Plant Science 265 (2017) 154–166

Contents lists available at ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

An ERF transcription factor from *Tamarix hispida*, ThCRF1, can adjust osmotic potential and reactive oxygen species scavenging capability to improve salt tolerance

Liping Qin[a,b,c], Liuqiang Wang[d], Yong Guo[d], Yan Li[a], Halik Ümüt[b], Yucheng Wang[a,d,*]

[a] Key Laboratory of Biogeography and Bioresource in Arid Land, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi, 830011, China
[b] Key Laboratory of Oasis Ecology, College of Resources & Environmental Sciences, Xinjiang University, Urumqi, 830046, China
[c] University of Chinese Academy of Sciences, Beijing 100049, China
[d] State Key Laboratory of Tree Genetics and Breeding, Northeast Forestry University, Harbin, 150040, China

ARTICLE INFO

Keywords:
Cytokinin response factor
Ethylene responsive factor
Tamarix hispida
Transcription factors
Salt stress
Transient genetic transformation
Cis-acting elements

ABSTRACT

Ethylene-Responsive Factors (ERFs) are plant-specific transcription factors (TFs) involved in multiple biological processes, especially in abiotic stress tolerance. However, the ERFs from woody halophytes that are involved in salt stress have been little studied. In the present investigation, we characterized a subfamily member of ERF TFs from *Tamarix hispida*, ThCRF1, which responds to salt stress. ThCRF1 is a nuclear protein that binds to the motifs including TTG, DRE and GCC-box. Transient transformation was performed to generate *T. hispida* overexpressing *ThCRF1* and RNA interference (RNAi)-silenced *ThCRF1* to analyze its function using gain- and loss-of-function methods. Overexpression of *ThCRF1* in *T. hispida* significantly improved tolerance to salt-shock-induced stress; by contrast, RNAi-silence of *ThCRF1* significantly decreased tolerance to salt-shock-induced stress. Further experiments showed that ThCRF1 induces the expression of genes including those encoding pyrroline-5-carboxylate synthetase (*P5CS*), trehalose-6-phosphate synthase (*TPS*), trehalose-6-phosphate phosphatase (*TPP*), superoxide dismutase (*SOD*) and peroxidase (*POD*), which lead to enhanced proline and trehalose levels and increased SOD and POD activities. These results were further confirmed by studying transgenic Arabidopsis plants overexpressing *ThCRF1*. Therefore, the results suggested that ThCRF1 improves tolerance to salt-shock-induced stress by enhancing trehalose and proline biosynthesis to adjust the osmotic potential, and by improving SOD and POD activities to increase reactive oxygen species scavenging capability.

1. Introduction

The AP2 (APETALA2)/ERF (Ethylene-Responsive Factor) family is a largest TF family of plants. For instance, there are 163 and 144 ERFs found in rice [1] and *Arabidopsis thaliana* [2], respectively. As a plant-specific TF family, the AP2/ERF TFs can be divided into three subgroups, including AP2 (containing two AP2/ERF domains), RAV (related to ABI3/VP1) (containing one AP2/ERF domain and one B3 domain) and ERF (containing one AP2/ERF domain). [3,4]. All AP2/ERF TFs include at least one AP2/ERF domain comprised by about sixty amino acids [2,5,6]. Rashotte et al. [7] further named six genes from the Arabidopsis ERF subfamily B-5 (Group VI) as a cytokinin response factor (CRF) 1–6, because the expressions of some of these genes can be induced by exogenous cytokinin. The CRF TFs include a CRF domain and a TEH (Thr, Glu, His) region in the N-terminal region, both of which are located N-terminal to the AP2/ERF conserved domain [8].

To date, previous studies showed that numerous AP2/ERF family TFs were involved in abiotic and/or biotic stress tolerance [9–14], especially, salt [15,16] and drought stress tolerance [17,18]. Among the ERF subfamily, the CRF members are also involved in salt or drought tolerance. Currently, 12 CRF TFs have been identified from *A. thaliana* [7,8,19], and many of them are involved in the abiotic stress response. For instance, CRF2, CRF3, and CRF4 play roles in coping with cold stress [20,21], while CRF6 responds to oxygen stress by inhibiting the expression of genes related to the cytokinin pathway [22]. Eleven CRF TFs identified in tomato were also studied [19]. Several tomato *SlCRFs* can be induced by cytokinin and can respond to salt stress. For instance, *SlCRF1* and *SlCRF2* show tissue development expression patterns, and are also responsive to cytokinin, drought, and other abiotic stress, indicating that they serve functions in plant environmental responses and growth [23,24].

