OsWRKY45 alleles play different roles in abscisic acid signaling and salt stress tolerance but similar roles in drought and cold tolerance in rice

Zeng Tao, Yanjun Kou, Hongbo Liu, Xianghua Li, Jinghua Xiao and Shiping Wang

Supplementary Data

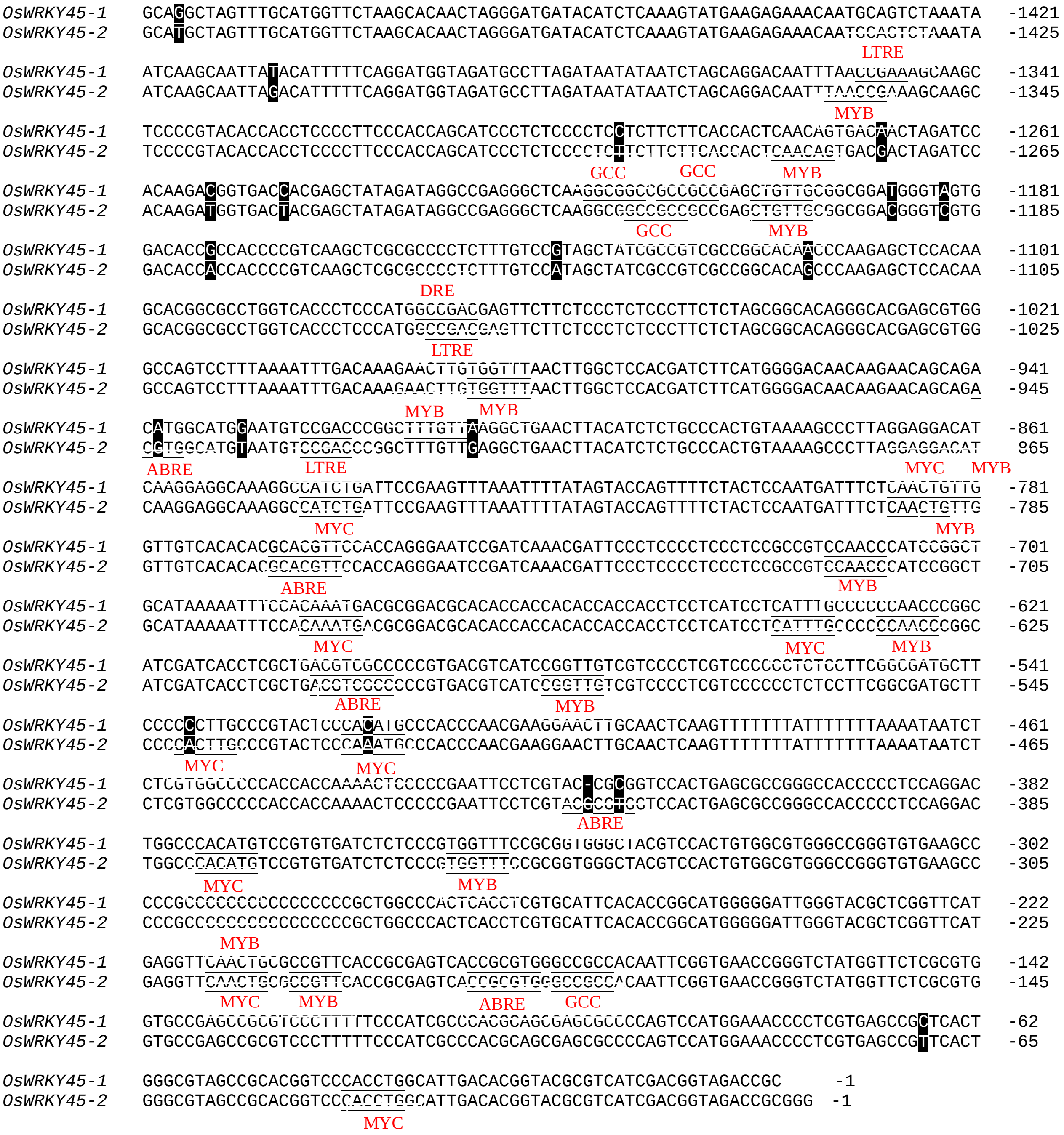

Figure S1. Alignment of the promoter regions of *OsWRKY45-1* and *OsWRKY45-2*. The nucleotide immediately upstream of the transcription initiation site are numbered as "-1". The putative ABRE, DRE, GCC, LTRE, MYB, and MYC *cis*-elements are underlined.

