

Overexpression of the stress-induced *OsWRKY08* improves osmotic stress tolerance in *Arabidopsis*

SONG Yu^{1,2}, JING ShaoJuan^{1,2} & YU DiQiu^{1†}

¹ Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Kunming 650223, China;

² Graduate School of Chinese Academy of Sciences, Beijing 100039, China

Previous Northern blotting analyses of rice seedlings have screened several WRKY genes among the transcripts that are differentially regulated in the following treatments: high salinity, cold stress, polyethylene glycol (PEG) and heat shock. Here, we report characterization of a WRKY gene, *OsWRKY08*, in rice, which was found to be inducible by PEG, NaCl, Abscisic acid (ABA), and naphthalene acetic acid (NAA) as its ortholog *AtWRKY28* in *Arabidopsis*. To determine whether overexpression of *OsWRKY08* alters abiotic stress tolerance, 35S::*OsWRKY08* recombinant was generated and transformed into *Arabidopsis*. Physiological tests indicated that 35S::*OsWRKY08* transgenic *Arabidopsis* displayed increased tolerance to mannitol stress through increasing the lateral root number and primary root length during seedling root development. Further, semi-quantitative RT-PCR showed that *AtCOR47* and *AtRD21*, two ABA-independent abiotic stress responded genes, were induced in 35S::*OsWRKY08* transgenic plants. These results suggest *OsWRKY08* improves the osmotic stress tolerance of transgenic *Arabidopsis* through an ABA-independent signaling pathway.

OsWRKY08, transcription factor, osmotic stress tolerance

WRKY transcription factors comprise a large superfamily which is widely present in all plants. Since the phylogenetic relationship among the *Arabidopsis* WRKY transcription factors was reported^[1,2], the phylogenetic trees of WRKY proteins have been constructed in moss^[3], rice^[4,5], tobacco^[6], barley^[7], cowpea^[8] and soybean^[9]. All of these phylogenetic trees were mainly calculated on the basis of the conserved WRKY domain defined by the conserved amino acid sequence WRKYGQK (WRKYGKK or WRKYGEK) at its N-terminal end and a novel Cys₂His₂ or Cys₂HisCys zinc finger motif at the C-termini^[1,4]. Both conserved elements of the domain are necessary for binding affinity of WRKY proteins to the consensus *cis*-acting element W box (C/T)TGAC(T/C)^[10,11].

Although the first cloned *WRKY* gene *SPFI* was involved in regulation of carbohydrate metabolism^[12], WRKY proteins have been shown to play important roles in the interaction between plants and pathogens^[13–15]

or herbivores^[16,17]. In *Arabidopsis*, *AtWRKY52* and *AtWRKY27* took part in the development of wilt disease symptoms caused by *Ralstonia solanacearum*^[18,19], and *AtWRKY23* was activated during the early stages of nematode feeding site establishment^[16]. In tobacco, NaWRKY3/6 mediated a plant's herbivore specific defenses via activating JA signaling pathway^[17], and NbWRKY1/2 was involved in N-mediated resistance to tobacco mosaic virus^[20]. In rice, overexpression of *OsWRKY13*, *OsWRKY53* and *OsWRKY71*, respectively enhanced the resistance against rice blast fungus (*Magnaporthe grisea*) through up-regulating the expressional

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†Corresponding author (email: ydq@xtbg.ac.cn)

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levels of *OsNPR1/NH1* and *OsPRI*^[21–23]. Overproduction of *OsWRKY45* enhanced blast resistance via *OsNPR1/NH1*-independent signaling pathway^[24]. Hence, WRKY transcription factors appear to play a major role in transcriptional reprogramming during a variety of defense responses.

