

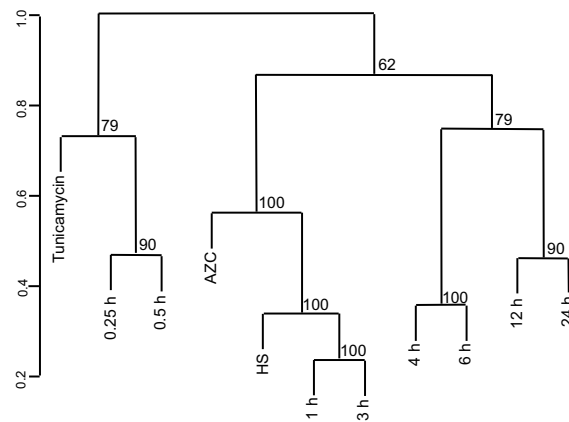
Supplemental Figure 1. Time course of heat-shock, CPR and UPR marker gene

(A) Time course of *HSP70A* expression during heat treatment (37°C) determined using qRT-PCR.

(B) Time course of *HSP70A* and *Bip3* expression after the infiltration of AZC.

(C) Time course of β *ZIP60* and *Bip3* expression after the infiltration of tunicamycin.

In each case, relative expression level of *HSP70A* to *EF1 α* is shown. Measurements are the means of three technical and three biological replicates. Bars = \pm SD

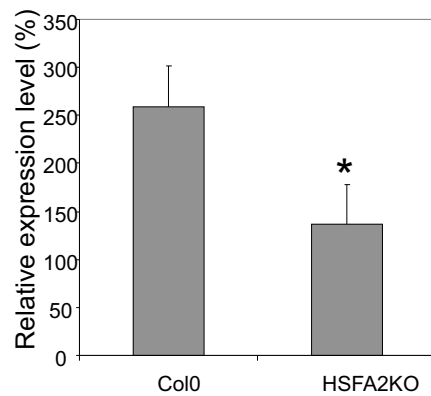


Supplemental Figure 2. Clustal analysis of public and recoded microarray datasets.

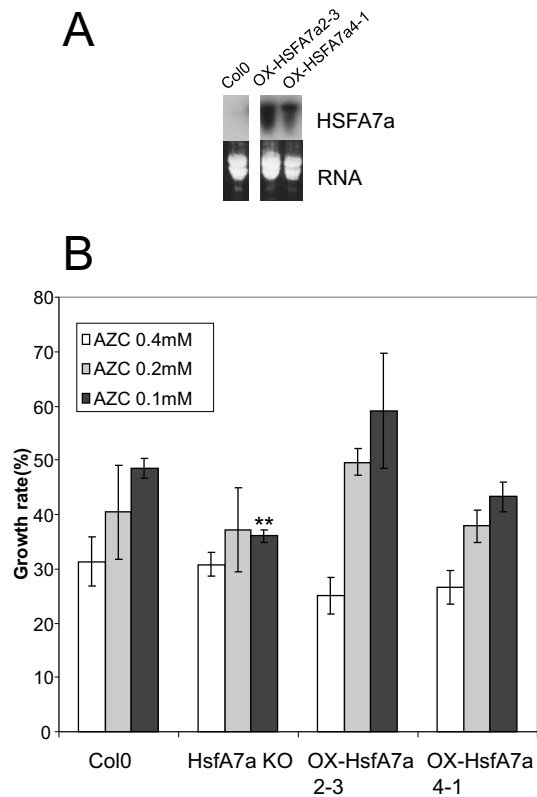
Public dataset transcript changes for heat-stressed shoots, determined within the AtGeneExpress project (Heat-Stressed samples: Nover et al. Gene Expression Omnibus (GEO) Accession GSE5628; Control: Townsend et al. GEO accession GSE5620) were compared with our microarray data for hHeat-shock (HS), AZC- and Tunicamycin-treated tissues. The analysis of the public dataset was carried out using the same statistical approaches as for our experiments but restricted to shoot samples (32 chips) with the following time points: [0 h = start heat stress] 0.25 h, 0.5 h, 1 h, 3 h, 4 h, 6 h, 12 h, 24 h. The analysis shows a close correlation of our heat-stressed samples with the public data and a clustering of our tunicamycin-treated samples with the earliest responses to heat stress. The left scale bar represents the correlation distance.

