Supplemental information

Exploring the genotoxic stress response in primed orphan legumes seeds challenged with heat stress

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Supplementary Table S1. List of oligonucleotide primers used for *q*RT-PCR analyses. For each oligonucleotide set, PCR efficiency (E) is reported. *Ls, Lathyrus sativus*. *EF2, ELONGATION FACTOR 2*. *GAPDH, GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE*. *SOD, SUPEROXIDE DISMUTASE*. *APX, ASCORBATE PEROXIDASE*. *MT, METALLOTHIONEIN*. *OGG1, 8-OXOGUANINGLYCOSYLASE/LYASE*. *FPG, FORMAMIDOPYRIMIDINE-DNA GLYCOSYLASE*. *Lig, DNA LIGASE*. *TOP, DNA TOPOISOMERASE*. *TFIIS, TRANSCRIPTION ELONGATION FACTOR IIS*. *SPMS, SPERMINE/SPERMIDINE SYNTHASE*. *SPDS, SPERMIDINE SYNTHASE*. *5.8S, 5.8S rRNA*. *5.8S-IS, 5.8S rRNA-INTERSPACE*. *PCNA, PROLIFERATING CELL NUCLEAR ANTIGEN*. *SOG1, SUPPRESSOR OF THE GAMMA RESPONSE 1*.

Gene (Accession n°)	**Oligonucleotide Forward**	**Oligonucleotide Reverse**	**E**
LsEF2a (GBSS01000402)	5'-CCTCCAGTGGTCTTGCTTCC-3'	5'-TGATTTCATGGGTGGGGCTG-3'	1.8
LsGAPDH (GBSS01000223)	5'-GCTCCCAGTAAAGATGCTCCC-3'	5'-AGCCCTTCAACAATGCCAAAC-3'	1.8
LsSODa (GBSS01003115)	5'-TCTCCACTGGTCCTCACTTC-3'	5'-CCCTCCCGAGTATGGAATGA-3'	1.8
LsSODb (GBSS01001045)	5'-GTGGACAACAACAGCCCTTC-3'	5'-TGCCCCTGAGGATGAGACTA-3'	1.8
LsAPXa (GBSS01001446)	5'-TTCAGAGCTTGGCTTTGTGC-3'	5'-ACATCCAACAGAGCTGAGCA-3'	1.8
LsAPXb (GBSS01019041)	5'-GTAGCGGTAGAGGTCACTGG-3'	5'-GCGATGCACCTTGTTTAGCA-3'	1.8
LsMT1 (GBSS01000013)	5'-ATCAAAGTGGATCTTCGCCG-3'	5'-GTTGTGGAAGCAGTTGC-3'	1.8
LsMT2 (GBSS01000484)	5'-TCTTGCTGTGGTGGAAACTG-3'	5'-CTCAGATCCGACTCCCATGA-3'	1.8
LsOGG1 (GBSS01016977)	5'-TTGAGGGCTACCAAACAGC-3'	5'-CGTAGTGTGTTACGCCTGAG-3'	1.8
LsFPG (GBSS01018706)	5'-GCGAGCTGCAACAATGGTTT-3'	5'-TAACCAAGTGCATCGTCGCA-3'	1.8
LsLig1 (GBSS01022069)	5'-AGCAACCAAAGGTGTAGCAG-3'	5'-TGCTGCAACAACATCAGGAA-3'	1.8
LsTOP1 (GBSS01013769)	5'-TCGTAGTGTTTGGCTGAGGTG-3'	5'-CACGGAAGGCACGCTGATA-3'	1.8
LsTFIIS (GBSS01005128)	5'-GCTCAACAGTACCGACCAGA-3'	5'-GCAGTGACAGTGGAGTCTC-3'	1.8
LsSPSD (GBSS01000983)	5'-TCAGAGAACCAGCCAGGAAT-3'	5'-GGCACCAGAAAACACACTTCA-3'	1.8
LsSPMS (GBSS01003422)	5'-GTGTGAATGTCGGTGAGAGG-3'	5'-ATGCACTGAGCCACCATTTT-3'	1.8
Ls5.8SrRNA (AY839389)	5'- TCCCGTGAACCATCGAGTCT -3'	5'- CCCTCAACCTAATGGCATCG -3'	1.8
Ls5.8SrRNA-IS (AY839389)	5'- GTTTCCGTGCGGGCTGTG -3'	5'- CCCTCAACCTAATGGCATCG -3'	1.8
LsPCNA (GBSS01017540)	5'-GAATCCCGAGGTGTTCACT-3'	5'-TATGGCTAAGATGTTGAAGTGCG-3'	1.8
LsSOG1	5'-CTTTTCCTCCTCCCGCACT-3'	5'-GATTTTGGTGATGTCCGCTGG-3'	1.8