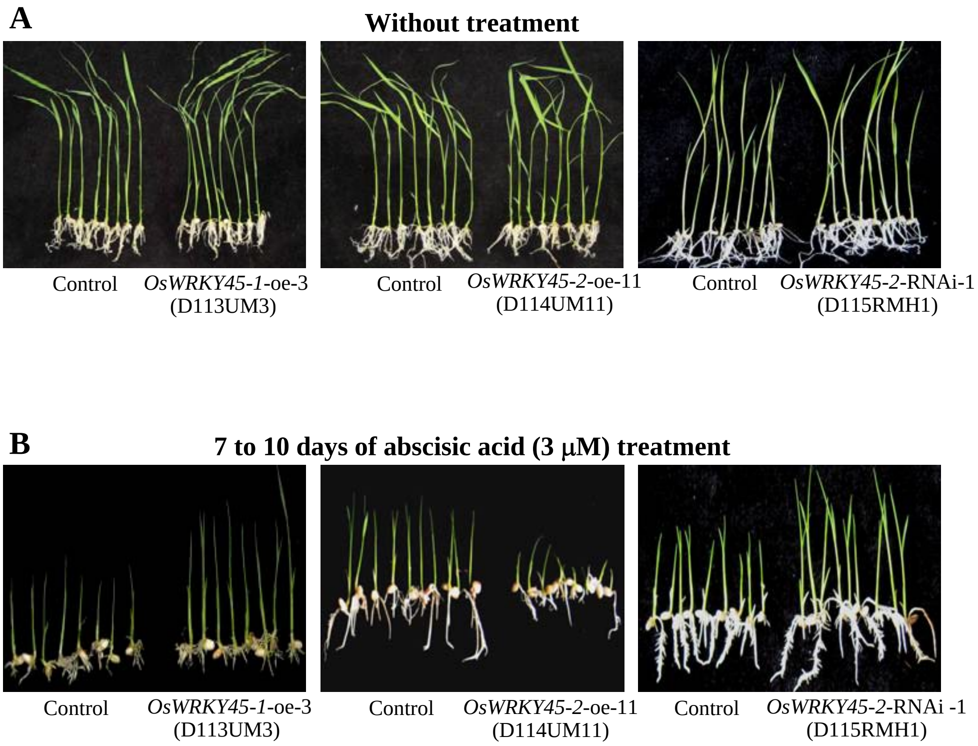

Figure S2. Modulating *OsWRKY45-1* and *OsWRKY45-2* expression influenced rice response to abscisic acid (ABA) treatment. Control, negative siblings from corresponding transgenic segregating populations; oe, overexpression; RNAi, RNA interference. (A) Rice seedlings before ABA treatment. Plant grown on MS medium for 10 d. (B) Phenotypes of transgenic plants after ABA treatment. Rice seedlings grown on MS medium containing ABA for 7 to 10 d (until the transgenic and control plants showed marked differences in growth rate) and then phenotypes were recorded. Because of the variant lengths of ABA-treatment time (7 to 10 d) to different groups of plants, the control plants with the same genetic background in the *OsWRKY45-1*-oe and *OsWRKY45-2*-oe groups exhibited different lengths of shoot and root.