ATGGAAGAACTGAAAAGTGGAAATGGAGGAAGAAACGG
TGACGTTTACTGGTTCTGTAGCGGCTTCTTCATCTGT
AGGATCCTCTTCTCTCCTAGACCAATGGAAGGGCTT
AACGAAACAGGGCCACCACCGTTTCTGACTAAGACTT
ACGAAATGGTGGAAAGATCCGGCGACGGACACGGTGGT
TTCTTGGAGTAATGGTCGTAACAGCTTTGTGGTGTGG
GATTCTCATAAGTTCTCAACAACCTCCTTCCACGTT
ACTTCAAGCATAGCAATTTCTCAAGTTTTATTTCGTCA
GCTCAATACTTATgtaagtacctctttgcttcottat
atctacttagtcttgtttctgtttttgtttctaattt
ttaattttggatttcttttagatgggtttcctatgagt
cttcagctataaaaggttagttctttaagcttttaagt
ggctaaattgaaattagatgcattgagattttggtca
tatgttaagagaatcatttaacataaaactctgttcaa
tcctctgtttctgaagttcaaaatgtgtttggtagta
aaaacagaatcttggaatgataagtaaggactctgcc
tcaagttcagtagctgatgaaactgttttggtgatt
gcagGGATTCAGAAAGATTGATCCAGATAGATGGGAA
TTTGCAAATGAAGGGTTTTTAGCAGGACAAAAGCATC
TCTTGAAGAACATCAAAAAGAAGGAGGAACATGGGTTT
GCAGAAATGTGAATCAGCAAGGATCTGGGATGTCATGT
GTTGAGGTTGGGCAATACGGTTTTCGACGGGGAGGTTG
AGAGGTTGAAGAGGGATCATGGTGTGCTTGTAGCTGA
GGTAGTTAGGTTGAGGCAACAGCAACACAGCTCCAAG
AGTCAAGTTGCAGCTATGGAGCAACGGTTGCTTGTTA
CTGAGAAGAGACAGCAGCAGATGATGACGTTCCCTTGC
CAAGGCGTTGAACAATCCGAACCTTTGTTTCAGCAGTTT
GCGGTTATGAGTAAAGAGAAGAAGAGTTTGTGTTGGTT
TGGATGTGGGGAGGAAACGGAGGCTTACTTCTACTCC
AAGCTTGGGGACTATGGAGGAGAATTTGTTACATGAT
CAAGAGTTTGATAGAATGAAGGATGATATGGAAATGT
TGTTTCGCTGCAGCAATCGATGATGAGGCGAATAATTC
GATGCCTACTAAGGAGGAACAATGTTTGGAGGCTATG
AATGTGATGATGAGAGATGGTAATTTGGAAGCAGCGT
TGGATGTGAAAGTGGAAAGATTTGGTTGGTTTCGCCTTT
GGATTGGGACAGCCAAGATCTACATGACATGGTTGAT
CAAATGGGTTTTCTTGGTTCGGAACCT**TAA**

Supplemental Figure 3. Nucleotide sequence of HSFA2-II.
The nucleotide sequence of *HSFA2-II* RNA is shown in black.
Mini-exon is identified in black small capitals. The premature
stop codon is indicated by an underline. Intron sequences are
shown in lowercase blue letters.



