

Supplementary Materials: ThWRKY4 from *Tamarix hispida* Can Form Homodimers and Heterodimers and Is Involved in Abiotic Stress Responses

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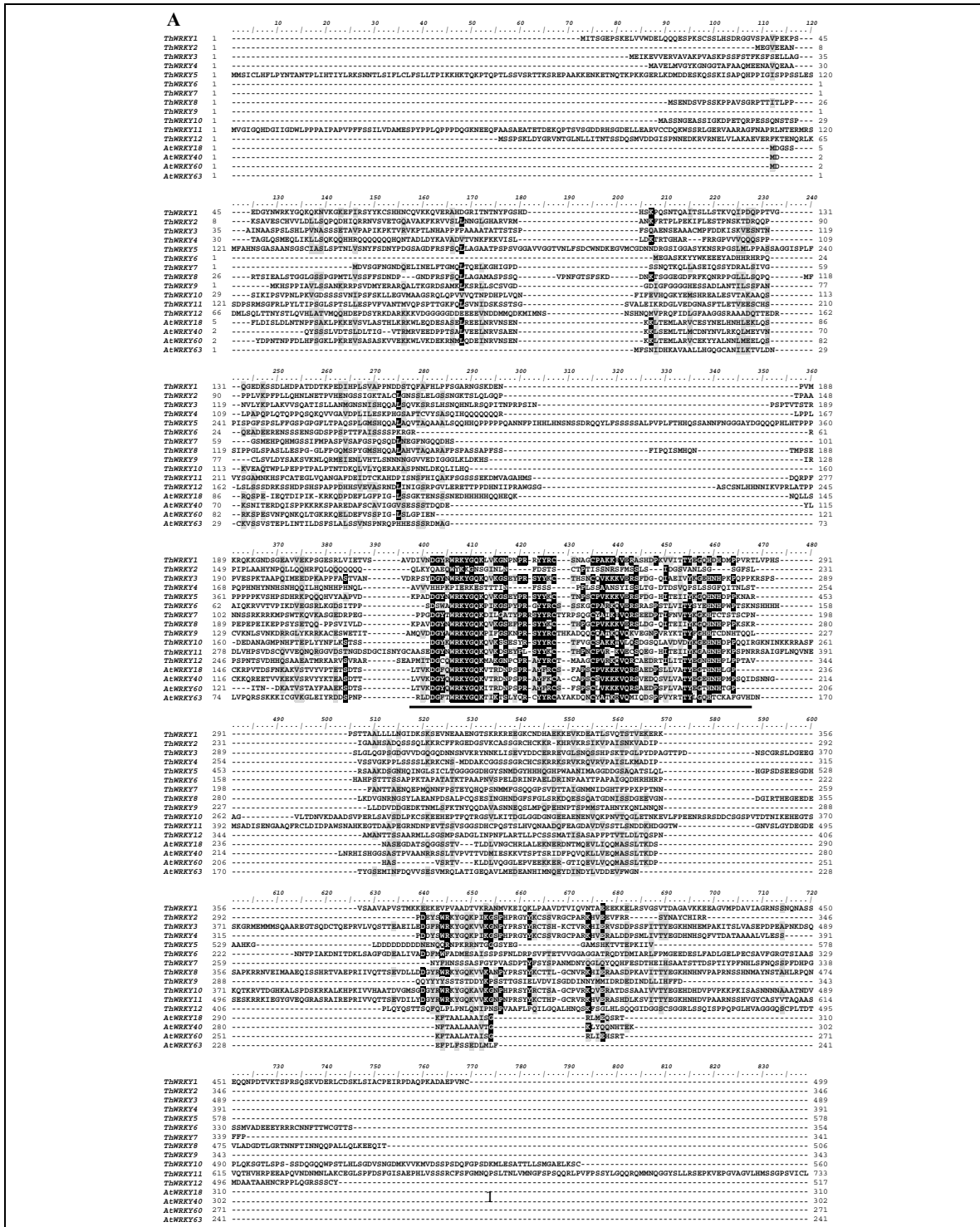


Figure S1. Cont.