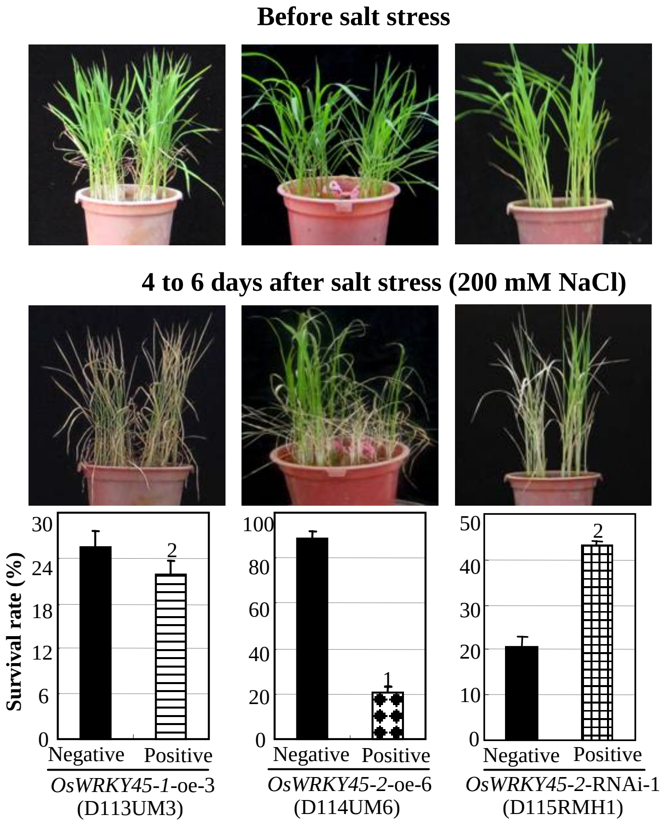

Figure S3. Modulating the expression of *OsWRKY45-2* but not *OsWRKY45-1* markedly influenced rice response to salt stress. Rice seedlings were irrigated with the solution containing 200 mM NaCl at four-to-five leaf stage. After 4 to 6 d of stress (until almost all the leaves of one group in the pot lost their green color and some leaves died) , the survival rates were recorded. Negative, negative siblings from corresponding transgenic segregating populations; positive, positive transgenic plants; oe, overexpression; RNAi, RNA interference. Bars represent mean (3 technical replicates with each replicate containing 16 to 20 plants) ± standard deviation. The “1" or “2" indicates a significant difference was detected between a positive transgenic line and its negative control at $P < 0.01$ or $P < 0.05$, respectively. Because of the variant lengths of salt-stress time (4 to 6 d) to different groups of plants, the control plants with the same genetic background in the *OsWRKY45-1*-oe and *OsWRKY45-2*-oe groups exhibited different survival rates.

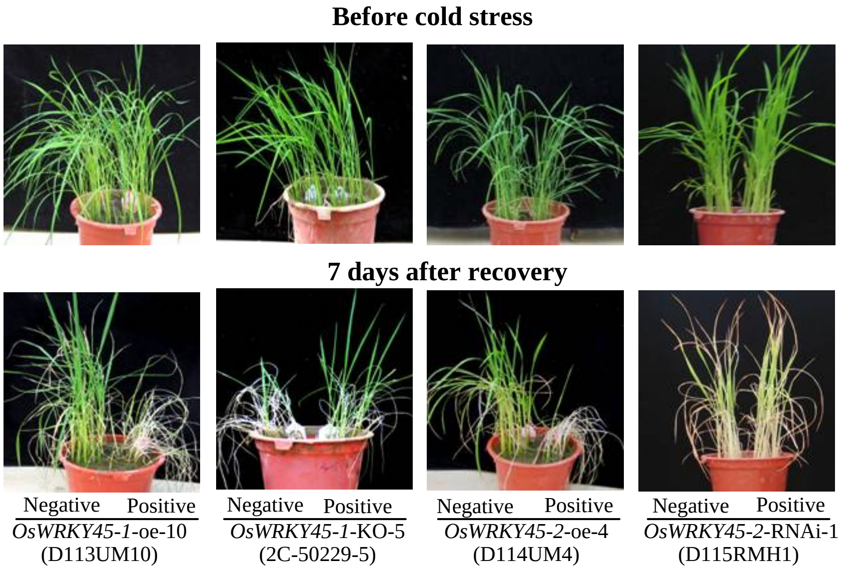

Figure S4. Modulating *OsWRKY45-1* and *OsWRKY45-2* expression influenced rice response to cold stress. Rice seedlings were kept at 4ºC for 4 to 6 days (until almost all the leaves of one group in the pot became completely rolled and some leaves died) and then transferred to room temperature (25 to 28ºC) for recovery. Negative, negative siblings from corresponding transgenic segregating populations; positive, positive transgenic plants; oe, overexpression; KO, knockout; RNAi, RNA interference.

