Biochemical and Biophysical Research Communications xxx (2018) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Arabidopsis non-TZF gene *AtC3H17* functions as a positive regulator in salt stress response

Hye-Yeon Seok [1], Linh Vu Nguyen [1], Hee-Yeon Park, Vaishali N. Tarte, Jimin Ha, Sun-Young Lee, Yong-Hwan Moon*

Department of Integrated Biological Science, Pusan National University, Busan, 46241, Republic of Korea

ARTICLE INFO

Article history:
Received 11 March 2018
Accepted 12 March 2018
Available online xxx

Keywords:
AtC3H17
Arabidopsis
Non-tandem CCCH-type zinc finger protein
ABA-dependent salt stress response

ABSTRACT

Functional studies of CCCH-type zinc finger proteins in abiotic stress responses have largely focused on tandem CCCH-type zinc finger (TZF) genes, whereas the study of functional roles of non-TZF genes in abiotic stress responses has largely been neglected. Here, we investigated the functional roles of *AtC3H17*, a non-TZF gene of Arabidopsis, in salt stress responses. *AtC3H17* expression significantly increased under NaCl, mannitol, and ABA treatments. *AtC3H17*-overexpressing transgenic plants (OXs) were more tolerant under NaCl and MV treatment conditions than the wild type (WT). *atc3h17* mutants were more sensitive under NaCl and MV treatment conditions compared with the WT. The transcription of the salt stress-responsive genes in ABA-dependent pathway, such as *RAB18*, *COR15A*, and *RD22*, was significantly higher in *AtC3H17* OXs than in WT both under NaCl-free condition and after NaCl treatment. Our results demonstrate that *AtC3H17* functions as a positive regulator in salt stress response, via the up-regulation of ABA-dependent salt stress-response pathway.

1. Introduction

Zinc finger proteins are categorized into several different kinds, depending on the order and number of Cysteine (Cys) and Histidine (His) residues, which bind to zinc ion in the secondary structure of the finger. Zinc finger proteins containing a CCCH-type zinc finger motif (consisting of three Cys residues and one His residue) are categorized as CCCH-type zinc finger proteins. CCCH-type zinc finger proteins are found in most eukaryotic organisms [1]. Sixty-eight and 67 CCCH-type zinc finger protein genes have been identified in Arabidopsis (*Arabidopsis thaliana*) and rice (*Oryza sativa*), respectively [1]. All 68 Arabidopsis CCCH-type zinc finger proteins contain 1–6 CCCH-type zinc finger motif(s) and are classified into 11 subfamilies based on the spacing between Cys and His in the zinc finger motifs as well as the number of zinc finger motifs [1,2]. In addition, CCCH-type zinc finger proteins are divided into tandem CCCH-type zinc finger (TZF) and non-TZF proteins: TZF proteins contain two tandem CCCH-type zinc finger motifs whereas non-TZF proteins have fewer or greater than two CCCH-type zinc finger motifs. There are 26 putative TZF proteins and 42 non-TZF proteins in the Arabidopsis genome [1]. Intriguingly, Arginine-rich (RR)-TZF proteins in plants have an RR-TZF domain comprising a plant-unique TZF motif of C-X_{7-8}-C-X_5-C-X_3-H-X_{16}-C-X_5-C-X_4-C-X_3-H preceded by an RR domain [3]. In Arabidopsis, 11 RR-TZF genes known as *AtTZF1-AtTZF11* represent the largest subfamily, IX, of CCCH-type zinc finger protein genes [4].

CCCH-type zinc finger proteins have been known as RNA-binding proteins involved in post-transcriptional regulation. For AtTZF1, the TZF motif is important for binding of RNA in a zinc-dependent fashion [5]. Moreover, AtC3H3 binds to RNA and acts as a nuclease [6]. However, it was also reported that plant CCCH-type zinc finger proteins function in transcriptional regulation. AtC3H14 and AtC3H15/AtCDM1 regulate transcription through DNA-binding and they exhibit transcriptional activation activity in yeast [7].

