The Arabidopsis Cys2/His2 zinc finger transcription factor ZAT18 is a positive regulator of plant tolerance to drought stress

Mingzhu Yin1,2, Yanping Wang3, Lihua Zhang1, Jinzhu Li1,2, Wenli Quan4, Li Yang1,2, Qingfeng Wang1,* and Zhulong Chan3,4,*

1 Key Laboratory of Aquatic Botany and Watershed Ecology, Wuhan Botanical Garden/Sino-Africa Joint Research Center, Chinese Academy of Sciences, Wuhan, Hubei 430074, China
2 University of Chinese Academy of Sciences, Beijing 100039, China
3 Key Laboratory of Horticultural Plant Biology, Ministry of Education, College of Horticulture and Forestry Sciences, Huazhong Agricultural University, Wuhan, Hubei 430070, China
4 Key Laboratory for Quality Control of Characteristic Fruits and Vegetables of Hubei Province, College of Life Science and Technology, Hubei Engineering University, Xiaogan, Hubei 432000, China

* Correspondence: qfwang@wbgcas.cn or zlchan@mail.hzau.edu.cn

Received 19 January 2017; Editorial decision 7 April 2017; Accepted 13 April 2017

Editor: Qiao Zhao, Tsinghua University

Abstract

Environmental stress poses a global threat to plant growth and reproduction, especially drought stress. Zinc finger proteins comprise a family of transcription factors that play essential roles in response to various abiotic stresses. Here, we found that ZAT18 (At3g53600), a nuclear C2H2 zinc finger protein, was transcriptionally induced by dehydration stress. Overexpression (OE) of ZAT18 in Arabidopsis improved drought tolerance while mutation of ZAT18 resulted in decreased plant tolerance to drought stress. ZAT18 was preferentially expressed in stems, siliques, and vegetative rosette leaves. Subcellular location results revealed that ZAT18 protein was predominantly localized in the nucleus. ZAT18 OE plants exhibited less leaf water loss, lower content of reactive oxygen species (ROS), higher leaf water content, and higher antioxidant enzyme activities after drought treatment when compared with the wild type (WT). RNA sequencing analysis showed that 423 and 561 genes were transcriptionally modulated by the ZAT18 transgene before and after drought treatment, respectively. Pathway enrichment analysis indicated that hormone metabolism, stress, and signaling were over-represented in ZAT18 OE lines. Several stress-responsive genes including COR47, ERD7, LEA6, and RAS1, and hormone signaling transduction-related genes including JAZ7 and PYL5 were identified as putative target genes of ZAT18. Taken together, ZAT18 functions as a positive regulator and plays a crucial role in the plant response to drought stress.

Key words: Drought stress, hormone, RNA sequencing, transcriptome analysis, ZAT18, zinc finger proteins.
Introduction

Drought stress has a negative effect on crop yield and plant distribution. Sesile plants have evolved complex mechanisms to sense and cope with drought conditions. During drought tolerance responses, many drought-responsive genes are activated. Transcription factors (TFs) serve as important mediators in the plants response to drought stress via transcriptional regulation of the downstream genes responsible for plant tolerance, including DREB, ERF, MYB, MYC, WRKY, bZIP, and zinc finger TFs (Rohit et al., 2016).

Zinc finger TFs have been classified into C2H2, C2C2, C2HC, C2C2C2C2, and C2HCC2C2 types according to the number and location of the cysteine and histidine residues (Laity et al., 2001). Genome-wide analysis showed that there were 68 and 67 CCCH family zinc finger TF genes in Arabidopsis and rice (Wang et al., 2008), while 176 and 189 C2H2-type zinc finger TFs were identified in these two species (Englbrecht et al., 2004; Agarwal et al., 2007), indicating that the C2H2-type zinc finger TFs were one of the most abundant TFs in eukaryotes (Laity et al., 2001). The functions of zinc finger proteins (ZFPs) have been well characterized in many plants. Studies revealed that zinc finger TFs played key roles during plant growth and development, and a number of zinc finger TFs were involved in plant abiotic and biotic stress responses (Wu et al., 2008; X.M. Liu et al., 2013). In pepper, a C2H2-type zinc finger TF gene, CaZFP1, was induced after inoculation with bacterial pathogens and treatments with ethylene and abscisic acid (ABA). Overexpression of CaZFP1 in Arabidopsis enhanced resistance against infection by Pseudomonas syringae and improved tolerance to drought stress (Kim et al., 2004). In Thellungiella halophila, the expression level of the ThZF1 gene increased after salinity and drought treatments, and overexpression of ThZF1 in the Arabidopsis azf2 mutant resulted in similar plant growth and reproductive development to that of the wild type (WT) in the presence of salt (Xu et al., 2007). GsZFP1, a C2H2-type zinc finger TF gene from Glycine soja, was induced by ABA and abiotic stress treatments. Transgenic GsZFP1 Arabidopsis plants showed increased tolerance to cold and drought stresses through activation of cold stress-responsive genes and ABA biosynthesis-related genes (Luo et al., 2012). Moreover, overexpression of CgZFP1, a C2H2 ZFP gene from Chrysanthemum, increased salt and drought stress tolerance in Arabidopsis. Under salt or drought conditions, genes involved in osmotic adjustment and reactive oxygen species (ROS) scavenging showed enhanced expression in CgZFP1 transgenic Arabidopsis plants (Gao et al., 2012). The detailed functions of several ZFP genes in rice were also characterized. The results indicated that OsZFP genes were induced by abiotic stress, and overexpression of these genes improved tolerance to a variety of abiotic stresses in rice (Xu et al., 2008; Huang et al., 2009; Zhang et al., 2012, 2014).

It was reported that the C1 family of C2H2-type zinc finger TFs in Arabidopsis were involved in stress response (Kodaira et al., 2011; W.X. Liu et al., 2013). Twenty members of C2H2-type ZFPs belonging to the C1-2i subclass family have been identified in Arabidopsis, namely AZF1, AZF2, AZF3, Zat5, Zat6, Zat7, Zat8, Zat10, Zat11, Zat12, Zat13, Zat14, Zat15, Zat16, Zat17, Zat18 (At3g53600), At5g04390, At1g02040, At2g26940, and At4g04404 (Ciftci-Yilmaz and Mittlar, 2008). AZF1 and AZF2 in Arabidopsis negatively regulated ABA-repressive and auxin-inducible genes under abiotic stress conditions (Kodaira et al., 2011). AZF2 was shown to be a negative regulator of ABA signaling during seed germination (Gabriele et al., 2010). Many studies have reported that ZAT6 co-ordinated phosphate homeostasis and root development, and mediated salt and osmotic stress responses (Devaiah et al., 2007; Mito et al., 2011; X.M. Liu et al., 2013). Further analysis showed that ZAT6 modulated salicylic acid (SA)-related genes and C-REPEAT-BINDING FACTOR (CBF) genes in response to biotic and abiotic stresses, and might be essential for melatonin-mediated freezing stress resistance in Arabidopsis (Shi and Chan, 2014; Shi et al., 2014a).

