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RESEARCH ARTICLE

A wheat gene *TaSAP17-D* encoding an AN1/AN1 zinc finger protein improves salt stress tolerance in transgenic *Arabidopsis*

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Abstract

The stress-associated protein (SAP) multigene family is conserved in both animals and plants. Its function in some animals and plants are known, but it is yet to be deciphered in wheat (*Triticum aestivum* L.). We identified the wheat gene *TaSAP17-D*, a member of the SAP gene family with an AN1/AN1 conserved domain. Subcellular localization indicated that TaSAP17-D localized to the nucleus, cytoplasm, and cell membrane. Expression pattern analyses revealed that *TaSAP17-D* was highly expressed in seedlings and was involved in NaCl response, polyethylene glycol (PEG), cold, and exogenous abscisic acid (ABA). Constitutive expression of *TaSAP17-D* in transgenic *Arabidopsis* resulted in enhanced tolerance to salt stress, confirmed by improved multiple physiological indices and significantly upregulated marker genes related to salt stress response. Our results suggest that *TaSAP17-D* is a candidate gene that can be used to protect crop plants from salt stress.

Keywords: SAP, multigene family, *TaSAP17-D*, salt tolerance, wheat

1. Introduction

As sessile organisms, plants are vulnerable to various abiotic stresses, such as drought, salinity, extreme temperature, high light intensity, and heavy metals. Crop production and productivity deteriorates under these adverse environmental conditions (Knight and Knight 2001). One important way to improve tolerances to multiple stresses is to overexpress transcription factor gene(s) that are able to control multiple genes from various pathways (Kasuga *et al*. 1999; Shi *et al*. 2015) or by overexpressing genes responsible for abiotic signal perception and transduction (Zhang *et al*. 2012; Chakraborty *et al*. 2015). The zinc finger protein (ZFP) is a multifunction protein that acts as a transcription factor, RNA-binding protein, as well as a protein modification enzyme protecting against environmental stresses (Jan *et al*. 2013; Hichri *et al*. 2014; Zhang *et al*. 2014; Baek *et al*. 2015). Stress-associated protein (SAP) is a group of ZFPs composed of two zinc-finger domains, an N-terminal A20 domain and/or a C-terminal AN1 domain.

The SAP gene family has been identified in eukaryotes and is well-characterized in animals (Gilles *et al*. 2011). In plants, the first SAP gene *OsiSAP1* isolated from rice and encodes an A20/AN1 ZFP induced by multiple abiotic stresses (Mukhopadhyay *et al*. 2004). Since then, *SAP*s have been identified in many plant species, including

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tomato, maize, *Arabidopsis*, *Aeluropus littpralis*, *Populus trichocarpa*, *Solanum lycopersicum*, and *Medicago truncatula* (Vij and Tyagi 2008; Ben Saad *et al*. 2010; Gilles *et al*. 2011). The majority of SAPs are involved in abiotic stress response and several of them improve tolerances to biotic and abiotic stresses (Giri *et al*. 2013). The AtSAP5 and OsSAP7 proteins have been shown to possess E3 ligase activity and increased drought tolerance (Kang *et al*. 2013; Sharma *et al*. 2015). In addition, *AtSAP12* can function as a redox sensor by changing its oligomeric conformation depending upon the cellular redox potential (Ströher *et al*. 2009). Plants transformed with the *MusaSAP1* gene have demonstrated strengthened defense mechanisms against biotic stresses by upregulating polyphenol oxidase (PPO) at the transcriptional level (Sreedharan *et al*. 2012). The *TaSAP1* and *TaSAP2* genes are found to be involved in multiple abiotic stress response, including drought, high salinity, cold, and exogenous abscisic acid (Wang 2011). Six haplotypes (Hap) of *TaSAP1-A1* have been identified in 300 wheat accessions, among them *TaSAP1-A1-Hap*III is significantly associated with thousand-grain weight in multiple growing environments (Chang *et al*. 2013).

