Transcriptional profiling of maturing tomato (*Solanum lycopersicum* L.) microspores reveals the involvement of heat shock proteins, ROS scavengers, hormones and sugars in the heat-stress response

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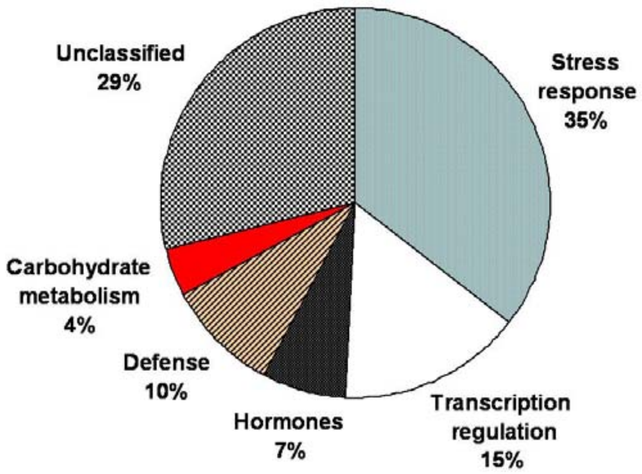

Fig. S1. Graphical representation of the percentage of genes belonging to a given functional group for the 104 heat-regulated genes that are presented in Table S3.

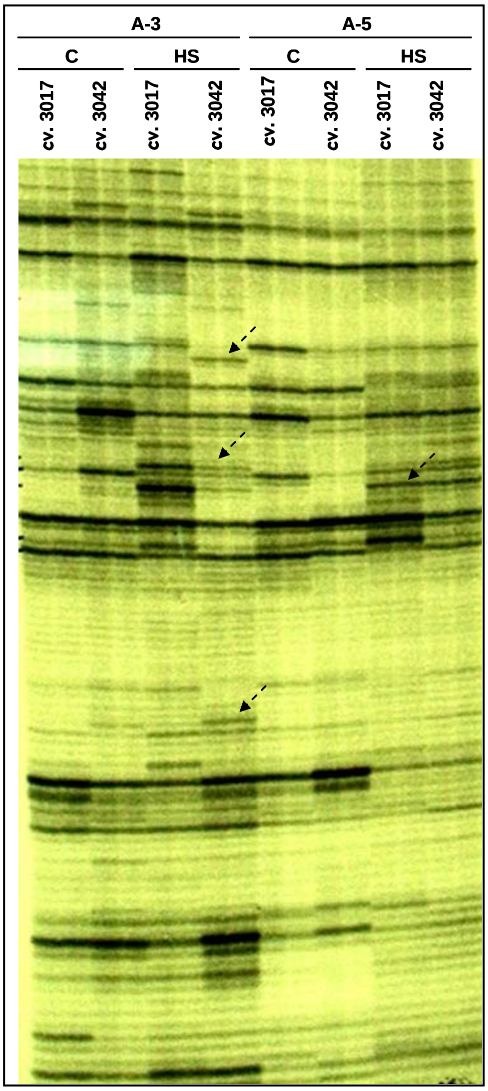

Fig. S2. Autoradiography of a representative cDNA-AFLP polyacrylamide gel. Amplicons resulting from one specific primer combination (*Eco*RI-ACT/ *Mse*I-CTT) were separated electrophoretically. There are 8 duplicated lanes (total of 16 lanes), corresponding to differentially expressed fragments of maturing microspores at developmental stages A-3 and A-5 (3 and 5 days before anthesis, respectively) derived from plants of *cv*. 3017 and *cv*. 3042, which had been either kept under control conditions (C, 26/22 ^{o}C day/night temperatures) or exposed to HS (2 h at 43-45 ^{o}C). Arrows point to TDFs that exhibit differential expression patterns.

Table S1. List of adapters and primers used for cDNA-AFLP application

A.	**Forward**	**Reverse**
EcoRI adapters	5' CTCGTAGACTGCGTACC 3'	3' AATTGGTACGCAGTCTAC 5'
MseI adapters	5' TACTCAGGACTCAT 3'	3' GACGATGAGTCCTGAG 5'
B.		
Pre-selective amplification primers	5' GACTGCGTACCAATTCA 3'	5' GATGAGTCCTGAGTAAC 3'
Selective amplification primers*	5' GACTGCGTACCAATTCACT 3'	5' CATGAGTCCTGAGTAACTG 3'
	5' GACTGCGTACCAATTCACA 3'	5' CATGAGTCCTGAGTAACTT 3'
	5'GACTGCGTACCAATTCACC '3	5' CATGAGTCCTGAGTAACAG 3'
	5' GACTGCGTACCAATTCAAC 3'	5' CATGAGTCCTGAGTAACAT 3'
		5' CATGAGTCCTGAGTAACTA 3'
		5' CATGAGTCCTGAGTAACTC 3'
		5' CATGAGTCCTGAGTAACAA 3'
		5' CATGAGTCCTGAGTAACAC 3'

*All possible *EcoR*I X *Mse*I primer combinations were used (except for *EcoR*I-AAC X *Mse*I-CTG/CAA/CAC), resulting in a total of 29 combinations.