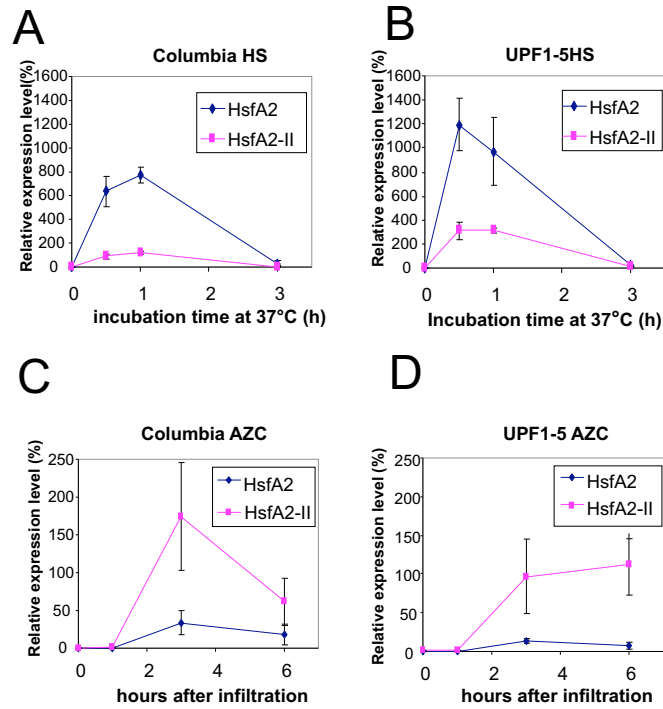
Supplemental Figure 4. Later *HSP70A* responses in the *hsfa2* knock-out line showing CPR. Expression analysis (qRT-PCR) for *HSP70A* RNA in the *hsfa2* insertional null (KO) mutant and a wild type (Col-0) control plant, 6 h after treatment with AZC. The data, expressed as values relative to the expression of *EF1a*, are the mean (Bars = \pm SD) of three biological and three technical replicates. *HSP70A* induction in the *hsfa2* mutant was significantly different from that of Col-0 plant at p value < 0.05.



Supplemental Figure 5. Characterization of *HSFA7a* knock-out and over-expression lines.

(A) Northern blot analysis of *HSFA7a* RNA in the lines over-expressing *pro35S:HSFA7a*.

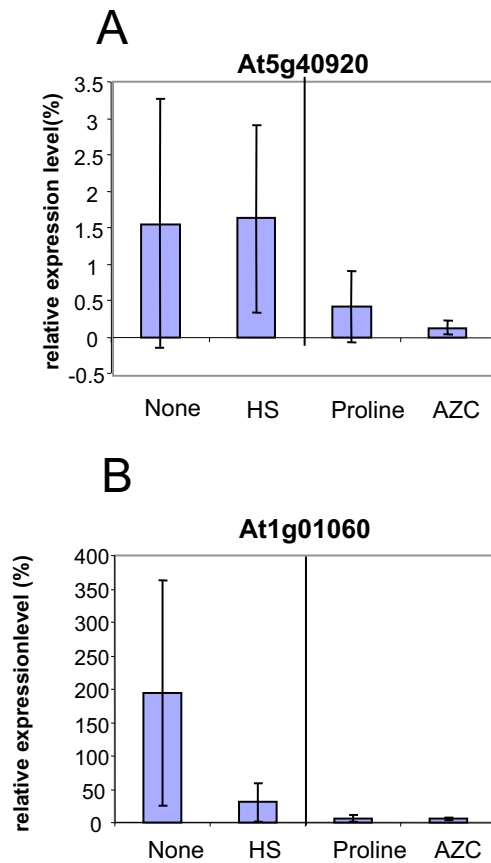
(B) The growth of *Arabidopsis* lines (Col-0 wild type, *HSFA7a* KO mutant, two *HSFA7a* over-expression lines) in the media with the indicated concentration of AZC. The percentage growth was calculated in comparison to the growth of the lines cultured without AZC. The data show the average of three sets of plants. The growth rates of mutant and transgenic lines were compared to Col-0 wild type *Arabidopsis* at each concentration of AZC (student's t-test; p value <0.05) is indicated by asterisks. Two asterisks show significant growth reduction. Only the *hsfa7a* mutant line at the highest AZC concentration showed any significant difference. Bars = \pm SD



Supplemental Figure 6. Time course of *HSFA2* and *HSFA2-II* transcript accumulation in wild-type and NMD defective plants.

