Fig. S*1*. Phylogenetic relationship of Hsfs A4 and A5.The phylogenetic tree is based on amino acid sequences of the N-terminal parts (DNA binding domains and HR-A/B regions) of Hsfs and was elaborated using the Clustalx 1-8_msw and Tree view softwares. Sequence data were derived from the following database accession numbers:

At *Arabidopsis thaliana* HsfA4a (At4g18880), HsfA4c (At5g45710), HsfA5 (At4g13980); Bv, *Beta vulgaris* HsfA5 (BQ488901); Cs, *Citrus sinensis* HsfA4a (DY270414), HsfA5 (DY300016); Gh, *Gossypium hirsutum* HsfA5 (DT548305); Gm, *Glyine max* HsfA4a (TC135284), HsfA5 (BM270993); Ha, *Helianthus annuus* HsfA5 (DY932755); Hv, *Hordeum vulgare* HsfA4a (TC42448), HsfA5 (TC151027); Le, *Lycopersicon esculentum* HsfA4a (BT014619), HsfA4b ((TC107140), HsfA5 (TC155271); Lj, *Lotus japonicus* HsfA4a (AP004978); Ls, *Lactuca sativa* HsfA4a (DY973605), HsfA5 (DY974369); Ms, *Medicago sativa* HsfA4a (AF494082); Mt, *Medicago truncatula* HsfA4a (TC79769), HsfA5 (TC79192); Nb, *Nicotiana benthamiana* HsfA5 (CK287755); Nt, N. *tabaccum* HsfA4a (AB014484); Os, *Oryza sativa japonica* HsfA4a (AP004879), HsfA4d (AC111015), HsfA5 (AP004999); Pa, *Phaseolus aureus* HsfA4a (AY052627); Sb, *Sorghum bicolor* HsfA4a (BM322601); So, Saccharum officinarum HsfA5 (CA264007); St, Solanum tuberosum HsfA4b (BG591987), HsfA5 (TC112724); Ta, *Triticum aestivum* HsfA4d (CV766704), HsfA5 (CJ655373); To, *Taraxacum officinarum* HsfA4a (DY824833); Zm *Zea mays* HsfA4a (X92943), HsfA5 (EE174627)

Fig. S*2*. Expression analysis of Arabidopsis Hsfs A4a, A4c and A5 based on microarray data
A, developmental series; *B*, abiotic stress series; *C*, pathogen infections, and elicitor treatments. The color code for the signal intensities is given at the bottom. Shown are the gcRMA normalized and averaged signal intensities (http://www.weigelworld.org/resources/microarray/AtGenExpress/) visualized as "heat maps" (with GeneSpring Version 7.2, Silicon Genetics) with re-transformed linear signal intensities for Hsfs A4a, A4c, and A5. Ubiquitin 11 mRNA levels were used as constitutive expression control.

Table S*1*: Sequence alignment of C-terminal signature sequences of HsfA4 and HsfA5 [1])