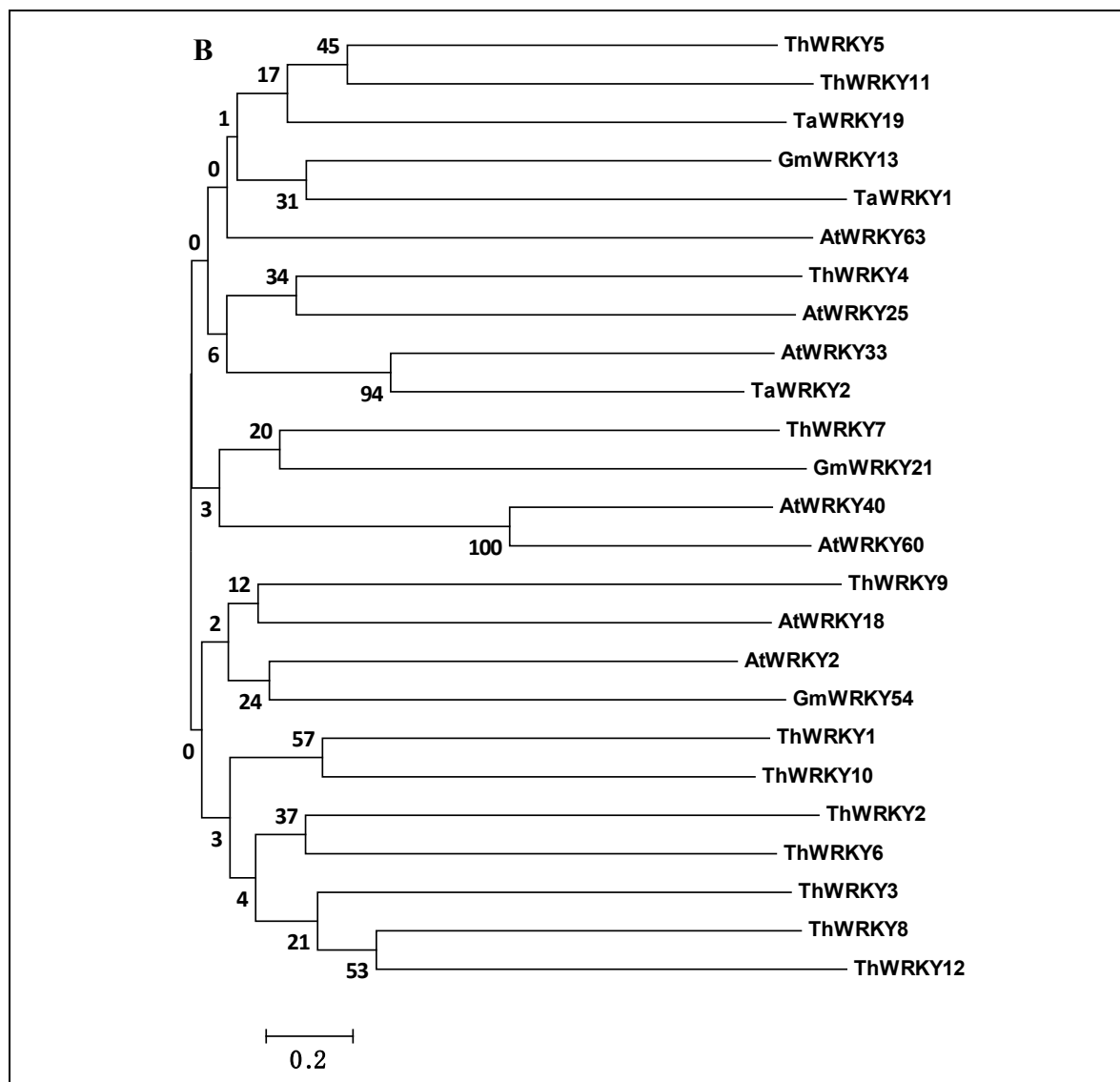


Figure S1. Sequence analysis of ThWRKYs. (A) Multiple sequence alignment of amino acid sequences of ThWRKYs with homologous WRKYs from *Arabidopsis* were performed with ClustalW using BioEdit software. The typical WRKY domain is underlined; The different boxes show the similar and identical of the sequences; (B) Phylogenetic tree of ThWRKYs and other plant stress-responsive WRKYs, which was constructed using MEGA 5.05 with 1000 bootstrap replicates.

Table S1. Primers used in constructing yeast expression vector.

