J Plant Res (2016) 129:955–962
DOI 10.1007/s10265-016-0833-0

REGULAR PAPER

Arabidopsis ATAF1 enhances the tolerance to salt stress and ABA in transgenic rice

Yongchang Liu[2] · **Jie Sun**[2] · **Yaorong Wu**[1]

Received: 10 January 2016 / Accepted: 23 March 2016 / Published online: 23 May 2016

Abstract NAC (NAM, ATAF1/2, CUC2) transcription factors are plant-specific and have diverse functions in many plant developmental processes and responses to stress. In our previous study, we found that the expression of *ATAF1*, an *Arabidopsis* NAC gene, was obviously induced by high-salinity and abscisic acid (ABA). The overexpression of *ATAF1* in *Arabidopsis* increased plant sensitivity to ABA and salt. To investigate whether *ATAF1* affects the sensitivity of monocotyledon plant to salt and ABA, *ATAF1* transgenic rice were generated. Transgenic rice exhibited significantly improved salt tolerance and insensitivity to ABA. The results of real-time PCR showed that *ATAF1* overexpression in rice elevated the transcription of *OsLEA3*, *OsSalT1* and *OsPM1*, which are stress-associated genes. Our results indicate that *ATAF1* plays an important role in response to salt stress and may be utilized to improve the salt tolerance of rice.

Keywords *ATAF1* · NAC · Rice · Salt stress

Electronic supplementary material The online version of this article (doi:10.1007/s10265-016-0833-0) contains supplementary material, which is available to authorized users.

✉ Yaorong Wu
yrwu@genetics.ac.cn

[1] State Key Laboratory of Plant Genomics, National Center for Plant Gene Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, No. 1 West Beichen Road, Beijing 100101, China

[2] College of Agriculture/The Key Laboratory of Oasis Eco-agriculture, Shihezi University, Beisi Road, Shihezi 832003, Xinjiang, China

Introduction

Abiotic stresses, such as drought, high-salinity, low-temperature and oxidative stresses, are principal causes limiting crop productivity throughout the world (Mahajan and Tuteja 2005). Under stressful conditions, the expression of many genes is induced, which encode dehydrins, kinases, phosphatases, transcription factors and detoxification proteins (Brini et al. 2007; Huang et al. 2009; Kim et al. 2012; Li et al. 2012; Zhang et al. 2012). The regulation of these genes leads to physiological and metabolic changes, which facilitate the survival of the plants (Zheng et al. 2009). Transcription factors play an important role in the response to abiotic stresses by regulating the expression of stress-related genes (Ding et al. 2015; Mao et al. 2015; Wang et al. 2015).

NAC(NAM, ATAF1,2 and CUC2) transcription factors belong to a plant-specific transcription factor family that comprises of 117, 151, 152 and 152 predicted members in *Arabidopsis*, rice, soybean and maize respectively (Shao et al. 2015). NAC proteins contain a conserved DNA-binding NAC domain within the N-terminal and a diverse transcription regulatory domain in the C-terminal (Hu et al. 2010). Previous studies have showed that NAC proteins are involved in many biological processes, such as formation of the apical meristem, flowering, initiation and elongation of lateral roots, construction of cell walls, response to hormones, and fruit ripening, etc. (Kim and Park 2007; Souer et al. 1996; Xie et al. 2000; Zhong et al. 2006; Zhu et al. 2014). Many NAC transcription factors have been found to modulate the response to abiotic stresses and ABA in both *Arabidopsis* and rice (He et al. 2005; Hu et al. 2008; Wu et al. 2009; Zheng et al. 2009). In *Arabidopsis*, *VNI2* (induced by high-salinity and ABA) is a positive regulator of resistance to salt stress. *VNI2* transgenic plants are less susceptible to high-salinity, while mutant seedlings

are severely affected by high-salinity (Yang et al. 2011). On the contrary, some salt stress-related NAC TFs play a negative role in salt tolerance. For example, *NTL8* encoding a membrane-bound NAC transcription factor, was induced by high-salt, but unaffected by ABA. *NTL8* negatively regulates the salt response in germination via the GA pathway (Kim et al. 2008). *ANAC092*, which is also known as *AtNAC2* and *ORE1*, also negatively regulates salt tolerance and salinity-induced chlorophyll loss (Balazadeh et al. 2010). In rice, some members of NAC TFs positively regulate the response to salt stress, such as *SNAC1* (Hu et al. 2006), *ONAC045* (Zheng et al. 2009), *OsNAC6* (Takasaki et al. 2010), *OsNAC5* (Song et al. 2011; Takasaki et al. 2010), *OsNAP* (Chen et al. 2014), *ONAC106* (Sakuraba et al. 2015) and *ONAC022* (Hong et al. 2016), etc.

