D	2046	1946	ATCACTGAACGGTCAGAACTG	TCTTCACTTCCTCGTAGTCAG
Bn17	BnaC03g13930D	1731	1731	AACAACACCTTGACCATCGTC	CATCTTGCTACCTTCAGCATC
Bn18	BnaC04g12620D	1692	1690	TGGCAGAACCAGCATACACAG	CACTTCCTCTGAAGAGTAACC
Bn19	BnaC06g23110D	2652	1800	CTATGTTAGCTAGAGGTCAGC	CCTCAATCCTCATCTTCTTCAC
			148	AGAGAGTGATCCAACAGATGG	
			487	ACTGTGTTGTGATTATGACCTC	
Bn12	BnaA09g55830D	2925	850	GTCTCTCTGATCCTAACCGTC	GTTGCTTGTCTGGTCAACATC
			1169	TCTGCTGAACGTGAATACGAC	
			1500	TACATCACTGAACGCTTCCTG	
Bn17	BnaC03g13930D	1731	1500	AACAACACCTTGACCATCGTC	CATCTTGCTACCTTCAGCATC

Note: A total of 19 Canola genes were used and each was coded with Bn and numbering. Five fragments on Bn12 ($Bn12_{148bp}$, $Bn12_{487bp}$, $Bn12_{850bp}$, $Bn12_{1169bp}$, and $Bn12_{1500bp}$) were used to analyze the correlations between the ΔCt values and mRNA fragment sizes. For the comparisons of different genes with similar fragment lengths, fragments $Bn11_{1490bp}$, $Bn12_{1500bp}$, $Bn13_{1486bp}$, $Bn14_{1500bp}$, and $Bn17_{1500bp}$ were used.

Table S4. The gene and primers used in qPCR analysis of changes in the stored mRNA of aged Arabidopsis seeds.

Gene name	Coding sequence length (bps)	Sequence length (bps)	Forward primer	Reverse primer
At1g74310	2736	2000	TAGTTGCTGGTGCTAAATACC	TTAATCCTCGATCATTTCCTCA

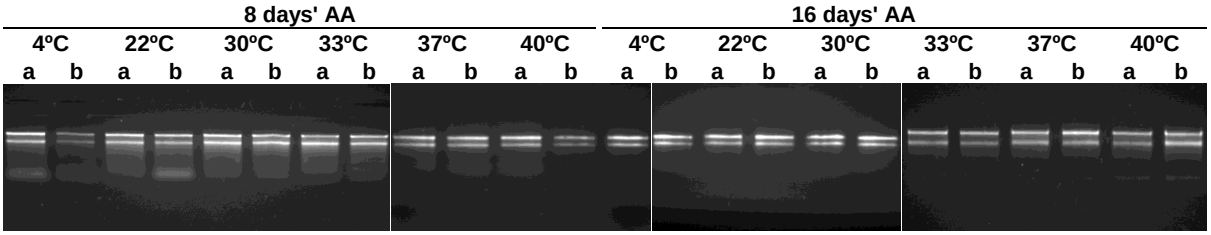
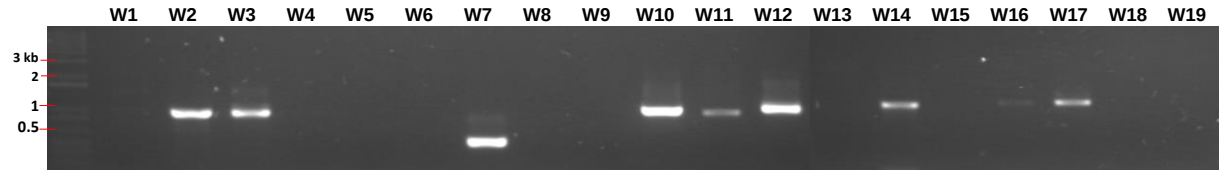


Figure S1. The integrity of total RNAs isolated from Arabidopsis seeds that acceleratedly aged under different temperatures. Total RNAs were isolated from dry seeds of Arabidopsis using the modified protocol as described in the Materials and Methods. The AA temperatures for the seeds were indicated on the top of the rRNA bands; there were six temperatures with two biological replicates of total RNAs, labeled as "a" and "b". The same amount of total RNA (0.5 μg) was loaded into each lane and subjected to electrophoresis.

