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Supplementary Material

The role of oxidative stress in determining the level of viability of black poplar (*Populus nigra*) seeds stored at different temperatures

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Table S1. Fatty acids composition in black poplar (*Populus nigra* L.) seeds

The percentage of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and α-linolenic acid (C18:3) determined using a GLC-10 FAME (Supelco) quantitative mix and heptadecanoic acid (17:0) as the internal standard

Fatty acid		**Content [% ± s.e.]**
C16:0	Palmitic acid	24.61 ± 0.31
C18:0	Steraic acid	9.72 ± 0.21
C18:1	Oleic acid	12.79 ± 0.19
C18:2	Linoleic acid	35.82 ± 0.19
C18:3	α-Linolenic acid	17.21 ± 0.17

Table S2. Phospholipids: phosphatidylinositol (PI), phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylethanolamine (PE) and phosphatidic acid (PA) analyzed in black poplar (*Populus nigra* L.) seeds stored for 3 months, 1 year and 2 years at LN, –20°C, –10°C, –3°C and 3°C

Data represent the mean ± s.e. of six independent replicates. The Kruskal–Wallis test was used to compare storage temperature treatment. Data marked with the same letter are not statistically significant according to the multiple range test ($P \leq 0.05$)

	PI	PC	PG	PE	PA
3 months at:					
LN	29.51 ± 2.55	43.32 ± 2.46	21.06 ± 4.00	32.54 ± 2.40	36.89 ± 2.14
–20 °C	28.58 ± 1.52	48.18 ± 1.07	19.38 ± 1.53	24.21 ± 2.91	23.07 ± 3.05
–10 °C	20.91 ± 2.45	29.82 ± 2.18	9.44 ± 0.15	18.92 ± 2.24	15.43 ± 2.28
–3 °C	13.91 ± 2.32	25.67 ± 2.39	7.21 ± 0.88	16.34 ± 0.92	16.51 ± 1.62
+3 °C	25.61 ± 2.89	33.58 ± 4.67	15.39 ± 0.65	21.72 ± 0.82	17.11 ± 0.90
	$P = 0.018$	$P = 0.022$	$P = 0.013$	$P = 0.283$	$P = 0.033$
1 year at:					
LN	19.52 ± 0.41	37.16 ± 3.20	16.71 ± 1.18	16.92 ± 5.18	24.00 ± 1.92
–20 °C	16.45 ± 1.14	25.82 ± 2.32	13.96 ± 2.67	18.99 ± 5.59	23.09 ± 0.79
–10 °C	12.49 ± 4.03	28.75 ± 0.37	12.76 ± 0.53	16.43 ± 6.27	23.66 ± 0.40
–3 °C	12.13 ± 0.25	12.96 ± 1.27	14.37 ± 0.12	23.45 ± 1.72	27.67 ± 1.65
+3 °C	19.23 ± 0.79	17.96 ± 2.93	2.93 ± 0.29	15.07 ± 1.74	9.27 ± 0.48
	$P = 0.081$	$P = 0.017$	$P = 0.454$	$P = 0.331$	$P = 0.062$
2 years at:					
LN	14.81 ± 2.01	24.26 ± 3.39	9.58 ± 0.74	16.61 ± 2.85	12.90 ± 2.10
–20 °C	18.93 ± 2.52	27.40 ± 1.53	16.66 ± 2.88	26.37 ± 6.14	19.50 ± 2.43
–10 °C	18.36 ± 3.03	41.20 ± 3.59	16.15 ± 2.57	23.11 ± 2.46	20.51 ± 1.33
–3 °C	27.91 ± 4.89	30.77 ± 9.88	15.19 ± 1.52	21.69 ± 5.02	30.99 ± 8.21
+3 °C	23.29 ± 1.79	39.54 ± 2.30	14.04 ± 1.22	38.82 ± 9.41	25.51 ± 1.15
	$P = 0.011$	$P = 0.221673$	$P = 0.029$	$P = 0.092$	$P = 0.0051$

