FEBS Letters 582 (2008) 1037–1043

Overexpression of a TFIIIA-type zinc finger protein gene *ZFP252* enhances drought and salt tolerance in rice (*Oryza sativa* L.)

Dong-Qing Xu[1], Ji Huang[1], Shu-Qiao Guo, Xia Yang, Yong-Mei Bao, Hai-Juan Tang, Hong-Sheng Zhang*

State Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing 210095, PR China

Received 7 January 2008; revised 19 February 2008; accepted 25 February 2008

Available online 4 March 2008

Edited by Julian Schroeder

Abstract **We previously identified a salt and drought stress-responsive TFIIIA-type zinc finger protein gene *ZFP252* from rice. Here we report the functional analysis of *ZFP252* using gain- and loss-of-function strategies. We found that overexpression of *ZFP252* in rice increased the amount of free proline and soluble sugars, elevated the expression of stress defense genes and enhanced rice tolerance to salt and drought stresses, as compared with *ZFP252* antisense and non-transgenic plants. Our findings suggest that *ZFP252* plays an important role in rice response to salt and drought stresses and is useful in engineering crop plants with enhanced tolerance to salt and drought stresses.**

Keywords: Drought; Functional analysis; Rice; Salt; ZFP252; Zinc finger protein

1. Introduction

Plant growth and crop productivity are largely affected by environmental stresses such as drought, salinity and low temperature. To date, many stress-related genes have been isolated and characterized from various plants. These genes encode products either directly protecting plant cells from abotic stresses or regulating expression of other genes to enhance plant tolerance to the stresses [1,2]. Under the stress conditions, the C-repeat binding factor/dehydration-responsive element binding factor (CBF/DREB) transcription factors induce the expression of downstream genes containing C-repeat/dehydration response elements (CRT/DRE) in their promoters to improve plant tolerance [3,4]. Many other transcription factors such as NAC, MYB, bZIP and zinc finger proteins have been well characterized with their roles in the regulation of stress-responses [5,6].

The TFIIIA-type zinc finger proteins, first discovered in *Xenopus*, represent an important class of eukaryotic transcription factors [7]. More than 40 TFIIIA-type zinc finger protein genes were identified from various plants including petunia, soybean, *Arabidopsis* and rice so far [8–12]. The SCOF-1, a TFIIIA-type zinc finger protein from soybean, functions as a positive regulator of cold-regulated (COR) gene expression mediated by abscisic acid responsive element (ABRE) via protein–protein interaction, which in turn enhances cold tolerance of transgenic plants [8]. A petunia zinc finger protein gene, *ZPT2-3*, was induced by cold and drought stresses, and its overexpression in transgenic petunia increased plant tolerance to drought stress [9]. The *Arabidopsis* STZ/ZAT10 functions as a transcription repressor under abiotic stresses and its gain- and loss-of-function mutants both improved plant tolerance to abiotic stresses [10].

Rice is one of the most important food crops in the world, which accommodates 60% of the population in the world [13]. It is valuable to survey the TFIIIA-type zinc finger proteins involved in rice responses to abiotic stresses, not only for our understanding the molecular mechanisms of stress-responses in monocots but also for improvement of plant tolerance to abiotic stresses by gene-transfer. Recently, we isolated three TFIIIA-type zinc finger protein genes *ZFP245* [14], *ZFP182* [11] and *ZFP252* (renamed from *RZF71*) [15] from rice and found that these genes were induced by various abiotic stresses. As the expression of *ZFP182* in transgenic tobacco or overexpression in rice plants increased their tolerance to salt stress, ZFP182 might play a curial role in plant tolerance to salt [11]. Here we report the functional analysis of *ZFP252* using gain- and loss-of-function strategies. By stress assays, we found that overexpression of *ZFP252* in rice increased tolerance to salt and drought stresses. The contents of free proline and soluble sugars in sense-*ZFP252* transgenic rice plants were higher than those in the WT (wild-type) and antisense-*ZFP252* transgenic rice plants under salt and drought stress. Our results suggest that *ZFP252* might play a key role in stress-responsive signal transduction pathway, and be useful in engineering crop plants with enhanced tolerance to salinity and drought stresses.

2. Materials and methods

2.1. Plant materials and growth

The seeds of japonica rice (*Oryza sativa* L.) cultivar Zhonghua 11 and its transgenic lines (T_2) with *ZFP252* gene were sterilized with 0.1% $HgCl_2$ for 15 min, germinated in 30 °C in darkness for 2 d, and were cultured in the nutrition solution [16] or sown on plastic pots (12 cm in height and 15 cm in diameter) filled with a mixture of soil and sand (1:1) [17] in the greenhouse at 28 °C/22 °C (day/night) with a 16 h photoperiod. The seedlings at the four-leaf stage were used for subsequent stress tolerance assays.

*Corresponding author.
E-mail address: hszhang@njau.edu.cn (H.-S. Zhang).

[1]These authors contributed equally to this work.

Abbreviations: CBF/DREB, C-repeat binding factor/dehydration-responsive element binding factor; CRT/DRE, C-repeat/dehydration response elements; COR, cold-regulated gene; ABRE, abscisic acid responsive element

0014-5793/$34.00
doi:10.1016/j.febslet.2008.02.052

2.2. Generation of ZFP252 transgenic rice

The full-length *ZFP252* gene has been isolated from rice seedlings [15]. The full-length sequences of sense- and antisense-*ZFP252* were obtained by PCR and inserted into BglII and BstEII sites of pCAMBIA1301 vector by replacing the GUS coding region. The pCAMBIA1301 harboring sense or antisense-*ZFP252* gene was transformed into the calli of rice cultivar Zhonghua 11 by *Agrobacterium*-mediated method [18], to generate transgenic rice lines with sense-*ZFP252* and antisense-*ZFP252* gene driven by CaMV 35S promoter, respectively. We obtained four lines transformed with sense-*ZFP252* gene (ZFP252-ox) and two lines with antisense-*ZFP252* gene (ZFP252-kd).

