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ORIGINAL ARTICLE

Overexpression of a *Camellia sinensis* DREB Transcription Factor Gene (*CsDREB*) Increases Salt and Drought Tolerance in Transgenic *Arabidopsis thaliana*

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Abstract Dehydration-responsive element-binding protein (DREB) transcription factors play key roles in plant stress signal transduction pathways. We herein describe the functions of a *Camellia sinensis* DREB transcription factor (CsDREB) in response to abiotic stress. Subcellular localization analyses indicated that the CsDREB localizes to the nucleus. *CsDREB* expression in *C. sinensis* leaves was induced by heat, cold, drought, high salinity, H_2O_2, and exogenous abscisic acid (ABA). Additionally, CsDREB showed no transcriptional activation in *Saccharomyces cerevisiae*. Transgenic *Arabidopsis thaliana* plants overexpressing *CsDREB* exhibited enhanced tolerance to salt and drought stresses. The overexpression of *CsDREB* in *A. thaliana* plants resulted in the up-regulated expression of ABA-dependent stress-induced genes (i.e., *AtRD29B*, *AtRAB18*, *AtABI1*, and *AtABI2*) and ABA-independent stress-induced genes (i.e., *AtCOR15a* and *AtRD29A*). Furthermore, an analysis of the *CsDREB* promoter sequence revealed the presence of several abiotic and biotic stress-related motifs, along with the developmental stage- and tissue-specific elements. An examination of the transient expression of the *CsDREB* promoter in *Nicotiana benthamiana* leaves revealed that the promoter is highly responsive to ABA and methyl jasmonate. Collectively, these results suggest that *CsDREB* may increase plant tolerance to salt and drought stresses *via* both ABA-dependent and ABA-independent pathways.

Keywords: *Camellia sinensis*, DREB, Drought tolerance, Promoter, Salt tolerance, Transcription factor

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Introduction

Being sessile organisms, higher plants face variable forms of environmental stresses including drought, high salinity, and extreme temperatures. Plants respond and adapt to these stresses using an array of physiological, biochemical, and molecular mechanisms, thereby acquiring stress tolerance. Transcription factors (TFs) are crucial signal transmitters that regulate plant adaptations to stresses in plants through various signal transduction pathways (Agarwal and Jha 2010).

The dehydration-responsive element-binding proteins (DREBs) form one of the largest families of TFs in the plant genomes. They can specifically bind to the CRT/DRE elements (G/ACCGAC) in promoter regions of downstream target genes and activate or suppress the transcription of these genes (Zhao et al. 2012a). There are 57 and 52 DREB TFs in *Arabidopsis thaliana* (Iida et al. 2005) and *Oriyza sativa* (Nakano et al. 2006), respectively, and they play various roles in responses to abiotic and biotic stresses (Singh et al. 2002). For example, *A. thaliana* DREB2A has dual functions in both water and heat-shock stress responses (Sakuma et al. 2006b). The overexpression of *OsDREB1A* in transgenic *A. thaliana* plants up-regulates the expression of a stress-inducible gene (i.e., *AtDREB1A*) resulting in plants with increased tolerance to drought, salt, and freezing stresses (Dubouzet et al. 2003). In addition, the constitutive expression of *DREB* genes isolated from cotton (*Gossypium hirsutum*), sweet cherry (*Prunus avium*), maize (*Zea mays*), soybean (*Glycine max*), and *Caragana korshinskii* in non-host plants enhances the tolerance of transgenic plants to various environmental stresses (Kitashiba et al. 2004; Chen et al. 2007; Gao et al. 2009; Wang et al. 2010; Zhou et al. 2016). In addition to its role in stress responses, it is interesting to note that the overexpression of a cotton *GhDREB1* gene inhibits plant normal growth and development in transgenic

tobacco (*Nicotiana tabacum*) (Shan et al. 2007).

Tea plants [*Camellia sinensis* (L.) O. Kuntze or China type] are an important perennial evergreen woody crop used for the production of non-alcoholic caffeine-containing beverages around the world. Tea plants experience several biotic and abiotic stresses during their lifecycle, such as attacks by *Ectropis oblique* (Wang et al. 2016c) or *Colletotrichum camelliae* (Wang et al. 2016a), extreme temperatures (Wang et al. 2013; Liu et al. 2016b), heavy metals (Mukhopadhyay et al. 2013), and especially drought stress (Das et al. 2015; Liu et al. 2016a), which considerably decreases tea leaf production and quality (Wang et al. 2016b). The exposure of tea plants to drought conditions can result in oxidative damage due to the enhanced production of reactive oxygen species (ROS), ultimately causing cell death (Das et al. 2015). Hence, identifying the genetic components underlying drought tolerance in *C. sinensis* is of great importance. To the best of our knowledge, a total of 29 CsDREB TFs have been identified using the *C. sinensis* transcriptome database (Wu et al. 2015), but little information on the functional identification of CsDREB is available. In this study, we analyzed one *C. sinensis CsDREB* gene (Cs002-DREB-A1; GenBank Accession No. KF988866) and its promoter regarding their responses to abiotic stresses. The results indicate that the CsDREB plays a critical role in the development of *A. thaliana* tolerance to salt and drought stresses via both abscisic acid (ABA)-dependent and ABA-independent pathways.

Results

Cloning and Characterization of *CsDREB*

The complete *CsDREB* open reading frame (ORF) was isolated from *C. sinensis* cv. 'Longjing-changyecha' using RT-PCR. The *CsDREB* gene encoded 250 amino acids, and the predicted protein had a calculated molecular mass of 27.6 kDa and a theoretical isoelectric point of 6.5. An alignment of the amino acid sequences of CsDREB and its homologs revealed that the protein contained one conserved DNA-binding domain (AP2 domain) consisting of 59 amino acids (68–126) (Fig. 1). In addition, a putative nuclear localization signal (NLS) sequence (i.e., PKKRAGRKKFK) was detected in the N-terminal region using NLStradamus (Ba et al. 2009).