Besides several biotic stresses, *WRKY* genes were also induced or repressed by various abiotic stresses such as heat, cold, salinity, drought, injury and reactive oxygen species^[25,26]. Seki et al.^[27] screened several *WRKY* genes responding to high salinity, cold and osmotic stress from *Arabidopsis*. The expression of *AtWRKY25* and *AtWRKY33* was observably enhanced by salinity^[28], and their overexpression transgenic plants were sufficient to increase *Arabidopsis* NaCl tolerance^[29]. Recently, it has been shown that overexpression of *OsWRKY45*^[30], *OsWRKY11*^[31], *TcWRKY53*^[32] and *GmWRKY13/21/54*^[9] altered the drought tolerance, dry heat tolerance, osmotic stress tolerance, and multiple abiotic stresses tolerance of transgenic plants respectively. In addition, *LtWRKY21* was induced by drought and salinity stress^[33,34], *CaWRKY1* protein was thought to function in cold adaptation^[35,36], and *HvWRKY38* protein was involved in cold-, drought- and ABA-responses^[37,38]. Taken together, WRKY proteins are emerging as key regulators in abiotic stress defense responses.

To seek the function of rice *WRKY* genes, we previously screened several members from rice induced by high salinity, cold stress, PEG and heat shock^[4]. Here we showed that the expression of *OsWRKY08*, which encoded a typical group II WRKY protein, was upregulated immediately by PEG, NaCl, H₂O₂, ABA and naphthalene acetic acid (NAA) as its ortholog *AtWRKY28* in *Arabidopsis*. Physiological tests of the 35S::*OsWRKY08* transgenic *Arabidopsis* plants revealed an increased lateral root number and primary root length to mannitol treatment rather than to NaCl and ABA stresses. Furthermore, we showed that *AtCOR47* and *AtRD21*, two ABA-independent abiotic stress responded genes, were induced in 35S::*OsWRKY08* transgenic plants, but *AtRD29A*, *AtRD22*, *AtKINI*, and *AtABI4*, four ABA-dependent abiotic stress responded genes, were not. This suggests that *OsWRKY08* improves the osmotic stress tolerance of transgenic *Arabidopsis* through ABA-independent signaling pathway.

1 Materials and methods

1.1 Plant materials and treatments

Rice seeds (*Oryza sativa* ssp. *japonica* cv. Nipponbare, provided by the Rice Research Institute, Yunnan Agricultural University) were surface sterilized in culture dishes with soggy filter paper for three weeks, and then transferred into the soil in a greenhouse at 28°C–32°C. The young roots and young leaves of 14-day-old rice seedlings were cut off directly. The young panicles, mature leaves, flag leaves, and old leaves in the rice flowering stage were snipped. Two-week-old rice seedlings were harvested and exposed to 25% (w/v) PEG8000, and 300 mmol/L NaCl, 3% (v/v) H₂O₂ for 0, 1, 2, 4, 12 h; and 100 μmol/L ABA, 100 μmol/L NAA, the water control for 0, 1/4, 1/2, 1, 2 h. All of these plant materials were frozen rapidly in liquid nitrogen and stored at –80°C for RNA extraction and further analysis.

1.2 RNA gel blotting

Total RNA was isolated from rice samples as described by Logemann et al.^[39], electrophoretically separated in the denaturing formaldehyde agarose gel, and blotted onto nylon membranes. The nylon membranes were hybridized with ³²P-labeled specific *OsWRKY08* probes, which were amplified by PCR from constructed rice cDNA library^[4] with the following primers: 5'-CGCAC CAATCTCATTCTAGTT-3' and 5'-TTCGCCCTTTT TTATTCTT-3'. The ethidium bromide-stained rRNA was used as the loading control.

1.3 Transformation of *OsWRKY08* in *Arabidopsis*

The coding region of the *OsWRKY08* cDNA^[4] was cloned into the pOCA30 vector, which is derived from pOCA28 containing the modified CaMV 35S promoter^[40]. The fidelity of the construct was confirmed by restriction digestion and sequence analysis. The 35S::*OsWRKY08* construct was transformed into *Agrobacterium tumefaciens* strain GV3101 and selected on Luria-Bertani medium containing spectinomycin at 100 g/mL or gentamycin at 40 g/mL. Transgenic *Arabidopsis* plants (Columbia ecotype) were generated by the floral-dip method^[41]. Transformants were screened on 1/2 Murashige and Skoog (MS) medium containing 50 g/mL kanamycin. Two independent lines of T3 plants were used for detailed analysis.