Genes	GenBank Number	Construct	Forward and Reverse Primers (5'-3')	
ThWRKY1	JX416191	AD-ThWRKY1	TGGCCATTATGGCCCGGGATG ATTACTTCAGGGGAACC	GACATGTTTTTCCCGGGCTA GCAGTTAACAGGTTTC
		BD-ThWRKY1	CATGGAGGCCGAATTCATGAT TACTTCAGGGGAACC	GCAGGTCGACGGATCCCTAG CAGTTAACAGGTTTC
ThWRKY2	JX416192	AD-ThWRKY2	TGGCCATTATGGCCCGGGATG GAGGGGGTTGAGGAGGCT	GACATGTTTTTCCCGGGTCA CCTTCGTATGTGACAATAAG
		BD-ThWRKY2	CATGGAGGCCGAATTCATGGA GGGGGTTGAGGAGGCT	GCAGGTCGACGGATCCTCAC CTTCGTATGTGACAATAAG
ThWRKY3	JQ040808	AD-ThWRKY3	TGGCCATTATGGCCCGGGATG GAGATTAAAGAGGTGGTG	GACATGTTTTTCCCGGGTTA CTGGCTATCTTTGTTAGG
		BD-ThWRKY3	CATGGAGGCCGAATTCATGGA GATTAAAGAGGTGGTG	GCAGGTCGACGGATCCTTACT GGCTATCTTTGTTAGG
ThWRKY4	JX416193	AD-ThWRKY4	TGGCCATTATGGCCCGGGATG GCAGTGGAAATTAATGGTAG	GACATGTTTTTCCCGGGCTA CGATGATTCAAGCACG
		BD-ThWRKY4	CATGGAGGCCGAATTCATGGC AGTGGAAATTAATGGTAG	GCAGGTCGACGGATCCCTAC GATGATTCAAGCACG
ThWRKY5	JX416194	AD-ThWRKY5	TGGCCATTATGGCCCGGGATG TCAATCTGTCTTCATTTTCTCC	GACATGTTTTTCCCGGGAAC TATGATCTTGGGTTCAAGTCAC
		BD-ThWRKY5	CATGGAGGCCGAATTCATGTC AATCTGTCTTCATTTTCTCC	GCAGGTCGACGGATCCAACCT ATGATCTTGGGTTCAAGTCAC
ThWRKY6	JX416195	AD-ThWRKY6	TGGCCATTATGGCCCGGGATG GAAGGTGCAAGCAAGAAG	GACATGTTTTTCCCGGGTCA ACTAGTGGTCCCACACCAAG
		BD-ThWRKY6	CATGGAGGCCGAATTCATGGA AGGTGCAAGCAAGAAG	GCAGGTCGACGGATCCTCAA CTAGTGGTCCCACACCAAG
ThWRKY7	JX416196	AD-ThWRKY7	TGGCCATTATGGCCCGGGATG GATGTCAGCGGTTTCAATG	GACATGTTTTTCCCGGGTCA CGGGAAAAATCCTGGG
		BD-ThWRKY7	CATGGAGGCCGAATTCATGGA TGTCAGCGGTTTCAATG	GCAGGTCGACGGATCCTCAC GGGAAAAATCCTGGG
ThWRKY8	JX416197	AD-ThWRKY8	TGGCCATTATGGCCCGGGATG TCCGAAAACGACTCTGTTC	GACATGTTTTTCCCGGGCTA CGTGATTGTGTTCTTC
		BD-ThWRKY8	CATGGAGGCCGAATTCATGTC CGAAAACGACTCTGTTC	GCAGGTCGACGGATCCCTAC GTGATTGTGTTCTTC
ThWRKY9	JX416198	AD-ThWRKY9	TGGCCATTATGGCCCGGGATG AAGCACTCGCCGCCTATC	GACATGTTTTTCCCGGGCTA ATCAAAAAATGAATCAGC
		BD-ThWRKY9	CATGGAGGCCGAATTCATGAA GCACTCGCCGCCTATC	GCAGGTCGACGGATCCCTAA TCAAAAAATGAATCAGC
ThWRKY10	JX416199	AD-ThWRKY10	TGGCCATTATGGCCCGGGATG GCTTCATCAAACGGAG	GACATGTTTTTCCCGGGTCA GCAAGACTTGAGTTCTGC
		BD-ThWRKY10	CATGGAGGCCGAATTCATGGC TTCATCAAACGGAG	GCAGGTCGACGGATCCTCAG CAAGACTTGAGTTCTGC
ThWRKY11	JX416200	AD-ThWRKY11	TGGCCATTATGGCCCGGGATG GTTGGGATTGGTCAAC	GACATGTTTTTCCCGGGTTA TAAACAGATAACAGATG
		BD-ThWRKY11	CATGGAGGCCGAATTCATGGT TGGGATTGGTCAAC	GCAGGTCGACGGATCCTTATA AACAGATAACAGATG
ThWRKY12	JX416201	AD-ThWRKY12	TGGCCATTATGGCCCGGGATG TCCTCTCCTAGCAAATTG	GACATGTTTTTCCCGGGTTA ATAGCAGCTGCTAGACC
		BD-ThWRKY12	CATGGAGGCCGAATTCATGTC CTCTCCTAGCAAATTG	GCAGGTCGACGGATCCTTAAT AGCAGCTGCTAGACC

The words in different colors indicate a homology to the linear ends (5' and 3') of pGADT7 and pGBKT7 vectors.