Subcellular Localization of CsDREB

CsDREB was predicted to be localized to the nuclei by WoLF PSORT (Horton et al. 2007). To confirm the subcellular

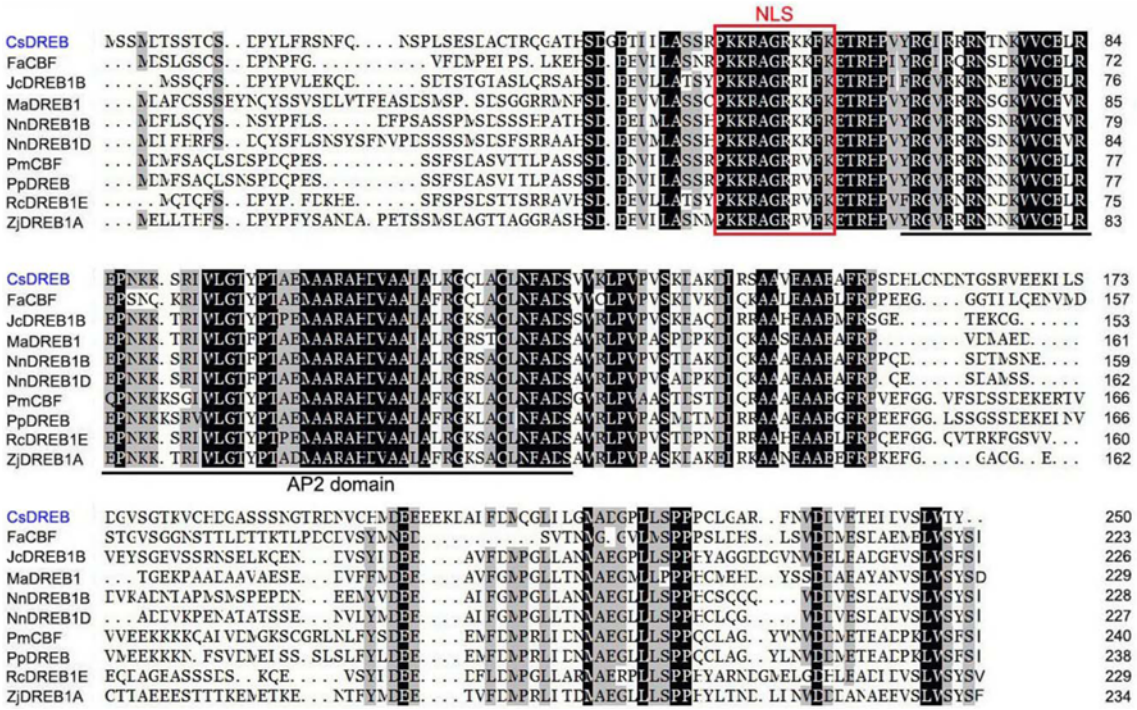

Fig. 1. Comparison of the deduced amino acid sequences of CsDREB and homologous proteins. The nuclear localization signal (NLS) sequence is indicated by a red rectangle, while the conserved AP2 domain is underlined. The amino acid sequences are shown as follows: CsDREB (AHL69786), *Camellia sinensis*; FaCBF (ADZ95598), *Fraxinus americana*; JcDREB1B (XP_012087006), *Jatropha curcas*; MaDREB1 (AFQ59977), *Morus alba*; NnDREB1B (XP_010255545), *Nelumbo nucifera*; NnDREB1D (XP_010278524), *N. nucifera*; PmCBF (ALN96413), *Prunus mume*; PpDREB (AIU39990), *Prunus pseudocerasus*; RcDREB1E (XP_002509703), *Ricinus communis*; ZjDREB1A (XP_015901870), *Ziziphus jujuba*.

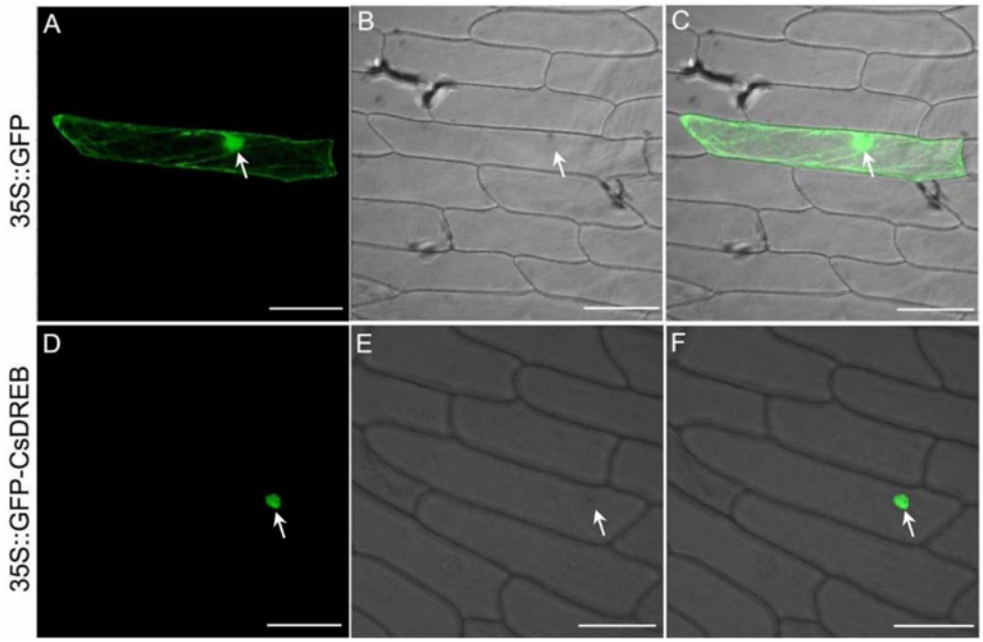

Fig. 2. Subcellular localization of CsDREB in onion epidermal cells. (A–C) Onion epidermal cells transformed with 35S::GFP. (D–F) Onion epidermal cells transformed with 35S::GFP–CsDREB. (A, D) Dark field images for detecting of green fluorescent protein (GFP) fluorescence. (B, E) Light field microscopy images for presenting morphological features. (C, F) Superimposed light and dark field images. White arrows indicate cell nuclei. Bar = 100 μm.