1.4 Stress tolerance tests for transgenic *Arabidopsis*

Seeds of the wild type (Columbia ecotype, from *Arabidopsis* Biological Resource Centre at Ohio State University) and transgenic *Arabidopsis* plants were surface sterilized with 20% (v/v) bleach for 15 min and washed three times with sterile distilled water. Sterile seeds were vernalized at 4°C for 72 h before being placed at 22°C for germination on 1/2 MS medium. Three days later the wild type and transgenic *Arabidopsis* were transferred to MS agar plates supplemented with 200 mmol/L mannitol, 100 mmol/L NaCl, 0.2 μmol/L NAA, 0.8 μmol/L ABA, and the MS agar plates wiping off sucrose for 7 d at (22±2)°C with 16 h of light and 8 h of darkness. Each assay was repeated three times with similar results obtained. Data were analyzed using SPSS soft.

1.5 RT-PCR

Total RNA was extracted from 3-week-old *Arabidopsis* using TRIzol Reagent (Invitrogen) and treated with DNase I (Ferments). The first-strand cDNA was synthesized from 2×10⁻⁷ g total RNA by reverse transcription with 2×10⁻⁸ g gene-specific primers using OneStep RT-PCR kit (Ferments). PCR was performed using 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min, followed by a final extension at 72°C for 7 min. PCR was run for 26–29 cycles using *AtActin2* gene as internal standards. The gene-specific primers for *AtCOR47* were 5'-ATCCCGAGGAAGAGAAGAAA-3' and 5'-TCAG TGGTCT TGGCATGATA-3', for *AtRD21* were 5'-CTCGTGTG GAACAAACCTAAAC-3' and 5'-TTCCTAAAAAGA AGGCATTCATAAT-3', for *AtKIN1* were 5'-AAGCCC ACATCTCTTCATC-3' and 5'-TCGTTTATTTGAA AATCCCAAC-3', for *AtRD22* were 5'-CCGATGCAG AAGTACAAAATC-3' and 5'-GTTCCAAGCTGAGG TGTCTT-3', for *AtRD29* were 5'-GATTTGACGGAG AACAGATT-3' and 5'-TCTCCGGAGTAACCTAGC ATT-3', for *AtABI4* were 5'-CCCAACATCAACACAA CCATCT-3' and 5'-CGGACCACCTTTGCCTTT-3', and for *AtACT2* were 5'-TCAACTCCTCCGCTCAACGC AAAC-3' and 5'-ACGGCGGTGGATGAGTTATTG AT-3. RT-PCR reactions were repeated 3 times.

2 Results

2.1 Sequence analysis and expression profiles of *OsWRKY08*

The *OsWRKY08* (Os05g50610) cDNA was cloned from

screening of the 4°C-treated rice leaf (*Oryza sativa* cv. Nipponbare) library^[4], which encodes a protein of 337-amino acids. We clarified its sequence homology by using FASTA program with the sequence of OsWRKY08 as query and found it shares 80.9% and 74.1% similarities with AtWRKY71 (gene ID At1g29860) and AtWRKY28 (gene ID At4g18170), respectively. According to the phylogenetic tree of Zhang et al.^[42], we also found that AtWRKY28 and AtWRKY71 are more closely related to OsWRKY08 than other WRKY TFs in *Arabidopsis*. Further sequence analysis indicated OsWRKY08, AtWRKY71 and AtWRKY28 had a typical WRKY domain (WRKYGQK) and a C₂H₂ zinc finger motif (Figure 1), falling into Group II of WRKY superfamily according to the classification of Eulgem et al.^[1]. Through the sequence comparison of the three typical WRKY proteins in rice and *Arabidopsis*, we found a close relationship among OsWRKY08, AtWRKY71, and AtWRKY28, suggesting a similar function for them.