Table S2. List of primer sequences used for semi-quantitative RT-PCR analysis for validation of (**A**) microarray and (**B**) cDNA-AFLP results

A. Sequences of semi-quantitative RT-PCR primers used for validating the microarray results

Gene symbol	**Accession**	**Affy ID**	**Forward primer**	**Reverse primer**	****Amp. length**
*LeHSP17.4- CII**	AF090115	Les.3578.1.S1_at	5' TCTCTTCTTCCCTCTTCCTT 3'	3' TAACACACCACTCTTCCACA 5'	251
Small heat shock protein (vis1)	AY128100	Les.3677.1.S1_at	5' ACTCTGTCCATTGTGTCCAT 3'	3' ATTCCAACTCCTCTCTTCCA 5'	223
LeHSP21	U66300	Les.269.1.S1_at	5' TGGGAGACTTAAACGAGTGT 3'	3' AGGAAGAAACAGAGCATCAG 5'	238
LeMTHSP23.8	AB017134	Les.4150.1.S1_at	5' CGTTATCGTCCTCCTTTACA 3'	3' CAGCTTATGACCAGGATGAT 5'	249
SlAPX3	DQ096286	LesAffx.3918.1.S1_at	5' TGTCTCCCAGGATGAAAAGG 3'	3' ATCGACGTTTGATCGGAGAA 5'	335
LpHSF30	X67601	LesAffx.24696.1.S1_at	5' CAACCTCAACACATGACATC 3'	3' GTGGTGTGGGATTCTCATAA 5'	250
LeMBF1 (ER24)	EU240881	Les.3551.1.S1_at	5' CACTGCCTTACCATTCTCAT 3'	3' CACAAGTTCAAACGGTGAAG 5'	242
LeDREB1	AF500011	Les.3985.1.S1_at	5' TGCACAAGGACCATACATAG 3'	3' AGTGCGTAAAGTTCCTGCTA 5'	235
LeACS3	U17972	Les.3642.1.S1_at	5' TCGAATCCAGGATAGTATGG 3'	3' CTGGGTTTAAGAGAAATGGA 5'	247
18S ribosomal RNA; *S. lycopersicum*	X51576		5' CTCCGGAATCGAACCCTAAT 3'	3' CTTCTGGAAGGGATGCATTT 5'	200

* The primers used may amplify LeHSP17.6-CII cDNA (accession U72396) as well.

B. Sequences of semi-quantitative RT-PCR primers used for validating the cDNA-AFLP results

TDF number	**Homologue gene**	**Accession**	**Forward primer**	**Reverse primer**	****Amp. length**
TDF3.2	*LeHSP90*	AF123259	5' GGATTTGCTGCTTACACAAC 3'	3' GGTTGAGCATAGACGAAGAA 5'	246
TDF3.1	*AtHSP81-1*	AT5G52640	5' CTGAGTAACAATCTGGGTACG 3'	3' CTCAACTGGTCTTCCTTGAG 5'	283
TDF31	*AtCDPK2*	AT1G35670	5' GAGTCCTGAGTAACATCACCA 3'	3' GCATTAAAAGGAGAGAGGAG 5'	131
TDF18.1	*AtVAMP725*	AT2G32670	5' GACCCAAATTCCCTATTGAGG 3'	3' GAAATTCCCCTTGCCTTCTT 5'	110
TDF4.2	*AtGAE5*	AT4G12250	5' TGGGCTTTCCTTTTGGTATG 3'	3' TGCAGGTCTCGTTACCCTTT 5'	308
TDF12.1	*LeTFT9*	X98865	5' TCACTCTGCATCTTCACCTC 3'	3' GGATAGCCTGAATGAGGATT 5'	160
TDF3.3	*AtCDC48*	AT3G53230	5' GCAGGGTCAATAATATCAGG 3'	3' GCTCTAGGAACTAGCATCCA 5'	532
TDF6.1	Adenylyl-sulfate reductase; *S. lycopersicum*	AY568717	5' TGATACTGGGAGGTTGAATC 3'	3' TCAAACGAAGGGTCTATCTG 5'	298
TDF10.1	*LAT56*; *S. lycopersicum*	X15500	5' ATGTCACCTCTTTCACCAAC 3'	3' CTGTTGCTGGTTCCACTAAT 5'	309
Reference gene	18S ribosomal RNA; *S. lycopersicum*	X51576	5' CTCCGGAATCGAACCCTAAT 3'	3' CTTCTGGAAGGGATGCATTT 5'	200

