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Research article

A *ThDREB* gene from *Tamarix hispida* improved the salt and drought tolerance of transgenic tobacco and *T. hispida*

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ABSTRACT

Dehydration-responsive element-binding (DREB) transcription factors are important abiotic stress tolerance related genes, and some reports on the roles of DREB have primarily addressed herbal plants. To explore the abiotic stress tolerance role of *DREB* (*ThDREB*) from *Tamarix hispida*, a *ThDREB* gene with a complete ORF of 783 bp that encodes a 28.74 kDa protein with 260 amino acids, was isolated and functionally annotated. *ThDREB* expression was highly induced by NaCl, PEG, $NaHCO_3$ and $CdCl_2$ treatments, and the highest expression level (369.2-fold of control) was found for the roots that were under NaCl stress for 6 h. The tobacco plants that were transformed by *ThDREB* were conferred with higher germination rates, fresh weights and root lengths than the wild type (WT) tobacco plants under NaCl and mannitol treatments. The total chlorophyll content (tcc), superoxide dismutase (SOD) and peroxidase (POD) activities were also higher in the transgenic lines in comparison with the WT, and the malondialdehyde (MDA) and H_2O_2 content, electrolyte leakage (EL) rate and ROS as tracked by staining were generated to a lesser degree in *ThDREB* transgenic plants than in the WT under NaCl and mannitol stress. Furthermore, the transient overexpression analysis of *ThDREB* in *T. hispida* also improved plant salt and drought tolerance in comparison with the empty vector-transformed lines. Our results indicated that *ThDREB* expression could effectively improve tolerance to salt and drought stress by enhancing the antioxidase activity that keeps the ROS at a low accumulation level and makes them easy to scavenge.

1. Introduction

Plant growth and development is always limited by salinity, drought, extreme temperature, heavy metal and other stresses. Many studies on the stress-resistant mechanisms of plants have revealed some tolerance-related genes, including some transcription factors that play essential roles in stress signaling pathways (Augustine et al., 2015; Fountain et al., 2015). *Tamarix hispida* is a shrub or small tree that grows primarily in arid and semi-arid regions, and it exhibits tolerance to salt, drought, and high temperatures. Therefore, *T. hispida* may be an ideal model plant for investigating physiological and molecular mechanisms of stress responses in trees and for the cloning of a stress tolerance gene. To date, some studies have aimed at revealing the stress-tolerant mechanism of *T. hispida* and have identified multiple stress-regulated genes, such as the V-ATPase c subunit (*ThVHAc1*), eukaryotic translation initiation factor (*eIF5A*), and WRKY transcription factor (*ThWRKY4*) (Wang et al., 2012, 2015; Yang et al., 2016). However, less is known about the DREB (dehydration responsive element binding protein) transcription factors in *T. hispida*. The signaling/regulatory pathways of stress responses in *T. hispida* require further intensive study.

The DREB transcription factor is one subfamily member of the AP2/ERF family. It plays important roles in plant tolerance to abiotic stress. Peng et al. (2013) cloned the CBF/DREB1 (C-repeat binding factor/DREB factor 1) homolog *AmCBF2* from the mangrove *Avicennia marina*. Expression analyses revealed that *AmCBF2* was induced by cold, drought, high salinity, and heavy metals as well as abscisic acid (ABA), indicating that *AmCBF2* may be involved in cold, drought and heavy metal stress response signaling pathways. *JcDREB* expression in the woody biodiesel plant *Jatropha curcas* showed that it was induced by cold, salt and drought stresses, but

Abbreviations: EL, Electrolyte leakage; MDA, Malondialdehyde; POD, Peroxidase; ROS, Reactive oxygen species; SOD, Superoxide dismutase; ABA, Abscisic Acid; COR, Cold-Responsive; qRT-PCR, quantitative Real-Time PCR; MW, Molecular Weight; pI, Isoelectric Point; Tcc, Total Chlorophyll Contents; DAB, 3,3′-Diaminobenzidine Tetrahydrochloride; H_2DCF-DA, 2, 7-Dichlorofluorescein Diacetate.