2.3. RNA isolation and first strand cDNA synthesis

Total RNA was extracted from various stress-treated seedlings of the non-transgenic (wild-type, WT) or transgenic rice lines (ZFP252-ox and ZFP-252-kd) using Trizol reagent (Invitrogen, USA) according to the manufacturer's protocol. The RNA was sequentially treated with DNase I (Promega, USA) at 37 °C for 15 min in order to remove the remaining genomic DNA. The first strand cDNA was synthesized with 4 μl of purified total RNA using the reverse transcription system (Promega, USA) according to the manufacturer's protocol.

2.4. Semi-quantitative RT-PCR and real-time qPCR

For semi-quantitative RT-PCR of *ZFP252*, 2 μg of total RNA was used with the reverse transcription system (Promega, USA) according to the manufacturer's protocol. PCR was performed with *ZFP252*-specific primers of 5′-GGTGGAGGCGGTTCTTGAGG-3′ and 5′-CGTCGTAGTGGCATCGCTTGT-3′. The reaction included an initial 5 min of denaturation at 94 °C, followed 40 s at 94 °C, 40 s at 56 °C and 40 s at 72 °C with 30 cycles, a final 10 min extension at 72 °C. The *RAC1* gene [19] was used as an internal control with the same PCR conditions as *ZFP252* gene, but 26 of cycles.

For real-time qPCR of *ZFP252* gene, 2 μg of total RNA was also used with the reverse transcription system (Promega, USA) according to the manufacturer's protocol. The real-time qPCR was performed in an optical 96-well plate with a BIO-RAD iQ5 real-time PCR system (BIO-RAD, USA) as previously described [20] for four stress-related genes *OsDREB1A* [21], *Oslea3* [22], *OsP5CS* [23] and *OsProT* [24], and an internal control*18sRNA* [25].

2.5. Analysis of abiotic stresses tolerance in transgenic plants

The T_2 generation of four ZFP252-ox lines (S3, S6, S7 and S10) and two ZFP252-kd lines (A14 and A17) was used in stress assays as well non-transgenic plants (WT). In order to evaluate their tolerance to salt stress, the seeds of WT and transgenic lines were germinated and grown in 1/2 Murashige and Skoog (MS) medium plus 0 mM and 200 mM NaCl for 12 d [11]. The fresh weight of each*ZFP252* transgenic or WT plant was measured respectively. In addition, the seedlings at the four-leaf stage of the WT and transgenic lines were grown on soil and sand (1:1) mixture, and watered with 500 ml of 100 mM NaCl in the greenhouse. The survival rates of rice seedlings were counted 2 weeks later [26]. As to their tolerance to drought stress, the germinated seeds of WT and transgenic lines were grown on soil and sand (1:1) mixture, supplied with the same volume of water. The seedlings at the four-leaf stage were withheld watering for 14 d, and then re-watering for 7 d, the survival rates of rice plants were counted [17,26].

2.6. Electrolyte leakage assay

The seedlings at the four-leaf stage of the WT and transgenic lines grown on soil and sand (1:1) mixture in the greenhouse were prevented from watering. After 10 d, the third leaves from the top were used for electrolyte leakage assay as described by Guo et al. [27].

2.7. Measurement of the contents of proline and soluble sugars

The seedlings at the four-leaf stage grown on soil and sand (1:1) mixture in the greenhouse, were watered with 500 ml of 100 mM NaCl or prevented from watering. After 10 d of NaCl or drought treatment when the top leaves of WT plants started to slightly roll, the contents of free proline and soluble sugars in WT and transgenic lines without or with stress treatments were determined by sulfosalicylic acid method [28] and anthrone method [29], respectively.

3. Results

3.1. Expression of ZFP252 in transgenic rice plants

We analyzed the expression of *ZFP252* in WT and transgenic lines (T_2 generation) by both semi-quantitative RT-PCR and quantitative real-time PCR. The results showed that the expression level of *ZFP252* in the four ZFP252-ox lines (S3, S6, S7 and S10) was significantly higher than that in WT plants (Fig. 1A and C). The expression of *ZFP252* in two ZFP252-kd lines (A14 and A17) was hardly detected (Fig. 1B and C).

3.2. Overexpression of ZFP252 increases rice tolerance to salt and drought stresses

There was no significant difference in growth rate and fresh weight of seedlings between sense-*ZFP252* or antisense-*ZFP252* transgenic plants and WT when cultured on the 1/2 MS medium (Fig. 2A and B). However when cultured on 1/2 MS medium supplied with 200 mM NaCl or grown in the pots watered with the solution of 100 mM NaCl, the ZFP252-ox lines grew much better than ZFP252-kd lines or WT plants (Fig. 2A and C). Meanwhile the ZFP252-ox lines had higher fresh weight and survival rates than the WT and ZFP252-kd lines (Fig. 2B and D). The results indicated that overexpression of *ZFP252* in rice seedlings enhanced plant tolerance to salinity.