** Amp. length – amplicon length in bp.

Table S3. *Complete list of genes with significant (p-value<0.05) differential expression (>2.0-fold) between heat-stressed and control maturing microspores*

Affy ID	Accession/ AGI No.[a]	E-value[b]	Gene description[c]	Arabidopsis ortholog[d]	Fold[e]
Stress response					
Hsps					
LesAffx.69957.1.S1_at[BH]	AT1G52560	8E-50	26.5 kDa class I small heat shock protein-like (AtHSP26.5-P-CI); *A. thaliana*		158
Les.269.1.S1_at[BH]	U66300	1E-132	Chloroplast heat shock protein (LeHSP21); *S. lycopersicum*	AT4G27670	155
Les.3739.1.S1_at[BH]	AB026983	5E-116	Endoplasmic reticulum-located small heat shock protein (LeERHSP21.5); *S. lycopersicum*	AT4G10250	154
LesAffx.10596.1.S1_at [BH]	AT5G59720	1E-63	18.2kDa heat shock protein (AtHSP18.2); *A. thaliana*		141
Les.3677.1.S1_at[BH]	AY128100	1E-134	Small heat shock protein (vis1); *S. lycopersicum*	AT4G27670	132
Les.4004.1.S1_a_at[BH]	AF123256	2E-66	17.8 kDa class I small heat shock protein (LeHSP17.8- CI); *S. lycopersicum*	AT1G07400	93
Les.4150.1.S1_at[BH]	AB017134	2E-131	Mitochondrial small heat shock protein (LeMTHSP23.8); *S. lycopersicum*	AT4G25200	82
Les.3578.1.S1_at[BH]	AF090115	2E-99	Cytosolic class II small heat shock protein HCT2 (LeHSP17.4- CII); *S. lycopersicum*	AT5G12020	75
LesAffx.70264.1.S1_at[BH]	AB333795	6E-49	Peroxisomal small heat shock protein (GmPHS-P); *Glycine max*	AT5G37670	64
Les.3581.1.S1_at	U72396	3E-68	Class II small heat shock protein (LeHSP17.6- CII) ;*S. lycopersicum*	AT5G12030	40
LesAffx.31298.1.S1_at	AF123255	2E-31	17.7 kDa class I small heat shock protein (LeHSP17.7- CI); ;*S. lycopersicum*	AT1G59860	5
Les.3550.1.S1_at[BH]	AF096251	7E-7	Ethylene-responsive heat shock protein cognate 70 (LeHSC70/ ER21); *S. lycopersicum*		83
LesAffx.34336.1.S1_at[BH]	AT2G32120	1E-112	HSP70T-2; *A. thaliana*		36
LesAffx.10807.1.S1_at	X13301	3E-29	Heat shock protein hsp70 (PhHSP70) ;*Petunia x hybrida*	AT5G02490	7
Les.3401.3.S1_at	EU404167	8E-57	Hsp70-interacting protein 1; *Vitis labrusca*	AT4G22670	6
Les.3401.2.S1_at	EU404167	8E-57	Hsp70-interacting protein 1; *Vitis labrusca*	AT4G22670	5
Les.3550.1.A1_s_at	AF096251		Ethylene-responsive heat shock protein cognate 70 (LeHSC70/ ER21); *S. lycopersicum*		5
Les.3134.1.S1_at	AF123259	7E-60	Heat shock protein 90 (LeHSP90); *S. lycopersicum*		5
LesAffx.56637.1.S1_at[BH]	AT3G09700	2E-29	DNAJ heat shock N-terminal domain-containing protein; *A. thaliana*		70
Les.4705.1.S1_at	AT4G21320	1E-134	Heat stress associated 32 (AtHSA32); *A. thaliana*		23