localization of CsDREB, the 35S::GFP–CsDREB and 35S::GFP (positive control) constructs were introduced into onion epidermal cells *via* particle bombardment. Fluorescence was observed throughout the cells transformed with 35S::GFP (Fig. 2A–C), while it was only detected in the nucleus of cells transformed with 35S::GFP–CsDREB (Fig. 2D–F). These results indicated that CsDREB is a nuclear localized protein.

CsDREB Expression Profiles in Response to Abiotic Stress and ABA

To clarify the CsDREB function, the *CsDREB* expression patterns following abiotic stress and ABA treatments were analyzed by qRT-PCR. Exposures to heat (Fig. 3A), drought (Fig. 3D), and cold (Fig. 3E) conditions increased the *CsDREB* transcript levels, with peak values at 1, 2, and 4 h after treatments, respectively. These increases were followed by decreases to the original levels over the remaining time points. After the H_2O_2 (Fig. 3B) and exogenous ABA (Fig. 3C) treatments, *CsDREB* transcript abundance sharply increased, peaking at 1 h after imposition of the treatments, but then decreased at 2, 4, and 6 h. The *CsDREB* expression level was up-regulated by 1.64- and 6.30-fold at 12 h after treatments, respectively, but then decreased considerably by the final time point. Exposure to high salinity stress significantly increased the abundance of *CsDREB* transcripts for the duration of the study period, with peaks at 1, 6, and 24 h (Fig. 3F). Our results revealed that *CsDREB* expression is up-regulated by several abiotic stresses, suggesting that *CsDREB* may participate in various abiotic stress responses in *C. sinensis*.

CsDREB-overexpressing Transgenic *A. thaliana* Exhibit Increased Tolerance to Salinity and Drought Stresses

As the tea plant transgenic system has not been established, *CsDREB*-overexpressing transgenic *A. thaliana* plants were generated to further investigate the biological function of *CsDREB*. Exogenous *CsDREB* expression was detected in transgenic *A. thaliana* lines (i.e., OE-8 and OE-12) but not in the wild-type (WT) and vector control (Vector) plants (Fig. 4A, Fig. S3). There were no obvious phenotypic differences among the WT, Vector, and transgenic plants under normal growth conditions (Fig. 4B). In contrast, after high salinity and drought treatments, the control plants (i.e., WT and Vector) were severely withered, whereas the transgenic plants continued to grow relatively normally (Fig. 4C, D). Only 14.8% of WT and 15.7% of Vector plants survived after high salinity treatment, whereas 85.3%–90.4% of transgenic plants survived (Fig. 4E). The survival rate of transgenic and control plants were 72.2–80.7% and 8.2%–10.7% after drought treatment, respectively (Fig. 4E). In addition, the *CsDREB* transgenic plants under high salinity or drought stresses showed significantly higher proline content and lower MDA content than the control plants ($P <$

