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Tamarix hispida zinc finger protein ThZFP1 participates in salt and osmotic stress tolerance by increasing proline content and SOD and POD activities

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ABSTRACT

Zinc finger proteins (ZFPs) are a large family that play important roles in various biological processes, such as signal transduction, RNA binding, morphogenesis, transcriptional regulation, abiotic or biotic stress response. However, the functions of ZFPs involved in abiotic stress are largely not known. In the present study, we cloned and functionally characterized a *ZFP* gene, *ThZFP1*, from *Tamarix hispida*. The expression of *ThZFP1* is highly induced by NaCl, mannitol or ABA treatment. To study the function of *ThZFP1* involved in abiotic stress response, transgenic *T. hispida* plants with overexpression or knockdown of *ThZFP1* were generated using a transient transformation system. Gain- and loss-of-function studies of *ThZFP1* suggested that ThZFP1 can induce the expression of a series of genes, including *delta-pyrroline-5-carboxylate synthetase* (*P5CS*), *peroxidase* (*POD*) and *superoxide dismutase* (*SOD*), leading to accumulation of proline and enhanced activities of SOD and POD. These physiological changes enhanced proline content and reactive oxygen species (ROS) scavenging capability when exposed to salt or osmotic stress. All the results obtained from *T. hispida* plants were further confirmed by analyses of the transgenic *Arabidopsis* plants overexpressing *ThZFP1*. These data together suggested that ThZFP1 positively regulates proline accumulation and activities of SOD and POD under salt and osmotic stress conditions.

1. Introduction

Zinc-finger proteins (ZFPs) constitute one of the largest protein families in plants. They are characterized by a common zinc finger domain, which is involved in protein–protein recognitions and sequence-specific binding to DNA/RNA [1]. The zinc finger domain is consisted by cysteines and/or histidines coordinating with zinc atoms to form the peptide structures essential for specific biological functions [2,3]. According to the number and order of the C and H residues (C and H represent cysteine and histidine, respectively) in the secondary structure of the finger, ZFPs are classified into nine groups, including the types of C2H2, C8, C6, C3HC4, C2HC, C2HC5, C4, C4HC3 and CCCH [4,5].

ZFPs are found to be involved in diverse biological processes, including transcriptional regulation, signal transduction, RNA binding and morphogenesis, and play important roles in many aspects of biological processes, such as plant growth, development and various biotic and abiotic stress responses [2,4,6]. For instance, some plant ZFPs serve as a component of the signal transduction chain linking light signals to development [7]. A C3HC4-type RING finger protein was found to target a photomorphogenesis-promoting transcription factor involved in ubiquitylation and degradation [8,9]. *Arabidopsis AtATL15* ZFP gene was shown to be responsive to ascorbate (AsA) and played a role in regulation of plant growth [10]. In *Capsicum annuum*, CaRZFP1, a heat-inducible C3HC4 type RING ZFP, can promote plant growth by inducing the expression of some growth related genes [11]. ZFPs also play important roles in biotic and abiotic stress tolerance. For instance, overexpression of a CCCH type zinc finger protein (*AtZFP1*) can confer salt tolerance to the transgenic plants by maintaining ionic balance and limiting oxidative and osmotic stress. Further studies indicated that AtZFP1 could regulate the expression of some stress related genes such as *SOS1*, *AtP5CS1*, *KIN1*, *RD29B* and *RD22* to mediate stress tolerance [12]. ZFP36, a rice C2H2-type zinc finger protein, is found to regulate the expression of *SOD* and *APX* genes to enhance their activities, and therefore plays a key role in antioxidant defence and oxidative stress tolerance [13]. A zinc finger gene from *Chrysanthemum morifolium*, *BBX24*, plays a dual role in modulating both flowering time and abiotic stress tolerance *via* influencing GA biosynthesis [14]. *Arabidopsis* AtZAT6 protein could regulate the expression of

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salicylic acid-related genes and C-REPEAT-BINDING FACTOR1 (*CBF*) to positively modulate biotic and abiotic stress tolerance [15]. *Arabidopsis* CCCH type zinc finger protein AtZFP1 is found to improve salt tolerance by adjusting ionic balance and decreasing oxidative and osmotic stress [16]. *OsRHC1*, a rice ZFP gene, was shown to be involved in plant disease resistance [17]. *Arabidopsis* CCCH tandem zinc finger proteins (AtTZF1) is associated with processing bodies (PBs), which can bind DNA and RNA *in vitro*, suggesting that AtTZF1 might play a role in the regulation of DNA and/or RNA [18]. These studies suggested that ZFPs play important roles in many biological processes, and are also involved in abiotic stress tolerance. However, there are still many ZFPs that have not been functionally characterized.

In the present study, we cloned a *ZFP* gene, *ThZFP1*, from *T. hispida* and characterized its function involved in abiotic stress tolerance. The findings showed that *ThZFP1* could increase proline content and activities of POD and SOD by inducing the expression of genes such as *delta-pyrroline-5-carboxylate synthetase* (*P5CS*), *superoxide dismutase* (*SOD*) and *peroxidase* (*POD*). Our study provides insights into the function of *ThZFP1* in the regulation of salt and osmotic stress tolerance in *T. hispida*.

2. Materials and methods

2.1. Plant materials

The seeds of *T. hispida* were planted into the mixture of turf peat and sand (2:1, v/v) in a greenhouse with the conditions of 70–75% relative humidity, 14 h light/10 h darkness photocycle and a stable temperature at 24 °C. For analysis of the expression of *ThZFP1*, well watered two-month-old seedlings were watered on their roots with a solution of 100 μM ABA, 200 mM mannitol or 200 mM NaCl, and were collected at 6 h post-watering. Meanwhile, the seedlings watered with fresh water were used as controls. *Arabidopsis* plants were grown in pots containing the mixture of perlite/soil (2:1, v/v) in a greenhouse under the conditions of 70–75% relative humidity, a 16 h light/8 h darkness photocycle and a stable temperature at 22 °C.