A: HsfA4	NLS AHA NES
1.LeA4b (TC107140)	ASRKRRLL 113aa NDVFWQQFLTETPGCTEPQQVENKGINE 12aa YWWNRGVNLENLAERMGHLSSPATGS*
2.StA4b (CV498961)	KRRLLV 136aa NDVFWQQFLTETPGCTEPQEVENKGINE 12aa YWWNSGVNVENLAERMGHLSSPATG
3.AtA4c (At5g45710)	HERRKRRF 83aa NDDFWEQCLTENPGSTE-QQEVQSERRD 13aa YWWNSGNVNNIT--------EKAS*
4.AtA4a (At4g18880)	NERKRRFP 127aa NDGFWQQFFSENPGSTE-QREVQLERKD 11aa CWWNSRNVNAITEQ-LGHLTSSERS*
5.LeA4a (BT014619)	NERKRRLP 130aa NDVFWEQFLTENPGSTDVKPEREDMESK 11aa FWWNRKTVISLTEQ-LGHLTPAE*
6.NtA4a (AB014484)	NDRKRRLP 132aa NDIFWEQFLTENPGSVD-ASEVQSERKD 15aa FWWNMKSVNSLAEQ-LGHLTPAEKT*
7.HaA4a (BQ966974+915996)	NSRKRRLL 113aa NDVFWEQFLTETPGSGD-TQEVQSERRD---VTKPLWGTHNLGKITEK-MGNLGPGKLDVR*
8.CsA4a (CX673366)	HDRKRRLP 128aa NDVFWEQFLTENPGSSD-AQEVQSERKE 15aa FWWNMRNVNSLAEQ-MGHLTPAERT*
9.InA4a (CJ759462)	PDRKRRLP 128aa NDLFWEQFLTENPGSTDAPTDVLSERKN 15aa FWWSVKSVNNLAEQ-LGHLTPAERT*
10.GmA4a (TC135284)	MDRKRRLP 128aa NDIFWERFLTENPGSSEMQEAQSEREDS 7aa FWWNIRNVNNPPEQ-MGHLSKAEQT
11.GmA4c (AW185379)	LDRKRRLP 128aa NDVFWEQFLTEDPGASE-TREVQSERKD 15aa FWWNKRNANNLPEQ-MGHVGQAEKT*
12.MtA4a (TC79769)	MERKRRLP 128aa NDVFWEQFLTEDPGASE-AQEVQSERKD 15aa FWWNMRKSNNHPEQ-MGHVSQVEKI*
13.ZmA4a (X82943)	HGKKRRLP 163aa NDGFWQQFLTEQPGPD-VHQEAQSERRD 16aa FWWGKKNVEQMREK-LGRLTSVEKT*
14.OsA4a (AP004879)	HRKKRRLP 167aa NDGFWQQFLTEQPGSSDAHQEAQSERRD 15aa LWWGKRNVEQITEK-LGLLTSTEKT*
15.SoA4a (CA262559)	HGKKRRLQ 162aa NDGFWQQFLTEQPGS-DAHHEAQSERRD 15aa FWWGKKNVEQMTEK-LGHLTSVEKT*
16.AcA4d (DV855873)	SSKKRRVP 161aa NDLFWERFLTETP 9aa HDADCKRETPEPKDHVRIGIDRNWFNRRGNVEQIIEQMEHL*
17.OsA4d (AC111015)	FSKKRRVP 180aa NDVFWERFLTETP 11aa SPKDDVKAELGCNGFHHREKVDQITEQMGHLASAEQTLHT*
18.ZmA4d (DN207970)	truncat.>136aa NDVFWERFLTDAA 7aa EAKEDVKTAVDRCCPRL-QDNVDQITEQMGQLDSA---SYAPENY*
19.SoA4d (CA286636)	truncat.>159aa NDVFWERFLTD-- 5aa EAKEDVKAAVNRSCLRL-QDNGDQITEQMGQLDSAENDSYAPQNY*

B: HsfA5	NLS	SNED	CLLLAS	AHA	NES
1.LeA5 (TC115271)	FSKKRRLP 52aa	STQSSNED 78aa	CQLNLSLAS 63aa	NDVFWEQFLTERPGCSDNEEASS	23aa RKVEHLTL*
2.StA5 (TC112724)	FSKKRRLL 52aa	STQSSNED 78aa	CQLNLSLAS 63aa	NDVFWEQFLTERPGCSDNEEASS	23aa RKVEHLTL*
3.AtA5 (At4g13980)	YNKKRRLP 53aa	SIQSSNEE 68aa	CHLNLTLAS 55aa	NDVFWEQFLTERPGSSDNEEASS	23aa KNIEQLTL*
4.AfA5 (DR915639)	INKKRRLP 50aa	SAQSSDED 67aa	CHLNLTLAS 73aa	NDGFWEQYLTERPGSPDTEEASS	24aa SDMEQLTL*
5.NbA5 (CK290990+287755)	FSKKRRLP 52aa	STQSSNED<70aa	xxLNLSLAS 63aa	NDVFWEQFLTERPGCSDNEEASS	23aa RKVEHLTL*
6.GmA5 (TC103978)	AYKKRRLP 52aa	STQSSNED 72aa	CQLNLTLAS 74aa	NDVFWEQFLTERPGCSDNEEAIS	23aa KNMDQLTL*
7.MtA5 (TC79192)	YNKKRRLP 52aa	STQGSNED 72aa	CQLNLTLAS 74aa	NDVFWENFLTERPGCSDNEEAIS	23aa KNMDNLTL*
8.ZmA5 (DT938817)	truncat.	STQSSHED 54aa	CHLNLSLAS 84aa	NDKFWEQFLTERPGCPEAEEASF	20aa RDMGQLKL*
9.OsjA5 (AP004999)	FNKKRRLP 54aa	STQSSNED 54aa	CHLSLTLAS 75aa	NDKFWEQFLTERPGCSETEEASS	24aa EDVEQLKL*
10.SbA5(AW680707+680643)	FHKKRRLP 54aa	STQSSHED 54aa	CHLNLSLAS 83aa	NDKFWEQFLTERPGCSEAEEASS	20aa RDMGQLKL*
11.SoA5(CA175344+109762)	truncat.>50aa	STQSSHED 54aa	CHLNLSLAS 82aa	NDKFWEQFLTERPGCSEAEEASS	19aa RDMGQLKL*
12.HvA5 (TC151027+ BQ759144)	LHKKRKLS 54aa	STQSSHED>50aa	CHLSLTLAS 67aa	NDNFWEQFLTERPGDASSGPRDP	15aa EATEQLKL*

[1]) Amino acid sequences were derived from the EST databases (see also legend to Figs. 1 and S*1*) and the corresponding accession numbers are given in brackets.