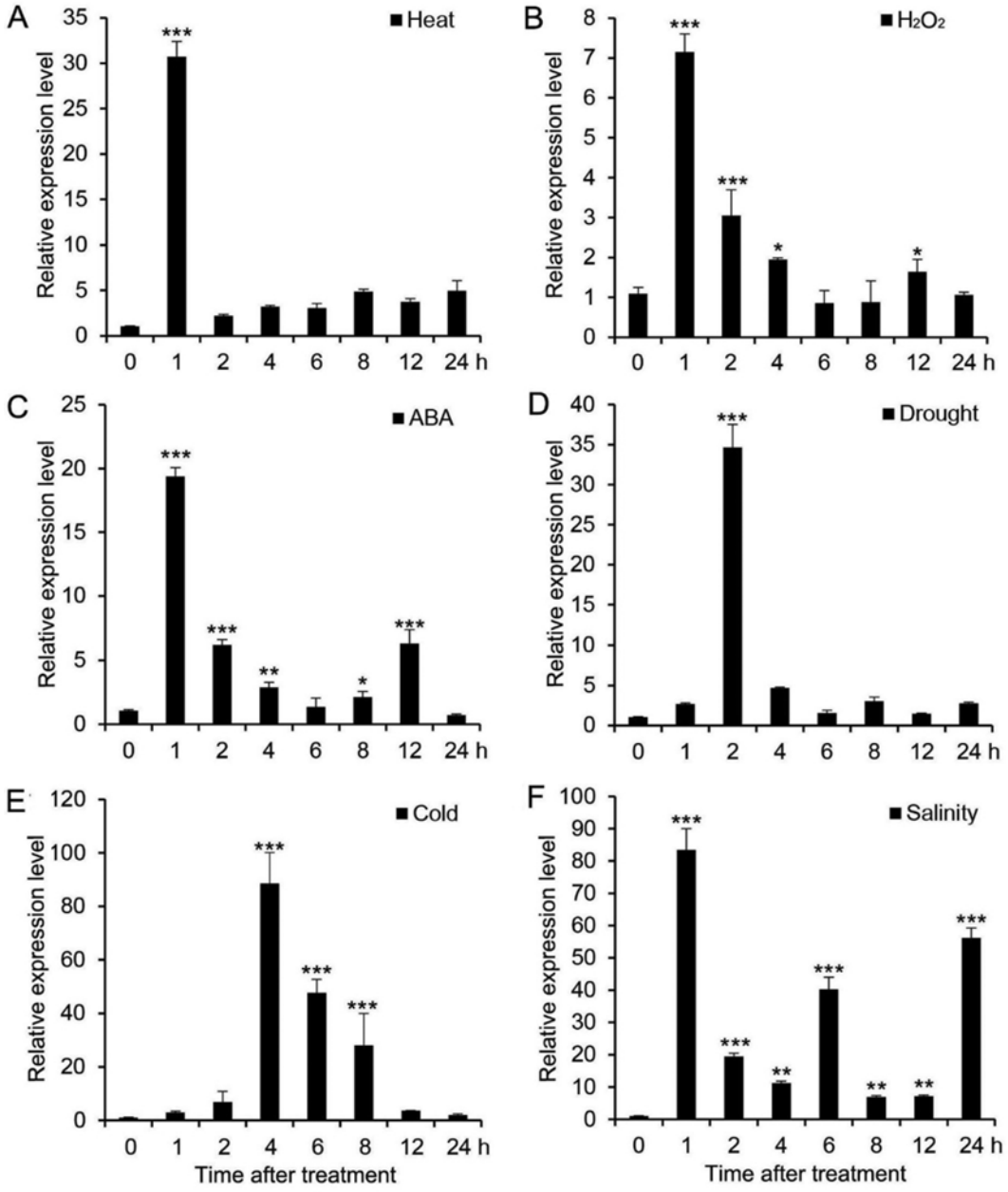

Fig. 3. *Camellia sinensis CsDREB* expression levels. Tea plants exposed to heat (38°C) (A), H_2O_2 (150 mM) (B), abscisic acid (200 μM) (C), drought (20% polyethylene glycol 6000) (D), cold (4°C) (E), and high salinity (200 mM NaCl) (F). The data are represented as the mean ± standard deviation from three independent measurements. Asterisks indicate significant differences between the treated plants and untreated controls (0 h) (* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$).

0.001) (Fig. 4F, G). Taken together, these results indicated that *CsDREB*-overexpressing *A. thaliana* plants were more tolerant to salt and drought stresses than the control plants.

CsDREB Overexpression Activates the Expression of ABA-dependent and ABA-independent Genes

To clarify the possible CsDREB-associated regulatory mechanisms influencing plant responses to salt and drought stresses, the expression levels of ABA-dependent stress-induced genes (i.e., *AtRD29B*, *AtRAB18*, *AtABI1*, and *AtABI2*) and ABA-independent stress-induced genes (i.e., *AtCOR15a* and *AtRD29A*) were compared between control lines (i.e., WT and Vector) and transgenic lines (i.e., OE-8 and OE-12). Under normal growth conditions, the expression levels of the six stress-induced genes were significantly higher in *CsDREB*-overexpressing lines than in the control lines (Fig. 5). These results suggested that *CsDREB* may increase plant tolerance to salt and drought stresses by activating the expression of