Affy ID	**Accession/ AGI No.**[a]	**E-value**[b]	**Gene description**[c]	**Arabidopsis ortholog**[d]	**Fold**[e]
Les.4955.1.S1_at	AT1G30070	2E-14	SGS domain-containing protein; *A. thaliana*		16
LesAffx.58365.1.S1_at	AT3G08970	2E-37	DNAJ heat shock N-terminal domain-containing protein; *A. thaliana*		6
Oxidative stress					
Les.5622.1.S1_at[BH]	AT3G16050	1E-143	Pyridoxal phosphate synthase protein (AtPDX1); *A. thaliana*		64
LesAffx.3918.1.S1_at[BH]	DQ096286	4E-108	Cytosolic Ascorbate peroxidase 3 (SlAPX3); *S.* lycopersicum	AT3G09640	53
Les.1132.1.A1_at[BH]	AT1G17870	2E-43	Ethylene-dependent gravitropism-deficient and yellow-green-like (ATEGY3); *A. thaliana*		32
LesAffx.33042.1.S1_at	X53393	4E-25	Cytochrome c oxidase subunit Vc; *Sweet potato*	AT2G47380	16
Les.3011.1.S1_at	AF243522	0	Light dependent NADH:protochlorophyllide oxidoreductase 2; *S. lycopersicum*	AT2G27680	12
Les.1936.1.S1_at	AY547273	1E-79	Early light-inducible protein (LeELIP); *S. lycopersicum*	AT3G22840	7
Les.4709.1.S1_at	AT2G27680	0	Aldo/keto reductase family protein; *A. thaliana*		6
Les.4223.1.S1_at	AY034148	0	Alternative oxidase 1a (LeAOX1a); *S. lycopersicum*	AT3G22370[P]	5
Les.1724.1.S1_at	AB087837	9E-65	Glutathione S-transferase (PsGST); *Pisum sativum*	AT5G02790	4
Les.1724.2.S1_at	AI776156	1E-12	Glutathione S-transferase (PsGST); *Pisum sativum*	AT5G02790	4
Others					
LesAffx.47187.1.S1_at[BH]	AT3G03270	4E-55	Universal stress protein (USP) family protein/ early nodulin ENOD18 family protein*;* *A. thaliana*		28
Les.2255.1.S1_at	AT1G77000	1E-177	Ubiquitin-protein ligase AtSKP2B; *A. thaliana*		6
LesAffx.1790.1.S1_at	AF452011	1E-142	Aquaporin-like protein (PIP1;2); *Petunia x hybrida*	AT4G00430	5
Les.3392.2.S1_at	AT1G62740	7E-59	Stress-inducible protein; *A. thaliana*		5
Les.3392.3.S1_at	AF512999	2E-84	TPR1 (MsTRP1); *Medigaco sativa*	AF512999	5
Hormone metabolism & response					
Ethylene					
Les.3551.1.S1_at[BH; BY]	EU240881*	5E-113	ethylene-responsive transcriptional coactivator multiprotein bridging factor ER24 (LeMBF1); *S. lycopersicum*	AT3G24500	156
Les.3550.1.S1_at	AF096251*	7E-7	Ethylene-responsive heat shock protein cognate 70 (ER21); *S. lycopersicum*		83
Les.3985.1.S1_at[BH]	AF500011*	3E-179	Dehydration responsive element binding protein (LeDREB1); *S. lycopersicum*	AT5G05410	14
Les.2322.1.A1_at	AY044235*	2E-103	Jasmonate and Ethylene responsive factor 1 (LeJERF1); *S. lycopersicum*	AT1G53910	9
Les.3642.1.S1_at	U17972	0	1-aminocyclopropane-1-carboxylate synthase 3/ ACC synthase 3	AT4G37770	6