Table S3. Pearson correlation coefficient calculated between germination capacity of black poplar seeds (stored for 3 months, 1 year and 2 years; Suszka *et al.* 2014) and superoxide anionradical ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2), protein carbonylation, electrolyte leakage, fatty acids including palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and α-linolenic acid (C18:3), phospholipids including phosphatidylinositol (PI), phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylethanolamine (PE) and phosphatidic acid (PA), reduced (GSH) and oxidized (GSSG) form of glutathione and their redox potential ($E_{GSSG/2GSH}$), reduced (AsA) and oxidized (DHA) form of ascorbate and their redox potential ($E_{AsA/DHA}$), enzymes of the ascorbate-glutathione cycle including ascorbate peroxidase (APX), glutathione reductase (GR), dehydroascorbate reductase (DHAR) and monodehydroascorbate reductase (MDHAR)

P-value was calculated from the R-score at 0.05 significance level. Strong correlation coefficient is indicated with bolded type

	Germination		
	Seeds stored for 3 months	**Seeds stored for 1 year**	**Seeds stored for 2 years**
$O_2^{-\bullet}$	R = 0.4745 *P* = 0.061842	**R = –0.8883** *P* < 0.00001	**R = –0.9373** *P* < 0.00001
H_2O_2	R = –0.4509 *P* = 0.091628	R = –0.3619 *P* = 0.185004	**R = –0.7855** *P* = 0.00052
Protein carbonylation	R = –0.5218 *P* = 0.046431	**R = –0.9227** *P* < 0.00001	R = –0.5901 *P* = 0.020575
Electrolyte leakage	R = 0.3847 *P* = 0.156814	R = 0.7110 *P* = 0.002962	**R = –0.9761** *P* < 0.00001
C16:0	R = –0.4005 *P* = 0.139055	R = 0.7085 *P* = 0.003112	**R = 0.7526** *P* = 0.001205
C18:0	R = 0.2425 *P* = 0.38385	R = 0.4750 *P* = 0.073571	**R = 0.7669** *P* = 0.00085
C18:1	**R = –0.8358** *P* = 0.000104	R = 0.7352 *P* = 0.00179	**R = 0.7500** *P* = 0.001284
C18:2	R = –0.4904 *P* = 0.063465	**R = 0.8723** *P* = 0.000496	**R = 0.7971** *P* = 0.000373
C18:3	R = –0.6626 *P* = 0.222972	**R = 0.8940** *P* = 6.2E-05	**R = 0.7804** *P* = 0.000579
PI	R = –0.1450 *P* = 0.606133	R = 0.3011 *P* = 0.275473	R = 0.7276 *P* = 0.002108
PC	R = –0.4874 *P* = 0. 06535	R = 0.7085 *P* = 0.003112	R = 0.6865 *P* = 0.004705
PG	R = –0.1405 *P* = 0.617472	**R = 0.8831** *P* = 1.3E-05	**R = 0.8558** *P* = 4.7E-05
PE	R = 0.031 *P* = 0.91267	**R = 0.7578** *P* = 0.001064	R = 0.3908 *P* = 0.149788
PA	R = 0.0012 *P* = 0.996614	**R = 0.8474** *P* = 6.7E-05	**R = 0.8232** *P* = 0.000163
GSH	R = 0.7181 *P* = 0.002568	R = 0.6530 *P* = 0. 008306	R = 0.5712 *P* = 0.026137
GSSG	R =0.5037 *P* = 0.055582	R = 0.4117 *P* = 0.127324	R = 0.5226 *P* = 0.045645
$E_{GSSG/2GSH}$	**R = –0.7584** *P* = 0.001049	R = 0.0871 *P* = 0.757581	R = –0.5265 *P* = 0.04377
AsA	R = 0.0421 *P* = 0.881576	R = –0.5586 *P* = 0.030432	R = 0.6831 *P* = 0.005001
DHA	R = 0.2803 *P* = 0.31156	**R = –0.8862** *P* = 1.1E-05	R = –0.6236 *P* = 0.012989
$E_{DHA/AsA}$	R = 0.5830 *P* = 0.022546	**R = –0.7611** *P* = 0.000982	**R = –0.8129** *P* = 0.000229
APX	R = 0.5373 *P* = 0.038875	**R = –0.8230** *P* = 0.000164	**R = –0.8962** *P* < 0.00001
GR	R = –0.5211 *P* = 0.046381	**R = –0.8606** *P* = 3.8E-05	**R = –0.8363** *P* = 0.000102
DHAR	R = 0.5544 *P* = 0.031976	**R = –0.7702** *P* = 0.000781	**R = –0.8333** *P* = 0.000114
MDHAR	R = 0.5634 *P* = 0.028737	**R = –0.8060** *P* = 0.000285	**R = –0.8182** *P* = 0.000193