a: Wheat genes



b: Canola genes

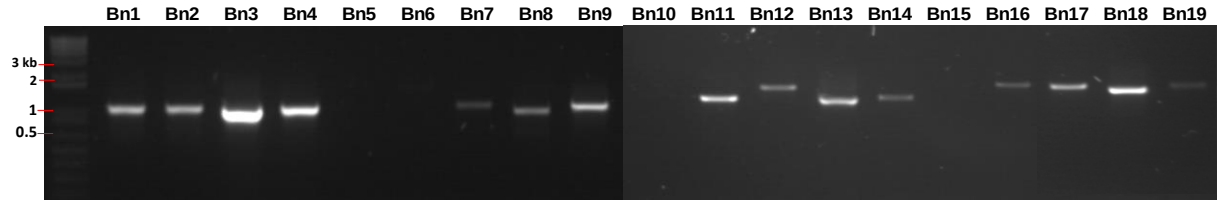


Figure S2. Presence of stored mRNAs of candidate genes in wheat and canola dry seeds. **a:** Nineteen wheat genes (as listed in Table S2) were used to determine the presence of stored mRNAs by RT-PCR. **b:** Nineteen canola genes (as listed in Table S3) were used to determine the presence of stored mRNAs by RT-PCR. cDNA reverse-transcribed from total RNA of unaged dry seeds was used in RT-PCR with primers specific for each of the genes. The PCR products were subjected to electrophoresis in 1% agarose gel.

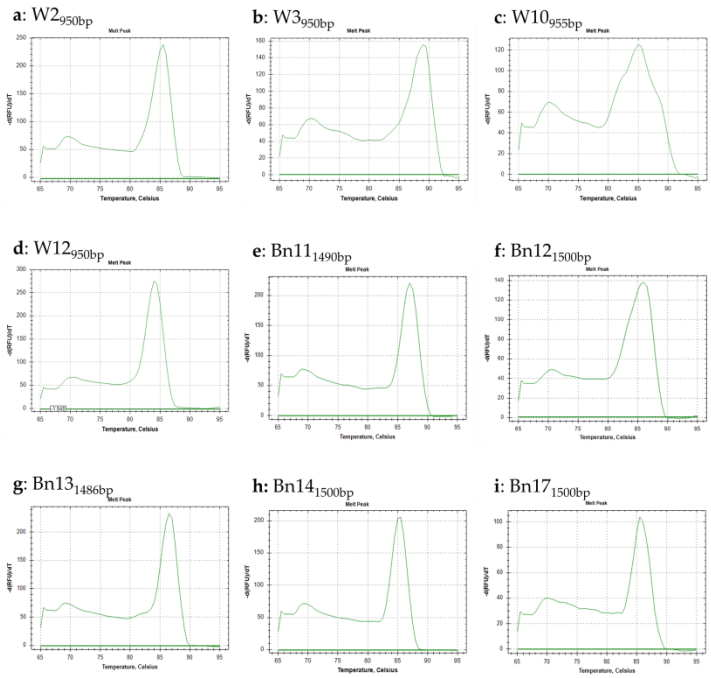


Figure S3. Melt-curves of candidate genes used for qPCR analysis. Figures **a-d** are melt-curves for the four wheat genes of W2$_{950bp}$, W3$_{950bp}$, W10$_{955bp}$ and W12$_{950bp}$, while figures **e-i** are melt-curves for the five canola genes of Bn11$_{1490bp}$, Bn12$_{1500bp}$, Bn13$_{1486bp}$, Bn14$_{1500bp}$, and Bn17$_{1500bp}$.

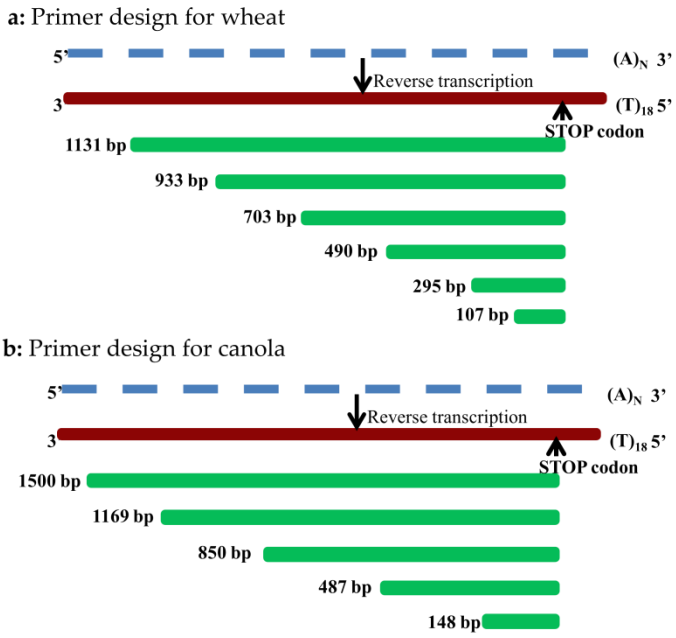


Figure S4. An illustration of amplifying fragments of different lengths from cDNA synthesized using total RNA and an oligo (dT)20 primer. The first-strand cDNA was firstly synthesized from total RNAs with an oligo (dT)20 primer. Then fragments of different lengths were amplified separately using the same reverse primer but different forward primers in PCR reactions.