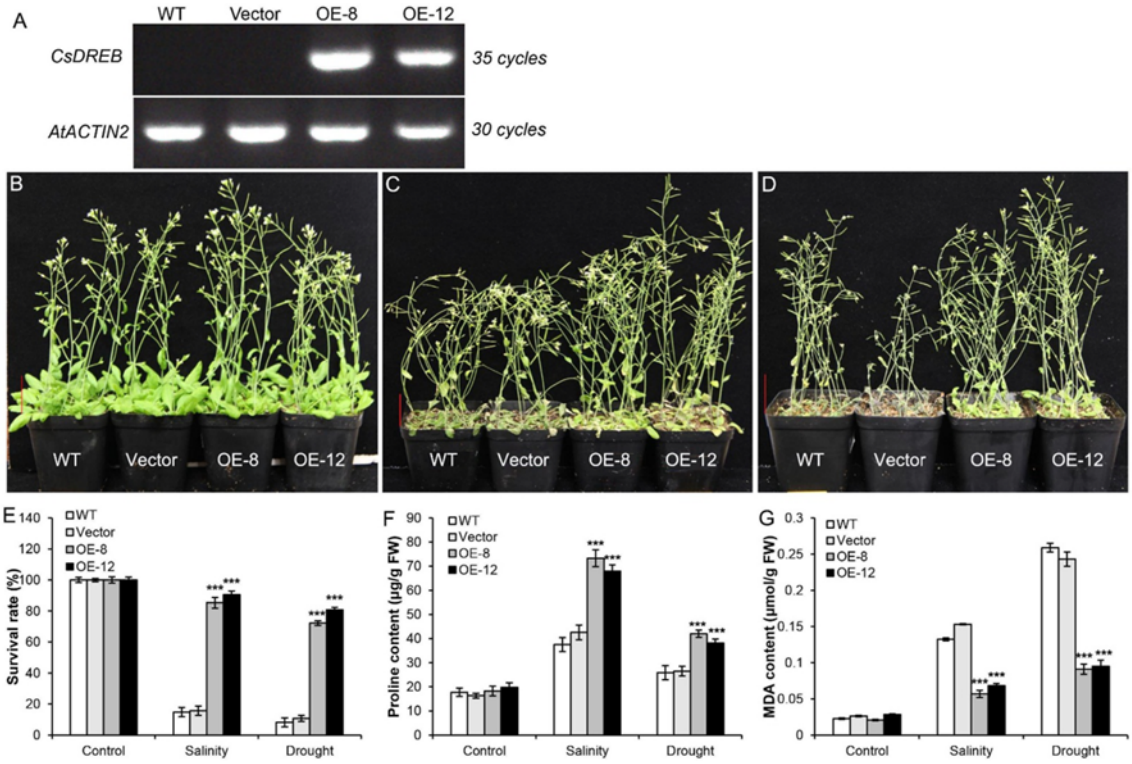

Fig. 4. Stress tolerance of *CsDREB*-overexpressing transgenic *Arabidopsis thaliana* plants. (A) *CsDREB* expression levels in wild-type (WT), vector control (Vector), and transgenic (i.e., OE-8 and OE-12) *A. thaliana* plants based on RT-PCR analysis. Phenotypes of 4-week-old WT, Vector, OE-8, and OE-12 plants under unstressed (B), high salinity (200 mM NaCl for 7 d) (C), and drought conditions (D). For drought treatment, 4-week-old seedlings withheld water for 10 d and then re-watered for 3 d. (E) Survival rate, (F) proline, and (G) MDA contents were measured before/after salinity/drought stresses. The survival rate of each line was the mean of at least 90 seedlings. Asterisks indicate significant differences between the transgenic plants and control plants (*** $P < 0.001$). Bar = 3 cm.

ABA-dependent and ABA-independent genes.

Transactivation Analysis of CsDREB

The ability of CsDREB to activate transcription was determined using a yeast one-hybrid assay. The yeast cells harboring pCL1 (positive control) grew well on minimal synthetic dropout medium without histidine and adenine (SD/–His–Ade). However, the yeast cells transformed with the pGBKT7 (negative control) or the pGBKT7–CsDREB vector were unable to grow on the same medium (Fig. S1), implying that CsDREB was unable to activate transcription in yeast cells.

Isolation and Characterization of *CsDREB* Promoter

The 839-bp putative *CsDREB* promoter sequence was obtained by thermal asymmetric interlaced (TAIL)-PCR (Fig. 6, Fig. S4), and the presence of *cis*-acting elements was assessed using the PlantCARE database (Lescot et al. 2002). Abiotic and biotic stress-related motifs [i.e., ABA-responsive element (ABRE), TGA, TGACG, and WUN] and developmental stage-specific elements (i.e., GCN4, Skn-1, and as1) were observed in the putative promoter sequence. These observations suggested that the *CsDREB* promoter region may be responsive to diverse abiotic and biotic stresses, and activated during specific developmental stages.

To experimentally confirm the responsiveness of the *CsDREB* promoter to various plant hormones, the P_{CsDREB}–GUS construct was transiently expressed in *Nicotiana benthamiana* leaves. We observed that GUS activity increased significantly when the leaves were treated with ABA and MeJA (Fig. 7).

Discussion

Many TFs have been identified in model plants, including AP2/ERF (Zhuang et al. 2008), MYB (Stracke et al. 2001), bHLH (Toledo-Ortiz et al. 2003), NAC (Ooka et al. 2003), HSF (Guo et al. 2008), and Dof (Lijavetzky et al. 2003). Regulating the expression of certain TF genes *via* genetic engineering techniques can greatly influence plant stress tolerance (Nishizawa et al. 2006; Jaffar et al. 2016; Jin et al. 2016). However, the functions of these TFs remain poorly characterized in non-model plants, including *C. sinensis*. Accordingly, this study focused on the effects of an AP2/ERF

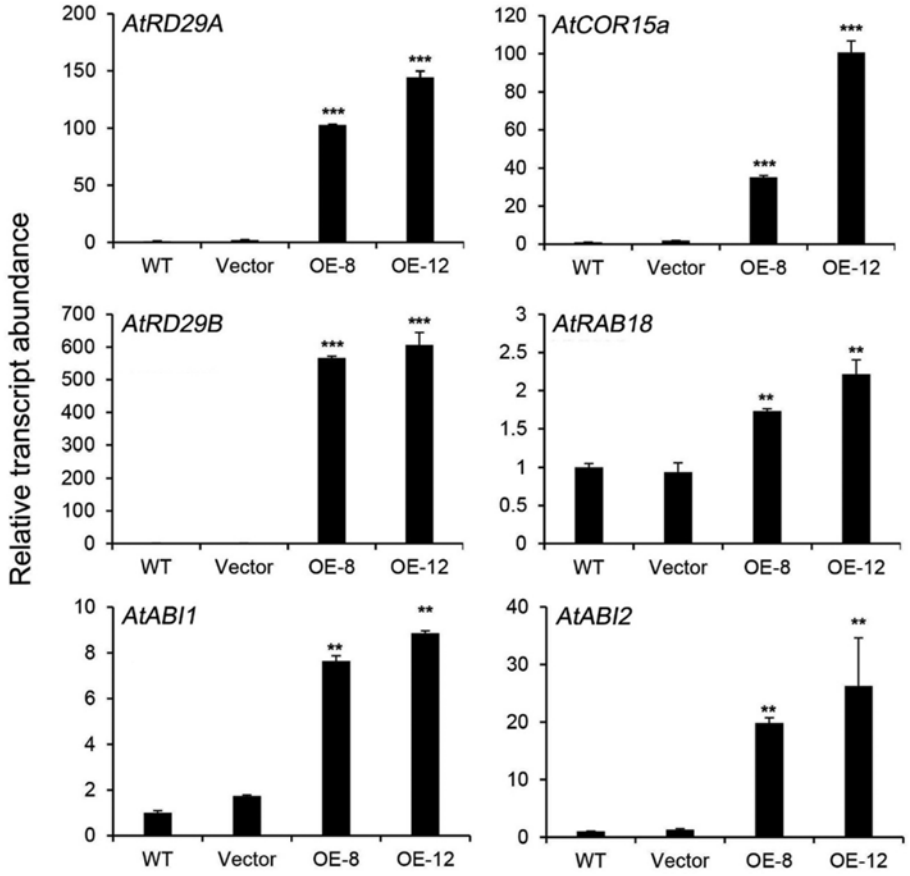

Fig. 5. Transcript levels of the downstream target genes in wild-type (WT), empty vector (Vector), and *CsDREB*-overexpressing (i.e., OE-8 and OE-12) *Arabidopsis thaliana* plants under unstressed conditions. Data are represented as the means ± standard deviation of three replicates. The transcript levels of each gene in WT plants were assigned a value of 1.0. Significant differences among WT, Vector, OE-8, and OE-12 plants are indicated by asterisks (** $P < 0.01$ and *** $P < 0.001$).