ATAF1 was one of the first NAC proteins identified in *Arabidopsis*. The expression of *ATAF1* was drastically induced by NaCl, drought, ABA and wound treatments (Lu et al. 2007; Wu et al. 2009). Induction of *ATAF1* expression indicated that *ATAF1* plays a vital role in response to abiotic stresses. Plants in which *ATAF1* is abolished displayed sensitivity to drought, but insensitivity to NaCl and ABA, while the overexpressors show reverse phenotypes (Wu et al. 2009). Further study shows that ATAF1 interacts with the catalytic subunits AKIN10 and AKIN11 of SnRK1, which is a key regulator of ABA signaling pathways (Kleinow et al. 2009). ATAF1 also directly regulates the expression of the key abscisic acid biosynthetic gene *NCED3* and ABA transport gene *ABCG4*0 (Garapati et al. 2015; Jensen et al. 2013). In addition, ATAF1 directly binds to the promoters of *ORE1*and *GLK1* which encode key chloroplast maintenance and senescence-promoting TFs, respectively, resulting in activation of *ORE1* and repression of *GLK1* transcription (Garapati et al. 2015). Briefly, ATAF1 regulates abiotic stress tolerance and senescence through the direct regulation of the photosynthesis and senescence transcriptional pathway mediated by ABA and H_2O_2. Meanwhile, *ATAF1* is up-regulated by jasmonic acid but down-regulated by salicylic acid, jasmonic acid, and 1-amino cyclopro-pane-1-carboxylic acid, all of which function in defense of plants against pathogens (Wang et al. 2009; Wu et al. 2009). The overexpression of *ATAF1* enhances susceptibility to *P. syringae pv. tomato DC3000*, *B. cinerea*, and *Alternaria brassicicola* (Wang et al. 2009). All of the above evidence implies that *ATAF1* is a key point of the crosstalk between biotic and abiotic stress pathways and functions as a switch between plant abiotic stress tolerance and defense (Mauch-Mani and Flors 2009). To study the function of *ATAF1* in monocotyledon plants, we generated transgenic rice of *ATAF1*. The transgenic rice significantly enhanced tolerance to salt and cold, as well as decreased sensitivity to ABA. *ATAF1* overexpression in rice elevated the transcription of some stress-related genes, which may account for the salt tolerance of the transgenic rice.

Materials and methods

Plant growth conditions

The seeds were surface sterilized with 70 % ethanol for 1 min and washed two times with sterile water. They were sterilized with 30 % NaClO for 50–60 min, and washed five times with sterile water. The sterile seeds were put on sterile filter paper to dry and then transferred to 1/2 MS medium containing 0.8 % Phytagel. Two-week-old seedlings were harvested for RNA extraction.

Constructs and transformation

The full length of *ATAF1* CDS was cloned into pCambia2300 under the *ubiquitin* promoter and *octopine synthetase* (*OCS*) terminal. The expression of *NPTII*, a selected marker gene, was driven under the control of the *CaMV* 35S promoter and *35S* poly A terminal. The whole expression region was located between left border (LB) and right border (RB) (Fig. 1a). The confirmed construct, pCAMBIA2300-*ATAF1*, was transformed into Huang Huazhan (*Oryza sativa L. subsp. indica*) by an *Agrobacterium*-mediated transformation method to generate transgenic rice.

RNA isolation and northern blot

Total RNA was isolated using the hot phenol method, and 10 μg was applied in each lane for RNA gel analysis with the α-32p-labeled *ATAF1* specific probe to check the transcription of *ATAF1* in transgenic rice. For semi-quantitative PCR, DNaseI-treated total RNA (2 μg) was denatured and reverse transcribed using the M-MLV reverse transcriptase at 42 °C for 60 min according to the manufacture's instruction (Promega). Real time-PCR was performed on the Bio-Rad real-time PCR system using SYBR mixture (Bio-Rad) with the flowing program: 95 °C for 3 s; 40 cycles of 95 °C for 5 s, and 60 °C for 5 s, the plates were then read.

Salt, cold and ABA tolerance assay

For the germination assay, seeds of the transgenic rice and vector controls were germinated in water and in water containing 150 mM NaCl and the number of germinated seeds was counted every day. To study the effect of salt stress on post-germination growth, the seeds were sown on 1/2 MS medium plus 0 or 150 mM NaCl. Two and three weeks later, the root and shoot length respectively, of transgenic

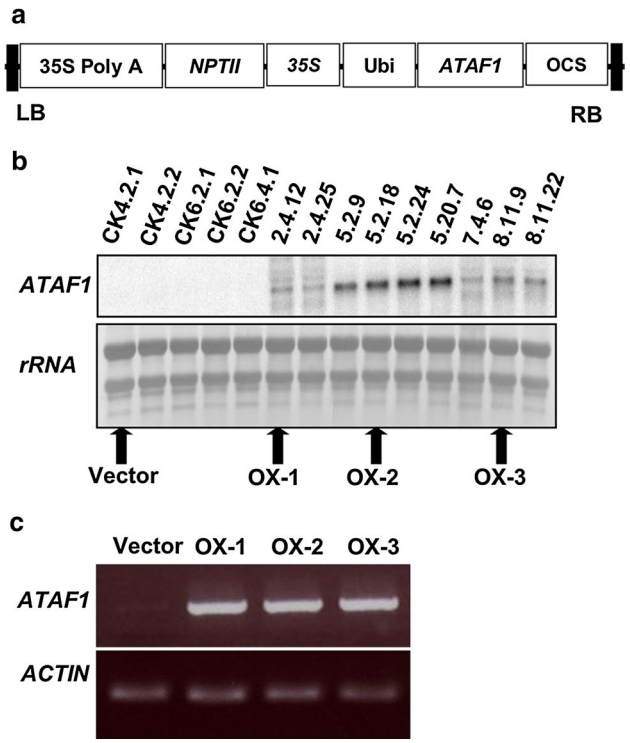

Fig. 1 The expression level of *ATAF1* in transgenic plants. **a** The schematic diagram of the *ATAF1* overexpression construct. **b** Northern blot was performed to check the transcription of *ATAF1*. *Arrows* indicated selected lines for further study. **c** RT-PCR was performed to confirm the expression level of the transgenic lines selected for further study