Overexpression of Zat7 resulted in inhibited growth and increased tolerance to salinity stress, while mutation of Zat7 abolished salinity tolerance without affecting growth suppression (Ciftci-Yilmaz et al., 2007). More interestingly, gain- and loss-of-function mutations of ZAT10 both functioned as positive regulators of plant tolerance to osmotic and salt stresses (Mittler et al., 2006). ZAT11 regulated paraquat-induced programmed cell death in Arabidopsis thaliana (Muhammad et al., 2013). Recently, overexpression of ZAT11 was shown to enhance the elongation of primary roots, but reduced resistance to nickel ion (Ni^{2+}) (Liu et al., 2014).

To date, the functions of ZAT18 in plant response to abiotic stress remain unclear. In this study, we found that ZAT18 positively modulated drought stress tolerance. Overexpression of ZAT18 improved seed germination after mannitol treatment and drought tolerance after withholding water. Physiological and transcriptomic changes were monitored after drought treatment. The results suggested that ZAT18 could play a crucial role in plant responses to drought stress.

Materials and methods

Plant materials and growth conditions

The A. thaliana Columbia-0 (Col-0) ecotypes, and the T-DNA insertion lines of SALK_027144C and SALK_132289C obtained from the Arabidopsis Biological Resource Center were used in this study. Arabidopsis seeds were surface-sterilized with 50% (v/v) bleach with 0.1% (v/v) Triton X-100, and then washed four times with sterile water. After stratification at 4 °C in the dark for 3 d, seeds were sown on Murashige and Skoog (MS) medium containing 3% (w/v) sucrose and incubated in a growth chamber. The growth chamber was controlled at 21–23 °C, 100 μmol photons m^{-2} s^{-1}, 60% relative humidity, and 16 h light/8 h dark cycles.

Constructs and generation of transgenic plants

For the ZAT18 plasmid construct, the coding sequence of ZAT18 was amplified using the primers ZAT18-F and ZAT18-R, then the PCR products was inserted into XbaI/KpnI sites of the pCAMBIA1305 vector. For the ProZAT18::GUS plasmid construct, a fragment of ~2.4 kb of the 5’ upstream region of the AT3G53600 gene was PCR amplified using the primers ZAT18-Pro-F and ZAT18-Pro-R, then the PCR products were inserted into SalI/Xmnl sites of the pBI101 vector. For the 35S::ZAT18-GFP plasmid construct, the coding sequence of ZAT18, in which the termination codon was removed, was amplified.
ZAT18 positively regulates plant drought stress

using the primers ZAT18-F and ZAT18-R, then the product was cloned into the pGFP vector using the restriction enzymes XbaI and KpnI. The resulting vectors were mobilized into Agrobacterium tumefaciens GV3101. Transformation of plants was achieved by the floral-dip method. The T1 transgenic seeds were selected on MS agar medium containing 50 mg l−1 hygromycin. Each T1 plant was individually collected. Selected T2 plants were propagated and confirmed by quantitative real-time PCR (qRT-PCR) analysis.

Subcellular localization analysis and β-glucuronidase (GUS) staining

For subcellular localization analysis, mesophyll protoplasts of 4-week-old Col-0 plants and polyethylene glycol-mediated transformation were performed according to the methods described by Yoo et al. (2007). Protoplasts were transfected with 35S::ZAT18-GFP plasmid. Transformed protoplasts were incubated at 22 °C in the dark for 16–24 h to allow accumulation of green fluorescent protein (GFP) or GFP fusion proteins. The fluorescence was examined at 488 nm by a laser scanning confocal microscope (TCS SP8, Leica, Germany). The method of GUS staining is described by Jefferson et al. (1987). ProZAT18::GUS plants after different periods were immersed in 90% acetone for 20 min, and then stained in GUS staining solution [1 mM X-Gluc, 0.1 M phosphate-buffered saline (PBS), pH 7.0; 2 mM potassium ferricyanide, 2 mM potassium ferrocyanide, 10 mM EDTA] at 37 °C overnight. The plants were decolorized by 70% ethanol overnight. Images were taken using an anatomical lens (SZX16 SZX10, Olympus, Japan). The primers are all listed in Supplementary Table S1 at JXB online.

Drought treatment, water loss, and leaf water content measurement

For the drought tolerance test, water was withheld from 7-day-old plants in pots. The plants were re-watered after 21 d drought treatment and survival rates were then measured. To analyze leaf water status, leaf water loss and leaf water content in vivo were determined. The detached leaves grown under control conditions were weighed at 1 h intervals for up to 8 h as described by Quan et al. (2016). For measurement of leaf water content, the leaf samples were collected at different time points (7, 14, and 21 d) under control and drought stress, and the FW was immediately measured. The DW was quantified after 16 h incubation at 80 °C, and the formula for calculation of leaf water content (LWC) was as follows: LWC(%)=(FW−DW)/FW×100 (Shi et al., 2012).

Electrolyte leakage (EL), malondialdehyde (MDA) and ROS contents, and measurement of the activities of antioxidant enzymes

For EL analysis, the detached leaves of control and drought-treated plants at different intervals were incubated in 15 ml of 0.1 M

---

**Fig. 1.** Expression and phylogenetic analysis of the C1-2i subfamily of C2H2 zinc finger transcription factors under abiotic stress conditions. (A) Expression level changes of the C1-2i subfamily of C2H2 zinc finger transcription factors by different abiotic stresses in the root and shoot. The publicly available microarray data were obtained from The Bio-Analytic Resource for Plant Biology [http://bar.utoronto.ca/affydb/cgi-bin/affy_dbi_express_browser_in.cgi]. (B) Maximum likelihood tree of C1-2i proteins. (C) Expression of ZAT18 affected by dehydration through real-time PCR.
water, and shaken at room temperature for 6 h. The $C_i$ was measured by a conductivity meter (Leici-DDS-307A, Shanghai, China). The detached leaves were boiled for 20 min and the $C_{max}$ was determined after cooling to room temperature: relative EL ($\%$) = ($C_i$/ $C_{max}$)×100 (Wang et al., 2013). The concentration of H$_2$O$_2$ was determined as described (Shi et al., 2012). Briefly, 1 ml of the above supernatant was dissolved in 1 ml of 0.1% titanium sulfate in 20% H$_2$SO$_4$ (v/v) thoroughly for 10 min, and then centrifuged at 12 000 g for 10 min at room temperature. The absorbance of the supernatant was measured at 410 nm using the known concentration of H$_2$O$_2$ as control. For the measurement of MDA, 0.5 g leaf samples were ground using liquid nitrogen, and then extracted in 5% trichloroacetic acid (TCA; w/v). The supernatants were centrifuged at 12 000 g for 10 min, then 2 ml of 0.67% thiobarbituric acid (TBA) (w/v) was added. Well mixed solutions were boiled at 100 °C for 30 min, and the absorbance of the supernatant was measured at 450, 532, and 600 nm after centrifugation. The concentration of MDA was calculated as described (Li et al., 2011). The catalase (CAT) and peroxidase (POD) activities in the samples were determined using a CAT Assay Kit (A007-1, Nanjing Jiancheng Bioengineering Institute, China) and a Plant POD Assay Kit (A084-3, Nanjing Jiancheng Bioengineering Institute), respectively, according to the manufacturer’s instructions.

Quantitative real-time PCR

qRT-PCR was performed with SYBR green fluorescence and used a CFX96TM Real Time System (Bio-Rad, California, USA). The expression levels of target genes were standardized with ubiquitin 10 (UBQ10, AT4G05320). The primers used in qRT-PCR were designed using the web tool Integrated DNA Technologies (http://sg.idtdna.com/scitools/Applications/RealTimePCR/) and are listed in Supplementary Tables S3 and S4. Experiments were repeated three times.