Table S4. Pearson correlation coefficient calculated between the ROS content: (*a*) superoxide anionradical ($O_2^{-\bullet}$), (*b*) hydrogen peroxide (H_2O_2) and parameters that are thought to be affected by ROS: protein carbonylation, electrolyte leakage, fatty acids including palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and α-linolenic acid (C18:3), phospholipids including phosphatidylinositol (PI), phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylethanolamine (PE) and phosphatidic acid (PA), reduced (GSH) and oxidized (GSSG) form of glutathione and their redox potential ($E_{GSSG/2GSH}$), reduced (AsA) and oxidized (DHA) form of ascorbate and their redox potential ($E_{AsA/DHA}$), enzymes of the ascorbate-glutathione cycle including ascorbate peroxidase (APX), glutathione reductase (GR), dehydroascorbate reductase (DHAR) and monodehydroascorbate reductase (MDHAR)

P-value was calculated from the R-score at 0.05 significance level. Strong correlation coefficient is indicated with bolded type

No.	Table S4*a*	$O_2^{-\bullet}$		
		Seeds stored for 3 months	**Seeds stored for 1 year**	**seeds stored for 2 years**
1.	**Protein carbonylation**	R = –0.1027 *P* = 0.715698	R = 0.6692 *P* = 0.006364	**R = 0.7951** *P* = 0.000396
2.	**Electrolyte leakage**	R = 0.4698 *P* = 0.07723	R = –0.4171 *P* = 0.121918	**R = 0.8842** *P* = 1.2E-05
3.	**C16:0**	R = 0.1754 *P* = 0.531797	**R = –0.7651** *P* = 0.000889	**R = –0.8272** *P* = 0.000142
4.	**C18:0**	**R = 0.9595** *P* < 0.00001	R = –0.7011 *P* = 0.00359	**R = –0.7549** *P* = 0.001024
5.	**C18:1**	R = –0.3239 *P* = 238909	**R = –0.8591** *P* = 4.1E-05	**R = –0.7544** *P* = 001155
6.	**C18:2**	**R = 0.9816** *P* < 0.00001	**R = –0.8652** *P* = 3.1E-05	**R = –0.8216** *P* = 0.000172
7.	**C18:3**	R = –0.1459 *P* = 0.603975	R = –0.6339 *P* = 0.011161	**R = –0.8100** *P* = 0.000252
8.	**PI**	R = –0.6690 *P* = 0.006386	R = –0.0267 *P* = 0.924749	R = –0.1804 *P* = 0.519975
9.	**PC**	**R = –0.8068** *P* = 0.000278	R = –0.5855 *P* = 0.021837	R = –0.5788 *P* = 0.023778
10.	**PG**	**R = –0.8101** *P* = 0.000251	**R = –0.9860** *P* < 0.00001	**R = –0.9013** *P* < 0.00001
11.	**PE**	R = –0.6217 *P* = 0.1335	R = –0.4661 *P* = 0.079912	**R = –0.7745** *P* = 0.000699
12.	**PA**	R = –0.6161 *P* = 0.01446	R = –0.6171 *P* = 0.014256	**R = –0.9148** *P* < 0.00001
13.	**GSH**	R = –0.1311 *P* = 0.641418	R = –0.0868 *P* = 0.758393	R = 0.4500 *P* = 0.092357
14.	**GSSG**	R =–0.2477 *P* = 0.373413	R =–0.3345 *P* = 0.222991	R =0.0205 *P* = 0.942192
15.	**$E_{GSSG/2GSH}$**	R = 0.0382 *P* = 0.892485	**R = 0.8123** ***P* = 0.000234**	**R = 0.7875** *P* = 0.000491
16.	**AsA**	**R = –0.8366** *P* = 0.000101	R = 0.2706 *P* = 0.329326	R = –0.0314 *P* = 0.911547
17.	**DHA**	**R = –0.9131** *P* <0.00001	**R = 0.9601** *P* < 0.00001	R = –0.5983 *P* = 0.018466
18.	**$E_{DHA/AsA}$**	**R = –0.8507** *P* = 5.8E-05	**R = 0.8850** *P* = 1.2E-05	**R = 0.7947** *P* = 0.0004
19.	**GR**	R = –0.2417 *P* = 0.385447	**R = 0.8922** *P* < 0.00001	**R = 0.8942** *P* < 0.00001
20.	**APX**	R = –0.4575 *P* = 0.086401	**R = 0.8547** *P* = 4.9E-05	**R = 0.9356** *P* < 0.00001
21.	**DHAR**	R = –0.4552 *P* = 0.088198	**R = 0.8882** *P* < 0.00001	**R = 0.8742** *P* = 2E-05
22.	**MDHAR**	R = –0.4524 *P* = 0.090421	**R = 0.8606** *P* = 3.8E-05	**R = 0.8946** *P* < 0.00001