CTAATCATCAATTTCTGTGAATGTATACTCGCCGCCGCCTCACCACCATTACCCTTTCT
ATTCGCCTCTTGTCGCCTCTCTTGGTTCATCTCATTATTGCCTCCCCTCGCCTCACCA
CAGCCTCGTCACTCCTCGCTTCGATAGGAAATTACGTCGACTGCATAGTCCTCTCTG
TTCATCGCCTCTTTGTTCGTCTATTGCAGAGGGGTTAGAGTTCAAAAAACAAAAAGAA
ACTAAAAAGTAAAATAAAAAAGTATATAAAAAATTAAAAACGACGTTGTTTGGAAAGGTT
TGA-element
GTCACATAGAACTTCTGAGCTCCTTCTAAACTAAGCAAAATGACGTCACACGTGCCA
TGACG-motif/as1 ABRE
GCATTTGAGGTACCAATCAAACGCATCTGGTCACGTCACATCTAGCTCCAAAAACAC
GCACTTTACGATGTTATTAAGACCTAACAAAAAGGTCCACGTGTTTTCAGCTGCTTCC
TACTATCAGTATTTCAAGACTTTGAAGTTTCGCCACGTCATCACTACCTCAACACGTGT
Skn-1_motif
TAAAATTCCACTGACAGGTTGACTAGAAAGAGTCAAAGATGTTTCCATTTTTGGGAAA
AAAAAAAGTAAGGGGACCTACTAGGTGGCCGCCCCCAAGCCATCAGCTTCCGTGTT
GCN4_motif
CGCGTCCTCATCCCAAATACGTGTCCCCCTACCCATCCAGACAATACCAGTACTTGCA
ABRE
TTTGCTTATATAAAAACCAAATCACAACTCACTTCACTTATAGTAAGCTGGCAAACCAC
AAAAAGCAAAACCAAATTCGAGTTCAGACCTACTTGATTATCATTACAAACACTATTTG
WUN-motif
GATCAAACACAAACACTATCTGGATC(ATG)

Fig. 6. Analysis of the *CsDREB* promoter sequence using the PlantCARE promoter motif analysis tool. The start codon is shown in parentheses. Functional elements are underlined or highlighted in different colors.

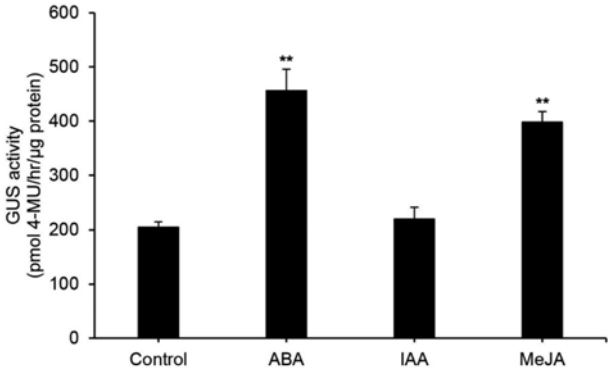

Fig. 7. β-glucuronidase (GUS) activity levels in transgenic tobacco (*Nicotiana benthamiana*) leaves in response to abscisic acid (ABA), auxin (IAA), and methyl jasmonate (MeJA). Control: the transgenic tobacco leaves treated with distilled deionized H_2O. Data are presented as the mean ± standard deviation of four replicates (** $P < 0.01$).

family gene (i.e., *CsDREB*) on *C. sinensis* stress tolerance.

Previous studies revealed that TFs must migrate to the nucleus to complete their functions (Wang et al. 2008; Yang et al. 2011). Our *in vivo* targeting experiment confirmed that the 35S::GFP–CsDREB fusion protein was localized to the nucleus of onion epidermal cells. This finding implies that

CsDREB is a nuclear protein that functions as a TF. However, we observed that CsDREB has no transcriptional activity in yeast cells, which is consistent with the findings of Sakuma et al. (2006a) and Zhao et al. (2012b). Hence, we speculate that a posttranslational modification is required to activate CsDREB.

The *CsDREB* expression level was significantly up-regulated in *C. sinensis* by heat, cold, high salinity, drought, H_2O_2, and exogenous ABA treatments. These results are inconsistent with those of other *DREB* genes. For example, *O. sativa OsDREB1F* expression is induced by high salinity, drought, cold, and ABA application, but not by H_2O_2 (Wang et al. 2008). Additionally, the *GhDREB1* expression level in cotton seedlings is up-regulated by low temperature and salt stresses, but was not induced by ABA or drought stress (Shan et al. 2007). The specific expression patterns suggest that *CsDREB* may act as a node linking several pathways. The ABA-inducible features imply that CsDREB may participate in an ABA-dependent signal transduction pathway. This suggestion was verified by detecting the expression levels of ABA-dependent stress-induced genes. The relatively high *CsDREB* transcript levels confirmed that CsDREB affects plant stress tolerance. The analysis of the *CsDREB*-overexpressing lines revealed a positive correlation between *CsDREB* expression and plant survival rates following exposures to salt and drought stresses. These results are in accordance with previous findings. For example, the overexpression of *OsDREB1F* in plants can greatly enhance plant tolerance to high salinity, drought, and cold stresses in rice and *A. thaliana* (Wang et al. 2008). In addition, *CsDREB* overexpression induced the expression of *AtCOR15a* and *AtRD29A* whose promoters harbor the DRE sequence (Wang and Hua 2009). This is consistent with the findings of Yang et al. (2011), and suggests that CsDREB may also participate in the ABA-independent pathways.