Transcriptomic analysis

Arabidopsis WT and two ZAT18 overexpression (OE) lines were grown in moist soil for 7 d. Drought stress was imposed by withholding water for 10 d. The rosette leaves of control and drought-treated WT and ZAT18 OE plants were then collected for RNA isolation. RNA purity and integrity were checked using the NanoPhotometer® spectrophotometer (IMPLEN, CA, USA) and RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, USA), respectively. RNA sequencing analysis was performed by the Novogene Corporation (Beijing, China). A total amount of 3 μg of RNA was used for generation of sequencing libraries using the NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA) following the manufacturer’s recommendations, and index codes were added to attribute sequences to each sample. After cluster generation, the library preparations were sequenced on an Illumina HiSeq platform and 125 bp/150 bp paired-end reads were generated. Clean reads were obtained by removing low quality reads, reads containing adaptor, and poly-N from raw data. At the same time, Q20, Q30, and the GC content of the clean data were calculated. The index of the Arabidopsis genome was built using Bowtie v2.2.3, and paired-end clean reads were aligned to the reference genome using TopHat v2.0.12. HTSeq v0.6.1 was used to count the read numbers mapped to each gene. Then the FPKM (fragments per kilobase of transcript sequence per millions base pairs sequenced) of each gene was calculated based on the length of the gene and the read counts mapped to this gene. Differential expression analysis of drought stress versus control conditions was performed using the DESeq R package (1.18.0). The resulting $P$-values were adjusted using the Benjamini and Hochberg’s approach for controlling the false discovery rate. Genes with an adjusted $P$-value ≤0.05 found by DESeq and fold change ≥2 were assigned as differentially expressed. Two biological replicates were used for each sample. The clean data were submitted to the Gene Expression Omnibus (GEO) database with the accession number GSE 93979.

Gene Ontology (GO) and pathway enrichment analyses

All differentially expressed genes with a $P$-value ≤0.05 and fold change ≥2 were loaded and annotated in the Classification SuperViewer Tool (http://bar.utoronto.ca/ntools/cgi-bin/ntools_classification_superviewer.cgi) (Provart and Zhu, 2003). Functional categories of every gene and pathway were assigned using MapMan (http://mapman.gabipd.org/web/guest/mapmanstore) as the classification source (Thimm et al., 2004). For GO term enrichment

Fig. 2. GUS staining of ProZAT18::GUS transgenic plants in different organs. In the transgenic ProZAT18::GUS plants, ZAT18 was detected in hypocotyls on the fourth day of seed germination (c), it was partially expressed in leaves, hypocotyls and roots at 8-d-old seedling (a, b, h), ZAT18 was found to be strongly expressed in leaves and stem apex at 10-d-old seedling (d). During flowering period, ZAT18 was predominantly expressed in stigmas, styles, siliques and stems (e–g). Scale bar, 1 mm.
ZAT18 positively regulates plant drought stress

Hierarchical cluster analysis

The data sets of specific genes were imported for hierarchical cluster analysis. An uncentered matrix and complete linkage method were used with the CLUSTER program (http://bonsai.hgc.jp/~mdehoon/software/cluster/software.htm) (de Hoon et al., 2004). Resulting tree figures were displayed using Java Treeview (http://jtreeview.sourceforge.net/) as described by Chan et al. (2012).

Statistical analysis

All of the experiments in this study were repeated three times, and the values presented are means ±SEs. For each independent experiment, the leaf sample extract was derived from the leaves of at least 15 plants. Asterisks above the columns in figures indicate significant differences relative to the WT at $P<0.05$ (t-test).

Results

ZAT18 transcript was induced by abiotic stress treatment

Expression level changes of all 20 C1-2i subclass family C2H2 zinc finger TFs in Arabidopsis were analyzed based on publicly available microarray data (Winter et al., 2007). Eighteen genes, with the exception ZAT7 (At3g46090) and ZAT8 (At3g46080), were found in these data. The results showed that AZF2, ZAT6, ZAT10, ZAT12, ZAT11, ZAT16, and ZAT18 (At3g53600) were highly up-regulated especially by cold, osmotic, salt, and drought stresses in root tissue, while AZF1, AZF2, ZAT6, ZAT10, ZAT11, ZAT12, and ZAT18 were highly induced by cold, osmotic, salt, drought, UV-B, and wounding in shoot tissue (Fig. 1A). In both shoot and root tissue, expression of ZAT18 was activated by most of the abiotic stress treatments. Phylogenetic analysis showed that ZAT18 shared high similarity with ZAT11 (Fig. 1B).

qRT-PCR results revealed that the ZAT18 expression level increased significantly after dehydration treatment, and reached the highest level at 3 h of treatment (Fig. 1C).

In the ProZAT18::GUS transgenic plant, ZAT18 was preferentially expressed in stems, siliques, and vegetative rosette leaves, while lower expression levels could be detected in cotyledons, hypocotyl, and roots (Fig. 2). The subcellular location of ZAT18 was detected. The results revealed that ZAT18–GFP fusion protein fluorescence was predominantly localized in the nucleus. In contrast, the control GFP protein without ZAT18 was distributed in the nucleus and cytoplasm, indicating that ZAT18 was a nuclear-localized protein (Fig. 3).

Overexpression of ZAT18 enhanced plant tolerance to drought

To characterize the function of ZAT18 in response to drought stress, we generated ZAT18 OE plants (Supplementary Fig. S1). Germination assay showed that no significant difference was observed between transgenic and WT plants under normal growth conditions (Fig. 4C, D). However, in the presence of 200 mM and 300 mM mannitol, the ZAT18 OE lines showed less sensitivity than WT plants during the seed germination stage (Fig. 4E–H). On the fourth day after sowing in the presence of 300 mM mannitol, ~65% of WT seeds germinated with emerged radicles, while >95% of ZAT18 OE seeds germinated with emerged radicles (Fig. 4G). Additionally, the OE lines showed significantly higher percentages of green cotyledons after 200 mM and 300 mM mannitol for 6 d and 9 d, respectively (Fig. 4A, B). These results indicated that ZAT18 might act as a positive regulator in osmotic stress during seed germination.

Fig. 3. Subcellular location of ZAT18. GFP signals were detected in protoplasts transformed with 3SS::GFP-ZAT18.
We then investigated the drought tolerance of \textit{ZAT18} OE lines. Water was withheld for 3 weeks from 1-week-old WT and OE plants grown under normal conditions, and then they were rehydrated for 3 d. As shown in Fig. 5A, the Col-0 plants showed serious withering and damage after drought treatment, and the survival rate was 16.47\% after 3 d rehydration. \textit{ZAT18} OE lines appeared relatively healthy and exhibited high vigor after 3 weeks of drought treatment. The survival rate of \textit{ZAT18} OE lines was ~90\% after re-watering (Fig. 5B). The EL did not show significant changes in the control condition between \textit{ZAT18} OE line and the WT. However, 21 d of drought stress caused severe cell membrane damage in WT plants as evidenced by a significantly increased EL, while \textit{ZAT18} OE lines exhibited no significant EL changes (Fig. 5C). The MDA content in \textit{ZAT18} OE plants was significantly lower than in the WT at 21 d after drought treatment (Fig. 5D). Moreover, \textit{ZAT18} OE plants showed significantly lower water loss rates when compared with WT plants (Fig. 6A). The leaf water content of \textit{ZAT18} OE plants was significantly higher than that of Col-0 plants after 3 weeks of withholding water (Fig. 6B). Taken together, these data suggested that the \textit{ZAT18} OE plants showed improved tolerance to severe drought stress.