(A, B) The time course of the accumulation of *HSFA2-II* to *HSFA2* RNAs after heat-shock in Col-0 wild type (A) and *upf1-5* mutant (B). The *upf1-5* mutant lacks an essential component of NMD machinery. Transcript accumulation was measured by qRT-PCR relative to the expression of *EF1a*. The data are the same as the data used to show the relative accumulation of *HSFA2-II* RNA in Fig 6. The data are the mean of three replicates. Bars= \pm SD.

(C, D) Similar experiment to that in (A) and (B), except plants were infiltrated with AZC.



Supplemental Figure 7. The accumulation of NMD target RNAs were not generically influenced by heat-shock or AZC treatment

(A) The accumulation of RNA from the pseudogene *At5g40920* was measured 1 h after heat-shock and 3 h after infiltration of AZC. Transcript accumulation was measured by qRT-PCR relative to the accumulation of *EF1a* RNA. The data are the mean of three replicates. Bars = \pm SD. There was no statistical difference between the samples.

(B) Similar experiment to that in (A) except accumulation of RNA from the pseudogene *At1g01060* was measured. There was no statistical difference between the samples.

No clear trend was observed in the expression levels of two pseudogenes after heat-shock and AZC treatment.

Supplemental Table 1 List of the genes up-regulated by CPR.

Array element	Log2(Fold Change)			WGeneName	WAccNum
	AZC	HS	Tun		
Protein folding					
250351_at	6.353	8.339	-0.681	17.7 kDa class II HS protein 17.6A (HSP17.7-CII)	AT5G12030
248657_at	5.95	7.2857	-1.071	peptidyl-prolyl cis-trans isomerase	AT5G48570
262307_at	5.899	8.2432	-1.13	DNAJ HS N-terminal domain-containing protein	AT1G71000
248434_at	5.731	8.3537	-1.354	23.5 kDa mitochondrial small HS protein (HSP23.5-M)	AT5G51440
260248_at	5.51	8.1854	-0.997	HS protein 101 (HSP101)	AT1G74310
263374_at	5.271	6.6641	-0.732	DNAJ HS family protein	AT2G20560
266294_at	5.159	7.1345	-0.806	17.6 kDa class I small HS protein (HSP17.6B-CI)	AT2G29500
252515_at	5.142	8.3814	-0.404	17.4 kDa class I HS protein (HSP17.4-CI)	AT3G46230
256245_at	4.772	6.613	-1.636	Hsp70A	AT3G12580
260978_at	4.717	9.3093	-0.55	17.6 kDa class I small HS protein (HSP17.6C-CI)	AT1G53540
258336_at	4.602	6.3981	-0.063	stress-responsive protein	AT3G16050
250296_at	4.501	7.4787	-0.464	17.6 kDa class II HS protein (HSP17.6-CII)	AT5G12020
256518_at	4.489	5.709	-0.998	expressed protein	AT1G66080
254059_at	4.472	8.4883	-0.653	23.6 kDa mitochondrial small HS protein (HSP23.6-M)	AT4G25200
248332_at	4.266	5.9204	-1.309	AtHSP90.1.	AT5G52640
263150_at	4.262	4.7848	-0.74	17.4 kDa class III HS protein (HSP17.4-CIII)	AT1G54050
254839_at	4.203	8.8066	-0.584	stress-inducible protein, putative	AT4G12400
249575_at	3.705	8.0845	-0.054	15.7 kDa class I-related small HS protein-like (HSP15.7-CI)	AT5G37670
250994_at	2.778	2.9872	-0.78	Hsc70-2	AT5G02490
265675_at	2.607	7.1931	-0.097	Hsc70-1	AT2G32120
256983_at	2.283	4.9584	-0.639	chaperonin, putative	AT3G13470
256999_at	2.132	2.372	-0.386	DNAJ HS N-terminal domain-containing protein	AT3G14200
253778_at	1.583	2.4194	-0.244	DNAJ HS family protein	AT4G28480
261655_at	1.613	4.2709	-0.105	peptidyl-prolyl cis-trans isomerase cyclophilin-type family protein	AT1G01940
Transcription/translation					
266841_at	5.998	7.1184	-2.074	HsfA2	AT2G26150
252081_at	5.368	7.6477	-0.48	HsfA7a	AT3G51910
258133_at	4.707	5.5459	-0.823	multi-protein bridging factor 1, conserved transcriptional coactivator.	AT3G24500
250304_at	4.358	4.8165	-0.126	elongation factor 1B alpha-subunit 1 (eEF1Balpha1)	AT5G12110
250781_at	3.613	5.7006	-0.144	DREB2A	AT5G05410
267026_at	3.413	4.3261	0.017	a member of the DREB subfamily A-2	AT2G38340
261081_at	2.949	5.043	-0.705	transformer serine/arginine-rich ribonucleoprotein, putative	AT1G07350

