

Table 1Suppl. Primers for reverse transcription quantitative PCR analysis and construction of yeast expression vectors; the underlined sequences are restrictionenzymes.

Gene name	Forward primer	Reverse primer
<i>JrWRKY6</i>	5'-GATGAGAACTCGTTGTCT-3'	5'-ATCTGGGAACCTTATTAG-3'
<i>JrWRKY53</i>	5'-GTACAAACATCGTCATCAC-3'	5'-TTGAGATATCGTCAGTGC-3'
<i>18S rRNA</i>	5'-GGTCAAATCTTCTCGTTCCTT-3'	5'-TCGCATTTTCGTACGTTCTT-3'
<i>JrWRKY6-JM</i>	5'-ATCGGGTACCATGGAATCTTCGGCTGGCTAC-3'	5'-CGATTCTAGATTAAATTGCCTTGCAAACTTG-3'
<i>JrWRKY53-JM</i>	5'-ATCGGGTACCATGGAGAACGGTTGGAGCTG-3'	5'-ATCGGGTACCTAAGCGAAAAACGCTGAAG-3'
<i>pHIS2-W-box</i>	5'-AATTCCTTGACCTTGACCTTGACCGAGCT-3'	5'-CGGTCAAAGGTCAAAGGTCAAG-3'
<i>pHIS2</i>	5'-GCCTTCGTTTATCTTGCTGCTC-3'	CGATCGGTGCGGGCCTCTTC-3'
<i>pGAD-JrWRKY6</i>	5'-TGGCCATTATGGCCCGGGATGGATCTTCGGCTGGCTAC-3'	5'-GACATGTTTTTTTCCCGGGTTAAATTGCCTTGCAAACTTG-3'
<i>pGAD-JrWRKY53</i>	5'-TGGCCATTATGGCCCGGGATGGAGAACGGTTGGAGCTG-3'	5'-GACATGTTTTTTTCCCGGGTAAAGCGAAAAACGCTGAAG-3'
<i>pGAD</i>	5'-CTATTTCGATGATGAAGATACCCACCAAACCC-3'	5'-GTGAACCTTGCGGGGTTTTTTCAGTATCTACG-3'
<i>pROKII</i>	5'-TTTTCATTTGGAGAGAACACG-3'	5'-TGCCAAAATGTTTGAACGATC-3'
<i>pROKII-JrWRKY6</i>	5'-ATCGGGATCCATGGATCTTCGGCTGGCTAC-3'	5'-CGATGGTACCTTAATTGCCTTGCAAACTTG-3'
<i>pROKII-JrWRKY53</i>	5'-ATCGGGATCCATGGAGAACGGTTGGAGCTG-3'	5'-CGATGGTACCTTAAGCGAAAAACGCTGAAG-3'
<i>pCAM-W-box</i>	5'-AGCTTTTGACCTTGACCTTGACCAACCTTCCTCTATATAAGGA AGTTCAATTTTCATTTGGAGAGAACACGGC-3'	5'-CATGGCCGTGTTCTCTCCAAATGAAATGAACCTTCCTTATATA GAGGAAGGGTGGTCAAGGTCAAGGTCAAAA-3'
<i>pCAMBIA1301</i>	5'-TAGAGTCGACCTGCAGGCAT-3'	5'-ATCATCATCATAGACACACG-3'