\textbf{Overexpression of \textit{ZAT18} decreased ROS production in response to drought stress}

Drought stress could trigger ROS accumulation in plants, and an efficient ROS detoxification system was essential for plants
ZAT18 positively regulates plant drought stress to survive. In this study, we found that the H$_2$O$_2$ content of the WT was significantly higher than that in ZAT18 OE plants after drought treatment for 14 d and 21 d (Fig. 7A). Enzyme activities of CAT and POD were measured after drought stress treatment. The results showed that no significant differences were found for the CAT and POD activities in ZAT18 OE and WT plants under control and 7 d drought treatment conditions. However, CAT and POD activities in ZAT18 OE

---

**Fig. 5.** Overexpression of ZAT18 increased drought stress tolerance in Arabidopsis. (A) Growth of ZAT18 OE and WT plants after drought treatment and rehydration. (B) Survival rate of ZAT18 OE and WT plants after drought treatment. (C) Electrolyte leakage of ZAT18 OE and WT plants. (D) MDA content changes of ZAT18 OE and WT plants. (This figure is available in colour at JXB online.)

**Fig. 6.** Overexpression of ZAT18 affected water loss in Arabidopsis. (A) Leaf water loss of ZAT18 OE and WT plants. (B) Leaf water content of ZAT18 OE and WT plants after 21 d drought treatment.
plants were significantly higher than those in Col-0 plants after 14 d and 21 d drought treatment (Fig. 7B, C).

**Mutation of ZAT18 slightly decreased drought tolerance**

Two ZAT18 SALK lines were obtained from TAIR (Fig. 8A). Expression analysis indicated that both lines are knock-down lines, and SALK_132289C has a 90% decreased ZAT18 level (Fig. 8B). Drought tolerance test results showed that both SALK lines were relatively sensitive to drought stress and the survival rates of SALK lines were relatively lower than those of the WT (Fig. 8C, D). EL analysis indicated that SALK_132289C suffered from drought tolerance when compared with the WT and SALK_027144C, as evidenced by a significantly higher EL (Fig. 8E). These results indicated that mutation of ZAT18 impaired drought stress tolerance.

Transcriptomic profiling analysis of ZAT18 OE plants after drought treatment

To dissect ZAT18-modulated drought stress tolerance, RNA sequencing (RNA-seq) analysis was performed to identify candidate ZAT18 target genes. In total, eight samples with two biological replicates per genotype/treatment combination were used for RNA-seq analysis. At least 2 G clean bases were generated for each sample. Comparative analysis revealed that 1777 genes were transcriptionally affected by ZAT18 transgene or drought treatment (Supplementary Table S2). We selected 11 genes which were modulated by the ZAT18 transgene and performed qRT-PCR analysis. The expression ratios measured by RNA-seq and by qRT-PCR were highly correlated. The trends of both up-regulated and down-regulated expression for the comparisons were similar (Supplementary Fig.
Expression of ZAT18 (AT3G53600) showed a >60-fold change in ZAT18 OE lines, indicating that RNA-seq data are at least partially reliable (Supplementary Table S2). The results showed that overexpression of ZAT18 modulated expression level changes of 423 and 561 genes under control and drought stress conditions, respectively (Fig. 9A). Drought stress treatment changed expression of 971 genes, with 768 up-regulated and 203 down-regulated. Comparatively fewer genes (583 up-regulated and 184 down-regulated) were changed by drought treatment in ZAT18 OE lines, indicating that several genes were constitutively activated in ZAT18 OE lines (Fig. 9A).
Overlapping analysis indicated that 246 and 56 genes were up- and down-regulated in both ZAT18 OE lines and the WT after drought treatment (ZAT18–drought versus ZAT18–control; WT–drought versus WT–control), while 55 and 83 genes were up- and down-regulated by the ZAT18 transgene before and after drought treatment (ZAT18–control versus WT–control; ZAT18–drought versus WT–drought) (Fig. 9B, C). These co-regulated genes in ZAT18 OE lines were the candidate targets of ZAT18 and are listed in Supplementary Table S3.

Enriched GO terms and pathways

GO term analysis was performed for genes modulated by the ZAT18 transgene and drought treatment. For biological process GO terms, signal transduction, response to stress, other biological processes, and response to abiotic or biotic stimulus were enriched. For cellular components GO terms, cell wall, plasma membrane, other cellular components, and extracellular were enriched. For molecular function GO terms, transcription factor activity, receptor binding or activity, and kinase activity were enriched (Fig. 10). Moreover, pathways affected by the ZAT18 transgene and drought treatment were analyzed. The results showed that five pathways were over-represented for genes affected by both ZAT18 transgene and drought treatment, including hormone metabolism, miscellaneous, stress, RNA, and signaling. Interestingly, pathways of metal handling and biodegradation of xenobiotics were enriched after drought treatment, but not by the ZAT18 transgene (Table 1).

Identification of candidate target genes of ZAT18

In total, 138 genes were transcriptionally modulated by the ZAT18 transgene under both control and drought stress conditions (Fig. 11A; Supplementary Table S3). Functional analysis revealed that the enriched pathways included tetrapyrrole synthesis, signaling, cell wall, hormone metabolism, major CHO metabolism, stress, and lipid metabolism (Fig. 11B), and enriched GO terms included signal transduction, response to stress, response to abiotic or biotic stimulus, other biological processes, and transport (Fig. 11C).

Among these candidate genes, 22 of them were up-regulated and 1 of them was down-regulated in all four comparisons. Additionally five drought-inducible genes were down-regulated by the ZAT18 transgene (Table 2; Fig. 9B, C). Several genes including RAS1, ERD7, LEA6, COR47, ARCK1, and At4g35985 were directly involved in plant abiotic stress response. Other genes were key components in hormone signaling transduction including JAZ7 and SAUR79, while WRKY18, NAC081, DDF1, WRKY70, and MYB48 were TF genes which play vital roles in stress response and flavonol biosynthesis (Table 2).
Discussion

As transcription repressors, C2H2 ZFPs have been functionally well characterized. As a large gene family, ZFPs play key roles in response to various stresses. Expression of C2H2 zinc finger TFs was induced by different abiotic stresses. *AZF1* and *ZAT10* were strongly induced by NaCl or cold treatment, but weakly by ABA treatment. *AZF2* was induced by NaCl or ABA treatment but weakly by low temperature (Hideki et al., 2000). Overexpression of *ZAT16* enhanced drought, NaCl, and cold tolerance (Shi et al., 2014). Overexpression of *ZAT10* conferred salt tolerance (Sakamoto et al., 2004). Overexpression of *ZAT6* enhanced drought, NaCl, and cold tolerance (Shi et al., 2014). Overexpression of *ZAT6* conferred improved drought tolerance (Fig. 5A) and mutation of *ZAT10* produced relatively decreased drought tolerance in Arabidopsis. These results indicated that *ZAT18* functioned as a positive drought stress regulator.