Affy ID	Accession/ AGI No.[a]	E-value[b]	Gene description[c]	Arabidopsis ortholog[d]	Fold[e]
			(LeACS3)*; S. lycopersicum*		
Les.3766.1.S1_at	U77719	1E-108	Ethylene-responsive late embryogenesis-like protein (ER5); *L. esculentum*	AT2G46140[P]	4
ABA					
Les.4807.1.S1_at[BH]	AT5G13200	1E-111	ABA-responsive protein-related/ GRAM domain-containing protein; *A. thaliana*		25
Les.5956.1.S1_s_at	AF386075	3E-40	Dehydrin 2 (DHN2); *Solanum commer*sonii	AT1G20440	4
LesAffx.3568.1.S1_a_at	AB049338	4E-07	Dehydrin (NtERD10); *Nicotiana tabacum*	AT1G20450	4
Jasmonic acid					
Les.368.1.S1_at[BH]	DQ359730	6E-34	Jasmonate resistance 1 -like protein (NaJAR6*); Nicotiana attenuate*	AT2G46370	43
Les.13.1.S1_at	AJ271093	0	Allene oxide synthase (LeAOS); *L. esculentum*	AT5G42650	14
Les.2322.1.A1_at	AY044235*	2E-103	Jasmonate and Ethylene responsive factor 1 (LeJERF1); *S. lycopersicum*	AT1G53910	9
Defense					
LesAffx.66384.1.S1_at[BH]	AT1G12060	2E-20	BCL-2-Associated athanogene 5 (AtBAG5); *A .thaliana*		56
LesAffx.58019.1.S1_at[BH]	AT5G20740	1E-29	Invertase/pectin methylesterase inhibitor family protein; *A. thaliana*		44
Les.4462.1.S1_at	X59884		Wound induced protein; *S. lycopersicum*		35
LesAffx.1881.1.S1_at[BH]	AT4G36010	6E-73	Pathogenesis-related thaumatin family protein; *A. th*aliana		19
LesAffx.68306.1.S1_at	AF493074	1E-23	Susceptibility antioxidant protein (OsRREO); Oryza sativ	AT2G35050	9
Les.5510.1.S1_at	AT2G37970	1E-89	AtSOUL-1; *A. thaliana*		8
LesAffx.65922.1.S1_at	AT2G26040	3E-13	Bet v I allergen family protein; *A. thaliana*		6
Les.4099.1.S1_at	AY187634		Phosphoenolpyruvate carboxylase kinase 2 (LePPCK2); *S. lycopersicum*	AT1G08650	5
Les.3989.1.A1_at	AJ457049		Hero resistance protein 2 homologue; *S. lyco*persicum		5
LesAffx.53426.1.S1_at	AT5G67270	9E-74	Microtubule end binding protein 1 (AtEB1C); *A. thaliana*		4
Carbohydrate metabolism					
Les.3522.1.S1_at	AF071786		Sucrose-phosphate synthase; *S. lycopersicum*	AT5G20280	7
Les.3069.1.S1_at	AT2G22900	4E-22	Galactosyl transferase GMA12/MNN10 family protein; *A. thaliana*		5
Les.3696.1.S1_at	AF311943	0	Galactinol synthase 1 (LeGolS-1); *S. lycopersicum*	AT2G47180	4
LesAffx.10299.1.A1_at	DQ104196	2E-77	Sorbitol transporter (ST1); *Nicotiana langsdorffii x Nicotiana sanderae*	AT3G18830	4
Transcription regulation					
LesAffx.24696.1.S1_at[BH]	X67601	2E-119	Heat stress transcription factor 30 (LpHSF30); *L. peruvianum*	AT2G26150	200
Les.3551.1.S1_at[BH]	EU240881	5E-113	Ethylene-responsive transcriptional coactivator multiprotein bridging factor ER24 (LeMBF1); *S. lycopersicum*	AT3G24500	156
Les.3985.1.S1_at[BH]	AF500011	3E-179	Dehydration responsive element binding protein (LeDREB1); *S.*	AT5G05410	14

Affy ID	**Accession/ AGI No.**[a]	**E-value**[b]	**Gene description**[c]	**Arabidopsis ortholog**[d]	**Fold**[e]
			lycopersicum		
Les.4725.1.S1_at	AT5G43990	9E-30	Histone-lysine N-methyltransferase/ zinc ion binding (AtSUVR2); *A. thaliana*		14
LesAffx.71311.1.S1_at	AJ632082	7E-24	Zinc finger DNA-binding protein (ZCT1); *A. thaliana*	AT2G37430	12
LesAffx.3163.1.S1_at	AT4G12040[P]	5E-48	Zinc finger (AN1-like) family protein; *A. thaliana*		10
LesAffx.3163.2.S1_at	AT4G22820	2E-30	Zinc finger (AN1-like) family protein; *A. thaliana*		10
Les.5126.1.S1_at	AF053077	5E-69	Osmotic stress-induced zinc-finger protein (ZPF); *Nicotiana tabacum*	AT1G27730	10
Les.2322.1.A1_at	AY044235	2E-103	Jasmonate and Ethylene responsive factor 1 (LeJERF1); *S. lycopersicum*	AT1G53910	9
LesAffx.23563.1.A1_at	AT1G07350	2E-25	Transformer serine/arginine-rich ribonucleoprotein, putative; *A. thaliana*		9
Les.5292.1.S1_at	AT3G27330[P]	5E-03	Zinc finger (C3HC4-type RING finger) family protein; *A. thaliana*		8
LesAffx.44490.1.S1_at	EU200164	3E-32	Zinc finger-homeodomain protein 1 (ZHD1); *Cucumis sativus*	AT3G28920	7
Les.4102.1.S1_at	AY192368	4E-162	Ethylene response factor 2 (LeERF2); *S. lycopersicum*	AT2G47520	6
LesAffx.66062.1.S1_at	AT3G56510	1E-68	TBP-binding protein, putative*; A. thaliana*		6
Les.2876.2.S1_at	AF208544	3E-79	Heat stress transcription factor A3 (LpHSFA3); *L. peruvianum*	AT5G03720	4
LesAffx.70885.1.S1_at	AJ009594	9E-58	Dof protein BBF1 gene (NtBBF1); *N. tabacum*	AT4G24060	3
Unclassified					
LesAffx.15004.1.S1_at[BH]	AW737975		cDNA, clone: FC25AB05, HTC in fruit; *S. lyco*persicum		380
Les.3726.1.S1_at[BH]	AF204783	4E-112	Ripening regulated protein DDTFR8; *S. lycopersicum*		130
Les.3822.1.S1_at[BH]	AF204795	1E-33	Ripening regulated protein DDTFR17; *S. lycopersicum*		55
Les.1718.1.A1_at[BH]	AT2G18440[P]	6E-03	Gene with unttable transcript 15 (GUT15); *A. thaliana*		50
Les.195.1.S1_at[BH]	BM411019		Transcribed locus; *S. lyco*persicum		46
Les.5053.1.S1_at[BH]	BT013184		Clone 134261F, mRNA sequence; *S. lyco*persicum		43
Les.2329.1.A1_at	BG734860		Transcribed locus; *S. lyco*persicum		24
Les.1910.1.S1_at	AT2G18440[P]	6E-03	Gene with unttable transcript 15 (GUT15); *A. thaliana*		18
Les.1287.1.A1_at	BG630588		cDNA, clone: FC12DF06, HTC in fruit; *S. lyco*persicum		16
LesAffx.65171.1.S1_at	CN384746		NP_175087.1 unknown protein; *A. thaliana*		14
Les.1910.2.S1_at	BM409608		Transcribed locus; *S. lyco*persicum		13
LesAffx.37707.1.A1_at	AJ831866		Transcribed locus; *S. lyco*persicum		13
Les.1287.2.S1_at	AW621773		cDNA, clone: FC12DF06, HTC in fruit; *S. lyco*persicum		12
LesAffx.66316.1.S1_at	AT1G24530[P]	8E-41	Transducin family protein / WD-40 repeat family protein; *A. thaliana*		10
Les.2131.2.S1_at	BE433043		NP_201222.1 unknown protein; *A. thaliana*		10
Les.1910.3.A1_at	BG630113		Transcribed locus; *S. lyco*persicum		10
Les.1493.1.S1_at	AT5G22300	0	Nitrilase 4 (AtNIT4); *A. thaliana*		9