In plants, CCCH-type zinc finger proteins are required in multiple biological processes such as the regulation of plant growth, maintenance of homeostasis, acquisition of immunity against

Abbreviations: ABA, abscisic acid; DAG, days after germination; FW, fresh weight; *GAPc*, glyceraldehyde 3-phosphate dehydrogenase; LD, long-day; MS, Murashige and Skoog; MV, methyl viologen; NBT, nitroblue tetrazolium; OX, overexpressing transgenic plant; ROS, reactive oxygen species; RT-PCR, reverse transcription-PCR; SD, short-day; TZF, tandem CCCH-type zinc finger; WT, wild type.

* Corresponding author.
E-mail address: moonyh@pusan.ac.kr (Y.-H. Moon).
[1] These authors equally contributed to this paper.

https://doi.org/10.1016/j.bbrc.2018.03.088
0006-291X/

pathogens, and adaptation to hormone and stress responses [7–11]. *AtTZF2*/*AtOZF1*, *AtTZF3*/*AtOZF2*, and cotton (*Gossypium hirsutum*) *GhZFP1* are all associated with jasmonic acid-induced leaf senescence [2,12]. *AtTZF4*/*SOM* and *AtTZF6*/*PEI1* play roles in seed germination and embryo formation, respectively [8,10]. *AtC3H14* and *AtC3H15*/*AtCDM1* are involved in the regulation of secondary wall thickening, male fertility, and anther development [7].

Functional studies have revealed that some CCCH-type zinc finger protein genes are engaged in the regulation of abiotic stress responses. *AtSZF1*/*AtTZF11* and *AtSZF2*/*AtTZF10* negatively regulate salt stress response [13], whereas *AtTZF4*/*SOM*, *AtTZF5*, and *AtTZF6*/*PEI1* positively regulate abscisic acid (ABA) response [14]. Overexpression of *AtTZF2*/*AtOZF1* or *AtTZF3*/*AtOZF2* has shown to confer ABA hypersensitivity and drought tolerance [2]. In rice, the expression of *OsTZF1* is up-regulated by drought, salt stress, and hydrogen peroxide [15]. Overexpression of cotton *GhZFP1* enhances tolerance to drought and delays drought-induced senescence [12]. *AtCPSF30*/*AtC3H11* is a subunit of polyadenylation factor and is required for *Pseudomonas* resistance [16]. In addition, *oxt6*, a T-DNA insertion mutant of *AtCPSF30*/*AtC3H11*, alters the poly(A) site choice and mRNA profile, and enhances the tolerance to oxidative stress [17]. However, functional investigation of plant CCCH-type zinc finger protein genes in abiotic stress responses has largely focused on TZF genes, whereas the functional roles of non-TZF genes in abiotic stress responses remain uncharacterized, except for *AtCPSF30*/*AtC3H11*.

AtC3H17, a non-TZF gene, is a single copy gene in Arabidopsis. We previously reported that *AtC3H17* has pleiotropic effects during vegetative and reproductive development, and functions as a transcriptional activator in nucleus [18]. Here, we investigated the role of *AtC3H17* in salt stress response. Our results demonstrate that *AtC3H17* is involved in the salt stress response of Arabidopsis through the modulation of ABA-dependent salt stress response pathway.

2. Materials and methods

2.1. Plant material and growth conditions

All Arabidopsis plants in this study were the Columbia ecotype (Col-0). The seeds were surface-sterilized, water-imbibed, and plated on MS agar media at 22 °C under short-day (SD) conditions (8-h-light/16-h-dark) [18]. After 7 days of germination (DAG), the seedlings were transplanted into the soil and grown at 22 °C under long-day (LD) conditions (16-h light/8-h dark). Selection of transgenic plants was carried out on media containing 25 mg/L kanamycin. T_3 or T_4 progeny of the homozygous lines was used in this study.

2.2. Stress treatments

For stress treatments prior to semi-quantitative and quantitative RT-PCR analyses, seedlings at 10 DAG grown under SD conditions were subjected to filter papers soaked with MS solution containing 300 mM NaCl, 300 mM mannitol, or 100 μM ABA. The seedlings were harvested at 0, 1, 2, 4, and 8 h after the exposure.