Plants respond to drought stress from the cellular to the whole-plant level. Water loss is crucial for plant tolerance to drought stress. In this study, *ZAT18* OE plants had a lower water loss rate and higher leaf water content than the WT under drought stress conditions for 21 d (Fig. 7A, B). The results indicated that *ZAT18* OE lines maintained a higher water status to alleviate water deficiency and showed improved tolerance to drought stress when compared with the WT. The EL is the index of membrane injury. MDA is the final product of lipid peroxidation and its content can reflect stress tolerance of plants. Lower EL and MDA in *ZAT18* OE plants indicated that *ZAT18* OE plants suffered less cell injury than the WT (Fig 6D, E). These results were consistent with the higher survival rate of *ZAT18* OE plants after drought stress treatment (Fig. 6B).

In response to stress, plants activate metabolic processes and accumulate osmolytes that are beneficial to retain water and antioxidants that protect cells from stress-related ROS (Kang et al., 2011). A series of ROS, such as H$_2$O$_2$ and O$_2^-$, were produced under stress conditions, resulting in lipid peroxidation, protein oxidation, DNA fragmentation, enzyme inhibition, and cell death (Apel and Hirt, 2004; Wang et al., 2009). Significantly lower H$_2$O$_2$ levels of *ZAT18* OE plants were observed after 21 d of drought treatment, suggesting that the *ZAT18* OE plants suffered less oxidative damage from drought stress (Fig. 8A). In order to scavenge stress-induced ROS, plants develop a complex antioxidative defense system, which is composed of the non-enzymatic and enzymatic antioxidants. This system is crucially important for plants to survive under severe stress conditions (Mittler, 2002; Foyer and Noctor, 2005; Sharma

Table 1. MapMAN pathway enrichment analysis of genes affected by ZAT18 transgene and drought stress treatment

Differentially expressed genes (fold change ≥2 and P-value ≤0.05) were annotated using the Classification SuperViewer Tool. MapMAN was used as the classification source.

| MapMAN pathway                      | ZAT18–control versus WT–control | ZAT18–drought versus WT–drought | ZAT18–drought versus ZAT18–control | WT–drought versus WT–control |
|-------------------------------------|----------------------------------|---------------------------------|-------------------------------------|-------------------------------|
|                                     | NF  | P-value  | NF  | P-value  | NF  | P-value  | NF  | P-value  |
| hormone metabolism                 | 3.81 | 0.0000  | 2.10 | 0.0012  | 3.07 | 0.0000  | 2.30 | 0.0000  |
| misc                                | 1.54 | 0.0047  | 1.88 | 0.0000  | 2.34 | 0.0000  | 1.24 | 0.0140  |
| stress                              | 1.42 | 0.0240  | 2.58 | 0.0000  | 1.56 | 0.0010  | 1.49 | 0.0010  |
| RNA                                 | 1.59 | 0.0001  | 1.08 | 0.0470  | 1.35 | 0.0005  | 1.24 | 0.0022  |
| signalling                          | 2.38 | 0.0090  | 2.98 | 0.0000  | 1.31 | 0.0140  | 1.70 | 0.0000  |
| secondary metabolism                | –    | –  | 2.15 | 0.0021  | 2.36 | 0.0001  | 1.48 | 0.0240  |
| transport                           | –    | –  | 1.52 | 0.0095  | 1.71 | 0.0003  | 1.79 | 0.0000  |
| development                         | 1.41 | 0.0500  | –    | –    | 1.44 | 0.0150  | 1.66 | 0.0007  |
| cell wall                           | 1.73 | 0.0240  | 1.85 | 0.0061  | –    | –    | 1.70 | 0.0026  |
| minor CHO metabolism                | 3.20 | 0.0160  | –    | –    | –    | –    | 1.95 | 0.0410  |
| S-assimilation                      | –    | –  | –    | –    | 1.73 | 0.0320  | –    | –    |
| metal handling                      | –    | –  | –    | –    | 4.75 | 0.0001  | 4.16 | 0.0001  |
| Biodegradation of xenobiotics       | –    | –  | –    | –    | 1.69 | 0.0220  | 3.70 | 0.0380  |
| redox                               | –    | –  | –    | –    | 0.68 | 0.0370  | –    | –    |
| cell                                | –    | –  | –    | –    | –    | –    | 1.97 | 0.0110  |
| not assigned                        | 0.88 | 0.0081  | 0.73 | 0.0000  | 0.83 | 0.0001  | 0.91 | 0.0038  |
| micro RNA, natural antisense, etc   | 0.34 | 0.0500  | –    | –    | –    | –    | –    | –    |
| protein                             | 0.45 | 0.0000  | 0.49 | 0.0000  | 0.45 | 0.0000  | 0.65 | 0.0000  |
| PS                                  | –    | –  | 0.0310 | –    | –    | 0.83 | 0.0440  |
| DNA                                 | 0.17 | 0.0000  | 0.11 | 0.0000  | 0.16 | 0.0000  | 0.13 | 0.0000  |

Red font indicated up-regulation and blue font indicates down-regulation.
Scales of normalized frequency (NF) are as follows: ⬤ ≥2, ⬤ 1-2, ⬤ 0.5-1, ⬤ <0.5.
As antioxidant enzymes, POD and CAT are responsible for detoxification of H\textsubscript{2}O\textsubscript{2}. Increased enzymes activities would decrease ROS levels (Apel and Hirt, 2004; Ouyang et al., 2010). ZAT18 OE plants exhibited significantly higher POD and SOD activities after drought treatment (Fig. 8B, C). Transcriptomic analysis showed that the peroxidases AT5G64120 and AT3G01420 were highly induced by the ZAT18 transgene at the gene expression level (Supplementary Table S2).

Among 20 members of the C2H2-type ZFP genes belonging to the C1-2i subclass family, evidence showed that AZF1, AZF2, ZAT6, ZAT7, ZAT10, ZAT11, and ZAT12 were involved in plant abiotic stress response (Mittler et al., 2006; Ciftci-Yilmaz et al., 2007; Devaiah et al., 2007; Gabriele et al., 2010; Kodaira et al., 2011; Mito et al., 2011; X.M. Liu et al., 2013; Muhammad et al., 2013; Liu et al., 2014; Shi and Chan, 2014; Shi et al., 2014a). Previously we determined that ZAT6 binds directly to the TACAAAT motifs in the promoter region of pathogen-related genes and CBF1 (Shi and Chan, 2014; Shi et al., 2014a). However, the target genes of most C2H2-type zinc finger TFs remain elusive. In this study, we identified 423 and 561 genes which showed significant changes in ZAT18 OE lines before and after drought treatment through RNA-seq analysis (Fig. 9A). Several stress-responsive genes and hormone signaling transduction-related genes were transcriptionally modulated and characterized as putative target genes of ZAT18. Among them, ERD7, LEA6, COR47/IRD17, and RAS1 were up-regulated by the ZAT18 transgene. ERD genes are a group of plant genes induced by plant stress and ABA (Rai et al., 2016). LEA genes are not only highly expressed during late stages of seed development, but are also induced by abiotic stress conditions (Babu et al., 2004; Yu et al., 2016). COR47 is a cold- and drought-inducible gene which contains a cis-acting element called DRE/CRT (for drought/cold-responsive element) which is critical for drought-induced gene expression (Chinnusamy et al., 2004). CBF3/DREB1A and DREB2A showed 3.3- and 2.6-fold changes in the ZAT18 OE line under control conditions (Supplementary Table S2). Response to ABA and Salt (RAS1) is an ABA- and salt stress-inducible gene and encoded a previously undescribed plant-specific protein, which required a functional ABA signaling pathway (Ren et al., 2010). Moreover, several genes involved in hormone signaling transduction pathways were identified as candidate ZAT18 target genes including JAZ7 and PYL5. JAZ7 is one of the more enigmatic members of the family of 13 JAZ protein genes of Arabidopsis which mediated dark-induced senescence and abiotic stress response (Christine, 2016). PYL5 is an ABA receptor gene, and overexpression