The AP2/ERF family regulates its downstream genes through

* Corresponding author at: State Key Laboratory of Tree Genetics and Breeding, Northeast Forestry University, 26 Hexing Road, Harbin 150040, China.
E-mail address: wangyucheng@ms.xjb.ac.cn (Y. Wang).

http://dx.doi.org/10.1016/j.plantsci.2017.10.006
Received 18 August 2017; Received in revised form 2 October 2017; Accepted 9 October 2017
Available online 13 October 2017
0168-9452/ © 2017 Elsevier B.V. All rights reserved.

binding to certain motifs that present in the promoters [13]. To date, the DRE/CRT (A/GCCGAC) and GCC-box (AGCCGCC) are the two main *cis*-acting elements that bound by ERF proteins to regulate the plant's response to environmental stress; additionally, a TTG element was also found to be bound by ERF proteins and involved in salt sress response [13,25–30]. Xu et al. [31] studied the *TaERF1* gene of wheat (*Triticum aestivum*) and showed that it could regulate pathogen and abiotic stress related genes through binding to the DRE and/or GCC-box motifs in their promoters. The *ERF1* gene from Arabidopsis could positively regulate its downstream genes through binding to DRE and GCC-box elements in their promoter regions of them, thereby conferring the salt tolerance to the transgenic Arabidopsis [26]. In addition to binding to DRE and GCC-box, ERF could also bind to other *cis*-acting elements. For instance, Wang et al. [30] showed that ThERF1 from *T. hispida* mainly binds to a novel *cis*-acting element, TTG motif, to regulate gene expression, and DRE and GCC-box were found rarely in the promoters of ThERF1-regulated genes when exposed to salt stress conditions.

T. hispida is a shrub or small tree that belongs to the Genus *Tamarix* Linn. It is particularly well known because of its strong tolerance to salt and alkali. *Tamarix* species could thrive in arid and salinized lands suggested that they are ideal woody halophytes for the study of abiotic stress tolerance.

In the current investigation, an ERF transcription factor was cloned from *T. hispida*, *ThCRF1*, whose transcripts is increased under salt and drought conditions. To characterize its function, we transiently transformed *T. hispida* for overexpression and RNAi-silence of *ThCRF1* for gain- and loss-of-function study. In addition, *ThCRF1* transformed *A. thaliana* plants were also generated, and were used to verify the results from *T. hispida*. A working model of ThCRF1′s role in response to salt stress was proposed. These results will be helpful in understanding the function of ERF TFs involved in abiotic stress tolerance.

2. Materials and methods

2.1. Plant material and abiotic stress

The seeds of *T. hispida* were collected from the Turpan Botanical Garden of the Chinese Academy of Sciences. The seeds were seeded in a sand and turf peat mixture (1:2 v/v) and grown in green house with the conditions of 14 h light, 10 h dark, and 70–75% relative humidity at 24 °C. The 2-month-old seedlings were treated with fresh water (as controls), 400 mmol/L NaCl or 400 mmol/L mannitol, respectively. After treatments for 3, 6, 9, 12, and 24 h, the roots and the aerial parts treated with fresh water, NaCl or mannitol were respectively harvested at the indicated time points, and were used for RNA isolation and determination of MDA and reactive oxygen species (ROS) contents. To identify whether *ThCRF1* was induced by cytokinin, the *T. hispida* seedlings were grown in the above-described growth conditions and then placed in 50 mL of 1/2MS liquid medium and cultured for 2 h. Then, 10 μM 6-Benzyladenine (6-BA) was added, and incubation was continued with shaking for 2 h, with the addition of 0.1% (v/v) DMSO as a control according to Rashotte and Goertzen [8]. After treatment, the seedlings were harvested for RNA isolation. To culture *T. hispida* in tubes, seeds were surface sterilized for 10 min in 5% NaClO, rinsed with sterile water for five times, and then seeded in 1/2 MS solid (2% sucrose) medium.

Arabidopsis seeds were plated on 1/2 MS solid medium. Seven-day-old seedlings were moved to pot containing a soil/perlite mixture under the conditions of 8 h dark/16 h light cycle, around 70% relative humidity, and with the temperature of 22 °C.