Affy ID	**Accession/ AGI No.**[a]	**E-value**[b]	**Gene description**[c]	**Arabidopsis ortholog**[d]	**Fold**[e]
Les.4752.1.S1_at	BT013346		Clone 135282R, mRNA sequence; *S. lyco*persicum	AT5G63100[P]	9
LesAffx.57312.1.S1_at	AB061261	2E-110	Ankyrin-like protein; *Solanum tuberosum*	AT3G04710	7
Les.1197.1.S1_at	AF272710	2E-146	7-transmembrane G-protein-coupled receptor; *Solanum chacoense*	AT5G65280	7
Les.1121.1.S1_at	AT3G63310	E-131	Glutamate binding; *A. thaliana*		7
Les.2846.3.A1_at	AT5G11090	2E-06	Serine-rich protein-related; *A. thaliana*		7
Les.2846.2.S1_at	AT5G11090	8E-36	Serine-rich protein-related; *A. thaliana*		6
Les.1409.3.S1_at	BE461946		cDNA, clone: FC21BA06, HTC in fruit; *S. lyco*persicum		6
LesAffx.17663.2.A1_at	CK715113		NP_563626.1 unknown protein; *A. thaliana*		6
LesAffx.17663.1.S1_at	AY822468	2E-93	AT-rich element binding factor 3 (PsATF3); *Pisum sativum*	AT4G01030	5
Les.2131.1.A1_at	BG627099		NP_201222.1 unknown protein; *A. thaliana*		5
LesAffx.71454.1.S1_at	AT2G17440	1E-88	Leucine-rich repeat family protein; *A. thaliana*		4
Les.4258.3.S1_at		E-134	ABC2 homolog 13 (AtATH13); *A. thaliana*		4
Les.4225.1.S1_at	BG631475		cDNA, clone: FC06BB09, HTC in fruit; *S. lyco*persicum		4

Results are presented as the fold difference between the mean-value derived from 12 replicates of heat-stressed vs. the mean value derived from 12 replicates of control microspores. Genes found to be statistically significant (p-value<0.05) are presented in this table.

[a] Either tomato NCBI accession number, GenBank homolog or *Arabidopsis* genome initiative number of best hit from TBLASTX search, using a significance threshold of 1E-4 are given for each Affy ID number.

[b] *E*-value indicating the significance level of homology to the corresponding gene.

[c] Gene function predicted by the Affymetrix annotation, TIGR definition, and NCBI database.

[d] Arabidopsis genome initiative number is given for *Arabidopsis* gene orthologs.

[e] The RMA-normalized ratio of gene expression in heat-stressed (n=12) vs. control (n=12) microspores.