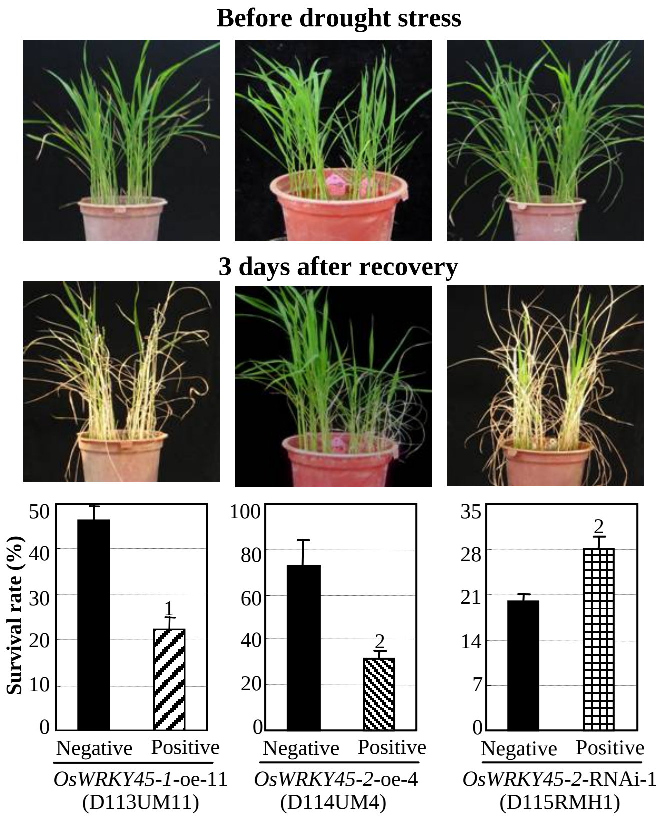

Figure S5. Modulating *OsWRKY45-1* and *OsWRKY45-2* expression influenced rice response to drought stress. Rice seedlings were withheld from water at four-to-five leaf stage for 3 to 5 d (until almost all the leaves of one group in the pot became completely rolled). After three days of recovery, the survival rates were recorded. Negative, negative siblings from corresponding transgenic segregating populations; positive, positive transgenic plants; oe, overexpression; RNAi, RNA interference. Bars represent mean (2-3 replicates with each replicate containing 16 to 20 plants) ± standard deviation. The "1" or "2" indicates a significant difference was detected between a positive transgenic line and its negative control at $P < 0.01$ or $P < 0.05$, respectively. Because of the variant lengths of water-stress time (3 to 5 d) to different groups of plants, the control plants with the same genetic background in the *OsWRKY45-1*-oe and *OsWRKY45-2*-oe groups exhibited different survival rates.

Table S1. Primers used for PCR amplification

Gene (GenBank accession no.)	Primer name	Forward primer (5’-3’)	Reverse primer (5’-3’)	Product size (nt)	Use
OsWRKY45 (GQ331927, GQ331930)	w45F4/w45R4	ATGGTACCGCCTACGCATCATCTCCTTC[a]	CAGGATCCTTATGGCACAACATTTAGCA[b]	1778-2293	Amplifying genomic fragment of different *OsWRKY45* alleles and sequencing
OsWRKY45	w45F6/w45R6	ATCACAAAGCATAGCATCATCT	CTCAGCACCTCCTCCTGGTCGG		Sequencing of *OsWRKY45*
OsWRKY45	w45F/R	TTCCTTGTTGATGTGTCGTCTCA	CCCCCAGCTCATAATCAAGAAC	80	Analyzing *OsWRKY45* expression by RT-PCR
NCED2 (AK120176)[c]	NCED2F/R	TCCGTTGCCCAAGATCAAG	CGTCCAACCGTGCAATCAC	75	Analyzing *NCED2* expression by RT-PCR
NCED4 (AK119780)	NCED4F/R	GATTGCACGGCACCTTCATT	CTCTGTAATTTGATTTTTCACTGGCTAAT	98	Analyzing *NCED4* expression by RT-PCR
NCED5 (AK107649)	NCED5F/R	CCCAGCTTGAAGCTTTTGCT	ACAACACTGCAACTATCCCTATCACT	97	Analyzing *NCED5* expression by RT-PCR
ABI5 (AK070998)	ABI5 F/R	AGTGGCACACTCATCCTGCTT	TGAAAGAAGACTTAATGTGCAAATCC	83	Analyzing *ABI5* expression by RT-PCR
Rab16D (AK109096)	RAB16D F/R	CGGGTAAACAATAAAGTCGTGATG	GCGCACTTACATACAGTGCTACGT	74	Analyzing *RAB16D* expression by RT-PCR
Rab21	RAB21	CACACCACAGCAAGAGCT	TGGTGCTCCATCCTGCTT	101	Analyzing *RAB21*