Ac, *Agrostis capillaris*; Af, *Aquilegia formosa*; In, *Ipomea nil*; for abbreviations of other plant names see legend to Fig. S*1*.

Table S2 – Overview of Hsf expression constructs and primers for DNA amplification.

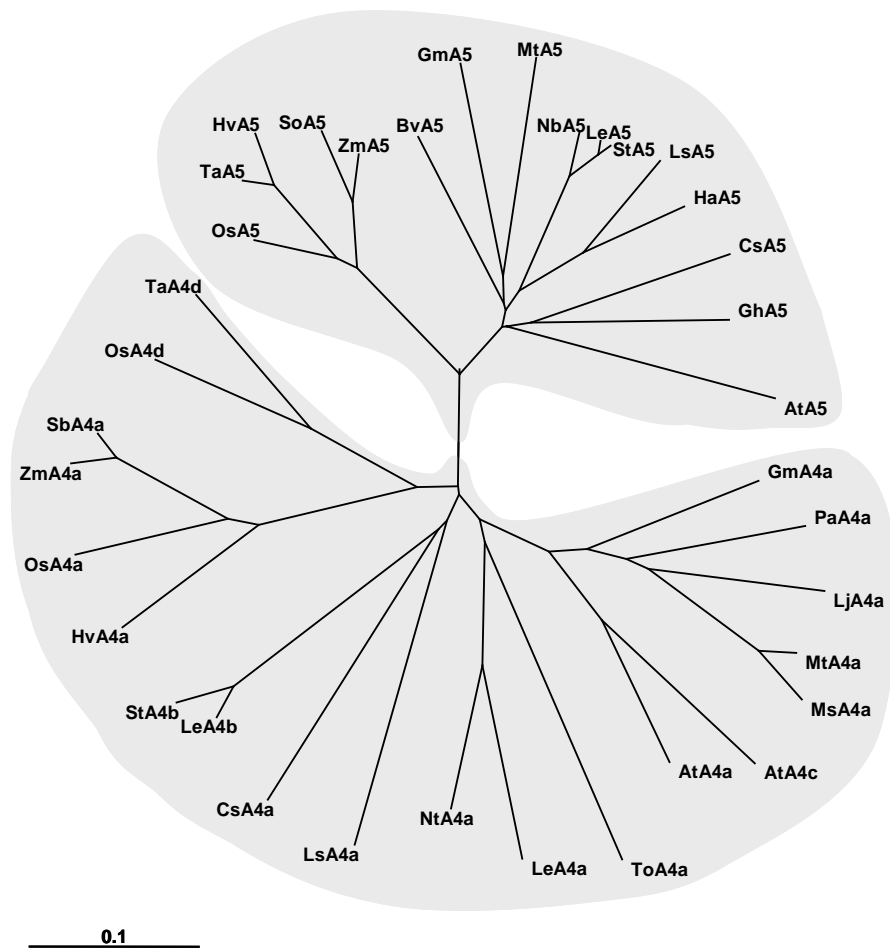| No.	Construct	Forward (fw) and reverse(re) primer sequences
1	pRTdS-HsfA4b-3HA	fw (*Nco*I) 5'-gcatag<u>ccatgg</u>ataactgtaatgg-3' and re (*Nhe*I) 5'-gaat<u>cagctag</u>cgcttcctgttgc-3'
2	pRTdS-3HA-HsfA5	fw (*Kpn*I) 5'–ctca<u>ggtacc</u>taaaatggatgtgatttcagc-3' and re *(Sal*I) 5'–gttaat<u>gtcgac</u>ttcatatactcaaagg-3'
3	pRTdS-3HA-HsfA5 (Δ1-109)	fw (*Kpn*I) 5'-cgtag<u>ggtacc</u>cattcacagtcatag-3' and re 5'-cggggg tgtgtaataagacctg-3' ligation via *Kpn*I and *Xba*I
4	pRTdS-3HA-HsfA5 (Δ1-199)	fw (*Kpn*I) 5'-ctcga<u>ggtacc</u>ggatatttctgca-3' and re same as for No. 3 ligation via *Kpn*I and *Sal*I
5	pRTdS-3HA-HsfA5 (Δ110-200)	fw (*Kpn*I) 5'-ctca<u>ggtacc</u>taaaatggatgtgatttcagc-3' and re (*Sal*I) 5'-cctcaaa<u>gtcgac</u>tctttctggatcaactgtgg-3' fw (*Sal*I) 5'-gacttt<u>gtcgac</u>cgtcttgctcagaaactcg-3' and re same as for No. 3 triple ligation via *Kpn*I, *Sal*I, and *Nhe*I
6	pRTdS-3HA-HsfA5 (110-200)	fw (*Kpn*I) 5'-cgtag<u>ggtacc</u>cattcacagtcatag-3' and re (*Xba*I) 5'-catggtctagagtttctgagcaagac-3'
7	pRT-HsfA1(A4bOD)	fw (*Xho*I) 5'-tgtagctccattg<u>ctcgag</u>tctgagagac-3' and re (*Sal*I) 5'-ctgctcgcgct<u>gtcgac</u>gaaatcagatg-3' ligation into pRT-LpHsfA1(ΔOD) cut with *Sal*I (22).
8	pRTdS-GFP-HsfA4b-3HA	fw (*Bgl*II) 5'-gatagc<u>agatct</u>taactgtaatgg-3' and re same as for No. 3 ligation via *Bgl*II and *Xba*I.
9	pRTdS-GFP-HsfA5	fw (*Sac*I) 5'-ggtacct<u>gagctc</u>gatgtgatttc-3' and re same as for No. 3 ligation via *Sac*I and *Xba*I.
10	pRTdS-myc-YN	fw (*Nhe*I) 5´-ccgctgctagcatggagcaaaagttg-3´ and re (*Xba*I) 5´-gaaattctagatcttaggccatgatatag-3´
11	pRTdS-HA-YC	fw (*Nhe*I) 5´-ccgctgctagcatgtacccatacgat-3´ and re (*Xba*I) 5´-gaaattctagatcttacttgtacagctcg-3´
12	pRTdS-3HA-HsfA5-myc-YN	fw (*Pag*I) 5'-ggcca<u>tcatgat</u>ctttacccatac-3' and re (*Bgl*II); 5'-gcagg<u>agatct</u>gccatctcg-3' fw (*Bgl*II) 5'-gatggc<u>agatct</u>cctgcacatagg3' and re (*Xba*I) 5'-gctcgtcgacttctca<u>tctaga</u>agcggtagcgtgttcagcc-3' triple ligation into No.10 cut *Nco*I and *Nhe*I
13	pRTdS-3HA-HsfA5-HA-YC	triple ligation using same PCR fragments as for No. 12 into No.11 cut *Nco*I and *Nhe*I
14	pRTdS-HsfA4b-mYC-YN	*Nco*I-*Nhe*I fragment from No. 1 cloned into No 10
15	pRTdS-HsfA4b-HA-YC	*Nco*I-*Nhe*I fragment from No. 1 cloned into No 11
16	pGST-HsfA4bCTD (112-393)	fw (*Sal*I) 5'-cgaaa<u>gtcgac</u>ccatagccactctgc-3' and re (*Not*I) 5'-gacattttctagagtcc<u>gcggccgc</u>cacc-3'
17	pGST-HsfA5CTD (110-478)	fw (*Sal*I) 5' ccatc<u>ggtcgac</u>acccatt-3' and re (*Not*I) 5'-ta<u>gcggccgc</u>cttgttgattcgacg-3'
18	pJC-3HA-HsfA5-6His	fw (*Xho*I) 5'-ggcgagg<u>acgtcgac</u>tggcc-3' and re (*Apa*I) 5'-cgtcttcatg<u>ggccc</u>aaagggtgagg-3' PCR fragment cloned into pJC-HsfB1CTD-6His (22)