Table S2. Primer sequences employed in the yeast one-hybrid assay and transient expression assays.

Construct	Forward and Reverse Primers (5'–3')	
pHIS2-W-box	<u>AATTCTTGACCTTGACCTTGACCGAGCT</u>	<u>CGGTCAAGGTCAAGGTCAAG</u>
pHIS2	GCCTTCGTTTATCTTGCTGCTC	CGATCGGTGCGGGCCTCTTC
pGAD-ThWRKY2	TGGCCATTATGGCCCGGGATGGAGGGG GTTGAGGAGGCT	GACATGTTTTTCCCGGGTCACCTT CGTATGTGACAATAAGC
pGAD-ThWRKY3	TGGCCATTATGGCCCGGGATGGAGATT AAAGAGGTGGTG	GACATGTTTTTCCCGGGTTACTG GCTATCTTTGTTAGG
pGAD-ThWRKY4	TGGCCATTATGGCCCGGGATGGCAGTG GAATTAATGGTAG	GACATGTTTTTCCCGGGCTACGA TGATTCAAGCACG
pGAD	CTATTCGATGATGAAGATACCCACCA AACCC	GTGAACTTGCGGGGTTTTTCAGTA TCTACG
pROKII-ThWRKY2	CTCTAGAGGATCCCCGGGATGGAGGGG GTTGAGGAGGCT	TCGAGCTCGGTACCCGGGTCACCT TCGTATGTGACAATAAGC
pROKII-ThWRKY3	CTCTAGAGGATCCCCGGGATGGAGATT AAAGAGGTGGTG	TCGAGCTCGGTACCCGGGTTACTG GCTATCTTTGTTAGG
pROKII-ThWRKY4	CTCTAGAGGATCCCCGGGATGGCAGTG GAATTAATGGTAG	TCGAGCTCGGTACCCGGGCTACG ATGATTCAAGCACG
pROKII	TTTCATTTGGAGAGAACACG	TGCCAAATGTTTGAACGATC
pCAM-W-box	AGCITTGACCTTGACCTTGACCCACCT TCCTCTATATAAGGAAGTTTCATTTCATT TGGAGAGAACACGGC	CATGGCCGTGTTCTCTCCAAATGA AATGAACTTCCTTATATAGAGGAA GGGTGGTCAAGGTCAAGGTCAAA
pCAMBIA1301	TAGAGTCGACCTGCAGGCAT	ATCATCATCATAGACACACG

The words in different colors and the underline indicate a homology to the linear ends (5' and 3') of pGADT7, pROKII or pCAMBIA1301 vectors and different sites of restriction endonucleases.

Table S3. Gene-specific primers used in real-time RT-PCR

Genes	GenBank Number	Forward and Reverse Primers (5'–3')	
<i>ThWRKY2</i>	JX416192	CCACCACCATTAGTCAA	AATCCCGCTATTACCCT
<i>ThWRKY3</i>	JQ040808	CCCAAGTTAAGTCGCGATC	GAATGCGTGCATTGTAGT
<i>ThWRKY4</i>	JX416193	CAGAATCACCACCCTCATAAT	TAGCATCGTCCATAGAGTTAC
<i>β-actin</i>	FJ618517	AAACAATGGCTGATGCTG	ACAATACCGTGCTCAATAGG
<i>α-tubulin</i>	FJ618518	CACCCACCGTTGTTCCAG	ACCGTCGTCATCTTCACC
<i>β-tubulin</i>	FJ618519	GGAAGCCATAGAAAGACC	CAACAAATGTGGGATGCT

Table S4. Primer sequences employed in subcellular localization analysis

Construct	Forward and Reverse Primers (5'–3')	
pBI121-ThWRKY2-GFP	TCTAGACTGGTACCCGGGATG GAGGGGGTTGAGGAGGCT	CTAGTCAGTCGACCCGGGTCA CCTTCGTATGTGACAATAAG
pBI121-ThWRKY3-GFP	TCTAGACTGGTACCCGGGATG GAGATTAAAGAGGTGGTG	CTAGTCAGTCGACCCGGGTTA CTGGCTATCTTTGTTAGG
pBI121-ThWRKY4-GFP	TCTAGACTGGTACCCGGGATG GCAGTGGAATTAATGGTAG	CTAGTCAGTCGACCCGGGCTA CGATGATTCAAGCACG
pBI121-GFP	TTTCATTTGGAGAGAACACG	CGACCAGGATGGGCACCAC

The red words indicate a homology to the linear ends (5' and 3') of pBI121 vectors.