[BH] To ensure no more than 5% false discovery rate, the BH approach for multiple comparison correction (Benjamini and Hochberg, 1995) was used. Genes that were less than the adjusted p-value of 5% are marked by 'BH', the rest (unmarked) are statistically significant ($p\text{-value}<0.05$, not corrected).

[P] Gene sequences found to be highly expressed in *Arabidopsis* pollen grains under control conditions (Zimmermann *et al.*, 2004).

Table S4. *Summary of HS-responsive transcript-derived fragments (TDFs) of maturing tomato pollen grains representing unknown proteins and unidentified sequences*

TDF[a]	Primer combination[b]	Length (bp)[c]	HS response[d]	Exp. pattern[e]	Gene description[f]	Accesion/ AGI No.[g]	E-value[h]
Unknown proteins							
TDF24.2	E-ACA/ M-CTT	215	+	A	Chromosome 1 BAC T8L23 genomic sequence: *A. thaliana*	AC079733	8E-04
TDF12.2	E-ACT/ M-CTG	278	+	A	Hypothetical protein; *A. thaliana*	AT1G15670	4 E-19
TDF14.3	E-ACA/ M-CAG	309	+	A	Protein binding; *A. thaliana*	AT1G44910	8E-04
TDF27.3	E-ACT/ M-CTG	186	–	B	Unknown protein; *A. thaliana*	AT3G54100	9E-09
TDF32.2	E-ACT/ M-CTT	173	+	C	Clone 135129R*; S. lycopersicum*	BT013338	1E-19
TDF36.1	E-ACA/ M-CAG	145	+	A	EST from severe drought-stressed opposite wood; *Populus*	CU233588	4E-15
TDF18.2	E-ACT/ M-CTT	243	+	A	DNA sequence from clone LE_Hba-24G5 on chromosome 4; *S.lycopersicum*	CU019619	3E-12
TDF32.3*	E-ACT/ M-CTG	147	+	B	DNA sequence from clone LE_Hba-331L22 on chromosome 4; *S.lycopersicum*	CU222538	1E-15
TDF51.1	E-ACT/ M-CAA	93	+	D	Chromosome 5 clone PGEC160O2; *S. demissum*	AC150162	0.006
TDF3.4	E-ACA/ M-CTT	556	+	A	Cchromosome 5 clone PGEC872C13; *S. demissum*	AC149266	3E-25
TDF4.6	E-ACT/ M-CTG	564	+	A	Chromosome 5 clone PGEC872C13; *S. demissum*	AC149266	2E-16
TDF4.7	E-ACT/ M-CTG	561	+	A	Chromosome 5 clone PGEC872C14; *S. demissum*	AC149267	2E-16
TDF17.5	E-ACT/ M-CTG	268	+	A	cDNA, clone: LEFL1004CE03, HTC in leaf*; S. lycopersicum*	K247357	1E-28
TDF4.8	E-ACT/ M-CTT	545	+	A	cDNA, clone: LEFL1008AH06, HTC in leaf*; S. lycopersicum*	AK247369	2E-84
TDF51.2	E-ACT/ M-CTT	473	+	A	cDNA, clone: LEFL1008AH06, HTC in leaf*; S. lycopersicum*	AK247369	2E-84
TDF17.6	E-ACT/ M-CTG	369	+	A	cDNA, clone: LEFL1008AH06, HTC in leaf*; S. lycopersicum*	AK247369	2E-84
TDF15.1	E-ACA/ M-CTG	330	+	A	Chromosome 3 clone C03Hba0233O20*; S. lycopersicum*	EU124739	2E-38
TDF15.2	E-ACA/ M-CTT	343	+	A	Chromosome 3 clone C03Hba0233O20*; S. lycopersicum*	EU124739	1E-42
TDF14.3	E-ACA/ M-CTT	314	+	A	Chromosome 9 clone C09Hba0165P17*; S. lycopersicum*	EF647605	0.001
TDF46.1	E-ACA/ M-CAG	104	+	A	Tomato chromosome 2, C02Sle0121H01*; S. lycopersicum*	AC215464	2E-6
Unidentified sequences							
TDF27.4	E-ACA/ M-CAG	159	+	A	Unidentified		
TDF36.2	E-ACA/ M-CAG	101	+	A	Unidentified		
TDF20.1	E-ACA/ M-CAG	208	+	A	Unidentified		
TDF14.5	E-ACA/ M-CTC	300	+	A	Unidentified		
TDF20.2	E-ACA/ M-CAG	250	+	A	Unidentified		