247509_at	2.68	2.0825	-0.433	HSFB2A	AT5G62020
254878_at	2.635	4.2306	-0.398	HSFB2B	AT4G11660
246981_at	2.408	2.9631	-0.071	bZIP protein	AT5G04840
246214_at	2.348	3.7324	-0.42	HSFB1	AT4G36990
264255_at	2.069	3.6136	0.1286	SF2/ASF-like splicing modulator (SRP30)	AT1G09140
262548_at	1.999	1.8639	-0.362	An Argonaute gene	AT1G31280
259649_at	1.982	2.5115	-0.075	TATA-binding protein-associated factor TAFII55 family protein	AT1G55300
250424_at	1.974	3.2877	0.9483	DNA-binding bromodomain-containing protein	AT5G10550
251793_at	1.945	2.9583	0.4054	regulator of chromosome condensation (RCC1) family protein	AT3G55580
259666_at	1.815	3.5904	-0.155	SC35-like splicing factor, 33 kD (SCL33)	AT1G55310
266715_at	1.699	1.0004	0.2144	RNA recognition motif (RRM)-containing protein	AT2G46780
253249_at	1.668	1.9142	-0.1	GATA transcription factor 3	AT4G34680
261663_at	1.575	4.6071	-0.632	myb family transcription factor	AT1G18330
260468_at	1.798	1.5905	-0.234	SNF2 domain-containing protein	AT1G11100
261564_at	1.538	2.0024	0.7574	no apical meristem (NAM) family protein	AT1G01720
Protein degradation					
261265_at	3.323	4.2637	-0.911	zinc finger (C3HC4-type RING finger) family protein	AT1G26800
262656_at	3.316	2.9735	-1.014	zinc finger (C3HC4-type RING finger) family protein	AT1G14200
248040_at	2.693	1.997	-0.342	zinc finger (C3HC4-type RING finger) family protein	AT5G55970
265077_at	2.477	3.2041	-0.338	zinc finger (C3HC4-type RING finger) family protein	AT1G55530
264465_at	2.383	2.4537	-0.328	E3 ubiquitin ligase SCF complex subunit SKP1/ASK1 (At18)	AT1G10230
264758_at	1.801	2.3901	0.4547	F-box family protein	AT1G61340
250036_at	1.729	5.6886	-0.02	U-box domain-containing protein	AT5G18340
257748_at	1.705	1.1005	0.3439	U-box domain-containing protein	AT3G18710
245828_at	1.575	1.3529	-0.406	zinc finger (C3HC4-type RING finger) family protein	AT1G57820
247125_at	1.53	1.5384	-0.346	zinc finger (C3HC4-type RING finger) family protein	AT5G66070

Supplemental Table 2 Validation of selected gene's expression by qRT-PCR.

