

Supplementary Materials

Table S1. Primers used for cloning of the *ThNAC4* gene.

Genes	Forward and reverse primers (5'–3')	
ThNAC4	ATGGAAAACATTCCTGGAT	TTAATAGTAACCCCAAAGGTC
M13	CGCCAGGGTTTTCCAGTCACGAC	GAGCGGATAACAATTTACACACAGG

Table S2. Primers used in constructing recombinant plasmid pROKII-ThNAC4.

Genes	Forward and reverse primers (5'–3')	
pROKII- <i>ThNAC4</i>	CTCTAGAGGATCCCCGGGATGGA AAACATTCCTGGAT	TCGAGCTCGGTACCCGGGTTAATA GTAACCCCAAAGGTC
<i>ThNAC4</i>	ATGGAAAACATTCCTGGAT	TTAATAGTAACCCCAAAGGTC

Table S3. Primers used in constructing recombinant plasmid pFGC5941-ThNAC4.

Genes	Forward and reverse primers (5'–3')	
ThNAC4-Cis	ATAAGGAAGTTCATTTTATTG	CAATCAAATGAAGAGCCAAT
ThNAC4-Anti	CTTACTTACACTTGCCTTGGAG	ATCTGAGCTACACATGCTCAG
pFGC5941-Cis	CATGATTTAAATCAACAGATGAG ATTCAGC	TTGGCGCCATGCCAAATTAGGAG GAGG
pFGC5941-Anti	CCTTAATTAATGCCAAATTAGG AGGAGG	CGCGGATCCGCAACAGATGAGAT TTCAGC

Table S4. Primers used in constructing recombinant plasmid pBI121-ThNAC4-GFP.

Genes	Forward and reverse primers (5'–3')	
pBI121-GFP	ATGGAAAACATTCCTGGAT	ATAGTAACCCCAAAGGTCTC
pBI121-ThNAC4-GFP	TCTAGACTGGTACCCGGGATGGA AAACATTCCTGGAT	CTAGTCAGTCGACCCGGGATAGT AACCCCAAAGGTCTC

Table S5. Gene-specific primers used in real-time PCR.

Genes	GenBank number	Forward and reverse primers (5'–3')	
<i>Tamarix hispida</i>			
<i>ThNAC4</i>	JQ974958	CTACTGGGAAGGACAAAG	ATTAGACATGATGGTGGGG
<i>β-actin</i>	FJ618517	AAACAATGGCTGATGCTG	ACAATACCGTGCTCAATAGG
<i>α-tubulin</i>	FJ618518	CACCCACCGTTGTCCAG	ACCGTCGTCATCTTACC
<i>β-tubulin</i>	FJ618519	GGAAGCCATAGAAAGACC	CAACAAATGTGGGATGCT
<i>Arabidopsis thaliana</i>			
<i>α-tubulin</i>	AT1G50010	GATGTACCGTGGTGTATGTC	GAGCCTCTGAAAATTCTCC

Table S6. Primer sequences of SOD, POD and Trehalose synthase genes used in real-time PCR.