It was reported that the expression of several *WRKY* genes including *AtWRKY28* responded to various abiotic stresses, such as high salinity, osmotic stress and several plant hormones^[27] (<http://urgv.evry.inra.fr/CATdb>). Also we examined the expression profiles of *OsWRKY08* under various treatments. Our results showed that *OsWRKY08* was induced strongly and rapidly following the treatment in 25% PEG8000, 300 mmol/L NaCl, 3% H₂O₂, 100 μmol/L ABA and 100 μmol/L NAA solutions (Figure 2(a)). *OsWRKY08* transcripts peaked at 2 h and then decreased gradually following exposure to PEG8000 and NaCl solution (Figure 2(a)). The expression of *OsWRKY08* was increased and reached the maximal level of expression following ABA, NAA application at 2 h and oxidative stress (H₂O₂) application at 4 h (Figure 2(a)). ABA and H₂O₂ were considered as two important response signals in the plant response to various stress conditions^[43]. *OsWRKY08* was remarkably upregulated by ABA and H₂O₂, salt and osmotic stress, which implied a strong association of *OsWRKY08* with some abiotic stresses.

Analysis of the expression profiles of *OsWRKY08* was also performed by Northern blotting analysis. Tissue-specific analysis showed that *OsWRKY08* was constitutively expressed in almost all the tissues and organs examined, including young roots, young panicles, young leaves, mature leaves, flag leaves and senescing leaves (Figure 2(b)).

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OsWRKY08      MSGPGGGGHLGYEDHPAAAGFLPFDHDDDUVASFFFGRSAASGGGAGAGAGAGDDDGUGL
AtWRKY71      -----MDDHVEHNYN-----TSLEEUH-----FK----S
AtWRKY28      -----MSNETRDLYNYQYPSSFSLHEMMLNLP TSNPSSYGNLPSQNGFNPSTYS
               . . . . .
OsWRKY08      ITPYSSITDYLQGF LQDPUYASSPLGGDAUKHETUVDHPSQAGGVAAPATPNSSULSS
AtWRKY71      LSDCLQSSLUMDYNLSLEKUFKFSYSSPFQSVSPSUNNPYLNL--TSNSPUVSSSSNEGE
AtWRKY28      FTDCLQSSPAAYESLLQKTFGLSPSS--EUFNSSIDQEPNRD--VTNDUINGGACNETE
               :: . . : . . : . . : . . : . . : . . : . . : . . : . . : . . : . .
OsWRKY08      SSEAAGGDDLRRCKKGRPEDEEEEEIDDEGSAUQSCKTNKMKNKKGAKKEREPRUAFMT
AtWRKY71      PKENTNDKSDQMEDNEGLHG-----UGESSKQLTKQGK--KKGEKKEREURUAFMT
AtWRKY28      TRUSPSNSSSEADHPGEDSGKSRKRELUGEDQISKKUGTKKTEUKKQREPRUSFMT
               . . . . .
OsWRKY08      KSEVDHLEDGYRWRKYGQKAUKNSYPRSYRCTAPRCGUKKRUEQDPSNUIITTYEG
AtWRKY71      KSEVDHLEDGYRWRKYGQKAUKNSPYRSPYRCTTQKCNUKKRUEFSQDPSIUITTYEG
AtWRKY28      KSEVDHLEDGYRWRKYGQKAUKNSPYRSPYRCTTQKCNUKKRUEFSQDPTUUITTYEG
               ***:*****:*****:*****:*****:*****:*****:*****:*****
OsWRKY08      QHHPSPUSYHMRQQLMHVS---ARGUMPGAAGAYQFGAPPPLLGFDEALAAURMT
AtWRKY71      KHHP IPSTLRGTVAAEHLLVHRGGGSLLSFPRHQDFLMHKHSPANYQSUGSLSYEH
AtWRKY28      QHHP IPTNLRGSSAAAAMFS----ADLMT--PRFAHDMFRTAAYTNGGSU--AAALDY
               :*.*.*.*.*.*.*.*.*.*.*.*.*.*.*.*.*.*.*.*.*.*.*.*.*.*.*.*.*.*.*.*.*.*
OsWRKY08      MNQQQQQQQLGFVP-----SIHAAAARPTMPPLHLTYAQQDLFLP
AtWRKY71      GHGTSSYNFNHNQP-----UUDYGLLQDIUPSFMFSKNES-----
AtWRKY28      GYGQSGYGSUNSNPSSHQVYHQGGEYELLREIFPSIFFKQEP-----
               . . . . .