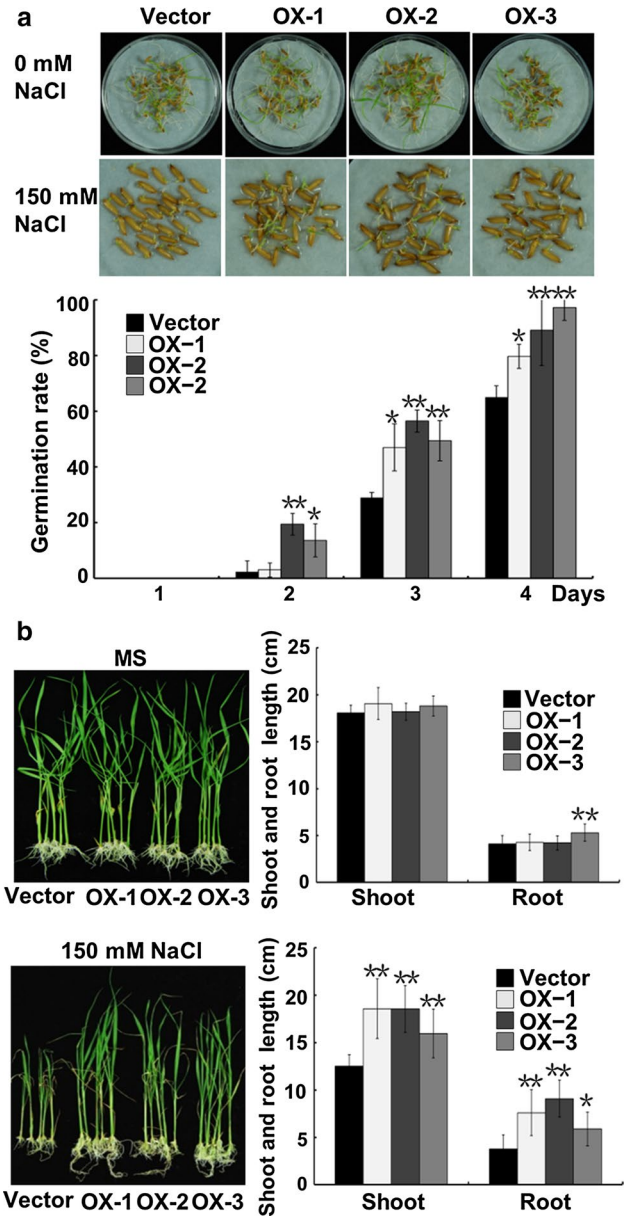

Fig. 2 Response to salt treatment of *ATAF1* transgenic rice. **a** Growth performance (*upper*) and germination rate (*lower*) of transgenic plants and vector control in water containing 150 mM NaCl. Photographs were taken after 8 days. The data represent the mean ± SE, n = 3. **b** Root length and shoot height of the transgenic and vector control plants on MS medium with 0 mM NaCl for 2 weeks and 150 mM NaCl for 3 weeks. The data represent the mean ± SE, n ≥ 20. *Asterisks* indicate significant differences (*$P \leq 0.05$; **$P \leq 0.01$) between the vector control and transgenic plants

rice growing on 1/2 MS medium plus 0 mM or 150 mM NaCl were measured. To study the response to ABA, seeds from the transgenic rice and vector controls were germinated in water containing 0, 4 and 8 μM ABA. After 8 days, the height of the transgenic rice was measured. To check the response of young seedlings to cold stress, seeds were germinated for 2 days at 37 °C in water. The most uniformly germinated seeds were sown into a 96-well plate from which the bottom had been cut off. The plate was floated on Hoagland's nutrient solution for 4 weeks in a greenhouse. For cold treatment, two-week-old vector control and transgenic seedlings were incubated in a growth chamber at 4 °C for 30 h and then recovered for 5 days.

Results

Identification of transgenic rice by northern blot and RT-PCR

To investigate the functions of *ATAF1* in rice, we generated transgenic rice by *Agrobacterium*-mediated transformation method to overexpress *ATAF1*. More than ten independent lines were screened with G418 and then transferred to the field for T2 seeds. To check the transcripts of *ATAF1*, we performed northern blot with an *ATAF1*-specific probe. Most of the transgenic rice exhibited a high expression level, but similar results were not observed in the vector

control plants. Four homozygous lines, including one line for vector control were selected for further studies, named Vector, OX-1, OX-2 and OX-3 (Fig. 1b). To confirm the northern blot results, we amplified *ATAF1* using specific primers by RT-PCR. As shown in Fig. 1C, we can detect the transcripts of *ATAF1* in transgenic rice, but not in the vector control plants (Fig. 1c).

Overexpression of *ATAF1* improved the salt tolerance of transgenic rice

ATAF1 was involved in the response to salt stress in *Arabidopsis*. The seeds of the transgenic rice and the vector control were germinated in water containing 0 mM and 150 mM NaCl and the number of germinated seeds was counted every day. After 3 days of germination in water containing 150 mM NaCl, only 29 % of the vector control seeds were germinated, whereas more than 47 % of the transgenic seeds germinated rapidly (Fig. 2a). The higher germination rate of the transgenic seeds compared with that of WT under high-salinity condition indicated the superior salt tolerance of *ATAF1*-overexpressing rice. To test the salt tolerance in post-germination growth, germinated seeds were transferred to 1/2 MS medium plus 150 mM NaCl and 0 mM NaCl as a control, and then grown in a growth chamber (16 h light/8 h dark, 28 °C). Fifteen days later, the root and shoot length of the plants that grew on 1/2 MS medium without NaCl were measured. There was no difference in growth performance between the transgenic and vector control plants. After 3 weeks, these same parameters were measured for plants that grew on 1/2 MS medium containing 150 mM NaCl. We found that not only the root length, but also the shoot length of the transgenic rice (shoot length: 18.57, 18.55, 15.95 cm; root length: 7.60, 9.06, 5.88 cm) was significantly longer than that of the control plants (shoot length: 12.5 cm; root length: 3.78 cm) (Fig. 2b). The overexpression of *ATAF1* improved the salt resistance compared with that of the vector control not only in the germination stage but also in the post-germination stage.