Genes	GenBank number	Forward and reverse primers (5'–3')	
<i>α-tubulin</i>	At1G50010	GATGTACCGTGGTGATGTC	GAGCCTCTGAAAATTCTCC
<i>Ubiquitin</i>	AT1G55060	GGAAAGCAGCTCGAAGATG	AAGCTTCCACCGCGGAGAC
<i>SOD1</i>	AT1G12520	GTCACCCGGAACCCACAGC	CCGAATAAAAAGGCCTCTCC
<i>SOD2</i>	AT3G56350	GAAGGAGGTGGCAAACCAC	TCTTGTAAGTGTGGATAGTAG
<i>SOD3</i>	AT5G23310	CGCTGCACAGGTCTATAACC	AATATCGTCCCACACGAGTG
<i>SOD4</i>	AT5G51100	CCTGGAGGTGGAGGAAAGC	CTGCATTGGGCGTCTTCAC
<i>POD1</i>	AT1G05260	CTTTCACAAACCGTCTCTAC	AGTGGTGAGAGCAGAGTCTG
<i>POD2</i>	AT1G14550	CCATAGGACAATCTCAATGC	TGATCGGTTACTAATAGTC
<i>POD3</i>	AT1G24110	TCTGACCGTTCAAGAAATGG	TGGAGCAACCCGTAACCGTG
<i>POD4</i>	AT1G30870	TGTGGCACCATCCAGTCGAG	CTGCGAAAGTCTTTACAAGC
<i>POD5</i>	AT1G65970	CAGTATGAGCCATGTGCCTG	CAAGCAACAAAGCGAATCTC
<i>POD6</i>	AT2G18140	TCCGGGAGCCACACCATTGG	TGGTCGGAATTCAACAGTC
<i>POD7</i>	AT2G18150	CCAATCCGGAAACGGAAGTC	TCTGCATACTTCTTGACGAG
<i>POD8</i>	AT3G49110	GCAACACTGGATTACCTGAC	CCATCAGCATATGCTCTCAC
<i>POD9</i>	AT3G50990	AGGTTATAACAACCATACTGG	CGTAATACTTGACCATCTC
<i>POD10</i>	AT4G11290	TCGACAGCGAATATGCCGAC	GAACTCTTGCTCCGATCCTC
<i>POD11</i>	AT4G17690	GAATGGTTTCACTCTAAAGG	GGAAGCTAACAGTCCAAGAC
<i>POD12</i>	AT4G25980	GAACAACGGCCTGCTTCTTC	TCCACGACCTGTCTGGTCCG
<i>POD13</i>	AT4G26010	TCCAGGACAGGCTTTCCGAC	GAAGAGTGTATTGCTTGATG
<i>POD14</i>	AT4G30170	AGCCGTCACGGCCTCTCTC	CAAGATTTGATCTGACGTG
<i>POD15</i>	AT5G47000	GACTGTTCTGACATCCAC	CTTGAAGTACATGTTGTCCG
<i>POD16</i>	AT5G51890	CTTGTCGGTGAAAGACATG	GACCCAAACACTCCTTTCC
<i>POD17</i>	AT5G58390	ATCCCTCCTCCGATCACTAC	GTCGAACCTATCGGGAGAG
<i>POD18</i>	AT5G58400	GGCAAGCCAGGTGCGTCAC	TCCGGCTGTAGGATACGAC
<i>POD19</i>	AT5G66390	CTCACTAAGTTCAAGCGTC	GAATAGGGTCTGGTCACCTC
<i>POD20</i>	AT5G64110	CTGGACATACGATAGGAACG	GACTCGAGGAGACCTCGAC
<i>TPS1</i>	NM106505	TCCGACATGCCAGCCATTGC	TCTCTCCTTTGAGGTCAAGC
<i>TPS7</i>	NM001331627	CCAGATGGCTAAAGAAGAGG	GCAAACACATTTCCCTGATG
<i>TPS8</i>	NM001334443	GAAAGTAATCCGAGAAATGG	AGCACGTCGGCTTCATCGTC
<i>TPS11</i>	NM127426	TAAACCTCAGGGAGTAAGC	ACACTTGGGGTATCATCGAG
<i>TPPB</i>	NM106458	ATGGGACAAGGCCAGGCAC	ACTTGTTAACCTGAGAAGG
<i>TPPC</i>	NM102071	TATCCCTGGAGCTACGGTC	GCATCTTCATCAGTACGGTC
<i>TPPD</i>	NM103289	TCAAAAGGACTGGGGATTGG	CCTTGAAAGCATCCTCGTC
<i>TPPF</i>	NM117313	GTTTGCCTCTAACTCATGG	CTCGGTTCCCATCTCTCAG
<i>TPPG</i>	NM118385	TCTCGGATTAAGCAACAAC	CCCCCATTTACCAAAGTC
<i>TPPH</i>	NM001342553	AAGTTTTGGAGGTTCTGTC	CCTCATCGGGTTCTTGACG
<i>TPPI</i>	NM121048	GAATGGGATAAAGGAAAGG	TCTTGCAAAGAATACGAAGC
<i>TPPJ</i>	AK221501	GTTGAAACTGTCTCAAGGTC	AGCCTTGCTCTCTCCCTCG

Table S7. Primer sequences used in construction pGBKT7-ThNAC4 vector.

