Biochemical and Biophysical Research Communications 499 (2018) 1039–1043

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

H2A.Z-containing nucleosomes are evicted to activate *AtMYB44* transcription in response to salt stress

Nguyen Hoai Nguyen, Jong-Joo Cheong*

Center for Food and Bioconvergence, Seoul National University, Seoul 08826, Republic of Korea

ARTICLE INFO

Article history:
Received 6 April 2018
Accepted 7 April 2018
Available online 10 April 2018

Keywords:
Arabidopsis
AtMYB44
H2A.Z
Nucleosome
Salt stress
Transcription

ABSTRACT

Transcripts of the Arabidopsis transcription factor gene, *AtMYB44*, accumulate rapidly to mediate a tolerance mechanism in response to salt stress. The *AtMYB44* promoter is activated by salt stress, as illustrated in *AtMYB44pro::GUS* transgenic plants. Chromatin immunoprecipitation (ChIP) assays revealed that RNA polymerases were enriched on the *AtMYB44* gene, especially on TSS-proximal regions, and nucleosome density was markedly reduced in the *AtMYB44* gene-body region in response to salt stress. In addition, H2A.Z occupation was significantly decreased at the *AtMYB44* promoter, transcription start site (TSS), and gene-body regions. Histone modifications including histone H3 lysine 4 trimethylation (H3K4me3) and histone H3 and H4 acetylation (H3ac and H4ac) were not affected under the same stress conditions. We found a decrease in the number of AtMYB44 proteins bound to their own gene promoters in response to salt stress. These results suggest that salt stress induces the eviction of H2A.Z-containing nucleosomes from the *AtMYB44* promoter region, which may weaken its affinity for binding AtMYB44 protein that acts as a repressor for *AtMYB44* gene transcription under salt stress-free conditions.

© 2018 Elsevier Inc. All rights reserved.

1. Introduction

Under drought conditions, the salt concentration in the soil increases as the moisture content decreases, and this can inflict serious stresses on growing plants. To overcome cellular ion imbalance under salt stress conditions, plants express a group of transcription factor genes at an early stage of the stress. Expression of the Arabidopsis transcription factor gene *AtMYB44* increases in leaf epidermal guard cells in response to salt stress [1]. Transgenic Arabidopsis [1] and soybean [2] overexpressing this gene exhibit enhanced tolerance to a sudden increase in the salt concentration in the soil.

In the chromatin of eukaryotic cells, genomic DNA is wrapped around a histone octamer consisting of H2A, H2B, H3, and H4, and together these components form a nucleosome. In gene transcription mediated by RNA polymerase, promoter activity is modulated by dynamic competition between nucleosomes and transcription factors. Thus, gene transcription is accompanied by nucleosome eviction or replacement with other nucleosomes with different compositions [3,4]. For instance, replacement of H2A with histone variant H2A.Z promotes variable gene expression without affecting gene DNA methylation [5,6]. In yeast, the chromatin remodeling SWR1 complex replaces H2A/H2B with H2A.Z/H2B dimers in nucleosomes, while the INO80 complex reverses this process [7]. H2A.Z-related nucleosomal reorganizations can change nucleosome structure, stability, and dynamics, leading to alterations in gene expression. In addition to alterations in nucleosome density and composition, gene transcription is also associated with other chromatin remodeling activities such as histone modification, DNA methylation, and small RNA-based chromatin modifications that can take place at the promoter, transcription start site (TSS), and gene-body regions [8,9]. In general, histone modifications including H3 lysine 4 trimethylation (H3K4me3) and H3 acetylation of lysine 9 (H3K9ac) or lysine 27 (H3K27ac) around the TSS are strongly associated with gene expression [10,11].

In the present study, we investigated the chromatin modifications that result in a significant increase in the number of *AtMYB44* transcripts in response to salt stress. We observed that H2A.Z occupation at the *AtMYB44* gene was significantly decreased at the promoter region. Furthermore, the binding of AtMYB44 proteins to their own gene promoters was clearly diminished under salt stress conditions. This suggests that salt stress induces the eviction of H2A.Z-containing nucleosome from the promoter region. This, in turn, alters the binding affinity of AtMYB44 protein, which acts as a repressor of *AtMYB44* gene transcription.