Fig. S*1*

0.1

Fig. S2

A

Seedling gr. | Rosette leaves | Stem | Apex | Flower | P | Silique/Seeds | Roots

Ubq11
HsfA4a
HsfA4c
HsfA5

B

shoot | root | culture

control, cold, osmotic, salt, drought, genotoxic, oxidative, UV-B, wounding, heat | control, cold, osmotic, salt, drought, genotoxic, oxidative, UV-B, wounding, heat | heat

Ubq11
HsfA4a
HsfA4c
HsfA5

C

Set A | Set B | Set B

control_0h, MgCl2_2h, MgCl2_6h, MgCl2_24h, DC3000_2h, DC3000_6h, DC3000_24h, avrRpm1_2h, avrRpm1_6h, avrRpm1_24h, HrcC-_2h, HrcC-_6h, HrcC-_24h, Psph_2h, Psph_6h, Psph_24h | H2O_1h, H2O_4h, Mg/Ca_1h, Mg/Ca_4h, HrpZ_1h, HrpZ_4h, Flg22_1h, Flg22_4h, LPS_1h, LPS_4h, GST_1h, GST_4h, GST-NPP1_1h, GST-NPP1_4h | H2O_6h, H2O_12h, H2O_24h, Pinf_6h, Pinf_12h, Pinf_24h

Ubq11
HsfA4a
HsfA4c
HsfA5

Signal intensities: 0 100 250 500 12,000

24