(GBSS01010054)			

Supplementary Table S2. Relative expression values of target genes in the tested experimental conditions in *L. sativus* Maleme-107 accession. For each gene, expression levels without common letters are significantly different (*p*-value < 0.05) as analyzed by one-way ANOVA and Duncan test. Values are expressed as mean ± standard error. DS, dry seeds; DB, seeds subjected to hydropriming and dry-back; UP, unprimed seeds; HP, hydroprimed seeds; NT, seeds subjected to 8 h imbibition at 25 °C without heat wave; HW, seeds subjected to 4 h imbibition at 25 °C followed by 4 h heat wave at 40 °C. *Ls, Lathyrus sativus*. *EF2, ELONGATION FACTOR 2. GAPDH, GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE. SOD, SUPEROXIDE DISMUTASE*. *APX, ASCORBATE PEROXIDASE. MT, METALLOTHIONEIN. OGG1, 8-OXOGUANINGLYCOSYLASE/LYASE*. *FPG, FORMAMIDOPYRIMIDINE-DNA GLYCOSYLASE. Lig, DNA LIGASE. TOP, DNA TOPOISOMERASE. TFIIS, TRANSCRIPTION ELONGATION FACTOR IIS. SPMS, SPERMINE/SPERMIDINE SYNTHASE*. *SPDS, SPERMIDINE SYNTHASE. 5.8S, 5.8S rRNA. 5.8S-IS, 5.8S rRNA-INTERSPACE. PCNA, PROLIFERATING CELL NUCLEAR ANTIGEN. SOG1, SUPPRESSOR OF THE GAMMA RESPONSE 1*.

