

Figure S1. Sequence alignment and phylogenetic analyses of ThHSFA1 and other plant HSF proteins. **(A)** Alignment of the amino acid sequences of some HSF proteins. Identity within the same amino acid is indicated by shading. The homeobox domains include the DBD, HR-A/B, NLS region, and AHA. **(B)** A phylogenetic tree of HSF proteins was constructed by MEGA 5.05 using the neighbor-joining method. The numbers beside each node represent bootstrap values based on 1000 replications.

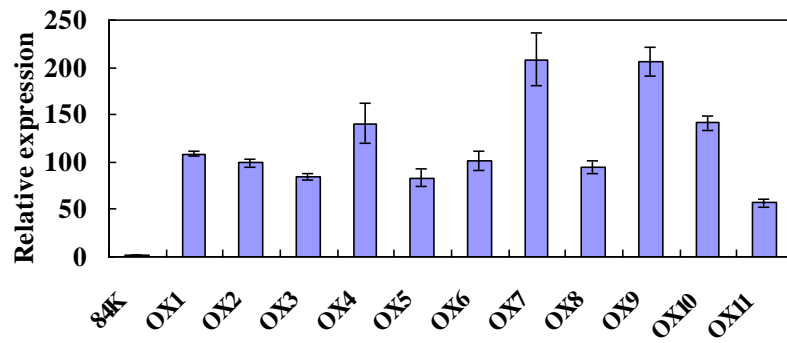


Figure S2. Expression analysis of *ThHSFA1* in transgenic poplar by RT-qPCR. RT-qPCR results showing *ThHSFA1* expression in 11 transgenic overexpression poplar lines (OX). Parallel analysis of the *PagActin* gene was carried out for normalization to the amount of added template. The error bars represent \pm SD, which were calculated from multiple biological replicates.

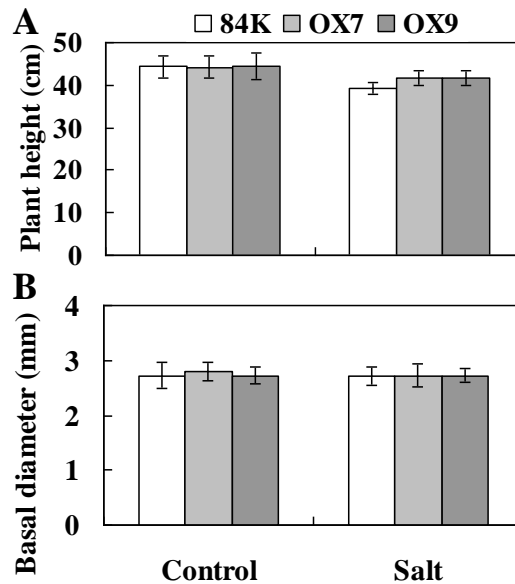


Figure S3. Effects on the growth of transgenic poplar overexpressing *ThHSFA1*. Plant height and basal diameter of nontransgenic 84K and *ThHSFA1*-transformed poplars under control and salt stress conditions. Poplar plants were cultivated and subjected to salt treatment every 2 days for 10 days as described in Figure 4. The error bars represent \pm SD from multiple biological replicates.

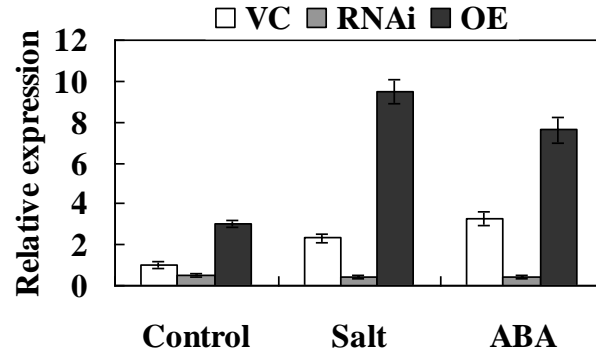


Figure S4. RT-qPCR analysis of the expression of *ThHSFA1* in different transgenic *T. hispida* plants. The expression of *ThHSFA1* was determined under normal control conditions and treatment with 150 mM NaCl or 40 μ M ABA for 24 h. The expression level of *ThHSFA1* in VC plants under control conditions was used as the calibrator (designed as 1). VC: the empty pROKII transformed *T. hispida* plants; RNAi: transiently RNAi-silencing *ThHSFA1* in *T. hispida* plants. OE: transiently overexpressing of *ThHSFA1* in *T. hispida* plants. The error bars represent \pm SD, which were calculated from multiple biological replicates.

Table S1. Primer sequences used in this study.