Genes	Forward and reverse primers (5'–3')	
pGBKT7-ThNAC4	CATGGAGGCCGAATTCATGGAAA ACATTCCTGG	GCAGGTCGACGGATCCTTAATAGT AACCCCAAAG
Rec2-1	CATGGAGGCCGAATTCATGGAAA ACATTCCTGGATT	GCAGGTCGACGGATCCCTGAAGA AATACCATTCC
Rec2-2	CATGGAGGCCGAATTCCTTCAGTGT CAGAGACAGG	GCAGGTCGACGGATCCCTCTGCA CTCTTCTGAAAG
Rec2-3	CATGGAGGCCGAATTCGCAGGAT GTAAGAAGTTC	GCAGGTCGACGGATCCCCATAGA ATGAGTTCAACGC
Rec2-4	CATGGAGGCCGAATTCATGGATC TGAATTCAATCCG	GCAGGTCGACGGATCCTTAATAGT AACCCCAAAGGTC
Rec2-5	CATGGAGGCCGAATTCATGGGAA TTGCGTGAACG	GCAGGTCGACGGATCCCCAACAA GTGGTTTTCC
Rec2-6	CATGGAGGCCGAATTCATGAATA TAGACTGGAGGG	GCAGGTCGACGGATCCGGTGAAG AGTCCGTCAATGG
Rec2-7	CATGGAGGCCGAATTCATGCCCG GTTATCATGCC	GCAGGTCGACGGATCCCGCCCTC CGTTCCGTTCC

Table S8. Primer sequences used in construction effector of pGADT7-rec2-ThNAC4.

Genes	Forward and reverse primers (5'–3')	
pGBKT7-ThNAC4	GCAGAGTGGCCATTATGGCCCAT GGAAAACATTCCTGG	GCGGCCGACATGTTTTTCCCTTA ATAGTAACCCCAAAG
pGADT7-Rec2	ATGAACATGGAGGCCAGTG	GATGGATCCCGTATCGATG

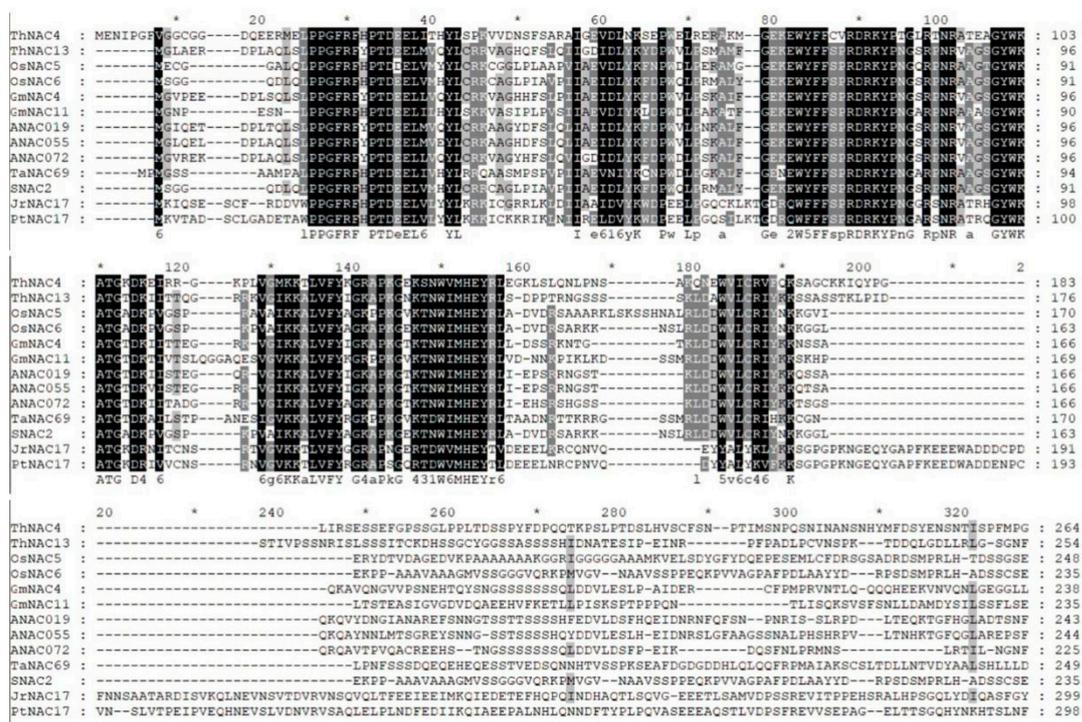


Fig. S1 Multiple sequence alignment analysis of NAC proteins from 12 other species