(AK121952)	F/R	AAGTG	AAG		expression by RT-PCR
RD22 (AK065358)	RD22 F/R	ATCTTGGCGCCTGATCGAG	AAAGAACCAAATTGCGTGCAT	120	Analyzing *RD22* expression by RT-PCR
SNAC1 (AK067690)	SNAC1 F/R	GGGTGCTGTGTCGGCTGTA	CGACTGCGACGTAACCATGT	100	Analyzing *SNAC1* expression by RT-PCR
OsDREB1B (AY166833)	OsDREB1B F/R	ACAGAGTAGGCAATGAGACTGAGGAT	TTACAGGAATTCATTGACTGCACAT	120	Analyzing *OsDREB1B* expression by RT-PCR
OsDREB1A (AY345233)	OsDREB1A F/R	TTACCACACTCGAGCAGAGCAA	CCGCTCATCTCCTGCTTGAT	100	Analyzing *OsDREB1A* expression by RT-PCR
Actin (X15865)	Actin-F/R	TGTATGCCAGTGGTCGTACCA	CCAGCAAGGTCGAGACGAA	121	Analyzing *actin* expression by RT-PCR

[a] The underlined nucleotides are the digestion site of *Kpn*I.

[b] The underlined nucleotides are the digestion site of *Bam*HI.

Table S2. The numbers of known abiotic stress-responsive *cis*-acting elements in the promoters of *OsWRKY45-1* and *OsWRKY45-2*

cis-elements	$P_{OsWRKY45\text{-}1}$	$P_{OsWRKY45\text{-}2}$	Stress	Reference
ABRE	3	5	ABA, drought, salt, cold	6, 9
DRE	1	1	drought, salt, cold	5, 7
GCC	4	4	ABA, drought	8
LTRE	3	3	ABA, drought, cold	3
MYB	13	13	ABA, drought, cold	1, 2
MYC	8	9	ABA, drought, cold	1, 2, 4

1. Abe H, Urao T, Ito T, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2003) Arabidopsis AtMYC2 (bHLH) and AtMYB2 (MYB) function as transcriptional activators in abscisic acid signaling. Plant Cell 15:63-78
2. Abe H, Yamaguchi-Shinozaki K, Urao T, Iwasaki T, Hosokawa D, Shinozaki K (1997) Role of arabidopsis MYC and MYB homologs in drought- and abscisic acid-regulated gene expression. Plant Cell 9:1859-1868
3. Baker SS, Wilhelm KS, Thomashow MF (1994) The 5'-region of Arabidopsis thaliana cor15a has cis-acting elements that confer cold-, drought- and ABA-regulated gene expression. Plant Mol Biol 24:701-713
4. Chinnusamy V, Ohta M, Kanrar S, Lee BH, Hong X, Agarwal M, Zhu JK (2003) ICE1: a regulator of cold-induced transcriptome and freezing tolerance in Arabidopsis. Genes Dev 17:1043-1054
5. Dubouzet JG, Sakuma Y, Ito Y, Kasuqa M, Dubouzet EG, Miura S, Seki M, Shinozaki K, Yamaquchi-Shinozaki K (2003) *OsDREB* genes in rice, *Qryza sativa* L., encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression. Plant J 33:751-763
6. Fujita Y, Fujita M, Satoh R, Maruyama K, Parvez MM, Seki M, Hiratsu K, Ohme-Takagi M, Shinozaki K, Yamaguchi-Shinozaki K (2005) AREB1 Is a transcription activator of novel ABRE-dependent ABA signaling that enhances drought stress tolerance in Arabidopsis. Plant Cell 17:3470-3488
7. Liu Q, Kasuga M, Sakuma Y, Abe H, Miura S, Yamaguchi-Shinozaki K, Shinozaki K (1998) Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in Arabidopsis. Plant Cell 10:1391-1406
8. Song CP, Agarwal M, Ohta M, Guo Y, Halfter U, Wang P, Zhu JK (2005) Role of an Arabidopsis AP2/EREBP-type transcriptional repressor in abscisic acid and drought stress responses. Plant Cell 17:2384-2396
9. Uno Y, Furihata T, Abe H, Yoshida R, Shinozaki K, Yamaguchi-Shinozaki K (2000) Arabidopsis basic leucine zipper transcription factors involved in an abscisic acid-dependent signal transduction pathway under drought and high-salinity conditions. Proc Natl Acad Sci U S A 97:11632-11637