Primers used for RT-qPCR				
Genes	GenBank number	Forward and reverse primers (5'–3')		Reference
<i>ThWRKY4</i>	JX416193	GCCTTCGTTTATCTTGCCTGCTC	CGATCGGTGCGGGCCTCTTC	Zheng et al., 2013 [7]
<i>ThHSFA1</i>	MW881195	AGCAATCAAGCGATAACC	AGGAGGAATGCCGTCAAG	
<i>Thtubulin</i>		CACCCACCGTTGTTCCAG	ACCGTCGTCATCTTCACC	Zheng et al., 2013 [7]
<i>PagActin</i>		ACCTCCAATCCAGACACTG	TTGCTGACCGTATGAGCAAG	
Primers used for ChIP-PCR				
<i>ProThWRKY4</i>		TAGATATCTATACTTTAATAC	TGTTTTATGTTACGAATCTG	
<i>ProThtubulin</i>		TACCATAACCCGTATCCGTAC	ACCATAACCCGTATCCATATC	

Constructs	Forward and reverse primers (5'–3')	
Specific primers used for genetic transformation of poplar		
pMD TM 19-T-ThHSFA1	ATGAGTAGCGTTCAAGAAG	TCACATCCTTGTGTTCTCAGC
pDONR207-ThHSFA1	GGGGACAACCTTT- GTACAAAAAAGTTGGAATGAG- TAGCGTTCAAGAAG	GGCGGCCGCACAACCTTT- GTACAAGAAAGTTGGG- TATCACATCCTTGTGTTCTCAGC
pROKII-ThHSFA1	CTCTAGAGGATCCCCGG- GATGAGTAGCGTTCAAGAAG	TCGAGCTCGG- TACCCGGGTCACATCCTT- GTGTTCTCAGC
pFGC5941-ThHSFA1	ThHSFA1-Cis(AscI)-F: TTGGCGCGCCCCAC- CGGCCTCCATGAAACC ThHSFA1-Anti(PacI)-F: CCTTAATTAACCAC- CGGCCTCCATGAAACC	ThHSFA1-Cis(SwaI)-R: CATGATTTAAATCTCACCCCCAG- CAGAATCAG ThHSFA1-Anti(XbaI)-R: TGCTCTAGACTCACCCCCAG- CAGAATCAG
Genomic DNA PCR	GACGCACAATCCCCTATCC	TCACATCCTTGTGTTCTCAGC
Primers used for yeast one-hybrid assay		
pGADT7-Rec2-ThHSFA1	TGGCCATTATGGCCCGG- GATGAGTAGCGTTCAAGAAG AATTCCACGAAAAATTCAA- GCACGAAAAATTCAAGCAC- GAAAAATTCAAGGAGCT	GACATGTTTTTTCCCGGGTCACAT CCTTGTGTTCTCAGC CCTTGAATTTTTCGTGCTT- GAATTTTTCGTGCTT- GAATTTTTCGTGG
pHIS2-3×HSE		GCGAAC- GCGTGAGCTCTGTTTTATGTTAC- GAATCTG
pHIS2-Pro ^{ThWRKY4}	ACTATAGGGCGAATTCTAGA- TATCTATACTTTAATAC	
Primers used for dual-luciferase reporter assay		
pGreenII 62-SK-ThHSFA1	TCTAGAACTAGTG- GATCCATGAGTAGCGTTCAA- GAAG	GCGTACCGAATTGGTAC- CTCACATCCTTGTGTTCTCAGC
pGreenII 0800-Luc-Pro ^{ThWRKY4}	GACGGTATCGATAAGCTTTA- GATATCTATACTTTAATAC	TCTAGAACTAGTG- GATCCTGTTTTATGTTACGAATCTG
Primers used for subcellular localization and transcriptional activation assay		
pBI121-ThHSFA1-GFP	TCTAGACTGGTACCCGG- GATGAGTAGCGTTCAAGAAG	CTAGTCAGTCGACCCGGG- TACATCCTTGTGTTCTCAGC GCAGGTCGAC- GGATCCTCACATCCTT- GTGTTCTCAGC
pGBKT7-ThHSFA1	CATGGAGGCCGAATTCATGAG- TAGCGTTCAAGAAG	GCAGGTCGAC- GGATCCAATCTGTCCATCTGATA- TAG
pGBKT7-ThHSFA1-N	CATGGAGGCCGAATTCATGAG- TAGCGTTCAAGAAG	GCAGGTCGAC- GGATCCTCACATCCTT- GTGTTCTCAGC
pGBKT7-ThHSFA1-C	CATGGAGGCCGAATTCGTGAAGT ATCAGCCCTTAAT	