Overexpression of *ATAF1* decreased ABA sensitivity

ABA is an important phytohormone and plays an essential role in the response to salt stress. The expression of *ATAF1* was clearly induced in *Arabidopsis* by high-salinity and ABA. Therefore, we investigated the response of transgenic plants to ABA in this study. The height of the *ATAF1* overexpression showed no large difference from that of the vector control plants. However, the growth inhibition caused by ABA was reduced in the overexpressor plants. The shoot elongation values of three transgenic lines grown in water containing 4 μM ABA were 2.16, 2.37 and 2.29 cm,

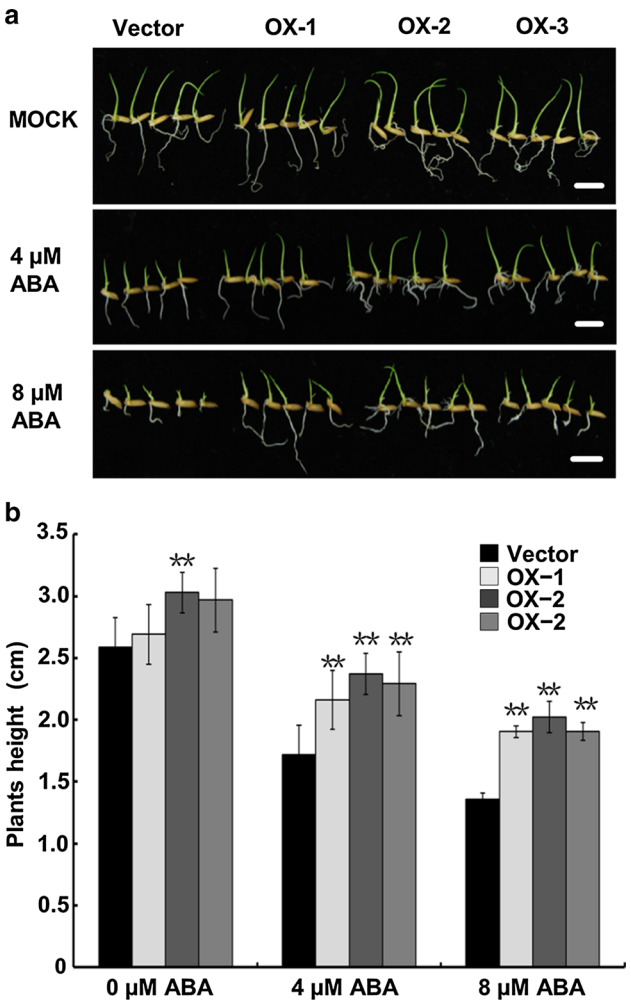

Fig. 3 Response to ABA of *ATAF1* transgenic rice. **a** Growth performance of transgenic plants and vector control in water containing 0, 4 and 8 μM ABA. **b** Plant height of transgenic rice and vector control after 8 days, *bar* = 1 cm. The data represent the mean ± SE, n ≥ 52. *Asterisks* indicate significant differences (**$P \leq 0.01$) between the vector control and transgenic plants

respectively, and these values were longer than those of vector control plants (1.72 cm). When the concentration of ABA was increased to 8 μM, the shoot length of the vector control plants decreased to 1.36 cm, which was significantly shorter than that of the transgenic plants (1.90, 2.02 and 1.91 cm, respectively) (Fig. 3a, b). Our results showed that *ATAF1* decreased the sensitivity of transgenic rice to ABA.

ATAF1 elevated the expression of *OsLEA3*, *OsSalT1* and *OsPM1*

To understand the mechanism of salt tolerance mediated by *ATAF1*, real-time PCR was performed to screen some marker genes involved in salt stress using two independent lines OX-1 and OX-2. Three genes were induced