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Figure 1 Alignment of OsWRKY08 with related WRKY proteins from *Arabidopsis*. The deduced amino acid sequence of OsWRKY08 (Os05g50610) was aligned with AtWRKY28 (At4g18170) and AtWRKY71 (At1g29860) using Clustal-X program.

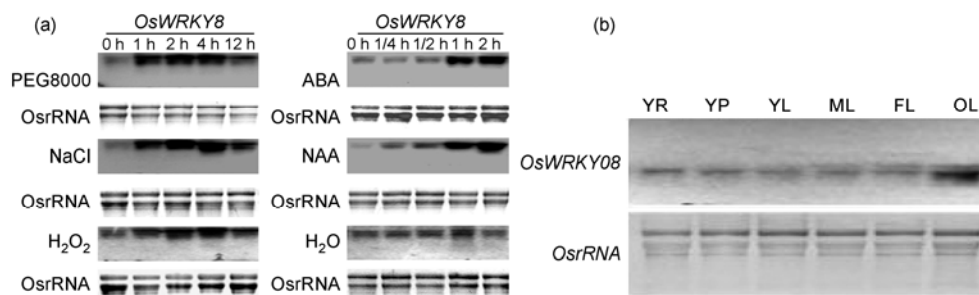


Figure 2 Induced and tissue expression patterns of *OsWRKY08* in rice. Expression of *OsWRKY08* was inspected in different organs, at different developmental stages, and after phytohormones or abiotic stresses treatments. For RNA gel blot analysis, 20 µg of total RNA was denatured and separated on agarose-formaldehyde gels and blotted to nylon membranes. ³²P-labeled cDNA of *OsWRKY08* fragment was used for hybridizations. Ethidium bromide was included in the loading buffer to confirm equal sample loading. (a) The transcript level of *OsWRKY08* after PEG8000 (25% w/v), NaCl (300 mmol/L), H₂O₂ (3% v/v), ABA (100 µmol/L), NAA (100 µmol/L), and water control treatment. (b) The transcript level of *OsWRKY08* in young roots (YR), young panicles (YP), young leaves (YL), mature leaves (ML), flag leaves (FL), and old leaves (OL).

2.2 Overexpression of *OsWRKY08* in *Arabidopsis* improves osmotic stress tolerance

To determine the *in vivo* function of *OsWRKY08* in plant abiotic stress response, we generated transgenic *Arabidopsis* plants overexpressing the *OsWRKY08* gene under the CaMV 35S promoter. Among eleven primary T1 transformants, seven plants showed the same phenotype as wild-type control plants, and four plants died at different stages. From the seven surviving transgenic lines we selected four individual overexpressing lines of *OsWRKY08* using Northern blotting analyses for collecting the T3 generation seeds (Figure 3). At the first abiotic

stresses screen, #05 and #11 lines showed the same results as #02, and another three lines in which the expression level of *OsWRKY08* was not higher than #06 showed similar results as #06. Therefore we used #02 and #06 lines to do the second and third abiotic stresses treatments.

For the abiotic tolerance assay, transgenic and wild type plants were germinated and grown on 1/2 × MS medium plates for 6 d, and then transferred to the new normal MS agar plates with or without 0.8 µmol/L ABA, 0.2 µmol/L NAA, 200 mmol/L mannitol, 100 mmol/L NaCl or 3% sucrose for 7 d. As shown in Figure 4, the number of the lateral roots and primary root length of