AGI number / gene name		log ₂ (FC)		
		Heat shock	AZC	Tunicamycin
At1g10230 SKP1/ASK1	Replicate 1	4.4527	2.1027	0.5147
	Replicate 2	2.4807	2.3017	0.7477
	Replicate 3	2.3358	2.1833	0.2135
	p-value	0.0228	0.0003	0.0431
At5g66070 zinc finger (C3HC4-type RING finger) family protein	Replicate 1	2.4553	1.3558	0.6783
	Replicate 2	1.9522	2.2533	0.6067
	Replicate 3	1.8287	0.8018	-0.7828
	p-value	0.0042	0.0368	0.3792
At1g26800 zinc finger (C3HC4-type RING finger) family protein	Replicate 1	6.4757	3.0843	-0.2750
	Replicate 2	3.9540	3.6727	0.3120
	Replicate 3	5.5190	3.6483	-0.6595
	p-value	0.0093	0.0015	0.7305
At1g14200 zinc finger (C3HC4-type RING finger) family protein	Replicate 1	5.7810	4.1443	-1.5913
	Replicate 2	4.2325	3.9800	-0.1997
	Replicate 3	4.3037	4.2147	-1.2383
	p-value	0.0055	0.0001	0.9316
At1g55530 zinc finger (C3HC4-type RING finger) family protein	Replicate 1	5.5077	3.3430	-0.3723
	Replicate 2	4.8818	2.0833	0.4240
	Replicate 3	4.5188	2.0997	-0.4210
	p-value	0.0017	0.0133	0.6514
At5g04840 bZIP protein	Replicate 1	6.8277	4.0638	0.8600
	Replicate 2	5.4743	3.4560	0.4650
	Replicate 3	5.4063	3.0130	0.0580
	p-value	0.0030	0.0037	0.0924
At1g11100 SNF2 domain-containing protein	Replicate 1	3.0420	4.0097	-0.0253
	Replicate 2	4.3707	2.8303	0.6660
	Replicate 3	1.1897	2.0603	-1.2200
	p-value	0.0449	0.0173	0.6203
At1g09140 SF2/ASF-like splicing modulator (SRP30)	Replicate 1	5.1927	3.3607	1.3083
	Replicate 2	5.2823	1.8280	0.5665
	Replicate 3	4.1763	2.3760	0.1040
	p-value	0.0026	0.0151	0.1004
At1g55310 SC35-like splicing factor, 33 kD (SCL33)	Replicate 1	4.4338	1.6637	0.8370
	Replicate 2	5.4192	1.4917	1.3812
	Replicate 3	3.9757	0.6913	-0.6797
	p-value	0.0042	0.0252	0.2465
At1g07350 transformer serine/arginine-rich ribonucleoprotein, putative	Replicate 1	6.9460	4.2900	0.6587
	Replicate 2	6.7397	2.6297	0.5482
	Replicate 3	5.6807	2.0610	-1.9650
	p-value	0.0018	0.0232	0.6021
At5g62020 HSFB2A	Replicate 1	3.9107	3.6507	-0.6540
	Replicate 2	1.7153	3.7423	0.2123
	Replicate 3	2.0720	3.5600	-1.3123
	p-value	0.0318	0.0001	0.8418

At2g38340 a member of the DREB subfamily A-2 of ERF/AP2 transcription factor family	Replicate 1	5.1270	2.5740	0.2197
	Replicate 2	5.4787	3.8757	0.0082
	Replicate 3	3.8450	3.3290	-0.0790
	p-value	0.0052	0.0066	0.3161
At5g05410 DREB2A	rep1	7.9750	4.8930	-0.3457
	rep2	4.6360	3.7282	-0.9575
	rep3	6.6103	5.7670	-1.7787
	p-value	0.0111	0.0074	0.9341

Supplemental Table 3 HSE representation in genes up-regulated by both HS and AZC.