To investigate the mechanisms regulating *CsDREB* expression, we isolated the *CsDREB* promoter region from *C. sinensis* genome. Previous studies indicated that the ABA-responsive element (ABRE) is highly conserved among ABA-responsive genes (Hobo et al. 1999). In our study, *CsDREB* promoter-driven GUS activity increased significantly in response to ABA and MeJA treatments (Fig. 7). This can be due to the presence of two ABRE motifs and one TGACG motif in the *CsDREB* promoter. However, exogenous auxin had no effect on the GUS activity, despite of the presence of a TGA element in the *CsDREB* promoter region. One possible explanation for this observation is that MeJA-induced responses may depend on concentration or time (Zhang and Xing 2008). In addition to the hormone-responsive elements, we also identified a GCN4 motif, which is essential for endosperm-specific gene expression (Wu et al. 1998). The Skn-1 motif is another endosperm-specific expression element present in the *CsDREB* promoter. This motif enables seed-specific gene expression in transgenic tobacco plants (Depater et al. 1993). Additionally, the *CsDREB* promoter contains the as1 motif, which helps to regulate gene expression levels in the root tips of transgenic tobacco plants (Verdaguer et al. 1998). The presence of a wound-responsive element (i.e., WUN-motif) indicates that CsDREB may also be regulated by wound stress (Wang et al. 2008).

In conclusion, our results indicate that *CsDREB* expression is rapidly induced by heat, cold, high salinity, drought, H_2O_2, and exogenous ABA. The *CsDREB*-overexpressing transgenic *A. thaliana* plants exhibited increased tolerance to salt and drought stresses. Functional analyses revealed that the enhanced tolerance to salt and drought stresses may be due to both ABA-dependent and ABA-independent pathways. Additional investigations of the transgenic *C. sinensis* plants will likely help to more comprehensively characterize the CsDREB functions related to stress tolerance.

Materials and Methods

Plant Materials and Treatments

Two-year-old cutting seedlings of tea plants (*C. sinensis* cv. 'Longjing-changye') were grown in a growth chamber under a 12-h light (220 μmol m^{-2} s^{-1}; 24°C)/12-h dark (20°C) photoperiod with a relative humidity of 75% for 45 d before treatments. For oxidative stress and hormone treatments, leaves of tea plants were sprayed with 10 mM H_2O_2 and 50 μM ABA, respectively. For high salinity and drought treatments, plants were irrigated with 200 mM NaCl and 100 g L^{-1} PEG 6000, respectively. Heat and cold stresses were implemented by incubating the plants at 38°C and 4°C, respectively. The fourth leaves from the top buds of tea plants were individually collected at 0, 1, 2, 4, 6, 8, 12, and 24 h after the initiation of treatments. Samples were immediately frozen in liquid nitrogen and stored at –80°C.

Cloning of *CsDREB* and Sequence Analyses

Total RNA was extracted from *C. sinensis* leaves using the RNAiso Plus reagent (TaKaRa, Dalian, China). The purified RNA was reverse transcribed to cDNA using the PrimeScript™ 1st Strand cDNA Synthesis Kit (TaKaRa) according to the manufacturer's instructions. Based on the *CsDREB* (GenBank: KF988866) sequence details available in the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/), a primer pair (i.e., CsDREB-ORF-F/R; Table S1) was designed to amplify the complete *CsDREB* ORF using the first-strand cDNA as the template. Specific PCR products were isolated and sub-cloned into the pEASY-T1 Simple Cloning Vector (TransGen, Beijing, China) for sequencing (Genscript, Nanjing, China). The amino acid sequences of CsDREB and its homologs were aligned using the DNAMAN 6.0.3.99 program and BLAST online tools (http://www.ncbi.nlm.gov/blast).

Subcellular Localization of CsDREB

To construct the 35S::GFP–CsDREB vector, the *CsDREB* ORF lacking a termination codon was amplified using the CsDREB-EGFP-F/R primers (Table S1). The confirmed PCR product was double-digested with *Bam*HI and *Xba*I, and then inserted into the

pCAMBIA2300–C–EGFP vector (Wang et al. 2014) upstream of the green fluorescent protein (GFP) sequence. Both the recombinant plasmid (35S::GFP–CsDREB) and the empty vector (35S::GFP) were transiently introduced into the onion (*Allium cepa*) epidermal cells using the PDS-1000/He particle delivery system (Bio-Rad, Hercules, CA, USA) for transient expression experiments. The onion peels were subsequently incubated for 16 h at 22°C on Murashige and Skoog plates in darkness (Li et al. 2015). The GFP signals were monitored using a confocal laser scanning microscope (LSM 710; Carl Zeiss, Jena, Germany).

Gene Expression Analysis by Quantitative Real-time PCR (qRT-PCR)

Total RNA was extracted using the *EasyPure*® Plant RNA Kit (TransGen) following the manufacturer's instructions. Approximately 1 μg of total RNA was reverse transcribed to cDNA using the *TransScript*® All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (One-Step gDNA Removal) (TransGen) following the manufacturer's instructions. The qRT-PCR using the *TransStart*® Tip Green qPCR SuperMix (TransGen) was used to analyze the expression levels of *CsDREB* gene and the downstream target genes. The PCR solution (20 μL) contained 10 μL of 2 × *TransStart*® Tip Green qPCR SuperMix, 0.2 μM forward and reverse primers, 100 ng cDNA template, and nuclease-free water. The qRT-PCR was conducted in a LightCycler 480 Real-Time PCR System (Roche Applied Science, Indianapolis, IN, USA) with the following program: 95°C for 2 min; 40 cycles of 95°C for 10 s, 58°C for 15 s, and 72°C for 20 s; 72°C for 3 min. *β-actin* (GenBank Accession No. HQ420251) and *ACTIN2* (GenBank Accession No. AT3G18780) were used as the internal reference genes for *C. sinensis* and *A. thaliana*, respectively. The relative transcript abundances were calculated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001). The resulting data are presented as the mean ± standard deviation of three biological experimental replicates. The qRT-PCR primer sequences are listed in Table S1.