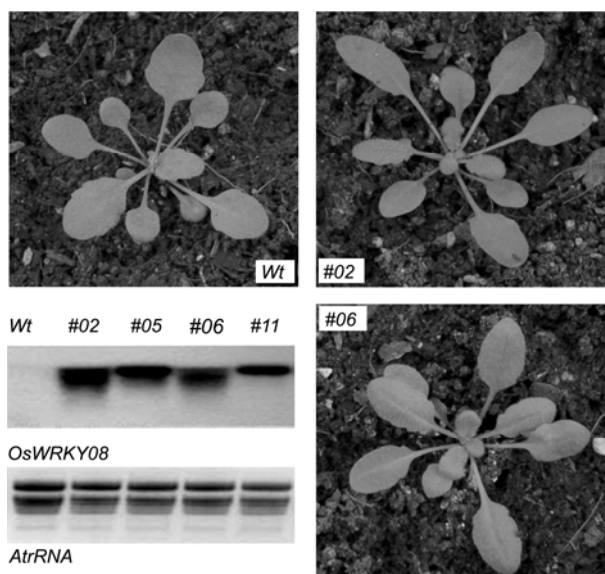


Figure 3 Phenotype of 21-day-old 35S::*OsWRKY08* lines and wild type plants. Analysis of *OsWRKY08* mRNA levels in wild type and four independent 35S::*OsWRKY08* plants. The levels of *OsWRKY08* mRNA were determined by RNA gel blot. #06 and #02 are two selected independent transgenic lines.

wild type plants were noticeably inhibited on MS agar plates with 100 mmol/L NaCl, 200 mmol/L Mannitol, 0.8 μ mol/L ABA, and without sucrose (Figure 4). Except for the 200 mmol/L mannitol condition, the growth of 35S::*OsWRKY08* plants was restrained as the wild type. The comparison of 35S::*OsWRKY08* and wild type seedlings on MS agar plates with 200 mmol/L mannitol showed that the transgenic seedlings were stronger and

healthier than the control seedlings (Figure 4(a)). The number of the lateral roots (Figure 4(b)) ($P < 0.001$) and relative primary root length (Figure 4(c)) ($P < 0.01$) of the 35S::*OsWRKY08* transgenic lines were more numerous and longer than the wild type seedlings on MS agar plates with 200 mmol/L Mannitol. These results indicated that overexpression of *OsWRKY08* in *Arabidopsis* can greatly enhance plant tolerance to osmotic stress through generating more lateral roots and growing longer primary roots.

2.3 Altered expression of osmotic stress response-related genes in *OsWRKY08* transgenic plants

To explore the molecular mechanism of the observed enhanced osmotic stress tolerance in the *OsWRKY08* overexpressing transgenic *Arabidopsis* plants, we monitored the expression of osmotic stress responsive genes by RT-PCR analysis. Under normal conditions, the test marker genes including *AtCOR47*, *AtRD21*, *AtKIN1*, *AtRD22*, *AtRD29A*, and *AtABI4*^[44,45] showed two different cases: the expression level of *AtRD21* and *AtCOR47* in 35S::*OsWRKY08* plants was higher than that in wild-type plants, whereas there was no significant induction of *AtKIN1*, *AtRD29A*, *AtRD22*, and *AtABI4* in both transgenic and wild type plants (Figure 5). *AtABI4*, *ABSCISIC ACID-INSENSITIVE 4* gene, encodes an AP2 transcription factor that binds to a CE1-like element present in lots of promoters of ABA and sugar regulated genes^[46]. *AtRD29A*, *AtRD22* and *AtKIN1* genes are drought-