Multiple sequence alignments of ThNAC4 and 12 representative plant NACs were performed with ClustalX. The consensus NAC subdomains are shown in black color. Their corresponding accession numbers are as the follows: *Tamarix hispida* ThNAC4 (JQ974958) and ThNAC13 (JQ974967); *Oryza sativa* OsNAC5 (BAA89799), OsNAC6 (BAA89800) and SNAC2 (CBX55846); *Glycine max* GmNAC4 (AAY46124) and GmNAC11 (ACC66315); *Arabidopsis thaliana* ANAC019 (NP_175697.1), ANAC055 (NP_188169.1), and ANAC072 (NP_567773.1); *Triticum aestivum* TaNAC69 (AAU08785); *Juglans regia* JrNAC17 (XP_018848079.1); *Populus trichocarpa* PtNAC17 (AOF43232.1).

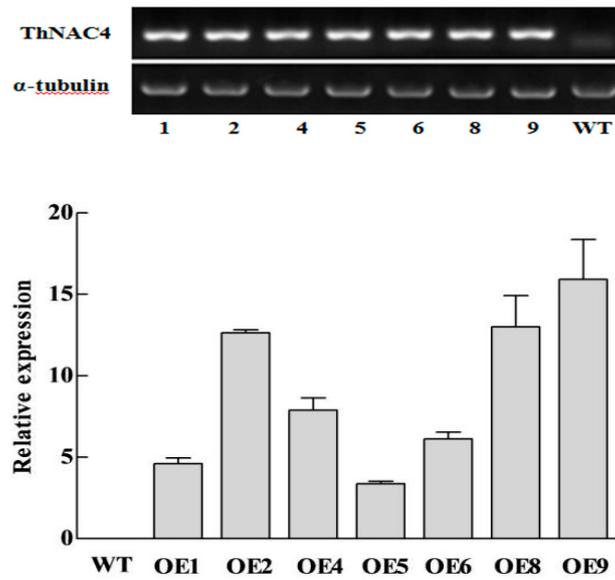


Fig. S2 Quantitative RT-PCR analysis of ThNAC4 expression in the WT and 9 homozygous overexpression lines (Line1-9) of ThNAC4-transformed *Arabidopsis*

Parallel reactions using *α-tubulin* (AT1G50010, as an internal control) in primers were carried out to normalize the amounts of added template.

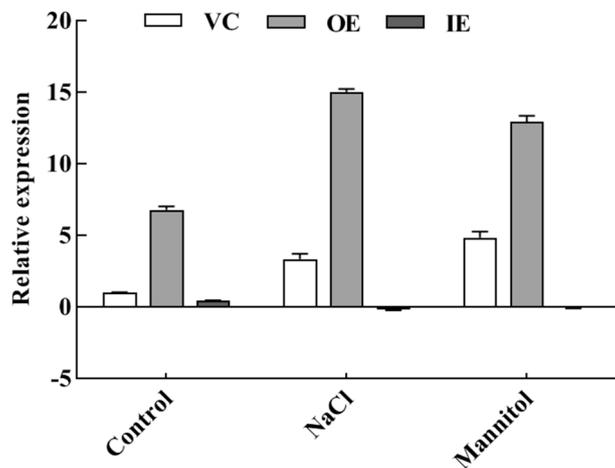


Fig. S3 Expression of *ThNAC4* in the different kinds of transgenic *T.hispida* plants

The expression of *ThNAC4* was determined under normal growth conditions or treatment with 150 mM NaCl or 200 mM mannitol for 24 h. The expression level of *ThNAC4* in control plants under normal growth conditions was used as the calibrator (designed as 1). VC: the pROKII vector control transformed *T. hispida* plants; OE: overexpressing of *ThNAC4* in *T. hispida* plants; IE: *ThNAC4* RNAi-silenced *T. hispida* plants. The error bars were standard deviations, which were calculated from multiple replicates of the real-time PCR.