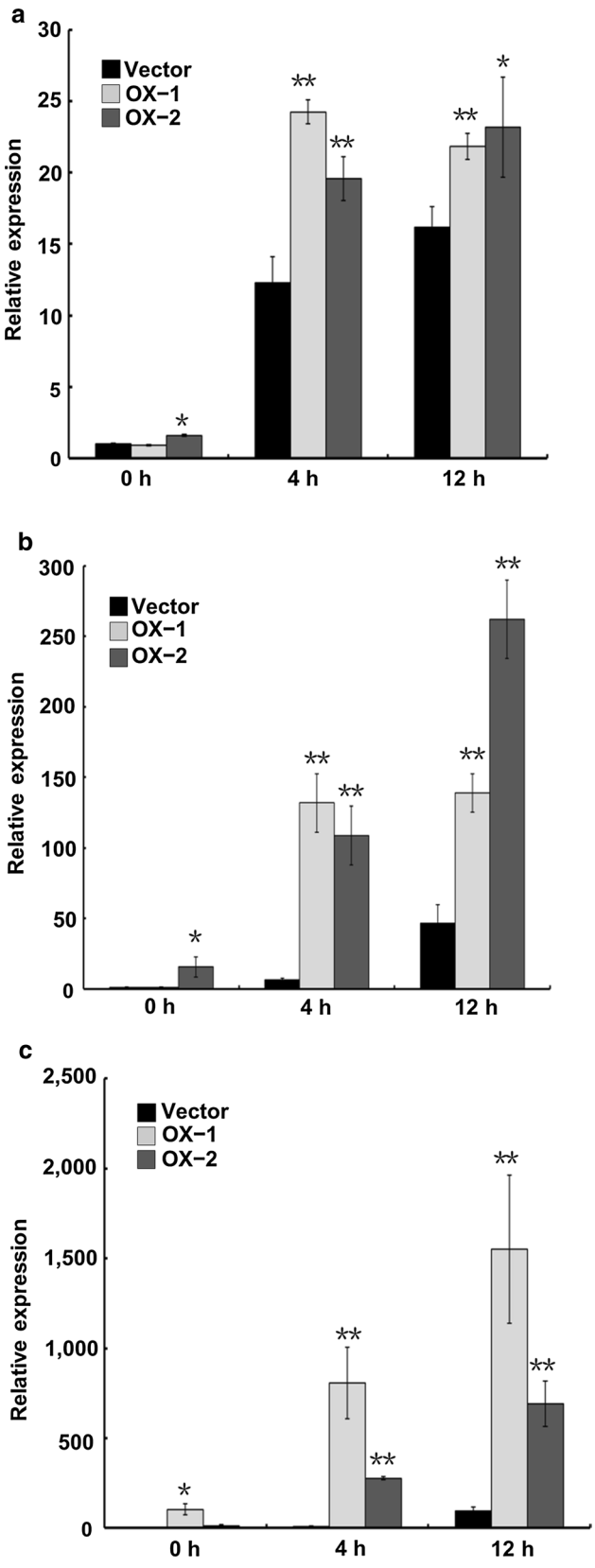

Fig. 4 Expression analysis of salt tolerance-associated genes by real-time-PCR. Two-week-old seedlings were treated with 250 mM NaCl for 0, 4 and 12 h. Real-time-PCR was performed using *OsLEA3* (**a**), *OsPM1* (**b**) and *OsSalT1* (**c**) specific primers. Two independent lines and a vector control line were used. The data represent the mean ± SE, n = 3. *Asterisks* indicate significant differences (*$P \leq 0.05$; **$P \leq 0.01$) between the vector control and transgenic plants

significantly after salt treatment in transgenic plants, including *OsLEA3*, *OsSalT1* and *OsPM1* (Fig. 4). The expression level in the overexpressor was much higher than that in the vector control plants. Promoters of these genes were analyzed. It was found that promoters of the three genes contained 5, 7 and 6 ATAF1-binding motifs, TT[A,C,G]CGT or T[A,C,G]CGT[A,G] (Jensen et al. 2013), respectively (Table S1), suggesting that *ATAF1* might bind to the promoters of *OsLEA3*, *OsSalT1* and *OsPM1* to activate the transcription of these genes.

Discussion

The overexpression of *ATAF1* in *Arabidopsis* leads to an increased sensitivity to NaCl and ABA but enhances plant tolerance to drought (Wu et al. 2009). In this study, the overexpression of *ATAF1* in rice enhances salt and ABA tolerance but has no effect on drought tolerance (Fig. 2). The mechanisms of the response to salt, drought and ABA in *Arabidopsis* and rice are speculated to be different. Many NAC TFs of *Oryza sativa* and *Zea mays* show relatively low homologies with NAC family members from dicot species (Mao et al. 2012). Therefore, the biological functions of homologous NAC TFs in *Arabidopsis* and rice are not the same absolutely. In rice, *OsNAC5* and *OsNAC6* belong to the ATAF subfamily, as do *ATAF1* and *ATAF2* in *Arabidopsis* (Ohnishi et al. 2005). The expression of *OsNAC5* and *OsNAC6* are induced by ABA, drought and salt stresses (Ohnishi et al. 2005; Takasaki et al. 2010). The overexpression of both in rice improves salt and drought tolerance (Nakashima et al. 2007; Song et al. 2011; Takasaki et al. 2010). OsNAC5 and OsNAC6 could bind to the promoter of *OsLEA3*, which is a stress-related gene (Takasaki et al. 2010). In this study, three genes, including *OsLEA3*, *OsSalT1* and *OsPM1*, are induced at higher expression levels in transgenic plants than in the vector control. *OsLEA3* encoding dehydrin is induced by drought, salt and ABA. After salt treatment, an accumulation of *OsLEA3* maintains the structure and function of the cell to facilitate its subsequent recovery (Chourey et al. 2003). *OsSalT1*, which encodes mannose-binding jacaline-like lectin, is

induced by various stress treatments, including MS salt, air drying, PEG, NaCl and KCl. OsSalT is responsible for changing the concentration of Na^+ to prevent damage and enhance salt tolerance (Claes et al. 1990; Zhang et al. 2000). Wheat *WPM1* encodes a hydrophobic polypeptide and is involved in ABA-mediated freezing tolerance (Koike et al. 1997). *OsPM1* is a homologue gene of *WPM1* and induced by ABA, drought and salt (Zheng et al. 2009). *OsLEA3*, *OsSalT1* or *OsPM1* are related to improving the tolerance of rice to abiotic stresses and have been used in several studies as gene markers (Chen et al. 2014; Chourey et al. 2003; Hong et al. 2016; Yang et al. 2012; Zheng et al. 2009). We therefor analyzed the promoters of these three genes and found that there are several ATAF1-binding motifs in promoters of *OsLEA3*, *OsSalT1* and *OsPM1*, respectively (Table S1). One possibility for the mechanism of improving salt tolerance might be that *ATAF1* directly binds to the promoters of *OsLEA3*, *OsSalT1* and *OsPM1* and transactivates their expression.