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not by ABA. The transgenic *Arabidopsis* overexpression of *JcDREB* led to enhanced salt and freezing stress (Tang et al., 2011). The overexpression of *AtDREB2A CA* in sugarcane also enhanced plant drought tolerance without a biomass penalty (Reis et al., 2014). *BdDREB2*, which was isolated from *Buchloe dactyloides*, was involved in responses to drought and salt stresses. The overexpression of *BdDREB2* in tobacco seedlings led to higher relative water and proline contents and lower MDA contents under drought stress (Zhang et al., 2014). A *PeDREB2a* gene isolated from desert-grown tree *Populus euphratica* was greatly induced by drought, NaCl, low temperature, 1-naphthaleneacetic acid (NAA), 6-benzylaminopurine (6-BA) and gibberellic acid (GA3) treatments. The overexpression of *PeDREB2a* resulted in enhanced tolerance to salt and drought stresses (Wu et al., 2015). The T_2 and T_3 transgenic lines that overexpressed *OsDREB2A* were found to have improved survival rates under severe drought and salt stress conditions in comparison with non-transgenic rice plants or rice plants that were transformed with an empty vector control (Cui et al., 2011). *SbDREB2A* was overexpressed in tobacco plants, and these transgenic plants showed better seed germination and growth characteristics under both hyperionic and hyperosmotic stresses, and they exhibited a higher water content, membrane stability and less electrolyte leakage under stress conditions (Gupta et al., 2014).

DREB transcription factors confer stress tolerance to plants primarily by binding to C-repeat dehydration-responsive (DRE/CRT) *cis*-acting elements in gene promoters and activating the transcription of downstream genes (Shavrukov et al., 2016). For instance, yeast one-hybrid assay showed that SsDREB, a DREB protein isolated from the succulent halophyte *Suaeda salsa*, specifically binds to the DRE sequence and could activate the expression of reporter genes in yeast. This finding suggests that the SsDREB protein is a CBF/DREB transcription factor. A semi-quantitative PCR of the *SsDREB* transgenic tobacco seedling showed the higher expression of some stress-responsive genes, namely *GST*, *Cu/ZnSOD*, and *Lea5* (Zhang et al., 2015). BpDREB2, an AP2/DREB-type transcription factor that was cloned from the woody plant *Broussonetia papyrifera*, could specifically bind to the DRE sequence and activate the expression of reporter genes (Sun et al., 2014). A yeast *in vivo* analysis showed that the conserved Val (14th) and Glu (19th) residues were crucial in the regulation of DREB1A binding activity in the DRE *cis*-element (Cao et al., 2001). The overexpression of a *DREB* gene from *Limonium bicolor* could regulate the transcription of *GST*, V-ATPase subunit G (*vag2*), *POD*, *TOBPXD*, and *poxN1* (Ban et al., 2011). In comparison with the wild type *Arabidopsis*, the overexpression of *VrDREB2A* activated the expression of downstream genes in the transgenic *Arabidopsis*, resulting in an enhanced tolerance to drought and high-salt stresses and no growth retardation (Chen et al., 2016). Overexpression of *AhDREB1* in transgenic tobacco led to the accumulation of its putative downstream genes that include *rd17, rd29A,* and *LHCP (*Shen et al., 2003). A relative mRNA expression level analysis by qRT-PCR indicated that the improved freezing tolerance of transgenic *Arabidopsis* plants that were overexpression of *PpCBF3* was conferred by the sustained activation of downstream cold-responsive (COR) genes, such as *COR15a*, *COR6.6*, *COR47*, *COR78*, and *P5CS* (Zhuang et al., 2015).