Accumulating evidence shows that SAPs involved in various environmental stresses responses, while their underlying molecular and physiological mechanisms are poorly understood. Wheat is one of the most important staple crops yet only two SAPs (A20/AN1 domain) have been characterized (Wang 2011; Chang *et al*. 2013). In this research, we identified a typical AN1/AN1 type SAP gene, *TaSAP17*, which is highly conserved in wheat nucleotide and amino acid sequences. The transcript of *TaSAP17-D* was induced by various environmental stresses. Over-expression of *TaSAP17-D* altered the expression patterns of stress responsive genes and led to strengthened salt stress tolerance in *Arabidopsis*.

2. Materials and methods

2.1. Plant materials, growth and treatment conditions

Wheat variety Hanxuan 10 that is known for its remarkable drought and salt tolerance was used in this study. For abiotic stress treatments, 11-d-old seedlings (at the two-leaf stage) grown in 1/2 Murashige and Skoog (MS) liquid culture at 23/18°C in a 16/8 h (light/dark) photoperiod were subjected to the following treatments: polyethylene glycol-6000 (16.1% PEG, –0.5 MPa), 250 mmol L^{-1} NaCl, low temperature (4°C), and 50 μmol L^{-1} abscisic acid (ABA). Leaf samples were harvested at 0.5, 1, 1.5, 2, 3, 6, 12, 24, 48, and 72 h. To investigate the expression of target genes at different developmental stages in wheat (germination, seedling, and heading stages), different tissues including plumule, root base, root, leaf, internode, flag leaf, stem, node, and depth of root at 0–30, 30–50, 50–70, 70–90, and 90–100 cm were collected.

Two wheat genotypes, the salt-tolerant variety Taishan 23 and the salt sensitive variety Nongda 20074, were selected to detect the expression patterns of *TaSAP17-D* under salt stress conditions (Peng *et al*. 2017).

Arabidopsis thaliana ecotype Col-0 was used in the transgenic experiment. *Arabidopsis* plants were grown in the chamber at 23/18°C with a 16/8 h (light/dark) photoperiod and 70% humidity.

2.2. Real-time quantitative PCR

Real-time quantitative PCR (qRT-PCR) was performed to determine gene expression. The *TaActin* (forward primer, 5′-CTCCCTCACAACAACAACCGC-3′; reverse primer, 5′-TACCAGGAACTTCCATACCAAC-3′) and *AtACTIN2* (forward primer, 5′-AGCACTTGCACCAAGCAGCATG-3′; reverse primer, 5′-ACGATTCCTGGACCTGCCTCATC-3′) were used as an internal control to quantify the relative transcript level of wheat and *Arabidopsis* target genes, respectively. The qRT-PCR was performed in triplicate with a Roche LightCycler 96 Real-Time PCR System (Roche, Switzerland) using the SYBR Green PCR Master Mix Kit (TaKaRa, Japan). Thermal cycling conditions were pre-incubated at 95°C for 2 min, followed by 95°C for 10 s, 60°C for 30 s, and 72°C for 30 s for 45 cycles. The relative transcription level for each gene was calculated using the $2^{-\Delta\Delta C_T}$ method (Schmittgen and Livak 2008).

2.3. Isolation of the full-length cDNA of *TaSAP17-D*

The full-length cDNA of *TaSAP17-D* was amplified using primers *TaSAP17* (forward primer, 5′-ATGGGCACG CCGGAGTTCC-3′; reverse primer, 5′-TTATGCTTTT GAAGTTCCTC-3′), and the PCR products were ligated with pEASY-Blunt vectors (TransGen, Beijing, China). The positive clones were selected after transformation, and then sequenced with an ABI 3730XL DNA Analyzer (Life Tech, USA).

2.4. Phylogenetic analyses

To understand the relationship of TaSAP17 and other SAP members, the amino acid sequence of TaSAP17 was used as a query in a BLASTX search to gather sequences with high similarity. The putative sequences with higher similarity were downloaded from NCBI for further analysis. Sequence alignments and comparisons were implemented by the