Previous studies show that ectopic expression of stress-related genes significantly improves stress tolerance in crops. Overexpression of *ZmCBF3*, which encodes a maize C-repeat-binding TF in rice, improves tolerance to drought, high-salt, and low-temperature stresses without growth retardation under normal growth conditions (Xu et al. 2011). *TaMnSOD*, which encodes manganese superoxide dismutase, was cloned from *Tamarix androsso*wii (Wang et al. 2010). *TaMnSOD* transgenic cotton plants improve drought tolerance through enhanced development of the root and leaf systems and the regulation of superoxide scavenging, thereby increasing the biomass as well as the root and leaf systems compared with the controls (Zhang et al. 2014). *AtCKX1* from *Arabidopsis thaliana* encoding cytokinin dehydrogenase 1 is ectopically expressed in barley under the control of a mild root-specific beta-glucosidase promoter from maize. Under severe drought stress, all transgenic genotypes maintained higher water contents and showed better growth and yield parameters during revitalization (Pospisilova et al. 2016). Thus, ectopic expression of foreign genes is an effective method to enhance stress resistance in crops by transgenic technology. In this study, the overexpression of *ATAF1* improves the salt and cold tolerance (Fig. S1), but reduces the grain yield of rice in the field (Fig. S2b). Moreover, transgenic plants have a shorter plant height than that of the vector control (Fig. S2a). The number of valid tillers and filled grain rate are not affected by overexpression of *ATAF1* (Fig. S2c, d). *ATAF1* is overexpressed in rice under the control of the *ubiquitin* promoter which is a constitutive expression promoter. Constantly high expression level in all tissues might explain the growth retardation and low grain yield. The overexpression of many stress-related transcription factors leads to growth retardation, including *AtDREB1A*, *AtDREB2A*, *OsWRKY89*, *MusabZIP53* (Kasuga et al. 1999; Qin et al. 2007, 2008; Shekhawat and Ganapathi 2014; Wang et al. 2007). A previous study showed that driving expression using a stress-inducible promoter can improve the stress tolerance without a negative effect on plant growth and development (Kasuga et al. 1999). *LIP9*, which encodes a low-temperature-induced protein, is induced by cold, drought, high-salinity stresses and ABA (Aguan et al. 1991). The overexpression of *OsNAC6* driven by the promoter of *LIP9* enhances the tolerance of rice to abiotic stress but does not cause defects in productivity (Nakashima et al. 2007). The *ubiquitin* promoter can be changed to a stress-induced promoter to increase the yield of transgenic rice. Therefore *ATAF1* can serve as a candidate gene to enhance salt tolerance in rice as long as the spatial–temporal expression is controlled.

References

Aguan K, Sugawara K, Suzuki N, Kusano T (1991) Isolation of genes for low-temperature-induced proteins in rice by a simple subtractive method. Plant Cell Physiol 32:1285–1289

Balazadeh S, Siddiqui H, Allu AD, Matallana-Ramirez LP, Caldana C, Mehrnia M, Zanor MI, Kohler B, Mueller-Roeber B (2010) A gene regulatory network controlled by the NAC transcription factor ANAC092/AtNAC2/ORE1 during salt-promoted senescence. Plant J 62:250–264

Brini F, Hanin M, Lumbreras V, Amara I, Khoudi H, Hassairi A, Pages M, Masmoudi K (2007) Overexpression of wheat dehydrin DHN-5 enhances tolerance to salt and osmotic stress in *Arabidopsis thaliana*. Plant Cell Rep 26:2017–2026

Chen X, Wang Y, Lv B, Li J, Luo L, Lu S, Zhang X, Ma H, Ming F (2014) The NAC family transcription factor OsNAP confers abiotic stress response through the ABA pathway. Plant Cell Physiol 55:604–619

Chourey K, Ramani S, Apte SK (2003) Accumulation of LEA proteins in salt (NaCl) stressed young seedlings of rice (*Oryza sativa* L.) cultivar Bura Rata and their degradation during recovery from salinity stress. J Plant Physiol 160:1165–1174

Claes B, Dekeyser R, Villarroel R, Van den Bulcke M, Bauw G, Van Montagu M, Caplan A (1990) Characterization of a rice gene showing organ-specific expression in response to salt stress and drought. Plant Cell 2:19–27

Ding Z, Yan J, Li C, Li G, Wu Y, Zheng S (2015) Transcription factor WRKY46 modulates the development of *Arabidopsis* lateral roots in osmotic/salt stress conditions via regulation of ABA signaling and auxin homeostasis. Plant J 84:56–69

Garapati P, Xue GP, Munne-Bosch S, Balazadeh S (2015) Transcription factor ATAF1 in *Arabidopsis* promotes senescence by direct regulation of key chloroplast maintenance and senescence transcriptional cascades. Plant Physiol 168:1122–1139

He X, Mu R, Cao W, Zhang Z, Zhang J, Chen S (2005) AtNAC2, a transcription factor downstream of ethylene and auxin signaling pathways, is involved in salt stress response and lateral root development. Plant J 44:903–916