* Corresponding author.
E-mail address: cheongjj@snu.ac.kr (J.-J. Cheong).

https://doi.org/10.1016/j.bbrc.2018.04.048
0006-291X/© 2018 Elsevier Inc. All rights reserved.

2. Materials and methods

2.1. Plant growth and treatment

Arabidopsis thaliana (ecotype Columbia) plants were grown on half-strength Murashige and Skoog (1/2 × MS) medium supplemented with 2% sucrose (w/v) and 1.2% phyto agar (w/v). The growth chamber conditions included a light cycle of 16-h on/8-h off, a light intensity of ~100 μmol photons m^{-2} s^{-1}, and a temperature of 23 ± 1 °C. To test the salt stress responses, two-week-old plants were transferred to liquid 1/2 × MS medium without NaCl (control) or supplemented with 250 mM NaCl, and grown for a further 6 h in the same growth chamber.

2.2. Histochemical β-glucuronidase (GUS) assay

Two-week-old transgenic Arabidopsis plants, *AtMYB44pro::GUS* #22 [1], were treated and used for GUS assays following procedures reported previously [1]. GUS activity was visualized as the presence of a blue precipitate in the plant tissue and photographed under a microscope (DE/Axio Imager A1, Carl Zeiss).

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

Total plant RNA was prepared using a Spectrum Plant Total RNA kit (Sigma-Aldrich), and used for first-strand cDNA synthesis using SuperScript III First-Strand Synthesis SuperMix (Invitrogen). For quantitative PCR (qPCR), SolGent 2 × Real-Time Smart Mix (SolGent) was used with specific DNA-primers (Supplementary Table 1). The Mx3005P qPCR system (Agilent Technologies) was used for qPCR and the thermocycling conditions were as follows: 95 °C for 15 min (1 cycle), 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s (40 cycles). The qPCR internal control was *AtACTIN2* (At3g18780).

2.4. Chromatin immunoprecipitation (ChIP) assay

Two-week-old Arabidopsis plants were used for cross-linking in 1% formaldehyde buffer, and then for the ChIP assay using an Epi-Quik Plant ChIP kit (Epigentek). Antibodies to RNA polymerase II (RNAPII) CTD repeat YSPTSPS (4H8), histone H3, histone H3 acetyl K9 (H3K9ac), histone H3 trimethyl K4 (H3K4me3), histone H2A.Z, and green fluorescent protein (GFP) were purchased from Abcam Co. Antibodies to acetyl-histone H3 (H3ac) and acetyl-histone H4 (H4ac) were from Merck Millipore. Normal rabbit IgG (Merck Millipore) was included as a negative control in the ChIP assay. The ChIP-qPCR experiments were repeated independently two or three times. Statistical analysis was performed using Duncan's test [12] at a 95% confidence level.

3. Results and discussion

3.1. Activation of the AtMYB44 promoter in response to salt stress

The qRT-PCR assays showed that treatment of two-week-old Arabidopsis with 250 mM NaCl strongly increased the level of *AtMYB44* transcripts, as well as those of the stress marker genes *RD29A* and *COR15A* (Fig. 1A). This was consistent with previous northern blot results [1]. In addition, *GUS* expression in the leaf epidermal guard cells of the *AtMYB44pro::GUS* transgenic plants was greatly enhanced when grown under salt stress conditions (Fig. 1B). These results indicate that the *AtMYB44* promoter is activated to induce the accumulation of gene transcripts in response to salt stress.