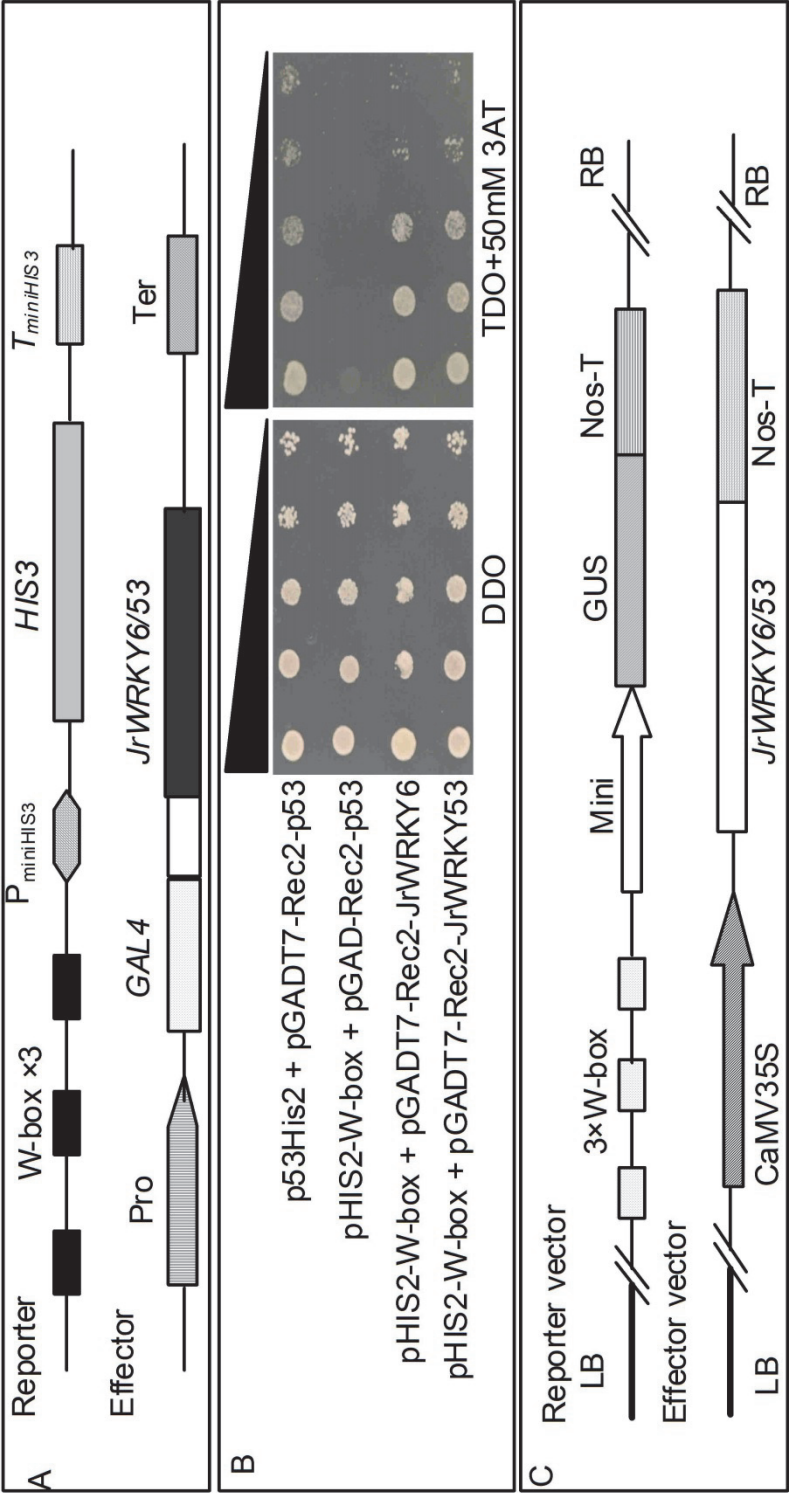


Fig. 1Suppl. The binding analysis of JrWRKY6 and JrWRKY53 to the W-box motif. *A* -A diagram of reporter and effector vectors. Three tandem copies of the W-box were inserted into the pHIS2 vector as the reporter construct. The coding DNA sequences (CDSs) of *JrWRKY6* and *JrWRKY53* were cloned into pGADT7-Rec2 as the effector constructs. *B* - The effector and reporter constructs were co-transformed into the yeast strain Y187. Positive transformants were further identified by spotting serial dilutions (1:1, 1:10, 1:100, 1:1000, and 1:10000) of yeast onto a DDO (SD/Leu/-Trp, SD means the Synthetic Dropout Medium)) plate and TDO (SD/-His/-Leu/-Trp) with 50 mM 3-AT plate. p53His2 + pGADT7-Rec2-p53 was used as a positive control, whereas the pHIS2-W-box + pGAD-Rec2-p53 was used as a negative control. *C* -A diagram of the reporters and effectors. Triple tandem copies of the W-box were fused with the 35SCaMV-46 minimal promoter and cloned into pCambia1301 for driving the β -glucuronidase (*GUS*) gene as the reporter construct. The CDS of *JrWRKY6* and *JrWRKY53* were cloned into pROKII under control of the 35S promoter as the effector constructs.