---

**Fig. 11.** Identification of candidate target genes of ZAT18. In total, 138 genes were transcriptionally modulated in ZAT18 OE lines under both control and drought stress conditions. (A) Cluster analysis of ZAT18 candidate target genes. (B) Pathway enrichment analysis of ZAT18 candidate target genes. (C) GO term enrichment analysis of ZAT18 candidate target genes.
ZAT18 positively regulates plant drought stress

of PYL5 improved drought stress tolerance and increased antioxidant enzyme activity and osmolyte levels (Shi et al., 2014b). Transcriptomic analysis indicated that GO terms including signal transduction, response to stress, and response to abiotic or biotic stress were enriched in ZAT18 OE lines (Fig. 10). Pathway analysis results showed that hormone metabolism, stress, and signaling were over-represented in ZAT18 OE lines (Table 1). These results indicated that the ZAT18 transgene modulated hormone signaling transduction pathways which might activate the downstream stress response.

In conclusion, we partially dissected the functions of ZAT18 in drought stress responses. ZAT18 overexpression enhanced osmotic stress responses in seed germination and improved drought stress tolerance. The ZAT18 transgene increased leaf water content and decreased ROS content. Transcriptomic analysis showed that genes involved in the hormone signaling transduction pathway and stress response were putative targets of ZAT18. Further research on the detailed mechanisms of how ZAT18 modulates the target genes is needed.

Supplementary data

Supplementary data are available at JXB online.

Fig. S1. ZAT18 expression level in wild-type and ZAT18 OE plants by qRT-PCR.

Fig. S2. Expression level changes of ZAT18 candidate target genes by qRT-PCR.

Table 2. Genes affected by both the ZAT18 transgene and drought stress treatment

Red font means up-regulation while blue font means down-regulation

| AGI     | ZAT18–control versus WT–control | ZAT18–drought versus WT–drought | WT–drought versus WT–control | ZAT18–drought versus ZAT18–control |
|---------|---------------------------------|---------------------------------|------------------------------|-----------------------------------|
|         | log2 FC | P-value | log2 FC | P-value | log2 FC | P-value | log2 FC | P-value |
| AT1G09950 | 3.86    | 0.0003  | 2.31    | 0.0000  | 3.69    | 0.0000  | 2.15    | 0.0000  |
| AT2G17840 | 2.12    | 0.0000  | 1.69    | 0.0000  | 1.43    | 0.0000  | 1.01    | 0.0000  |
| AT2G23120 | 1.03    | 0.0000  | 1.31    | 0.0004  | 1.11    | 0.0000  | 1.40    | 0.0002  |
| AT1G20440 | 1.15    | 0.0001  | 1.59    | 0.0004  | 1.21    | 0.0000  | 1.65    | 0.0004  |
| AT4G35985 | 1.72    | 0.0000  | 1.31    | 0.0000  | 1.61    | 0.0000  | 1.21    | 0.0000  |
| AT2G34600 | 3.21    | 0.0002  | 2.26    | 0.0000  | 4.54    | 0.0000  | 3.60    | 0.0000  |
| AT4G31800 | 1.76    | 0.0032  | 2.54    | 0.0000  | 1.43    | 0.0030  | 2.21    | 0.0001  |
| AT5G08790 | 2.76    | 0.0000  | 2.10    | 0.0000  | 2.28    | 0.0000  | 1.64    | 0.0000  |
| AT2G35290 | 2.12    | 0.0000  | 1.37    | 0.0001  | 1.80    | 0.0008  | 1.06    | 0.0009  |
| AT2G39975 | 1.56    | 0.0036  | 1.16    | 0.0000  | 1.64    | 0.0000  | 1.26    | 0.0000  |
| AT3G16857 | 1.88    | 0.0000  | 1.73    | 0.0000  | 1.23    | 0.0060  | 1.08    | 0.0001  |
| AT5G63450 | 5.03    | 0.0000  | 2.31    | 0.0000  | 3.91    | 0.0000  | 1.20    | 0.0000  |
| AT2G37940 | 2.12    | 0.0000  | 1.62    | 0.0000  | 1.52    | 0.0000  | 1.03    | 0.0000  |
| AT2G43230 | 1.60    | 0.0102  | 1.34    | 0.0000  | 1.26    | 0.0000  | 1.01    | 0.0142  |
| AT4G20860 | 1.26    | 0.0016  | 1.16    | 0.0020  | 1.21    | 0.0023  | 1.12    | 0.0037  |
| AT4G34412 | 2.75    | 0.0000  | 1.97    | 0.0000  | 3.71    | 0.0003  | 2.94    | 0.0000  |
| AT1G10260 | 1.05    | 0.0099  | 1.12    | 0.0000  | 1.38    | 0.0000  | 1.45    | 0.0000  |
| AT3G47720 | 3.05    | 0.0009  | 1.23    | 0.0126  | 2.83    | 0.0032  | 1.02    | 0.0239  |
| AT1G12610 | 3.12    | 0.0000  | 2.36    | 0.0000  | 1.87    | 0.0008  | 1.13    | 0.0000  |
| AT1G15010 | 2.67    | 0.0000  | 2.18    | 0.0000  | 3.21    | 0.0000  | 2.72    | 0.0000  |
| AT1G72520 | 1.39    | 0.0000  | 1.90    | 0.0000  | 1.50    | 0.0006  | 2.03    | 0.0000  |
| AT2G32150 | 1.03    | 0.0000  | 1.74    | 0.0000  | 1.49    | 0.0000  | 2.22    | 0.0000  |
| AT3G56400 | 1.66    | 0.0000  | 2.10    | 0.0000  | 1.02    | 0.0000  | 1.45    | 0.0036  |
| AT3G46130 | 1.11    | 0.0042  | 1.06    | 0.0001  | 1.35    | 0.0000  | 1.41    | 0.0001  |
| AT2G34940 | 1.02    | 0.0281  | 1.42    | 0.0000  | 1.41    | 0.0000  | 1.02    | 0.0273  |
| AT1G76960 | 1.73    | 0.0000  | 2.01    | 0.0000  | 1.91    | 0.0000  | 1.63    | 0.0061  |
| AT5G45380 | 1.49    | 0.0181  | 2.06    | 0.0000  | 1.64    | 0.0000  | 1.08    | 0.0449  |
| AT4G11890 | 1.80    | 0.0000  | 2.16    | 0.0000  | 1.48    | 0.0000  | 1.13    | 0.0218  |

Red font means up-regulation while blue font means down-regulation.

of PYL5 improved drought stress tolerance and increased antioxidant enzyme activity and osmolyte levels (Shi et al., 2014b). Transcriptomic analysis indicated that GO terms including signal transduction, response to stress, and response to abiotic or biotic stress were enriched in ZAT18 OE lines (Fig. 10). Pathway analysis results showed that hormone metabolism, stress, and signaling were over-represented in ZAT18 OE lines (Table 1). These results indicated that the ZAT18 transgene modulated hormone signaling transduction pathways which might activate the downstream stress response.