Gene	**DS**	**DB**	**UP-NT**	**UP-HW**	**HP-NT**	**HP-HW**
LsSODa	0.138 ± 0.00299ab	0.107 ± 0.00825c	0.153 ± 0.00125a	0.132 ± 0.00733b	0.0468 ± 0.00195d	0.0555 ± 0.00277d
LsSODb	0.958 ± 0.0448a	0.705 ± 0.0316b	0.598 ± 0.0177c	0.536 ± 0.0181c	0.272 ± 0.00958d	0.565 ± 0.0092c
LsAPX3a	0.0214 ± 0.0051b	0.0831 ± 0.00306a	0.0215 ± 0.00153b	0.0222 ± 0.00109b	0.0267 ± 0.001b	0.0236 ± 0.00165b
LsAPX3b	0.0138 ± 0.00228b	0.00827 ± 0.00136c	0.00708 ± 0.00107c	0.039 ± 0.0025a	0.00424 ± 0.000155c	0.00533 ± 6.33e-05c
LsMT1	0.13 ± 0.0144a	0.116 ± 0.018ab	0.076 ± 0.00879c	0.037 ± 0.00303d	0.122 ± 0.00751a	0.0829 ± 0.00569bc
LsMT2	2.13 ± 0.16b	2.38 ± 0.044a	1.27 ± 0.0587d	1.72 ± 0.0284c	0.503 ± 0.00634e	1.24 ± 0.0157d
LsSPDS	0.00448 ± 0.000344b	0.0104 ± 0.000632a	0.00977 ± 0.000279a	0.0105 ± 0.000676a	0.00495 ± 0.000524b	0.0044 ± 0.000248b
LsSPMS	0.00943 ± 0.00172d	0.014 ± 0.00117bc	0.0171 ± 0.00186ab	0.0197 ± 0.000875a	0.00838 ± 0.000411d	0.0113 ± 0.000347cd
LsSOG1	0.00069 ± 0.00015b	0.0014 ± 0.000354a	0.000101 ± 2.98e-05c	0.000132 ± 2.8e-05c	0.000803 ± 2.84e-05b	7.17e-05 ± 0c
LsOGG1	0.021 ± 0.00168c	0.00639 ± 0.00203e	0.0617 ± 0.00215a	0.0537 ± 0.00296b	0.0159 ± 0.000759cd	0.0136 ± 0.000313d
LsFPG	0.0107 ± 0.00204c	0.0138 ± 0.00154c	0.0567 ± 0.00295b	0.0956 ± 0.00767a	0.0146 ± 0.000705c	0.0122 ± 0.000586c
LsLig	0.00364 ± 0.000503e	0.0101 ± 0.0014e	0.0597 ± 0.000786b	0.0819 ± 0.00842a	0.0447 ± 0.00188c	0.0223 ± 0.000768d
LsTop1	0.0508 ± 0.00429b	0.0346 ± 0.0019c	0.0563 ± 0.0027b	0.0899 ± 0.00124a	0.0321 ± 0.00201c	0.0214 ± 0.00171d
LsTFIIS	0.0897 ± 0.00799b	0.0508 ± 0.00107b	0.168 ± 0.027a	0.204 ± 0.0149a	0.0547 ± 0.00967b	0.0825 ± 0.0187b
Ls5.8S	1240 ± 7.88c	2160 ± 115a	1740 ± 165b	601 ± 43.7d	245 ± 47.8e	529 ± 110de
Ls5.8S-IS	219 ± 10.1b	156 ± 6.45c	290 ± 14.5a	182 ± 8.43c	123 ± 6d	161 ± 2.44c
LsPCNA	0.0292 ± 0.0014d	0.0229 ± 0.000494d	0.11 ± 0.0089b	0.144 ± 0.00408a	0.0567 ± 0.000755c	0.0493 ± 0.00147c

Supplementary Table S3. Relative expression values of target genes in the tested experimental conditions in *L. sativus* Sofades accession. For each gene, expression levels without common letters are significantly different (*p*-value < 0.05) as analyzed by one-way ANOVA and Duncan test. Values are expressed as mean ± standard error. DS, dry seeds; DB, seeds subjected to hydropriming and dry-back; UP, unprimed seeds; HP, hydroprimed seeds; NT, seeds subjected to 8 h imbibition at 25 °C without heat wave; HW, seeds subjected to 4 h imbibition at 25 °C followed by 4 h heat wave at 40 °C. *Ls*, *Lathyrus sativus*. *EF2*, *ELONGATION FACTOR 2*. *GAPDH*, *GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE*. *SOD*, *SUPEROXIDE DISMUTASE*. *APX*, *ASCORBATE PEROXIDASE*. *MT*, *METALLOTHIONEIN*. *OGG1*, *8-OXOGUANINGLYCOSYLASE/LYASE*. *FPG*, *FORMAMIDOPYRIMIDINE-DNA GLYCOSYLASE*. *Lig*, *DNA LIGASE*. *TOP*, *DNA TOPOISOMERASE*. *TFIIS*, *TRANSCRIPTION ELONGATION FACTOR IIS*. *SPMS*, *SPERMINE/SPERMIDINE SYNTHASE*. *SPDS*, *SPERMIDINE SYNTHASE*. *5.8S*, *5.8S rRNA*. *5.8S-IS*, *5.8S rRNA-INTERSPACE*. *PCNA*, *PROLIFERATING CELL NUCLEAR ANTIGEN*. *SOG1*, *SUPPRESSOR OF THE GAMMA RESPONSE 1*.