Hong Y, Zhang H, Huang L, Li D, Song F (2016) Overexpression of a stress-responsive NAC tanscription factor gene *ONAC022* improves drought and salt tolerance in rice. Front Plant Sci 7:4

Hu H, Dai M, Yao J, Xiao B, Li X, Zhang Q, Xiong L (2006) Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor

enhances drought resistance and salt tolerance in rice. Proc Natl Acad Sci USA 103:12987–12992

Hu H, You J, Fang Y, Zhu X, Qi Z, Xiong L (2008) Characterization of transcription factor gene *SNAC2* conferring cold and salt tolerance in rice. Plant Mol Biol 67:169–181

Hu R, Qi G, Kong Y, Kong D, Gao Q, Zhou G (2010) Comprehensive analysis of NAC domain transcription factor gene family in *Populus trichocarpa*. BMC Plant Biol 10:145

Huang X, Chao D, Gao J, Zhu M, Shi M, Lin H (2009) A previously unknown zinc finger protein, DST, regulates drought and salt tolerance in rice via stomatal aperture control. Gene Dev 23:1805–1817

Jensen MK, Lindemose S, de Masi F, Reimer JJ, Nielsen M, Perera V, Workman CT, Turck F, Grant MR, Mundy J, Petersen M, Skriver K (2013) ATAF1 transcription factor directly regulates abscisic acid biosynthetic gene NCED3 in *Arabidopsis thaliana*. FEBS Open Bio 3:321–327

Kasuga M, Liu Q, Miura S, Yamaguchi-Shinozaki K, Shinozaki K (1999) Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. Nat Biotechnol 17:287–291

Kim SG, Park CM (2007) Membrane-mediated salt stress signaling in flowering time control. Plant Signal Behav 2:517–518

Kim SG, Lee AK, Yoon HK, Park CM (2008) A membrane-bound NAC transcription factor NTL8 regulates gibberellic acid-mediated salt signaling in *Arabidopsis* seed germination. Plant J 55:77–88

Kim IS, Kim YS, Yoon HS (2012) Rice ASR1 protein with reactive oxygen species scavenging and chaperone-like activities enhances acquired tolerance to abiotic stresses in *Saccharomyces cerevisiae*. Mol Cells 33:285–293

Kleinow THS, Krenz B, Jeske H, Koncz C (2009) NAC domain transcription factor ATAF1 interacts with SNF1-related kinases and silencing of its subfamily causes severe developmental defects in *Arabidopsis*. Plant Sci 177:360–370

Koike M, Takezawa D, Arakawa K, Yoshida S (1997) Accumulation of 19-kDa plasma membrane polypeptide during induction of freezing tolerance in wheat suspension-cultured cells by abscisic acid. Plant Cell Physiol 38:707–716

Li Y, Feng D, Zhang D, Su J, Zhang Y, Li Z, Mu P, Liu B, Wang H, Wang J (2012) Rice MAPK phosphatase IBR5 negatively regulates drought stress tolerance in transgenic *Nicotiana tabacum*. Plant Sci 188–189:10–18

Lu P, Chen N, An R, Su Z, Qi B, Ren F, Chen J, Wang X (2007) A novel drought-inducible gene, *ATAF1*, encodes a NAC family protein that negatively regulates the expression of stress-responsive genes in *Arabidopsis*. Plant Mol Biol 63:289–305

Mahajan S, Tuteja N (2005) Cold, salinity and drought stresses: an overview. Arch Biochem Biophys 444:139–158

Mao X, Zhang H, Qian X, Li A, Zhao G, Jing R (2012) *TaNAC2*, a NAC-type wheat transcription factor conferring enhanced multiple abiotic stress tolerances in *Arabidopsis*. J Exp Bot 63:2933–2946

Mao H, Wang H, Liu S, Li Z, Yang X, Yan J, Li J, Tran LS, Qin F (2015) A transposable element in a NAC gene is associated with drought tolerance in maize seedlings. Nat Commun 6:8326

Mauch-Mani B, Flors V (2009) The ATAF1 transcription factor: at the convergence point of ABA-dependent plant defense against biotic and abiotic stresses. Cell Res 19:1322–1323

Nakashima K, Tran LS, Van Nguyen D, Fujita M, Maruyama K, Todaka D, Ito Y, Hayashi N, Shinozaki K, Yamaguchi-Shinozaki K (2007) Functional analysis of a NAC-type transcription factor OsNAC6 involved in abiotic and biotic stress-responsive gene expression in rice. Plant J 51:617–630

Ohnishi T, Sugahara S, Yamada T, Kikuchi K, Yoshiba Y, Hirano HY, Tsutsumi N (2005) *OsNAC6*, a member of the NAC gene family, is induced by various stresses in rice. Genes Genet Syst 80:135–139

Pospisilova H, Jiskrova E, Vojta P, Mrizova K, Kokas F, Cudejkova MM, Bergougnoux V, Plihal O, Klimesova J, Novak O, Dzurova L, Frebort I, Galuszka P (2016) Transgenic barley overexpressing a cytokinin dehydrogenase gene shows greater tolerance to drought stress. New Biotechnol. doi:10.1016/j.nbt.2015.12.005

Qin F, Kakimoto M, Sakuma Y, Maruyama K, Osakabe Y, Tran LS, Shinozaki K, Yamaguchi-Shinozaki K (2007) Regulation and functional analysis of *ZmDREB2A* in response to drought and heat stresses in *Zea mays* L. Plant J 50:54–69