TDF[a]	Primer combination[b]	Length (bp)[c]	HS response[d]	Exp. pattern[e]	Gene description[f]	Accesion/ AGI No.[g]	E-value[h]
TDF27.5	E-ACA/ M-CAG	239	+	A	Unidentified		
TDF45.1	E-ACA/ M-CAG	100	+	A	Unidentified		
TDF57	E-ACA/ M-CAG	130	+	A	Unidentified		
TDF61	E-ACA/ M-CAG	198	+	A	Unidentified		
TDF11.2	E-ACA/ M-CAA	289	+	A	Unidentified		
TDF42.2	E-ACA/ M-CAA	61	+	A	Unidentified		
TDF49	E-ACA/ M-CAA	54	+	A	Unidentified		
TDF50	E-ACA/ M-CAA	51	+	A	Unidentified		
TDF35	E-ACA/ M-CTC	130	+	A	Unidentified		
TDF40.1	E-ACA/ M-CTC	106	+	A	Unidentified		
TDF14.6	E-ACA/ M-CTA	131	+	A	Unidentified		
TDF10.2	E-ACT/ M-CTG	304	+	A	Unidentified		
TDF60.1	E-ACA/ M-CTT	59	+	A	Unidentified		
TDF5.1	E-ACT/ M-CTT	407	+	A	Unidentified		
TDF12.3	E-ACT/ M-CTT	300	+	A	Unidentified		
TDF5.2	E-ACT/ M-CTT	405	+	A	Unidentified		
TDF12.4	E-ACT/ M-CTT	307	+	A	Unidentified		
TDF10.3	E-ACT/ M-CTG	306	+	A	Unidentified		
TDF60.2	E-ACA/ M-CTT	63	+	A	Unidentified		
TDF12.5	E-ACT/ M-CTT	375	+	A	Unidentified		
TDF17.7	E-ACT/ M-CTG	316	+	A	Unidentified		
TDF46.2	E-ACA/ M-CAG	160	+	A	Unidentified		
TDF12.6	E-ACT/ M-CTG	361	+	A	Unidentified		
TDF23.2	E-ACA/ M-CAG	230	+	C	Unidentified		
TDF23.1	E-ACA/ M-CAG	189	+	C	Unidentified		
TDF5.3	E-ACA/ M-CTT	425	+	E	Unidentified		
TDF51.3	E-ACT/ M-CAA	87	+	D	Unidentified		
TDF53	E-ACT/ M-CTT	143	+	D	Unidentified		
TDF30	E-ACT/ M-CAC	136	+	D	Unidentified		
TDF38.1	E-ACT/ M-CAG	79	+	A	Unidentified		
TDF38.2	E-ACT/ M-CAG	129	+	A	Unidentified		
TDF18.3	E-ACT/ M-CTT	302	+	A	Unidentified		

TDF[a]	Primer combination[b]	Length (bp)[c]	HS response[d]	Exp. pattern[e]	Gene description[f]	Accesion/ AGI No.[g]	E-value[h]
TDF45.2	E-ACT/ M-CAG	100	+	A	Unidentified		
TDF27.6	E-ACT/ M-CTT	192	–	B	Unidentified		
TDF40.2	E-ACA/ M-CAA	96	–	B	Unidentified		
TDF42.3	E-ACT/ M-CTT	97	–	B	Unidentified		
TDF42.4	E-ACT/ M-CTT	115	–	B	Unidentified		

a, TDF number, representing the relative location on the acryl-amid gel. Fragments with the same location, representing different treatment groups or primer combinations are marked .n.

b, primer combination used for the fragment amplification: E indicates *EcoR*I, M indicates *Mse*I.

c, length of the TDF sequence.

d, induction (+) or repression (-) of expression by heat-stress, detected in at least one of the tested stages of microspore development and one of the cultivars.

e, gene expression (Exp.) pattern. Expression patterns were divided into five categories: A-E. A, represents HS-induced expression in either or both developmental stages tested, in both cultivars. B, represents HS-down-regulated expression in both cultivars in microspores at either A-3 or A-5 developmental stage. C, represents constitutive expression in microspores of *cv*. 3017 and HS-induced in maturing microspores of *cv*. 3042 at either or both developmental stages. D, represents constitutive expression in *cv*. 3042 and HS-induced expression in microspores of *cv*. 3017 at either or both developmental stages. E, represents constitutive expression in microspores of *cv*. 3017 and HS-down-regulation in microspores of *cv*. 3042 at A-3.

f, gene description according to NCBI, TAIR or TIGR databases annotation.

g, either tomato NCBI accession number (No.), GenBank homolog or *Arabidopsis* genome initiative number (AGI) of best hit from TBLASTX search, using a significance threshold of 1.00E-4 are given for each TDF.

h, E-value indicating the significance level of homology to the corresponding gene.

*, TDF sequences found on the Affymetrix GeneChip® Tomato Genome Array. All TDFs sequences were checked using NETAFFX program of the Affymetrix service site (http://www.affymetrix.com/analysis/index.affx) for identifying TDFs which are presented on the Affymetrix chip.