In conclusion, we partially dissected the functions of ZAT18 in drought stress responses. ZAT18 overexpression enhanced osmotic stress responses in seed germination and improved drought stress tolerance. The ZAT18 transgene increased leaf water content and decreased ROS content. Transcriptomic analysis showed that genes involved in the hormone signaling transduction pathway and stress response were putative targets of ZAT18. Further research on the detailed mechanisms of how ZAT18 modulates the target genes is needed.

Supplementary data

Supplementary data are available at JXB online.

Fig. S1. ZAT18 expression level in wild-type and ZAT18 OE plants by qRT-PCR.

Fig. S2. Expression level changes of ZAT18 candidate target genes by qRT-PCR.
Table S1. The primers used for plasmid construction and identification of SALK_027144C and SALK_132289C in this study.

Table S2. List of genes affected by the ZAT18 transgene and drought stress.

Table S3. List of candidate target genes of ZAT18.

Table S4. The primers of ZAT18 candidate target genes used for qRT-PCR.

Acknowledgements

The mutants were provided by the Arabidopsis Biological Resource Center (ABRC). We thank Dr Pingfang Yang for help with analysis by qRT-PCR. We are grateful to the editor and reviewers for their comments and suggestions. This research was supported by Huazhong Agricultural University Scientific & Technological Self-Innovation Foundation (Program No. 2016RC010) and the National Natural Science Foundation of China (31370302) to ZC and a Sino-Africa Joint Research Project Grant to QW.

References

Agarwal P, Arora R, Ray S, Singh AK, Singh VP, Takatsuji H, Kapoor S, Tyagi AK. 2007. Genome-wide identification of C2H2-zinc-finger gene family in rice and their phylogeny and expression analysis. Plant Molecular Biology 65, 467–485.

Apel K, Hirt H. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annual Review of Plant Biology 55, 373–399.

Babu RC, Zhang J, Blum A, et al. 2004. HvA1, a LEA gene from barley confers dehydration tolerance in transgenic rice (Oryza sativa L.) via cell membrane protection. Plant Science 166, 855–862.

Chan Z, Bigelow PJ, Loescher W, Grumet R. 2012. Comparison of salt stress resistance genes in transgenic Arabidopsis thaliana indicates that extent of transcriptomic change may not predict secondary phenotypic or fitness effects. Plant Biotechnology Journal 10, 284–300.

Chinnusamy V, Schumaker K, Zhu JK. 2004. Molecular genetic perspectives on cross-talk and specificity in abiotic stress signalling in plants. Journal of Experimental Botany 55, 225–236.

Ciftci-Yilmaz S, Mittler R. 2008. The zinc finger network of plants. Cellular and Molecular Life Sciences 65, 1150–1160.

Ciftci-Yilmaz S, Morsy MR, Song L, Coutu A, Krizek BA, Lewis MW, Warren D, Cushman J, Connolly EL, Mittler R. 2007. The EAR-motif of the Cys2/His2-type zinc finger protein Zat7 plays a key role in the defense response of Arabidopsis to salinity stress. Journal of Biological Chemistry 282, 9260–9268.

de Hoon MJ, Imoto S, Nolan J, Miyano S. 2004. Open source clustering software. Bioinformatics 20, 1453–1454.

Devaiah BN, Nagarajan VK, Raghothama KG. 2007. Phosphate homeostasis and root development in Arabidopsis are synchronized by the zinc finger transcription factor ZAT76. Plant Physiology 145, 147–159.

Englbrecht CC, Schoof H, Böhm S. 2004. Conservation, diversification and expansion of C2H2 zinc finger proteins in the Arabidopsis thaliana genome. BMC Genomics 5, 39.

Foyer CH, Noctor G. 2005. Oxidant and antioxidant signaling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. Plant, Cell and Environment 28, 1056–1071.

Gabrièl D, Sabine R, Stefan H. 2010. Arabidopsis zinc-finger protein 2 is a negative regulator of ABA signaling during seed germination. Journal of Plant Physiology 167, 1418–1421.

Gao H, Song A, Zhu X, et al. 2012. The heterologous expression in Arabidopsis of a Chrysanthemum Cys2/His2 zinc finger protein gene confers salinity and drought tolerance. Planta 235, 979–993.

Hideki S, Takashi A, Tetsuo M, Masaki I. 2000. Expression of a subset of the Arabidopsis Cys2/His2-type zinc-finger protein gene family under water stress. Gene 248, 23–32.

Huang J, Sun SJ, Xu DQ, Yang X, Bao YM, Wang ZF, Tang HJ, Zhang H. 2009. Increased tolerance of rice to cold, drought and oxidative stresses mediated by the overexpression of a gene that encodes the zinc finger protein ZFP245. Biochemical and Biophysical Research Communications 389, 556–561.

Jefferson RA, Kavanagh TA, Bevan MW. 1987. GUS fusions: beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO Journal 6, 3901–3907.

Kang Y, Han Y, Torres-Jerez I, Wang M, Tang Y, Monteros M, Udvardi M. 2011. System responses to long-term drought and re-watering of two contrasting alfalfa varieties. The Plant Journal 68, 871–889.

Kim SH, Hong JK, Lee SC, Sohn KH, Jung HW, Hwang BK. 2004. CAF2P1, Cys2/His2-type zinc-finger transcription factor gene functions as a pathogen-induced early-defense gene in Capsicum annum. Plant Molecular Biology 55, 883–904.

Kodaira KS, Qin F, Tran LS, Maruyama K, Kidokoro S, Fujita Y, Shinozaki K, Yamaguchi-Shinozaki K. 2011. Arabidopsis Cys2/His2 zinc-finger proteins AZF1 and AZF2 negatively regulate abscisic acid-repressive and auxin-inducible genes under abiotic stress conditions. Plant Physiology 157, 742–756.

Laity JH, Lee BM, Wright PE. 2001. Zinc finger proteins: new insights into structural and functional diversity. Current Opinion in Structural Biology 11, 39–46.

Li Y, Zhao H, Duan B, Korpelainen H, Li C. 2011. Effect of drought and ABA on growth, photosynthesis and antioxidant system of Cotinus coggygria seedlings under two different light conditions. Environmental and Experimental Botany 71, 107–113.

Liu WX, Zhang FC, Zhang WZ, Song LF, Wu WH, Chen YF. 2013. Arabidopsis Di19 functions as a transcription factor and modulates PR1, PR2, and PR5 expression in response to drought stress. Molecular Plant 6, 1487–1502.

Liu XM, An J, Han HJ, Kim SH, Lim CO, Yun DJ, Chung WS. 2014. ZAT11, a zinc finger transcription factor, is a negative regulator of nickel ion tolerance in Arabidopsis. Plant Cell Reports 33, 2015–2021.

Liu XM, Nguyen XC, Kim KE, Han HJ, Yoo J, Lee K, Kim MC, Yun DJ, Chung WS. 2013. Phosphorylation of the zinc finger transcriptional regulator ZAT6 by MPK6 regulates Arabidopsis seed germination under salt and osmotic stress. Biochemical and Biophysical Research Communications 430, 1054–1059.