In order to test their tolerance to drought stress, the seedlings of WT, ZFP252-ox lines and ZFP252-kd lines at the four-leaf stage were withheld watering for 14 d, then re-watered for 7 d. Almost all leaves of the WT and transgenic lines A14 and A17 rolled after 14 d of un-watered, while only a few of leaves of transgenic lines S3, S6, S7 and S10 rolled (Fig. 3A).

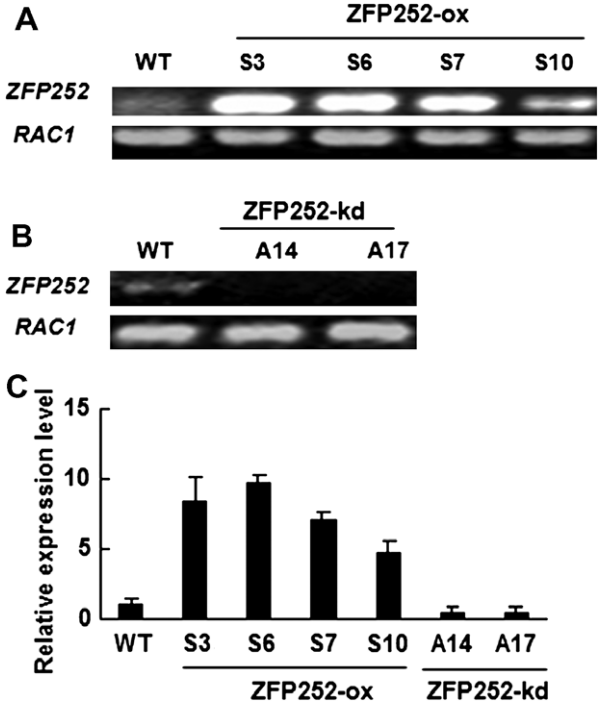

Fig. 1. Expression of *ZFP252* in transgenic rice and WT. (A) and (B) The results of semi-quantitative RT-PCR. (C) The result of real-time qPCR. WT represents non-transgenic plants.

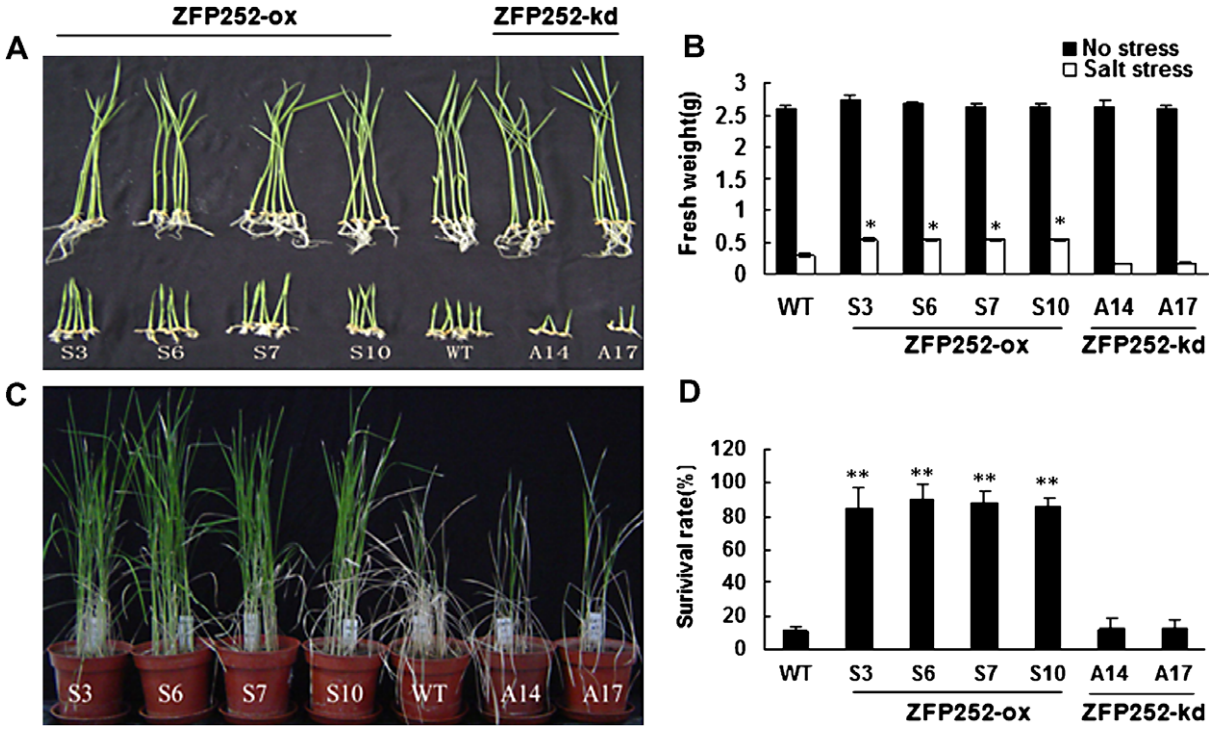

Fig. 2. *ZFP252* conferred salt tolerance in rice. (A) and (B) The seedlings and corresponding fresh weight of ZFP252-ox lines, ZFP252-kd lines and WT germinated and grown on 1/2 MS medium supplied with 0 mM and 200 mM NaCl for 12 d, respectively. (C) and (D) The seedlings and their survival rates of ZFP252-ox lines, ZFP252-kd and WT at the four-leaf stage watered with 500 ml of 100 mM NaCl for 14 d, respectively. Error bars are based on three replicates. The values with significant difference according to *t*-test are indicated by asterisks (*, $P \leqslant 0.05$ and **, $P \leqslant 0.01$).