No.	Table S4*b*	H_2O_2		
		Seeds stored for 3 months	**Seeds stored for 1 year**	**Seeds stored for 2 years**
1.	**Protein carbonylation**	R = –0.4839 *P* = 0.067599	R = 0.1920 *P* = 0.493014	R = 0.6331 *P* = 0.011295
2.	**Electrolyte leakage**	R = –0.2257 *P* = 0.418622	R = –0.2592 *P* = 0.350888	R = 0.7096 *P* = 0.00045
3.	**C16:0**	R = –0.1187 *P* = 0.67351	R = –0.5308 *P* = 0.041769	**R = –0.8841** *P* = 1.2E-05
4.	**C18:0**	R = –0.4851 *P* = 0.066822	R = –0.4518 *P* = 0.090902	**R = 0.9981** *P* < 0.00001
5.	**C18:1**	R = –0.4133 *P* = 125706	R = –0.6792 *P* = 005357	**R = 0.9815** *P* < 0.00001
6.	**C18:2**	R = –0.3295 *P* = 0.230414	R = –0.5766 *P* = 0.024444	**R = 0.9746** *P* < 0.00001
7.	**C18:3**	R = –0.2473 *P* = 0.37421	R = –0.4029 *P* = 0.136482	**R = –0.9471** *P* < 0.00001
8.	**PI**	R = 0.3031 *P* = 0.272138	R = –0.1986 *P* = 0.477297	**R = –0.7582** *P* = 0.001054
9.	**PC**	R = 0.5757 *P* = 0.02472	R = –0.3470 *P* = 0.205099	**R = –0.9289** *P* < 0.00001
10.	**PG**	R = 0.4381 *P* = 0.102393	R = –0.5982 *P* = 0.01848	**R = –0.8668** *P* = 2.9E-05
11.	**PE**	R = 0.1068 *P* = 0.704809	R = –0.6045 *P* = 0.016984	**R = –0.7743** *P* = 0.000702
12.	**PA**	R = 0.0091 *P* = 0.974323	R = –0.1134 *P* = 0.687392	R = –0.4390 *P* = 0.101609
13.	**GSH**	R = –0.1537 *P* = 0.584446	R = 0.2779 *P* = 0.315926	R = 0.6329 *P* = 0.011329
14.	**GSSG**	R = –0.2148 *P* = 0.442021	R = –0.0511 *P* = 0.85648	R =0.6046 *P* = 0.016961
15.	$\mathbf{E_{GSSG/2GSH}}$	R = 0.1272 *P* = 0.651451	R = –0.4623 *P* = 0.082735	R = 0.4313 *P* = 0.108458
16.	**AsA**	R = 0.5988 *P* = 0.018343	R = –0.5677 *P* = 0.027281	R = –0.0850 *P* = 0.76327
17.	**DHA**	R = 0.5389 *P* = 0.038486	R = 0.3612 *P* = 0.185918	R = –0.5420 *P* = 0.036878
18.	$\mathbf{E_{DHA/AsA}}$	R = 0.4430 *P* = 0.098173	R = 0.2353 *P* = 0.398557	R = 0.4208 *P* = 0.118306
19.	**GR**	R = 0.7163 *P* = 0.002664	**R = 0.7606** *P* = 0.000994	R = 0.6398 *P* = 0.010208
20.	**APX**	R = –0.3083 *P* = 0.263583	**R = 0.7579** *P* = 0.001062	R = 0.6312 *P* = 0.011619
21.	**DHAR**	R = 0.2861 *P* = 0.301255	**R = 0.8723** *P* = 2.2E-05	R = 0.6169 *P* = 0.014297
22.	**MDHAR**	R = 0.2879 *P* = 0.298089	**R = 0.7991** *P* = 0.000352	R = 0.6207 *P* = 0.013543