1000 bp upstream of start codon	Total number of the gene	HSE			
		gene	percentage	Z score	p-value
HS-only upregulated genes	785	35	4.46	6.15	3.87E-10
HS-AZC upregulated genes	153	23*	15.03	12.86	3.78E-38
Genome	33282	553	1.66		

* 38% of these genes encode transcription factors. These are: -

At2g26150 *HSFA2*

At3g51910 *HSFA7a*

At4g11660 *HSFB2b*

At5g62020 *HSFB2a*

At1g18330 *Early phytochrome responsive-1, EPR1, MYB-TF.*

At5g04410 *NAC family hypothetical TF*

At5g05410 *DREB2, DREB2a*

Supplemental Table 4 Primers used for this study.

Primers used for (q)RT-PCR		
EF1a	EF1aF*	TGAGCACGCTCTTCTTGCTTTCA
	EF1aR	GGTGGTGGCATCCATCTTGTTACA
HSP70	HSP70ARTF	CTCTGTTTGCTTGAGATTCTAGTTGG
	HSP70ARTR	ATGCCCAGTCGTCTTTCATAGGTC
HSP70B	HSP70BRTF	GCGTGTTTAGTGTTAAGACGAA
	HSP70BRTR	CTCGAAACAACGAAACAGCTGAA
Bip3	Bip3RTF	GAGTGGTTAGAAGAGAATGTGAATG
	Bip3RTR	CAAAATCCTATAGCTAACCGACTC
HsfA2II	HsfA2-AZCF	TTATTCGTCAGCTCAATACTTATATGGGTTTC
	HsfA2-exonR	CACATGACATCCAGATCCTTGC
HsfA2	HsfA2-HSF	TCGTCAGCTCAATACTTATGGATTC
	HsfA2-exonR	CACATGACATCCAGATCCTTGC
HsfA3	HsfA3F	CTGCAGAAACAACAAGTGATG
	HsfA3R	TAGTTCACCCGATAAGAAACG
HsfA7a	HsfA7aF	GAGTCAGCAAAGAGGAAAAGAGG
	HsfA7aR	CCATCTAGTTAGGAGGTGGAAG
TuMV	TuMV-RTF	GATCCACCACCGTCACCACAG
	TuMV-RTR	CTTAGCAAGGGACCCTGCTCCAC
TCV	TCVP28-RTF	CCAGGGATCTGGGCTAGTAGTAGTG
	TCVP28-RTR	CCTCTTCCGTTCCCTACCAACCTATC
at1g01060 (Arciga-Reyes et al., 2006)	qRTat1g01060F	ACAGGATGATTACCGTTCGTTTC
	qRTat1g01060R	CAATGGCAGTTATACTTGGAGGAG
at5g40920	qRTat5g40920F	GCTGGAGAACACTCTCTAAACTGG
	qRTat5g40920R	TGCCAATCATCTTCACATCATCACACTC