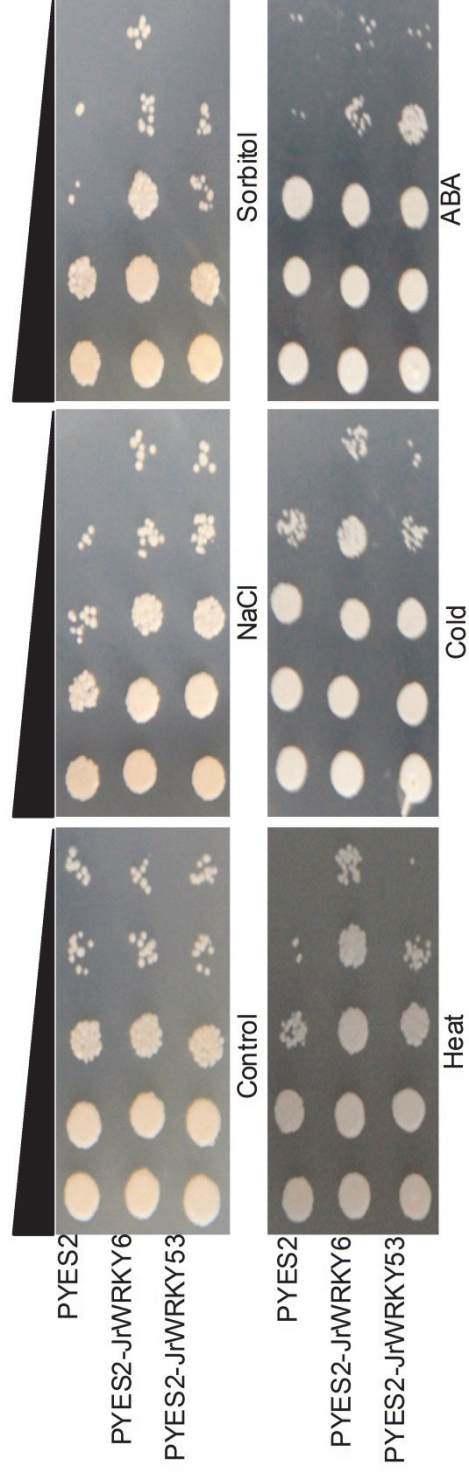


Fig. 2 Suppl. The abiotic stress tolerance analysis of *JrWRKY6* and *JrWRKY53* in a yeast expression system compared with the empty pYES2 (control) yeast. The two yeast cultures were independently grown in a synthetic complete (SC)-Ura liquid medium containing 2% (m/v) galactose at 30°C for 20 h up to $A_{600}=0.4$, then collected and adjusted the yeast with SC-Ura including 2% galactose and cultivated up to $A_{600}=1.6$ for stress analysis. The same number of cells were resuspended in 1 cm³ of 0.3 M NaCl, 0.3 M sorbitol, 100 μ M ABA, and treatments at 10 °C for 24 h, and 44°C for 1 h. Then, serial dilutions (10^0 , 10^1 , 10^2 , 10^3 , 10^4) were spotted onto SC-Ura agar plates and incubated at 30°C for 60 h. As a control, yeast with $A_{600}=1.6$ without any stress was also spotted onto SC-Ura agar plates with the same dilutions as the treatments and grown at 30°C for 40 h.