In the current study, a novel *DREB* gene (*ThDREB*) was cloned from *T. hispida*, the expression of *ThDREB* in response to different abiotic stresses was examined, and transgenic tobacco plants and *T. hispida* were generated to analyze their functions further. The results showed that *ThDREB* mediated some physiological processes that were involved in stress tolerance, especially the salt and drought response, which may provide valuable insights into the role of *DREB* in woody plants when they confront stress conditions.

2. Materials and methods

2.1. Plant materials and treatments

T. hispida seeds were planted in pots with a 12 cm diameter containing a mixture of turf peat and sand (2:1 *v*/*v*) in a greenhouse at an average temperature of 24 °C with 14 h light/10 h dark, 70–75% relative humidity and well-watered conditions. Each pot contained four seedlings. The four-month-old *T. hispida* plants were treated as follows: 0.4 M NaCl, 0.3 M $NaHCO_3$, 20% (*w*/*v*) PEG_{6000} and 150 μM $CdCl_2$ for 6, 12, 24, 48 or 72 h. Every treatment contained 8 pots and total 2.4 L of each of NaCl, $NaHCO_3$, PEG_{6000} and $CdCl_2$ water solution was poured in the pots that the plants absorbed them from bottom through holes. Every treatment was applied three independent times, and each replicate contained 32 plants. The rinsed root, stem and leaf samples from each replicate were independently harvested at the indicated time points and pooled for quantitative real-time RT-PCR (qRT-PCR) analyses. The plants that were watered with pure water were used as controls.

Wild-type tobacco (*Nicotiana tabacum*) (WT) and transgenic tobacco were used throughout the study. The seeds from T_3 generation were each sterilized with 75% ethanol for 1 min, rinsed with sterile deionized water for 2 min, then sterilized with 10% (v/v) sodium hypochlorite for 8 min, rinsed eight times with sterile deionized water and sown onto plates (90 mm diameter) containing half-strength Murashige and Skoog (1/2MS) agar medium; they were kept in a greenhouse under the above conditions for a related analysis.

2.2. Cloning the ThDREB gene and bioinformatics analysis

The *ThDREB* gene was cloned from the *T. hispida* leaf cDNA library (Gao et al., 2008), which was confirmed by clone sequencing. The molecular weight (MW) and isoelectric point (pI) predictions for the deduced protein were performed using the Compute pI/MW tool (http://www.expasy.org/tools/protparam.html). The phylogenetic tree of ThDREB and the *Arabidopsis* DREB proteins was constructed using the Neighbor-Joining method (Saitou and Nei, 1987).

2.3. RNA isolation and qRT-PCR assay

Total RNA was isolated from every pool samples using the CTAB method (Yang et al., 2016), and 0.5 μg of RNA was reverse-transcribed with the PrimeScript™ RT reagent Kit (Takara, Otsu, Shiga, Japan). The resulting cDNA product was diluted to 1/10 and used as a template for the qRT-PCR analyses, which were performed in an MJ Opticon™2 machine (Bio-Rad, Hercules, CA, USA). The qRT-PCR reaction system and procedures were performed according to the related *ThVHAc1* analysis (Yang et al., 2016). The primer sequences are listed in Table S1. The relative expression levels were calculated by delta-delta Ct method (Livak and Schmittgen, 2001), and the *ThDREB* expression in *T. hispida* was normalized to *β-actin* (FJ618517), *α-tubulin* (FJ618518), and *β-tubulin* (FJ618519). The qRT-PCR data in the transgenic tobacco lines were normalized to *tubulin* (AJ421412). The primers were shown in Table S1.

2.4. Generation of ThDREB transgenic tobacco

The full-length cDNA of *ThDREB* was amplified and cloned into prokII (Chen and Mills, 1987) and named *35S::ThDREB* (the primers are shown in Table S1). The expression vector containing the genetic construct *35S::ThDREB* was transformed into tobacco (*N. tabacum*) by *Agrobacterium*-mediated transformation method (Bastaki and Cullis, 2014). The kanamycin-resistant lines were