The survival rates of lines S3, S6, S7 and S10 were 85.32%–90.32%, and significantly higher than those of WT plants (11.23%) and ZFP252-kd lines A14 (17.62%), A17 (15.32%) (Fig. 3B). It was found that the third leaves from top of WT and ZFP252-kd transgenic plants severely rolled after 10 d of water withholding and their relative electrolyte leakages were higher than those from ZFP252-ox (Fig. 3C). These results suggested that damage degree to the cell membrane of WT and ZFP252-kd transgenic plants was higher than that to the ZFP252-ox transgenic under drought stress.

3.3. Accumulation of free proline and soluble sugars in ZFP252 transgenic rice plants

Under the control conditions, the contents of either free proline or soluble sugars had no significant difference between WT and ZFP252-ox or ZFP252-kd transgenic rice lines (Fig. 4A and B). After salt treatment with 100 mM NaCl for 10 d when the top leaves of WT plants began to slightly roll, ZFP252-ox transgenic lines S3, S6, S7 and S10 accumulated more free proline (Fig. 4A) and soluble sugars (Fig. 4B) than WT and ZFP252-kd transgenic lines A14 and A17. The contents of free proline and soluble sugars in the ZFP252-ox lines under salt stress were approximately 2.5–3.5 folds and 8.0–9.6 folds higher than those under the control conditions, respectively. However, the contents of free proline and soluble sugars in WT and ZFP252-kd transgenic plants under salt stress were only 1.3–1.6 folds and 3.3–4.0 folds higher than those under control conditions.

After water withholding for 10 d when the top leaves of WT plants started to slightly roll, it was observed that the contents of free proline and soluble sugars in ZFP252-ox transgenic lines were also significantly higher than those in WT and ZFP252-kd lines (Fig. 4C and D). The contents of free proline and soluble sugars in ZFP252-ox transgenic lines under drought stress were 1.8–2.5 and 4.6–6.8 folds higher than those under control conditions, respectively. However, the free proline and soluble sugars levels in WT and ZFP252-kd transgenic plants under drought stress were only 1.1–1.3 and 2.5–3.1 folds higher than those under control conditions respectively. There was no significant difference in the contents of free proline (Fig. 4C) and soluble sugars (Fig. 4D) between WT, ZFP252-ox and ZFP252-kd transgenic lines before the drought stress.

3.4. Expression of stress-related genes in ZFP252 transgenic rice plants

To further elucidate the possible role of *ZFP252* gene involved in responses to abiotic stresses, we analyzed the expression of several known stress-related genes in *ZFP252* transgenic lines and WT, including *OsDREB1A*, *Oslea3*, *OsP5CS* and *OsProT*. There was no significant difference in the expression levels of *Oslea3*, *OsP5CS* and *OsProT* between *ZFP252* transgenic lines and WT plants under non-treated conditions (Fig. 5B–D). However the *OsDREB1A* mRNA was much more accumulated in ZFP252-ox transgenic lines S3, S6, S7 and S10 as compared with that in ZFP252-kd lines A14, A17 or WT plants under normal conditions (Fig. 5A). Under salt or drought treatments the expression levels of all four stress-related genes in ZFP252-ox transgenic lines was increased more than that in ZFP252-kd lines and WT plants (Fig. 5A–D). It suggested that ZFP252 might be one upstream regulator of these genes mediating expression of some