Qin F, Sakuma Y, Tran LS, Maruyama K, Kidokoro S, Fujita Y, Fujita M, Umezawa T, Sawano Y, Miyazono K, Tanokura M, Shinozaki K, Yamaguchi-Shinozaki K (2008) *Arabidopsis* DREB2A-interacting proteins function as RING E3 ligases and negatively regulate plant drought stress-responsive gene expression. Plant Cell 20:1693–1707

Sakuraba Y, Piao W, Lim JH, Han SH, Kim YS, An G, Paek NC (2015) Rice ONAC106 inhibits leaf senescence and increases salt tolerance and tiller angle. Plant Cell Physiol 56:2325–2339

Shao H, Wang H, Tang X (2015) NAC transcription factors in plant multiple abiotic stress responses: progress and prospects. Front Plant Sci 6:902

Shekhawat UKS, Ganapathi TR (2014) Transgenic banana plants overexpressing *MusabZIP53* display severe growth retardation with enhanced sucrose and polyphenol oxidase activity. Plant Cell Tissue Organ Culture 116:387–402

Song S, Chen Y, Chen J, Dai X, Zhang W (2011) Physiological mechanisms underlying OsNAC5-dependent tolerance of rice plants to abiotic stress. Planta 234:331–345

Souer E, Houwelingen A, Van Kloos D, Mol J, Koes R (1996) The no apical meristem gene of Petunia is required for pattern formation in embryos and flowers and is expressed at meristem and primordia boundaries. Cell 85:159–170

Takasaki H, Maruyama K, Kidokoro S, Ito Y, Fujita Y, Shinozaki K, Yamaguchi-Shinozaki K, Nakashima K (2010) The abiotic stress-responsive NAC-type transcription factor OsNAC5 regulates stress-inducible genes and stress tolerance in rice. Mol Genet Genomics 284:173–183

Wang H, Hao J, Chen X, Hao Z, Wang X, Lou Y, Peng Y, Guo Z (2007) Overexpression of rice *WRKY89* enhances ultraviolet B tolerance and disease resistance in rice plants. Plant Mol Biol 65:799–815

Wang X, Basnayake BM, Zhang H, Li G, Li W, Virk N, Mengiste T, Song F (2009) The *Arabidopsis* ATAF1, a NAC transcription factor, is a negative regulator of defense responses against necrotrophic fungal and bacterial pathogens. Mol Plant Microbe Interact 22:1227–1238

Wang Y, Qu G, Li H, Wu Y, Wang C, Liu G, Yang C (2010) Enhanced salt tolerance of transgenic poplar plants expressing a manganese superoxide dismutase from *Tamarix androssowii*. Mol Biol Rep 37:1119–1124

Wang T, Tohge T, Ivakov A, Mueller-Roeber B, Fernie AR, Mutwil M, Schippers JH, Persson S (2015) Salt-related MYB1 coordinates abscisic acid biosynthesis and signaling during salt stress in *Arabidopsis*. Plant Physiol 169:1027–1041

Wu Y, Deng Z, Lai J, Zhang Y, Yang C, Yin B, Zhao Q, Zhang L, Li Y, Yang C, Xie Q (2009) Dual function of *Arabidopsis* ATAF1 in abiotic and biotic stress responses. Cell Res 19:1279–1290

Xie Q, Frugis G, Colgan D, Chua NH (2000) *Arabidopsis* NAC1 transduces auxin signal downstream of TIR1 to promote lateral root development. Gene Dev 14:3024–3036

Xu M, Li L, Fan Y, Wan J, Wang L (2011) *ZmCBF3* overexpression improves tolerance to abiotic stress in transgenic rice (*Oryza sativa*) without yield penalty. Plant Cell Rep 30:1949–1957

Yang SD, Seo PJ, Yoon HK, Park CM (2011) The *Arabidopsis* NAC transcription factor VNI2 integrates abscisic acid signals into leaf senescence via the COR/RD genes. Plant Cell 23:2155–2168

Yang A, Dai X, Zhang W (2012) A R2R3-type MYB gene, *OsMYB2*, is involved in salt, cold, and dehydration tolerance in rice. J Exp Bot 63:2541–2556

Zhang W, Peumans WJ, Barre A, Astoul CH, Rovira P, Rouge P, Proost P, Truffa-Bachi P, Jalali AA, Van Damme EJ (2000) Isolation and characterization of a jacalin-related mannose-binding lectin from salt-stressed rice (*Oryza sativa*) plants. Planta 210:970–978

Zhang L, Li Y, Lu W, Meng F, Wu C, Guo X (2012) Cotton *GhMKK5* affects disease resistance, induces HR-like cell death, and reduces the tolerance to salt and drought stress in transgenic *Nicotiana benthamiana*. J Exp Bot 63:3935–3951

Zhang D, Yang H, Li X, Li H, Wang Y (2014) Overexpression of *Tamarix albiflonum TaMnSOD* increases drought tolerance in transgenic cotton. Mol Breed 34:1–11

Zheng X, Chen B, Lu G, Han B (2009) Overexpression of a NAC transcription factor enhances rice drought and salt tolerance. Biochem Biophys Res Commun 379:985–989

Zhong R, Demura T, Ye Z (2006) SND1, a NAC domain transcription factor, is a key regulator of secondary wall synthesis in fibers of *Arabidopsis*. Plant Cell 18:3158–3170

Zhu M, Chen G, Zhou S, Tu Y, Wang Y, Dong T, Hu Z (2014) A new tomato NAC (NAM/ATAF1/2/CUC2) transcription factor, SlNAC4, functions as a positive regulator of fruit ripening and carotenoid accumulation. Plant Cell Physiol 55:119–135