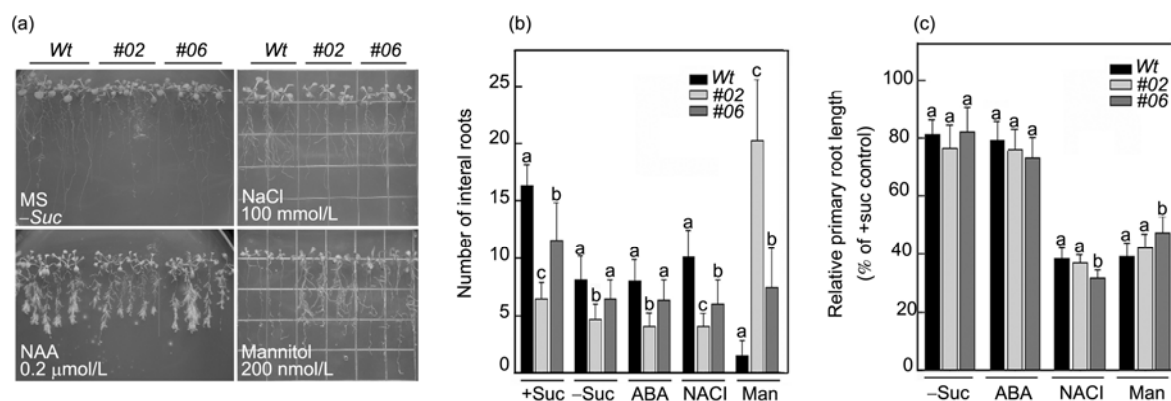


Figure 4 Response of 35S::*OsWRKY08* *Arabidopsis* lines to NAA, NaCl, and mannitol. (a) Seedlings of wild type (Wt) and 35S::*OsWRKY08* lines were germinated on a 1/2 MS agar plate for 3 d, then transferred to another MS agar plate supplemented with 0.2 μ mol/L NAA, 100 mmol/L NaCl, and 200 mmol/L mannitol for 7 d. #06 and #02 are two independent selected transgenic lines. (b) The total number of lateral roots per plant in different treatment. Values are means \pm standard deviation ($n = 12$). a, b and c, Student's *t*-test significant at $P \leq 0.001$ between two of Wt, #02 and #03. (c) The relative root length of different abiotic stress treated Wt and 35S::*OsWRKY08* plants was compared with control plants (3% sucrose). Values are means \pm standard deviation ($n = 12$). a and b, Mann-Whitney U-test significant at $P < 0.01$ between two of Wt, #02 and #03.

cold-, and ABA- inducible genes that contain the ABA-responsive element (ABRE) and dehydration responsive element (DRE) in their promoter sequences^[44]. However, the reductions in the four genes expression in 35S::*OsWRKY08* plants were slight compared with those in wild type plants (Figure 5). *AtRD21* and *AtCOR47* were involved in osmotic stress signaling by ABA-independent pathways^[47], and both of them were increased in 35S::*OsWRKY08* plants (Figure 5). Thus, the increased expression of *AtRD21* and *AtCOR47* in transgenic plants suggests that *OsWRKY08* may be involved in plant tolerance by ABA-independent pathways.

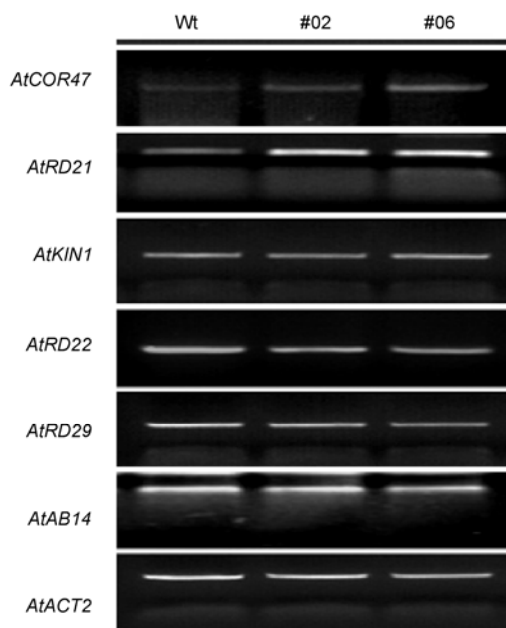


Figure 5 Expression of stress-responsive genes in wild type and 35S::*OsWRKY08* *Arabidopsis* lines was monitored by RT-PCR. Total RNA was extracted from 2-week-old wild type and 35S::*OsWRKY08* *Arabidopsis* plants. Transcript levels of *AtCOR47*, *AtRD21*, *AtKIN1*, *AtRD22*, *AtRD29*, and *AtABI4* were measured under normal conditions. #06 and #02 are two independently selected transgenic lines. *Actin2* was used as an internal control.