JrWRKY6

ATGGATCTTCGGCTGGCTACTAATGGTGATGCGGATGAGAACTCGTTGTCTTCGTCGGAAAGGAAATGGCCGGGACCGGTCTGGATCGCCCGCAATTAAAGGGA
GAAGTCATGGGTGCGAAGCAGCAGTTTGGGCTGGGAAGGATGGAATAGTAGGTTGCTTGTCTTCGATCAGGATAAGAAGGAACATGGTAGTAAATTT
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TTGACTCAATCCCCCAACCCGTTCCAACTCCAAAGGCCACCAACCCAATTCCTCCCAAACCCCTCACAGACACAGAACCTCGCCACCCGCCCAG
GCCACATTGCTGCCTCAAATAATTTGGTCAAGCACTCTACAATAATCAATCAAAAGTTCTCTGGTCTTCAGATGTCTCAAAGATATGGATTTCTGCTCTTCAAACCTTGGT
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GCAGCTCTCGCAGCAGCCATCACTTCTATTATTGGCGGTGCTGCTCAGACGAACAACATTAGCAACAATAATAATAGTAATAATAATGCCAACATCACACAACAC
CACCACCAGCAATGGCAACTTAACAAGTACTACTAGCAACAGCAATAGCAATGGCAACAATAAAATCAGCAATTCAAGTTTGCAAGGCAATTAA

JrWRKY53

ATGGAGAACGGTTGGAGCTGGGAGCAGAAACCACTGATTGGTGAACTAATACAGGGAATGGAGCTAGTGAAACAGTTAAGGGTACAATTTAGTACAACATCG
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TGAAAGGAGTAATGGCAGGTGTACCCGGGTCTCCAATTTCTGTCAACGGAAAGTCTCGGGACCGATGAATTTGACAAGGGTATCAAGGATCAATCAGGGCACTGA
CGATATCTCAAAGAAAGAGAAAGATAATTGCCCAGATGGACGGACCAATGTGAGAGTGAGATCTGAGAAATGGGATGGAAAGGACCCCATGAAGATGGCTATAGTTG
GAGAAATATGGTCAGAAAGACATCCTAGGAGGCCAAATATCCCAGAAAGCTACTATAGATGCACATTGCCGTAACTCTCAAAACTGCTGGGCGACAAAGCAAAGT
GCAAAAGATCAGATGATGACCCAAACCGCATTTGACATCACGTACCGAGGAAGGCACACCTGTTCCTCATGCTACCAATTTGGTTCCAGCACCATCATCACCAGA
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GGACAACAACACTAGTTTCTCTGGGCAAGTTTTCCTCCCAACCATTTTATCCCCGGCTACCCCTGAGTCAAACTTTCACTCAGTGTCCCCCATTCAGAACAGCAAC
TTCAAAAGGGGCTCACAAACGGGCATCGTTTCGGAATCTGACCTCACTGAGATCATCTCTGCCAAACACTTCAGCCACCAAGTTCTCCGATTCTAGACTTGGATTCT
CACTTGATCCAGCGGAAATTGGCCATTTCCTCATCTGACTCTTCAGCGGTTTTTCGGCTTAG

JrWRKY6

MDLRLATNGDADENSLSSSEGNRDRSGSPAIGEVMSKQQFGLGKDGNSDEVLAFDQDKKEHGSKIGREDSPDQTSQGWGPNKVPFRNSPKDVDQTEATMRK

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MNQGQHNSLSDTVSAATAAITADPNFTAALAAAITSIIGGAAQTNNISNNNNNNNNNITTTTTTSNGNLTSTTSNSNSNGNKNISNSSLQGN*

JrWRKY53

MENGWSWEQKPLIGELIQGMELVKQLRVQFSTTSSPETVETLLQRILASYEKALMILRWSGSIGQPQIEGVMAGVPGSPISVNGSLGTDEFDKGIKDHQGTDDISK
KRKILPRWTDHVRVRSENGMEGPHEDGYSWRKYGQKDILGAKYPRSYRYRCTLRNSQNCWATKQVQRSDDDDPNAFDITYRGRHTC}SHATNLVPAPSSPEKQEQKQ
NFNNNDNQDQRPILDILGNIGSSLRIVTELENKEMSYPFSPSTSFSGCTKSENSNFSLSTLDNNTSFLGSFSPFLSPATPESNFHSVSPFQNSNFKGAHNGHRSESDL
TEIISANTSATSSPILDLDLDFSLDPAEIGHFDPDSSAFFA*

Fig.3.Suppl. JrWRKY6 and JrWRKY53 nucleic acid and protein sequences. Sequences marked by yellow and a frame are WRKY domains.