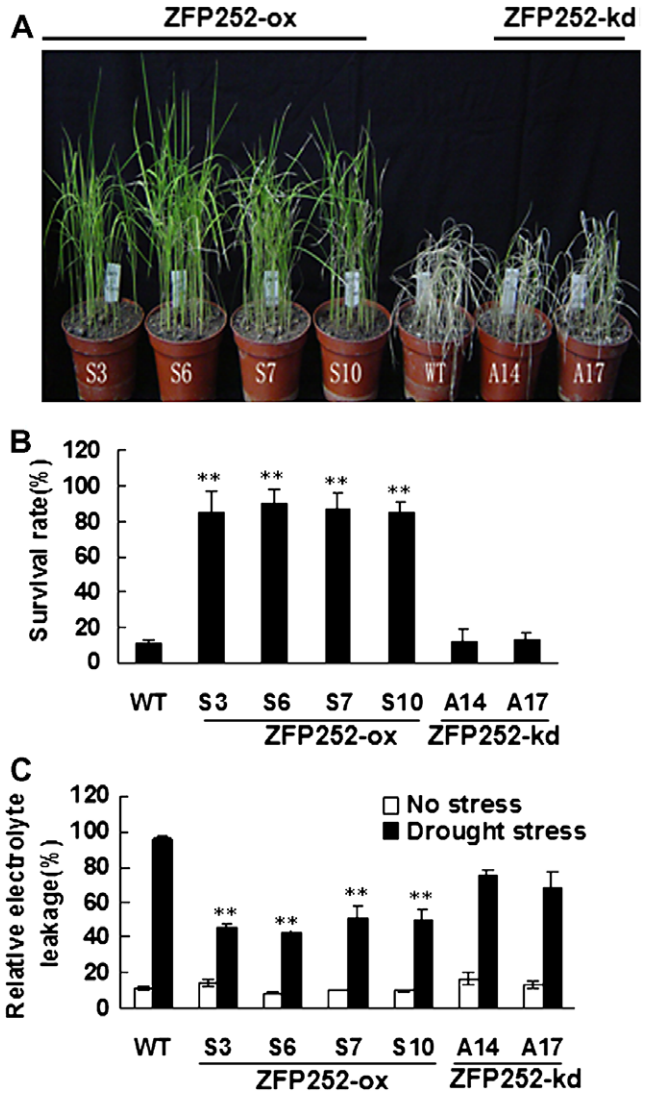

Fig. 3. *ZFP252* conferred drought tolerance in rice. (A) Recovery of the *ZFP252* transgenic plants and WT after 14 d of water withholding at the four-leaf stage, followed by 7 d of re-watering. (B) Survival rates of the plants were calculated for *ZFP252* transgenic plants and WT after drought stress. (C) Relative electrolyte leakage of *ZFP252* transgenic plants and WT after water withholding for 10 d. Error bars are based on three replicates. Values of transgenic plants significantly different from the WT according to *t*-test are indicated by asterisks (**, $P \leqslant 0.01$).

stress-related genes upon rice treated with salt or drought stresses. It acted as a master switch in stress tolerance, and was involved in the complicated network controlling stress-responsive genes.

4. Discussion

The ZFP252 is a TFIIIA-type zinc finger protein that was structurally similar with STZ/ZAT10, an extensively studied zinc finger protein in *Arabidopsis* [10,30]. Overexpression of *STZ/ZAT10* improved plant tolerance to salt and dehydration stresses in transgenic *Arabidopsis* plants and resulted in the growth retardation [30]. In this study, we generated transgenic plants overexpressing *ZFP252* (ZFP252-ox) or knocking-down *ZFP252* (ZFP252-kd) and found that ZFP252-ox plants were more tolerant to salt and drought stress as compared with WT and ZFP252-kd plants but not to cold (data not shown). There were no significant changes in morphological or agronomic traits among ZFP252-ox plants and ZFP252-kd and WT plants (Supplementary Fig.S1, Tables S1 and S2). It indicated that *ZFP252* gene might be more effective in engineering crops with enhanced stress tolerance. The *STZ/ZAT10* has been suggested as one of the downstream regulator of *DREB1A*, a CBF/DREB transcription factor in *Arabidopsis* [30–32]. As there were two CRT/DRE elements within *ZFP252* promoter region (data not shown), ZFP252 might likely be a downstream target of rice CBF/DREB proteins. We found that *OsDREB1A* encoding a DREB protein in rice was responsive to overexpression of *ZFP252* in ZFP252-ox plants, suggesting ZFP252 might be an upstream regulator of *OsDREB1A*. Similarly, another *Arabidopsis* TFIIIA-type zinc finger protein ZAT12 was suggested as an upstream regulator of CBF/DREB proteins [33].

The relative electrolyte leakage, as an index of membrane injury [34], from ZFP252-ox transgenic plants was lower than that from WT or ZFP252-kd transgenic plant under drought stress (Fig. 4C). It implied that the degree to membrane injury from ZFP252-ox transgenic rice plants was lower than from WT and ZFP252-kd transgenic rice plant under drought stress, and the ZFP252 protein could protect the cell membrane integrity of rice plants under drought stress. When suffered with abiotic stresses, many plants can accumulate more compatible osmolytes, such as free proline [35–37] and soluble sugars [38,39]. These osmolytes function as osmoprotectants in the stress tolerance of the plants. Our results showed that the salt and drought stress-induced increases of the contents of free proline and soluble sugars in the ZFP252-ox transgenic plants were higher than those in WT and ZFP252-kd transgenic plants (Fig. 4A–D). Further, the expressions of *OsP5CS* encoding proline synthetase [23] and *OsProT* encoding proline transporter [24] in ZFP252-ox plants were higher than that in WT and ZFP252-kd transgenic plants under salt and drought stress conditions by real-time qPCR analysis (Fig. 5C and D). These findings suggest that enhanced stress tolerance of ZFP252-ox plants might partially be through activating proline synthesis and transport pathways by *ZFP252* in rice under salt and drought stresses. Real-time qPCR showed that *Oslea3* gene encoding a late embryogenesis abundant protein [22] in ZFP252-ox transgenic rice plants was also higher than that in WT and ZFP252-kd transgenic plants under salt and drought stress conditions (Fig. 5B). However, we did not found that overexpression of *ZFP252* in rice could result in enhanced expression of these stress-related genes including *Oslea3*, *OsP5CS* and *OsProT* in *ZFP25* 2 transgenic plants under normal conditions (Fig. 5A–D). One possible explanation is that ZFP252 mediated activation of such stress-related genes accompanied with other stress-responsive regulator(s). Alternatively, there are stress negative regulator(s) with the role in repressing these stress-related genes in rice under normal conditions. The high level of negative regulator(s) may repress expression of stress-related genes activated by ZFP252 in transgenic rice plants under normal conditions, while they would be significantly accumulated in transgenic plants when negative regulators are down-regulated by abiotic stresses. By stress assays, we did not observe that ZFP252-kd lines showed more sensitive to salt and drought stress, as compared with WT plants. The possible explanation is some other zinc