3 Discussion

To date, a great number of studies have credibly shown that WRKY proteins have regulatory functions in plant immune responses^[13,14]. Whether their function includes the direct regulation of abiotic stress tolerance remains to be demonstrated. As the first step towards understanding the effects on plant abiotic stress tolerance of these *WRKY* genes, several groups analyzed their expression profiles and found that a majority of members were differentially induced by drought, high salinity,

cold, and heat^[26,27]. Consistently, many rice *WRKY* genes were inducible in drought, high salinity, cold, and heat stresses^[4]. Here, we focused on the *OsWRKY08* which is up-regulated by osmotic stress and identified as an important transcriptional regulator of transgenic *Arabidopsis* osmotic stress tolerance.

The abiotic tolerance assay of the wild type and the 35S::*OsWRKY08* transgenic *Arabidopsis* showed the growth (number of the lateral roots and primary root length) of 35S::*OsWRKY08* plants was strongly enhanced compared with wild type plants upon mannitol treatment. However, the growth of 35S::*OsWRKY08* plants was strongly inhibited compared with wild type plants in the NaCl treatment (Figure 4). These results suggest that the function of *OsWRKY08* is regulating the increase of osmotic stress tolerance and decrease of ionic stress tolerance. Several studies have proved there are ABA dependent and independent signaling pathways in response to osmotic stress^[44,45]. To confirm ABA-dependent or independent signaling pathways of *OsWRKY08*, the germination assay and ABA sensitivity assay were performed. The time course of germination rate (data not shown) and relative primary root length data upon ABA treatment (Figure 4) revealed no significant difference between wild type and 35S::*OsWRKY08* transgenic *Arabidopsis*. It seemed that the improved tolerance of osmotic stress was not potentiated by ABA-dependent signaling pathway in 35S::*OsWRKY08* transgenic *Arabidopsis*. In addition, some studies have demonstrated that Auxin plays an important role in lateral root development. We therefore put the wild type and 35S::*OsWRKY08* plants on MS medium with 0.2 mol/L NAA. The quantity of lateral roots on plates containing NAA showed no difference between 35S::*OsWRKY08* #06 lines and the wild type (Figure 4), and the lateral roots of 35S::*OsWRKY08* #02 lines were less than those of the wild type. Taken together, these results indicate that the improved tolerance of osmotic stress was not affected by ABA and NAA.

It has been suggested that *OsWRKY45*, which was previously screened from rice seedlings treated by abiotic stress just as *OsWRKY08*^[4], could enhance drought tolerance in transgenic *Arabidopsis* probably through ABA-dependent pathway^[30]. In our study, we monitored the ABA-dependent stress marker genes such as *RD22*, *RD29*, *KIN1*, and *ABI4* (Figure 5) in 35S:: *OsWRKY08* transgenic *Arabidopsis*. The results revealed no signifi-

cant difference between the wild type and 35S::*OsWRKY08* transgenic *Arabidopsis* (Figure 5). It seemed that the improved tolerance of osmotic stress was not potentiated by the ABA-dependent signaling pathway in 35S::*OsWRKY08* transgenic *Arabidopsis*. The expression of *OsWRKY08* responded to high salinity, osmotic, and oxidative damage, as do the *TcWRKY53* in *Thlaspi caerulescens*^[32] and *AtWRKY25/33* in *Arabidopsis*^[29]. *TcWRKY53* negatively regulates the osmotic stress tolerance of transgenic tobacco^[32], and overexpression of *AtWRKY25* or *AtWRKY33* was sufficient to enhance *Arabidopsis* NaCl tolerance^[29]. We report here that overexpression of *Os-*

WRKY08 improves the osmotic stress tolerance of transgenic *Arabidopsis* and two ABA independent stress marker genes *AtRD21* and *AtCOR47*^[47] were upregulated in transgenic *Arabidopsis*. *AtRD21* and *AtCOR47* were induced by drought and salt in *abi* (ABA-insensitive) or *aba* (ABA-deficient) *Arabidopsis* mutants^[44] but reduced in the *los5* (low expression of osmotically responsive genes) and *los6* *Arabidopsis* mutants^[48]. The expressional upregulation of both genes suggests that improved osmotic stress tolerance of 35S::*OsWRKY08* transgenic *Arabidopsis* is through potentiation of the ABA-independent signaling pathway.

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