To analyze the salt and oxidative stress responses of *AtC3H17* OXs and *atc3h17* mutants, seedlings at 7 DAG grown under SD conditions were transplanted to MS agar media supplemented with 0, 130, 140, 150, and 160 mM NaCl and 0, 1.5, 2.0, and 2.5 μM methyl viologen (MV). After 8, 11, and 12 d of the treatment, photograph was taken and fresh weight (FW) of the seedlings was measured.

To analyze the NaCl tolerance of mature *AtC3H17* OXs and *atc3h17* mutants, plants at 21 DAG grown under LD conditions were irrigated with 0, 250, 300, 350, and 400 mM NaCl for 3 weeks at 3- or 4-d intervals.

2.3. Histochemical staining of superoxide production

For histochemical staining of superoxide, seedlings at 10 DAG grown under SD conditions were placed on filter papers soaked with 0, 50, and 100 mM NaCl and 0, 0.1, 0.5, and 1.0 μM MV for 2 h. The seedlings were stained by nitroblue tetrazolium (NBT) solution as previously described [19].

2.4. Chlorophyll fluorescence (F_v/F_m) and chlorophyll content measurement

Photosynthetic activity (F_v/F_m) in the third or fourth rosette leaves of 6-week-old plants grown under LD conditions was measured using Handy PEA chlorophyll fluorimeter (Hansatech, King's Lynn, UK) as previously described [20].

Measurement of chlorophyll content in the fourth or fifth rosette leaves of 6-week-old plants grown under LD conditions was performed using SPAD-502 plus chlorophyll meter (Konica Minolta, Inc., Japan) according to Seok et al. [20].

2.5. Total RNA isolation and RT-PCR

The total RNA was extracted using a RNAqueous Plant Kit (Life Technologies, USA), supplemented with Plant RNA Isolation Aid (Life Technologies, USA) according to the manufacturer's protocols. The reverse-transcription reaction was performed as described in Seok et al. [20].

Quantitative PCR was performed in a reaction mixture containing gene-specific primers and Power SYBR Green PCR Master Mix (Applied Biosystems, USA) using QuantStudio 3 real-time PCR system (Applied Biosystems, USA), as described in Seok et al. [20]. Data were analyzed using QuantStudio™ Design and Analysis software (version 1.4). The normalized amount of target reflected the relative amount of target transcripts with respect to the endogenous reference gene *GAPc*.

Semi-quantitative PCR was carried out according to Seok et al. [20], in 30–33 cycles for *AtC3H17* and 22–25 cycles for *RD29A*, *RAB18*, *COR15A*, *RD22*, *DREB2A*, and *GAPc*, which were shown to be in the linear range of the amplification reaction. The sequences of primers used in the quantitative and semi-quantitative PCR are shown in Table S1.

3. Results and discussion

3.1. Transcription of AtC3H17 increases under osmotic stress conditions

In a previous study about the function of *AtC3H17*, we investigated differential gene expression between WT and *AtC3H17* OXs using a microarray experiment [18]. *RAB18* and *COR15A*, known as osmotic stress-responsive genes, displayed up-regulation in *AtC3H17* OXs compared with the WT [18]. To analyze the expression of *AtC3H17* under osmotic stress conditions, we carried out a quantitative RT-PCR analysis of *AtC3H17* using WT seedlings exposed to 300 mM NaCl, 300 mM mannitol, and 100 μM ABA for 0, 1, 2, 4, and 8 h. As a result, the transcript level of *AtC3H17* increased, starting from 1 h after NaCl, mannitol, and ABA treatments (Fig. 1A–C). *RD29A* transcript level was analyzed to confirm the proper treatment of NaCl, mannitol, and ABA (Fig. 1D–F). We obtained similar results from the semi-quantitative RT-PCR analysis, in which *AtC3H17* exhibited the increase of transcript level by NaCl, mannitol, and ABA treatments (Supplementary Fig. S1). These results suggest that *AtC3H17* might be involved in osmotic stress response. We further focused on the functional characterization of *AtC3H17* in salt stress response.

Please cite this article in press as: H.-Y. Seok, et al., Arabidopsis non-TZF gene *AtC3H17* functions as a positive regulator in salt stress response, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.03.088