Analysis of CsDREB Transcriptional Activity

The *CsDREB* ORF lacking a termination codon was amplified using the CsDREB-pGBKT7-F/R primers (Table S1). The amplified product was inserted into the *Eco*RI/*Bam*HI cloning sites of pGBKT7 to produce the yeast expression vector pGBKT7–CsDREB. The pGBKT7 (negative control), pCL1 (positive control), and pGBKT7–CsDREB vectors were used to transform *Saccharomyces cerevisiae* strain Y2HGold following the method described in the YEASTMAKER Yeast Transformation System 2 User Manual (Clontech, Mountain View, CA, USA). The transformants were selected according to the method described by Gao et al. (2015) and transferred to SD/-His-Ade medium supplemented with 20 mg mL^{-1} 5-bromo-4-chloro-3-indolyl-α-D-galactopyranoside (X-α-Gal) to analyze cell growth (Lei et al. 2016).

Plasmid Construction and Transformation of *A. thaliana*

The *CsDREB* coding sequence was amplified with the CsDREB-pBI121-F/R primers (Table S1) harboring the *Xba*I and *Bam*HI sites. The amplicons digested by *Xba*I and *Bam*HI were cloned into the plant expression vector pBI121 (Chen et al. 2003) to generate the 35S::CsDREB construct (Fig. S2A). The recombinant plasmid and empty vector were inserted into *Agrobacterium tumefaciens* strain EHA105 cells, which were then used to transform *A. thaliana* plants using a floral dip method (Zhang et al. 2006). The transgenic *A. thaliana* plants were screened on Murashige and Skoog medium containing 50 μg mL^{-1} kanamycin. Putative transformants were confirmed by RT-PCR using the CsDREB-ORF-F/R primers (Table S1). Only the homozygous T_3 plants were used for subsequent stress tolerance assays.

Evaluation of Salt and Drought Tolerance of Transgenic *A. Thaliana*

Homozygous transgenic *A. thaliana* lines (i.e., Vector, OE-8, and OE-12) and the wild-type (WT) (Columbia ecotype) plants were grown in a 3:1 (v/v) mixture of vermiculite and soilrite in a growth chamber under a 16-h light (220 μmol m^{-2} s^{-1}; 24°C)/8-h dark (20°C) photoperiod with a relative humidity of 75%. For the exposure to high salinity stress, 4-week-old seedlings were treated with 200 mM NaCl for 7 d. Drought conditions were stimulated by withholding water for 10 d. And then, the plants were re-watered for 3 d before being scored and photographed.

The proline content was quantified based on the method of Bates et al. (1973). MDA content was measured as described previously (Draper and Hadley 1990).

Cloning and Transient Expression of *CsDREB* Promoter

Genomic DNA was extracted from tea plants leaves using the Plant Genomic DNA Kit (Tiangen, Beijing, China). The *CsDREB* promoter sequence was then isolated with the Genome Walking Kit (TaKaRa). The Pro-GSP1, Pro-GSP2, and Pro-GSP3 primers (Table S1) were used during the primary, secondary, and tertiary reactions of TAIL-PCR (Liu and Whittier 1995), respectively. The final amplicon was ligated into the pEASY-T1 Simple Cloning Kit and transformed into *E. coli* strain *Trans*5α Chemically Competent Cell (TransGen) for sequencing. The resulting sequences were analyzed using the PlantCARE database (Lescot et al. 2002).

The *CsDREB* promoter was amplified using the primer pair Pro-F/-R (Table S1), and then double digested with *Hin*dIII and *Bam*HI. The resulting fragment was inserted into the *Hin*dIII and *Bam*HI cloning sites of the pBI121 vector to replace the 35S promoter (P_{CsDREB}–GUS; Fig. S2B). The transformation of *N. benthamiana* plants for transient gene expression analyses was conducted according to the method described by Pascual et al. (2015). Briefly, after a 48-h infiltration, *N. benthamiana* leaves were treated with 200 μM ABA, 100 μM MeJA, 50 μM IAA, or distilled deionized water (control), respectively. The infiltrated leaves were harvested 12 h after initiating the treatments. Quantitative assays to examine β-glucuronidase (GUS) activity were repeated three times according to the method described by Jefferson (1989).

Statistical Analysis

Data were analyzed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA) software. Significant differences between values were determined with Duncan's multiple range tests, and are indicated by asterisks (* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$).

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Author's Contributions

XL and MW conceived and designed the experiments; MW performed the experiments; MW, QL and HX analyzed the data; MW wrote the paper; MW, JZ and ZZ revised the manuscript.

Supporting Information

Fig. S1. Transactivation analysis of CsDREB.
Fig. S2. Diagrammatic representation of the 35S::CsDREB and P_{CsDREB}–GUS recombinant vectors.
Fig. S3. Expression level of *CsDREB* in wild-type (WT), vector control (Vector), and 8 transgenic (i.e., OE-1, OE-2, OE-4, OE-7, OE-8, OE-9, OE-12, and OE-13) *A. thaliana* plants.
Fig. S4. Isolation of *CsDREB* promoter by thermal asymmetric interlaced polymerase chain reaction (TAIL-PCR).
Table S1. Primers used in this study.

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