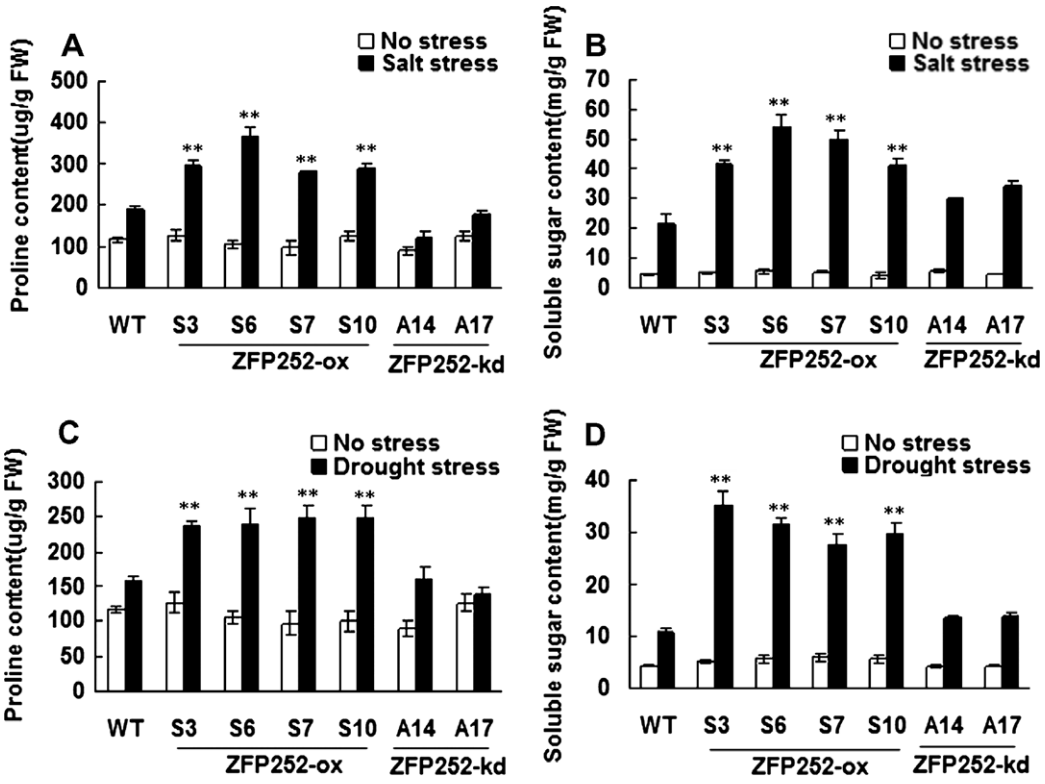

Fig. 4. The contents of free proline and soluble sugars in the *ZFP252* transgenic rice and WT under salt and drought conditions. (A) and (B) The contents of free proline and soluble sugars under 100 mM NaCl stress condition respectively. (C) and (D) The contents of free proline and soluble sugars under drought stress condition respectively. FW means fresh weight. Error bars are calculated on three replicates. Values of transgenic plants significantly different from the WT according to *t*-test are indicated by asterisks (**, $P \leqslant 0.01$).

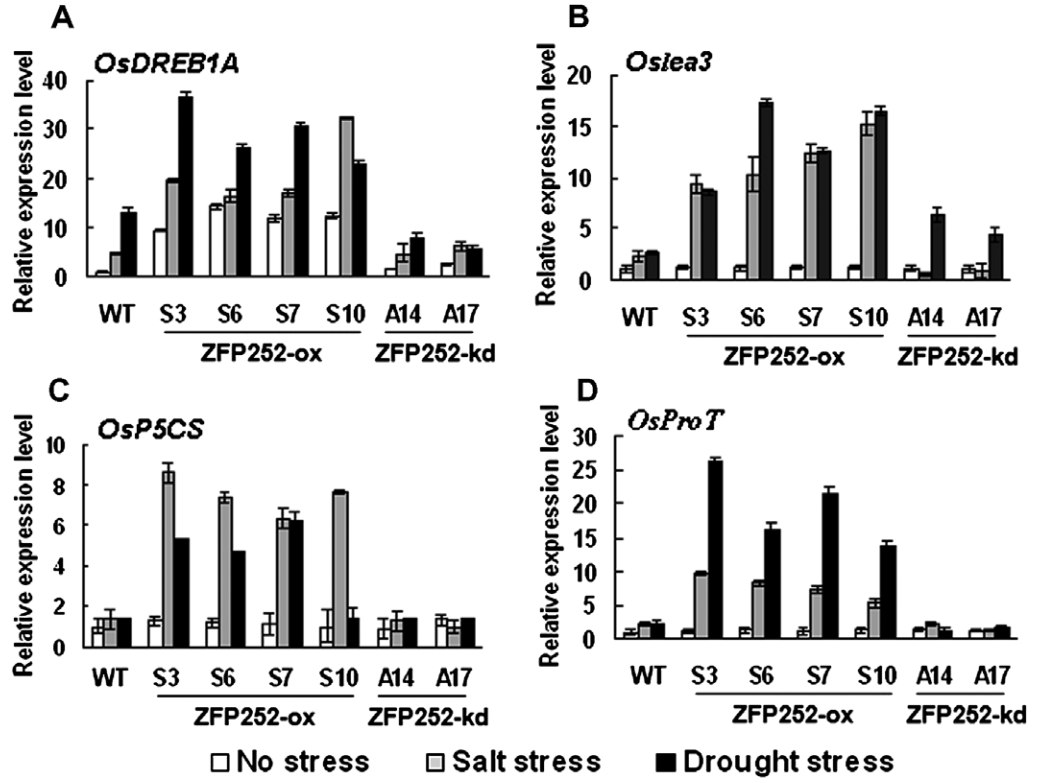

Fig. 5. Expression of stress-related genes in *ZFP252* transgenic and WT rice. Total RNA was extracted from the rice seedlings at four-leaf stage grown under control, salt or drought stress conditions. (A–D) The transcript levels of *OsDREB1A, Oslea3*, *OsP5CS* and *OsProT* were measured by real-time qPCR under normal conditions, 100 mM NaCl or drought treatments for 10 d, respectively. The amplification of *18sRNA* gene was used as an internal control. Error bars are calculated on three replicates.

finger protein could complement the function of ZFP252 in rice under salt and drought stress. Taken together, it was concluded that overexpression of *ZFP252* may contribute accumulation of compatible osmolytes, such as free proline, soluble sugars and LEA proteins that function as osmoprotectants, by regulating expression of stress-related genes in rice under salt and drought stress conditions.