2.2. Cloning of ThZFP1 and generation of ThZFP1 transformed plants

A full-length ZFP cDNA, *ThZFP1* (GenBank number: KM434773), was cloned from *T. hispida* by transcriptome analysis [19]. The amino acid sequences of ThZFP1 and 47 C3HC4-type RING type ZFPs from *Arabidopsis* retrieved from TAIR (http://www.arabidopsis.org/) database were analyzed with Clustal X software (version 1.81) using the neighbor-joining method, and a phylogenetic tree was built with the TreeView32 software.

The coding region (CDS) of *ThZFP1* was cloned into pROK2 under the control of *35S CaMV* promoter for overexpression of *ThZFP1* (35S:ZFP construct). An inverted repeat truncated cDNA of *ThZFP1* with 273 bp in length was cloned into the RNAi vector pFGC5941 [20] at the two sides of CHSA intron to generate the construct pFGC:ZFP for silence of the expression of *ThZFP1*. The genetic transformation of *T. hispida* plants was performed according a previously published protocol [21]. Briefly, plant seedlings were soaked in a transformation solution (1/2 MS + 150 μM acetosyringone + 3% (w/v) sucrose + 0.01% (w/v) Tween 20 + 0.6 OD *Agrobacterium tumefaciens*, pH 5.6) with shaking at 120 rpm at 25 °C. After soaking for 6 h, the plants were washed twice with distilled water, and were grown vertically on 1/2 MS agar medium (supplied with 150 μM acetosyringone and 2% (w/v) sucrose, pH 5.8). Three groups of transgenic *T. hispida* plants were generated by transient transformation with 35S:ZFP to overexpress *ThZFP1* (OX), with pFGC:ThZFP1 to silence the expression of *ThZFP1* (KD) or with the empty pROK2 plasmid as control (Con). The expression of *ThZFP1* in these kinds of transformed *T. hispida* plants was studied using real-time RT-PCR as described previously [21]. Briefly, total RNA was isolated from *T. hispida* and reversely transcribed into cDNA as the PCR template. *Agrobacterium*-mediated floral dip transformation with 35S:ZFP was used to generate *Arabidopsis* plants overexpression of *ThZFP1* [22]. The sequences of all primers used are shown in supplementary Table S1.

2.3. Subcellular localization analysis

For generation of the *ThZFP1* and *GFP* fusion gene, the coding region of *ThZFP1* without the termination codon was fused to the N-terminus of the green fluorescent protein (GFP) and was cloned into pROK2 under the control of the *35S CaMV* promoter (35S:ZFP-GFP). The GFP gene driven by 35S promoter (35:GFP) was used as a control. The constructs 35S:ZFP-GFP and 35:GFP were respectively transiently transformed into onion epidermis cells using particle bombardment according to the protocol (Bio-Rad, Hercules, CA, USA). After transformed for 48 h, the onion epidermis cells were visualized using a confocal laser scanning microscopy (LSM410, Zeiss, Jena, Germany).

2.4. Stress tolerance analysis in Arabidopsis plants

The *ThZFP1* transformed *Arabidopsis* plant T3 generation lines were randomly selected (Line 3 and 4) for stress tolerance. For germination rate assay, the seeds were sown on half-strength Murashige–Skoog (1/2MS) medium (as control), or 1/2 MS containing 2 μM ABA, 100 mM NaCl or 200 mM mannitol. After one week growth, the germination rates of the transgenic lines and wild type (WT) were calculated. For the stress tolerance experiment, seeds were sown on 1/2 MS medium for 3 d for germination and then transferred into 1/2 MS medium (as control condition) or 1/2 MS medium supplied with 2 μM ABA, 100 mM NaCl or 200 mM mannitol. After two weeks, the root length and fresh weight were measured.

2.5. NBT, DAB and Evans blue staining

For H_2O_2 and O^{2-} detection, NBT and DAB *in situ* staining were performed. The whole plants were exposed to 150 mM NaCl, 200 mM mannitol or 10 μM ABA for 24 h, and then young branches from *T. hispida* (OX, KD and Con) or leaves from *Arabidopsis* were incubated with nitroblue tetrazolium (NBT; 1 mg/ml NBT in 10 mM sodium azide and 10 mM phosphate buffer, pH 7.8) or 3′-diaminobenzidine (DAB) solutions (1 mg/ml DAB–HCl, pH 3.8), respectively. The procedures for NBT and DAB staining were followed by Zhang et al. [23]. Evans blue staining for examination of cell death was performed as described by Kim et al. [24]. Briefly, young branches from *T. hispida* or leaves from *Arabidopsis* were vacuum-infiltrated in 0.1% (w/v) Evans blue solution for 15 min, and were rinsed with a phosphate buffer for five times.

2.6. Analysis of physiological changes involves in abiotic stress tolerance

For the physiological studies, after transient transformation for 48 h, Con, OX and KD *T. hispida* plants were treated with 150 mM NaCl for 24 or 48 h, and the whole plants were harvested for study. The transgenic and WT *Arabidopsis* plants were treated with 150 mM NaCl, 200 mM mannitol or 10 μM ABA for 24 h, and the whole plants were harvested for study. A chlorophyll analyzer (Konica Minolta, Japan) was used to determinate