*F = forward primer; R = Reverse primer

Supplemental Table 4 continued

Primers used for qRT-PCR to validate the microarray data		
At1g10230	At1g10230RTF	TCGAGTACGCGAAGATGCAC
	At1g10230RTR	ATGTAATCAGCAACCGTCTGG
At5g66070	At5g66070RTF	CCACCTACCATGCATCGACAA
	At5g66070RTR	CAATGATTGGTGCATCAAATCTG
At1g26800	At1g26800RTF	GAAACAGTGATGGTGTGAGTCCG
	At1g26800RTR	GAGGATTATGGAGATTCTGAGC
At1g14200	At1g14200RTF	CGTGTCTATGTGTAGGTATGAG
	At1g14200RTR	AAACCTAGCCCCATGTTGCATC
At1g55530	At1g55530RTF	TTAGCACCTTGTTCTCCTCGTCG
	At1g55530RTR	ATGTAGCATTCAACCAAATCTGAGA
At5g04840	At5g04840RTF	TAGGCATGTTGCAGACGGTGGAAG
	At5g04840RTR	TGGCTATTCTCCAGGGACAGTGTT
At1g11100	At1g11100RTF	GAAGAGAAAGATGGTGGCTTCTGC
	At1g11100RTR	GTTTGTGAGAAGAGCTTCAATTGG
At1g09140	At1g09140RTF	CAAGATCGAATTCTCCAGTTTCACC
	At1g09140RTR	GGAGCCTGAGAAGCTTGAGACG
At1g55310	At1g55310RTF2	GCTTGACTCAATGAGGGGAAGGAGC
	At1g55310RTR2	GCAATCATGGCGTAGATTGCGAACC
At1g07350	At1g07350RTF	CGTAGCATATCCCCAAGGAAG
	At1g07350RTR	TGTTATGGGCTGACGGATCT
At5g62020	At5g62020RTF	GGACGAGAAGTGAAGGTGTTC
	At5g62020RTR	GACACGTGCTCTGCTTGTC
At2g38340	At2g38340RTF	TCAGAGGAAGGTTACAGCTATG
	At2g38340RTR	GATGCAGCATAAATCCTAAGCTAG
At5g05410	At5g05410RTF	GAACAGTGTTGCCAACGGTTCATAC
	At5g05410RTR	CAAATCCAAAAAGCTTCATATTG
At2g15130	At2g15130RTF	GGTGGTGATAGGTGGGATCAGGGTTACG
	At2g15130RTR	GTTGGCCTTGACTCTCTCCAGAGTTG

Supplemental Table 4 continued

Primers used for promoter::GUS constructs		
HSP70A	FA6	ACCCGCCATGGTTAGAGATCAG
337bp pro	FA27	TAAAGGTACCATTGGGTGATGAAATAAC
HSP70B	FA8	GATTTTCGTCGCCATGGTTGCTA
340bp pro	FA35	TAATGGTACCCTTACTGTTGGCCTTTTATTC
Primers used for site directed mutagenesis		
AtoB	AtoBF*	CTTTACTCGTGAACCTCTCTTGTAAGCTCTTTGCCGA CC
	AtoBR	GGTCGGCAAAGAGCTTACAAGAGAGTTCACGAGTAA AG
AKO	AKOF	CTTTACTCGTTAACGTTCTCTAAAGCTCTTTGCCGA CC
	AKOR	GGTCGGCAAAGAGCTTTAGAGAACGTTAACGAGTAA AG
BtoA	BtoAF	GGTGAATCCAGAACGTTCTCGAACGTTTGCGCGATT TCTC
	BtoAR	GAGAAATCGCGCAAACGTTGAGAACGTTCTGGATT CACC
BKO	BKOF	GGTGAATCCATAACTCTCTTTTACGTTTGCGCGATT TCTC
	BKOR	GAGAAATCGCGCAAACGTAAAAGAGAGTTATGGATT CACC
Primers used for cloning of Heat shock factors		
HsfA2	attB1HsfA2FLF	AAAAAGCAGGCTCCACCATGGAAGAAGTAAAGTGG AAATG
	attB2HsfA2FLR	AGAAAGCTGGTgTTAAGGTTCCGAACCAAG
HsfA2 truncated	attB2HsfA2DNR	AGAAAGCTGGGtTtCACTCCATAGTCCCCAAGCTTG
HsfA7a	attB1HsfA7aFLF	AAAAAGCAGGCTCCACCATGATGAACCCGTTTCTCC
	attB2HsfA7aFLR	AGAAAGCTGGGtTTAGGAGGTGGAAGCCAAACTC

* F= Forward primer; R=Reverse primer