Luo X, Bai X, Zhu D, Li Y, Ji W, Cai H, Wu J, Liu B, Zhu Y. 2012. Gs2ZFP1, a new Cys2/His2-type zinc-finger protein, is a positive regulator of plant tolerance to cold and drought stress. Planta 235, 1141–1155.

Mito T, Seki M, Shinozaki K, Ohme-Takagi M, Matsui K. 2011. Generation of chimeric repressors that confer salt tolerance in Arabidopsis and rice. Plant Biotechnology Journal 9, 736–746.

Mittler R. 2002. Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science 7, 405–410.

Mittler R, Kim Y, Song L, Coutu J, Coutu A, Ciftci-Yilmaz S, Lee H, Stevenson B, Zhu JK. 2006. Gain- and loss-of-function mutations in Zat10 enhance the tolerance of plants to abiotic stress. FEBS Letters 580, 6537–6542.

Muhammad KQ, Tsanko SG, Neerakkal S, Jacques H. 2013. The zinc finger protein ZAT11 modulates paraquat-induced programmed cell death in Arabidopsis thaliana. Acta Physiologiae Plantarum 35, 1863–1871.

Ouyang SQ, Liu YF, Liu P, Lei G, He SJ, Ma B, Zhang WK, Zhang JS, Chen SY. 2010. Receptor-like kinase OsSIK1 improves drought and salt stress tolerance in rice (Oryza sativa) plants. The Plant Journal 62, 316–329.

Provart NJ, Zhu T. 2003. A browser-based functional classification superviewer for Arabidopsis genomics. Currents Topics in Computational Molecular Biology 2003, 271–272.

Quan W, Liu X, Wang H, Chan Z. 2016. Comparative physiological and transcriptional analyses of two contrasting drought tolerant Alfalfa varieties. Frontiers in Plant Science 6, 1256.

Rai AN, Tamirisra S, Rao KV, Kumar V, Suprasanna P. 2016. Brassica RNA binding protein ERD4 is involved in conferring salt, drought tolerance and enhancing plant growth in Arabidopsis. Plant Molecular Biology 90, 373–387.

Ren Z, Zheng Z, Chinnusamy V, et al. 2010. RAS1, a quantitative trait locus for salt tolerance and ABA sensitivity in Arabidopsis. Proceedings of the National Academy of Sciences, USA 107, 5659–5674.
Rohit J, Shabir HW, Balwant S, et al. 2016. Transcription factors and plants response to drought stress: current understanding and future directions. Frontiers in Plant Science 7, 1–15.

Sakamoto H, Maruyama K, Sakuma Y, Meshi T, Iwabuchi M, Shinozaki K, Yamaguchi-Shinozaki K. 2004. Arabidopsis Cys2/His2-type zinc-finger proteins function as transcription repressors under drought, cold, and high-salinity stress conditions. Plant Physiology 136, 2734–2746.

Sharma P, Jha AB, Dubey RS, Pessarakli M. 2012. Reactive oxygen species, oxidative damage and antioxidative defense mechanism in plants under stressful conditions. Journal of Botany 2012, Article ID 217037, 26 pages. doi:10.1155/2012/217037.

Shi H, Chan Z. 2014. The cysteine2/histidine2-type transcription factor ZINC FINGER OF ARABIDOPSIS THALIANA 6-activated C-REPEAT-BINDING FACTOR pathway is essential for melatonin-mediated freezing stress resistance in Arabidopsis. Journal of Pineal Research 57, 185–191.

Shi H, Wang X, Ye T, Chen F, Deng J, Yang P, Zhang Y, Chan Z. 2014a. The Cysteine2/Histidine2-Type transcription factor ZINC FINGER OF ARABIDOPSIS THALIANA6 modulates biotic and abiotic stress responses by activating salicylic acid-related genes and C-REPEAT-BINDING FACTOR genes in Arabidopsis. Plant Physiology 165, 1367–1379.

Shi H, Wang Y, Cheng Z, Ye T, Chan Z. 2012. Analysis of natural variation in bermudagrass (Cynodon dactylon) reveals physiological responses underlying drought tolerance. PLoS One 7, e53422.

Shi H, Ye T, Zhu JK, Chan Z. 2014b. Constitutive production of nitric oxide leads to enhanced drought stress resistance and extensive transcriptional reprogramming in Arabidopsis. Journal of Experimental Botany 65, 4119–4131.

Shyu C. 2016. Unwinding JAZ7—enigma to harmony. Journal of Experimental Botany 67, 3183–3185.

Thimm O, Bläsing O, Gibon Y, Nagel A, Meyer S, Krüger P, Selbig J, Müller LA, Rhee SY, Stitt M. 2004. MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. The Plant Journal 37, 914–939.

Wang D, Guo Y, Wu C, Yang G, Li Y, Zheng C. 2008. Genome-wide analysis of CCH zinc finger family in Arabidopsis and rice. BMC Genomics 9, 44.

Wang WB, Kim YH, Lee HS, Kim KY, Deng XP, Kwak SS. 2009. Analysis of antioxidant enzyme activity during germination of alfalfa under salt and drought stresses. Plant Physiology and Biochemistry 47, 570–577.

Wang Y, Li L, Ye T, Lu Y, Chen X, Wu Y. 2013. The inhibitory effect of ABA on floral transition is mediated by AB5 in Arabidopsis. Journal of Experimental Botany 64, 675–684.

Winter D, Vinegar B, Nahal H, Ammar R, Wilson GV, Provart NJ. 2007. An ‘Electronic Fluorescent Pictograph’ browser for exploring and analyzing large-scale biological data sets. PloS One 2, e718.

Wu C, You C, Li C, et al. 2008. RDD1, encoding a Cys2/His2-type zinc finger transcription factor, acts as a master switch from vegetative to floral development in rice. Proceedings of the National Academy of Sciences, USA 105, 12915–12920.

Xu DQ, Huang J, Guo SQ, Yang X, Bao YM, Tang HJ, Zhang HS. 2008. Overexpression of a TFIIA-type zinc finger protein gene ZFP252 enhances drought and salt tolerance in rice (Oryza sativa L.). FEBS Letters 582, 1037–1043.

Xu S, Wang X, Chen J. 2007. Zinc finger protein 1 (ThZF1) from salt cress (Thellungiella halophila) is a Cys/2/His-2-type transcription factor involved in drought and salt stress. Plant Cell Reports 26, 497–506.

Yoo SD, Cho YH, Sheen J. 2007. Arabidopsis mesophyll protoplasts: a versatile cell system for transient gene expression analysis. Nature Protocols 2, 1565–1572.

Yu J, Lai Y, Wu X, Wu G, Guo C. 2016. Overexpression of OsEm1 encoding a group I LEA protein confers enhanced drought tolerance in rice. Biochemical and Biophysical Research Communications 478, 703–709.

Zhang H, Liu Y, Wen F, Yao D, Wang L, Guo J, Ni L, Zhang A, Tan M, Jiang M. 2014. A novel rice C2H2-type zinc finger protein, ZFP36, is a key player involved in abscisic acid-induced antioxidant defence and oxidative stress tolerance in rice. Journal of Experimental Botany 65, 5795–5809.

Zhang H, Ni L, Liu Y, Wang Y, Zhang A, Tan M, Jiang M. 2012. The C2H2-type zinc finger protein ZFP182 is involved in abscisic acid-induced antioxidant defense in rice. Journal of Integrative Plant Biology 54, 500–510.