2.2. Cloning and sequence analysis of ThCRF1

The full length of cDNA sequence of *ThCRF1* was cloned from *T. hispida* with the GenBank number of MF377537 [32]. For multiple sequence alignments, ThCRF1 was aligned with the amino acid sequences of other ERFs from Arabidopsis using the program Clustal W [33]. MEGA 5.0 was used to construct the phylogenetic tree based on the neighbor-joining method and bootstrap analysis with 1000 replications [34].

2.3. Quantitative real-time reverse transcription PCR (qRT-PCR)

Total RNA was isolated from *T. hispida* using a CTAB (hexadecyltrimethyl ammonium bromide) method, according to Chang et al. [35] with modifications. OligodT was used as initial primer to synthesis the cDNA with a TransStart® RT/RI Enzyme Mix kit (TransGen Biotech, China). Real-time PCR was carried out on an Opticon 2 System (Bio-Rad, Hercules, CA, USA). The conditions for qRT-PCRs were: 94 °C for 30 s; followed by 94 °C for 12 s, 58 °C for 30 s, 72 °C for 30 s, and 80 °C for 1 s (plate reading) for 45 cycles. Three independent biological replications were conducted and each replication was performed with three technical replications. The $2^{-\Delta\Delta CT}$ method was used to calculate the relative expression levels according to Livak and Schmittgen [36]. For *T. hispida* samples, *α-tubulin* (GenBank number FJ618518) and *Actin* (GenBank number FJ618517) were employed as the two internal controls to normalize RNA quantity; for Arabidopsis samples, *ATACT7* (GenBank number AT5G09810) and *TUB2* (GenBank number AT5G62690) were used as the internal controls to normalize RNA quantity. All primer sequences were shown as **Tables S1** and **S2**. For analysis of the expression of *ThCRF1* in *T. hispida* in response to NaCl and mannitol, the transcript level of ThCRF1 in T. hispida treated with fresh water at each time point was used to normalize that under salt or drought treatment at the corresponding time point.

2.4. Subcellular localization analysis

The coding region (CDS) of the *ThCRF1* cDNA, without the stop codon, was fused with the N-terminus of the GFP (green fluorescent protein) gene to obtain the *ThCRF1-GFP* fusion construct controlled by a 35S *CaMV* promoter (35S:ThCRF1-GFP). The gene of *GFP* under the control of 35S promoter (35S:GFP) was used as a control. These constructs were transformed separately into onion epidermal cells with a particle bombardment method (Bio-Rad,). After 24 h of culture on 1/2 MS solid medium in the dark, these transformed cells were visualized by a confocal laser scanning microscopy LSM410 (Zeiss, Jena, Germany). For nuclear location, 10 $\mu g \cdot mL^{-1}$ DAPI (4,6′-diamidino-2-phenylindole) dye was used to stain the cells for about 15 min. The primers used for plasmid construction are shown as **Table S3**.

2.5. Transcription activation assay

The CDS of ThCRF1, without the stop codon, was cloned into vector pGBKT7 (Clontech, USA) and introduced into yeast strain Y2HGold for transcription activation analysis. Yeast transformation and determination of blue/white colonies were conducted according to the instructions of manufacturer (Clontech, USA), and X-α-Gal was used as a substrate for the reporter gene MEL1. Primers used for plasmid construction are shown in **Table S3**.

2.6. Yeast one hybrid analysis

To determine whether ThCRF1 binds to the GCC-box, DRE, or TTG *cis*-acting elements, three tandem copies of TTG1, TTG2, DRE, GCC-box, and their base mutants as the main sequences were respectively cloned into pHIS2 (Clontech, USA) as reporters. The CDS of ThCRF1 was inserted into pGADT7-Rec2 plasmid (Clontech) to serve as an effector vector. Each reporter was co-transformed with the effector into Y187 for yeast one-hybrid (Y1H) assay according to the instructions for the Matchmaker™ Gold Yeast One-Hybrid System (Clontech, USA). The positive control was the interaction between pGADT7-Rec2-53 (the pGADT7-Rec2-53 plasmid harboring a murine p53 and GAL4 AD

Download English Version:

https://daneshyari.com/en/article/5515687

Download Persian Version:

https://daneshyari.com/article/5515687

Daneshyari.com