To further understand the ZFP252-mediated signaling pathway in response to abiotic stresses, it will be essential to identify and characterize the downstream and upstream molecules of ZFP252 by microarray analysis, yeast hybrid systems and so on.

Acknowledgements: This work was supported by National Natural Science Foundation of China (Grant Nos. 30571141 and 30600350), the Youth Foundation (Grant No. KJ06033) of Nanjing Agricultural University, the 111 Project (Grant No. B08025) and the Changjiang Scholars and Innovative Research Team in University (PCSIRT).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2008.02.052.

References

[1] Xiao, B., Huang, Y., Tang, N. and Xiong, L. (2007) Over-expression of a LEA gene in rice improves drought resistance under the field conditions. Theor. Appl. Genet. 115, 35–46.
[2] Martínez-Atienza, J., Jiang, X., Garciadeblas, B., Mendoza, I., Zhu, J.K., Pardo, J.M. and Quintero, F.J. (2007) Conservation of the salt overly sensitive pathway in rice. Plant Physiol. 143, 1001–1012.
[3] Xiong, L., Schumaker, K.S. and Zhu, J.K. (2002) Cell signaling during cold, drought, and salt stress. Plant Cell 14, 165–183.
[4] Nakashima, K. and Yamaguchi-Shinozaki, K. (2006) Regulons involved in osmotic stress-responsive and cold stress-responsive gene expression in plants. Physiol. Plantarum. 126, 62–71.
[5] Agarwal, P.K., Agarwal, P., Reddy, M.K. and Sopory, S.K. (2006) Role of DREB transcription factors in abiotic and biotic stress tolerance in plants. Plant Cell Rep. 25, 1263–1274.
[6] Chinnusamy, V., Zhu, J. and Zhu, J.K. (2006) Gene regulation during cold acclimation in plants. Physiol. Plant 126, 52–61.
[7] Miller, J., McLachlan, A.D. and Klug, A. (1985) Repetitive zinc-binding domains in the protein transcription factor IIIA from *Xenopus oocytes*. EMBO J. 4, 1609–1614.
[8] Kim, J.C., Lee, S.H., Cheong, Y.H., Yoo, C.M., Lee, S.I., Chun, H.J., Yun, D.J., Hong, J.C., Lee, S.Y., Lim, C.O. and Cho, M.J. (2001) A novel cold-inducible zinc finger protein from soybean, SCOF-1, enhances cold tolerance in transgenic plants. Plant J. 25, 247–259.
[9] Sugano, S., Kaminaka, H., Rybka, Z., Catala, R., Salinas, J., Matsui, K., Ohme-Takagi, M. and Takatsuji, H. (2003) Stress-responsive zinc finger gene ZPT2-3 plays a role in drought tolerance in petunia. Plant J. 36, 830–841.
[10] Mittler, R., Kim, Y., Song, L., Coutu, J., Coutu, A., Ciftci-Yilmaz, S., Lee, H., Stevenson, B. and Zhu, J.K. (2006) Gain- and loss-of-function mutations in Zat10 enhance the tolerance of plants to abiotic stress. FEBS Lett. 580, 6537–6542.
[11] Huang, J., Yang, X., Wang, M.M., Tang, H.J., Ding, L.Y., Shen, Y. and Zhang, H.S. (2007) A novel rice C2H2-type zinc finger protein lacking DLN-box/EAR-motif plays a role in salt tolerance. Biochim. Biophys. Acta 1769, 220–227.
[12] Huang, J. and Zhang, H.S. (2007) The plant TFIIIA-type zinc finger proteins and their roles in abiotic stress tolerance. Hereditas (Beijing) 29, 915–922.
[13] Akhtar, M.R. (2006) Impact of conservation technologies for sustainability of agriculture. J. Agric. Res. 44, 239–257.
[14] Huang, J., Wang, J.F., Wang, Q.H. and Zhang, H.S. (2005) Identification of a rice zinc finger protein whose expression is transiently induced by drought, cold but not by salinity and abscisic acid. DNA Sequence 16, 130–136.
[15] Guo, S.Q., Huang, J., Jiang, Y. and Zhang, H.S. (2007) Cloning and characterization of RZF71 encoding a C2H2-type zinc finger protein from rice. Hereditas (Beijing) 29, 607–613.
[16] Yoshida, S., Forno, D.A., Cock, J.H. and Gomez, K.A. (1976) Laboratory Manual for Physiological Studies of Rice, third ed, International Rice Research Institute, Philippines, pp. 61–64.
[17] Xiang, Y., Huang, Y. and Xiong, L. (2007) Characterization of stress-responsive CIPK genes in rice for stress tolerance improvement. Plant Physiol. 144, 1416–1428.
[18] Hiei, Y., Ohta, S., Komari, T. and Kumashiro, T. (1994) Efficient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA. Plant J. 6, 271–282.
[19] Reece, K.S., Mcelroy, D. and Wu, R. (1990) Genomic nucleotide sequence of four rice (*Oryza sativa* L.) *actin* genes. Plant Mol. Biol. 14, 621–624.
[20] Huang, J., Wang, M.M., Jiang, Y., Wang, Q.H., Huang, X. and Zhang, H.S. (2008) Stress repressive expression of rice SRZ1 and characterization of plant SRZ gene family. Plant Sci. 174, 227–235.
[21] Dubouzet, J.G., Sakuma, Y., Ito, Y., Kasuga, M., Dubouzet, E.G., Miura, S., Seki, M., Shinozaki, K. and Yamaguchi-Shinozaki, K. (2003) OsDREB genes in rice, *Oryza sativa* L., encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression. Plant J. 33, 751–763.
[22] Moons, A., de Keyser, A. and van Montagu, M. (1997) A group 3 LEA cDNA of rice, responsive to abscisic acid, but not to jasmonic acid, shows variety-specific differences in salt stress response. Gene 191, 197–204.
[23] Igarashi, Y., Yoshiba, Y., Sanada, Y., Yamaguchi-Shinozaki, K., Wada, K. and Shinozaki, K. (1997) Characterization of the gene for delta1-pyrroline-5-carboxylate synthetase and correlation between the expression of the gene and salt tolerance in *Oryza sativa* L. Plant Mol. Biol. 33, 857–865.
[24] Igarashi, Y., Yoshiba, Y., Takeshita, T., Nomura, S., Otomo, J., Yamaguchi-Shinozaki, K. and Shinozaki, K. (2000) Molecular cloning and characterization of a cDNA encoding proline transporter in rice. Plant Cell Physiol. 41, 750–756.
[25] Nakashima, K., Tran, L.S., Nguyen, D., Fujita, M., Maruyama, K., Todaka, D., Ito, Y., Hayashi, N., Shinozaki, K. and 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.
[26] Hu, H., Dai, M., Yao, J., Xiao, B., Li, X.H., Zhang, Q. and 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.
[27] Guo, Y., Xiong, L., Ishitani, M. and Zhu, J.K. (2002) An *Arabidopsis* mutation in translation elongation factor 2 causes superinduction of CBF/DREB1 transcription factor genes but blocks the induction of their downstream targets under low temperatures. Proc. Natl. Acad. Sci. USA 99, 7786–7791.
[28] Troll, W. and Lindsley, J. (1955) A photometric method for the determination of proline. J. Biol. Chem. 215, 655–660.
[29] Morris, D.L. (1948) Quantitative determination of carbohydrates with Drywood's anthrone reagent. Science 107, 254–255.
[30] Sakamoto, H., Maruyama, K., Sakuma, Y., Meshi, T., Iwabuchi, M., Shinozaki, K. and Yamaguchi-Shinozaki, K. (2004) *Arabidopsis* Cys2/His2-type zinc finger proteins function as transcription repressors under drought, cold and high-salinity stress conditions. Plant Physiol. 136, 2734–2746.
[31] Sakamoto, H., Araki, T., Meshi, T. and Iwabuchi, M. (2000) Expression of a subset of the *Arabidopsis* Cys(2)/His(2)-type zinc-finger protein gene family under water stress. Gene 248, 23–32.
[32] Maruyama, K., Sakuma, Y., Kasuga, M., Ito, Y., Seki, M., Goda, H., Shimada, Y., Yoshida, S., Shinozaki, K. and Yamaguchi-Shinozaki, K. (2004) Identification of cold-inducible downstream genes of the *Arabidopsis* DREB1A/CBF3 transcriptional factor using two microarray systems. Plant J. 38, 982–993.
[33] Vogel, J.T., Zarka, D.G., Van Buskirk, H.A., Fowler, S.G. and Thomashow, M.F. (2005) Roles of the CBF2 and ZAT12