Gene	**DS**	**DB**	**UP-NT**	**UP-HW**	**HP-NT**	**HP-HW**
LsSODa	0.108 ± 0.0062a	0.0438 ± 0.00774b	0.0886 ± 0.00642a	0.111 ± 0.00764a	0.0435 ± 0.000543b	0.0561 ± 0.0199b
LsSODb	1.79 ± 0.0604ab	0.934 ± 0.0639d	1.43 ± 0.0424bc	1.9 ± 0.061a	0.879 ± 0.0408d	1.24 ± 0.271cd
LsAPX3a	0.0244 ± 0.00165c	0.132 ± 0.00736a	0.0251 ± 0.000647c	0.047 ± 0.00144bc	0.0563 ± 0.00502bc	0.073 ± 0.0327b
LsAPX3b	0.0118 ± 0.00169b	0.0228 ± 0.00368a	0.0142 ± 0.00247b	0.0294 ± 0.00204a	0.00944 ± 0.0016b	0.00912 ± 0.000805b
LsMT1	0.0235 ± 0.00615b	0.21 ± 0.00903a	0.0127 ± 0.00491bc	0.000243 ± 8.98e-05c	0.00472 ± 0.00125c	0.0248 ± 0.00628b
LsMT2	1.89 ± 0.0349a	0.89 ± 0.0896d	1.48 ± 0.0553bc	1.58 ± 0.0646b	0.536 ± 0.0181e	1.31 ± 0.0644c
LsSPDS	0.00456 ± 0.000108	0.00418 ± 0.000745	0.00396 ± 0.00151	0.00324 ± 0.00117	0.00383 ± 0.000336	0.00559 ± 0.00154
LsSPMS	0.0105 ± 0.00117	0.0112 ± 0.00409	0.00786 ± 0.00238	0.0147 ± 0.00438	0.00479 ± 0.00106	0.00846 ± 0.00165
LsSOG1	0.00855 ± 0	0.065 ± 0.0278	0.0524 ± 0.00479	0.0812 ± 0.0119	0.0887 ± 0.0328	0.092 ± 0.0403
LsOGG1	0.0125 ± 0.0026cd	0.0109 ± 0cd	0.0387 ± 0.00244a	0.0328 ± 0.00493ab	0.00729 ± 0.00181d	0.0227 ± 0.00692bc
LsFPG	0.02 ± 0.00284b	0.0275 ± 0.00649b	0.0543 ± 0.00273a	0.0711 ± 0.0105a	0.0313 ± 0.0025b	0.0688 ± 0.012a
LsLig	0.00932 ± 0.00165c	0.0523 ± 0.00793a	0.0518 ± 0.00343a	0.0458 ± 0.00423a	0.0304 ± 0.000158b	0.0559 ± 0.00215a
LsTop1	0.0306 ± 0.00163b	0.0264 ± 0.00778b	0.0333 ± 0.00527b	0.0509 ± 0.00445a	0.0214 ± 0.00426b	0.0342 ± 0.00418b
LsTFIIS	0.11 ± 0.0067b	0.0523 ± 0.00277c	0.152 ± 0.00495a	0.15 ± 0.00806a	0.097 ± 0.00845b	0.162 ± 0.0143a
Ls5.8S	529 ± 33.6e	1430 ± 61.4b	837 ± 146d	2360 ± 53.8a	493 ± 0.477e	1130 ± 38.7c
Ls5.8S-IS	113 ± 0.384b	111 ± 2.08b	181 ± 15.7a	195 ± 9.62a	114 ± 3.77b	182 ± 1.22a
LsPCNA	0.0213 ± 0.00325c	0.0411 ± 0.0061bc	0.0847 ± 0.00737a	0.0939 ± 0.00648a	0.0515 ± 0.00154b	0.111 ± 0.0164a