transcription factors in configuring the low temperature transcriptome of *Arabidopsis*. Plant J. 41, 195–211.
[34] Morsy, M.R., Almutairi, A.M., Gibbons, J., Yun, S.J. and de Los Reyes, B.G. (2005) The OsLti6 genes encoding low-molecular-weight membrane proteins are differentially expressed in rice cultivars with contrasting sensitivity to low temperature. Gene 344, 171–180.
[35] Liu, J. and Zhu, J.K. (1997) Proline accumulation and salt-stress-induced gene expression in a salt-hypersensitive mutant of *Arabidopsis*. Plant Physiol. 114, 591–598.
[36] Armengaud, P., Thiery, L., Buhot, N., Grenier-De March, G. and Savouré, A. (2004) Transcriptional regulation of proline biosynthesis in *Medicago truncatula* reveals developmental and environmental specific features. Physiol. Plant 120, 442–450.
[37] Liu, K., Wang, L., Xu, Y., Chen, N., Ma, Q., Li, F. and Chong, K. (2007) Overexpression of OsCOIN, a putative cold inducible zinc finger protein, increased tolerance to chilling, salt and drought, and enhanced proline level in rice. Planta 226, 1007–1016.
[38] Gupta, A.K. and Kaur, N. (2005) Sugar signalling and gene expression in relation to carbohydrate metabolism under abiotic stresses in plants. J. Biosci. 30, 761–776.
[39] Chyzhykova, O.A. and Palladina, T.O. (2006) The role of amino acids and sugars in supporting of osmotic homeostasis in maize seedlings under salinization conditions and treatment with synthetic growth regulators. Ukr. Biokhim. Zh. 78, 124–129.