Genome-wide analysis (http://epigenomics.mcdb.ucla.edu) has

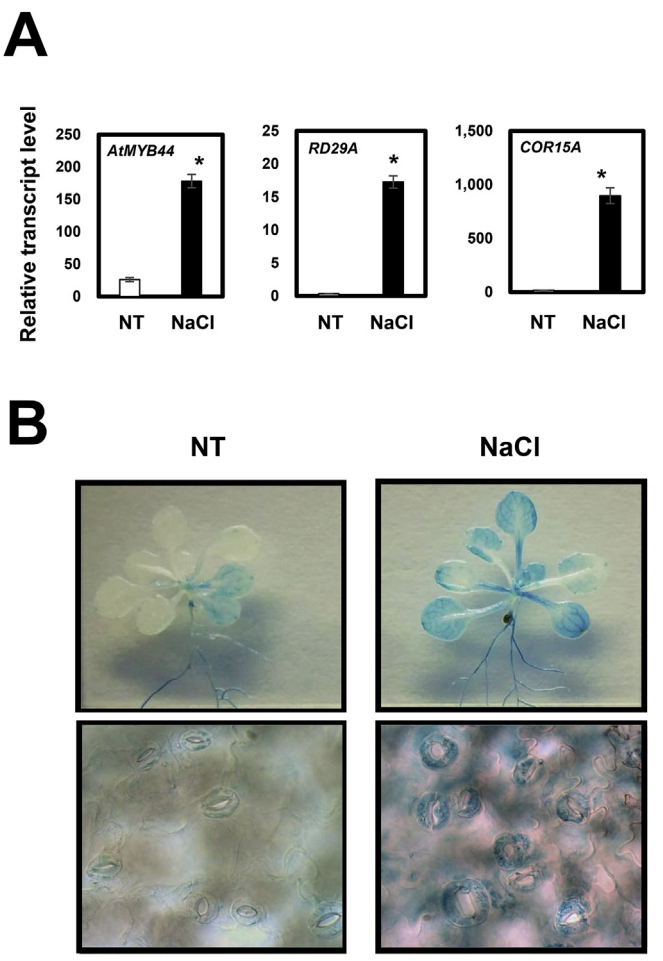

Fig. 1. Expression of *AtMYB44* gene in response to salt stress. A, Accumulation of *AtMYB44* gene transcripts under abiotic stress. Two-week-old wild-type (Col-0) plants growing on 1/2 × MS medium (2% sucrose) were carefully transferred to 1/2 × MS liquid medium (2% sucrose) without NaCl (NT) or supplemented with 250 mM NaCl (NaCl) for 6 h and used for total RNA extraction. qRT-PCR was performed using specific primers (Supplementary Table 1). *AtACTIN2* was used as an internal control. The experiments were performed three independent times and in triplicate for each. Columns marked with an asterisk indicate significant difference ($P < 0.05$). Bars represent standard error. B, Activation of the *AtMYB44* promoter in response to salt stress. Two-week-old *AtMYB44pro::GUS* #22 (Jung et al., 2008) transgenic Arabidopsis plants were used for histochemical β-glucuronidase (GUS) assays. GUS activity was visualized following the protocol described by Jung et al. (2008) using a microscope (DE/Axio Imager A1, Carl Zeiss).

shown that the *AtMYB44* promoter region is methylation-free and contains two nucleosome-rich regions on the *AtMYB44* gene-body [13,14]. Different primer sets (Supplementary Table 1) were designed to detect the *AtMYB44* promoter, TSS, and gene-body regions (as depicted in Fig. 2) and used in ChIP-qPCR assays. ChIP-qPCR with anti-RNAPII antibody revealed that RNA polymerases were enriched on the *AtMYB44* gene, especially on TSS-proximal regions, under salt stress conditions (Fig. 2A). In the control experiments, RNA polymerases were also enriched on two stress marker genes, *RD29A* and *COR15A* (Supplementary Fig. 1). These results indicate that the *AtMYB44* transcript is upregulated in response to salt stress via RNA polymerase-mediated transcription, and not by reducing the degradation of constitutively-expressed gene transcripts. Together, the results show that salt stress upregulates *AtMYB44* expression by enhancing its own promoter activity, leading to the recruitment of RNA polymerases to facilitate gene transcription.

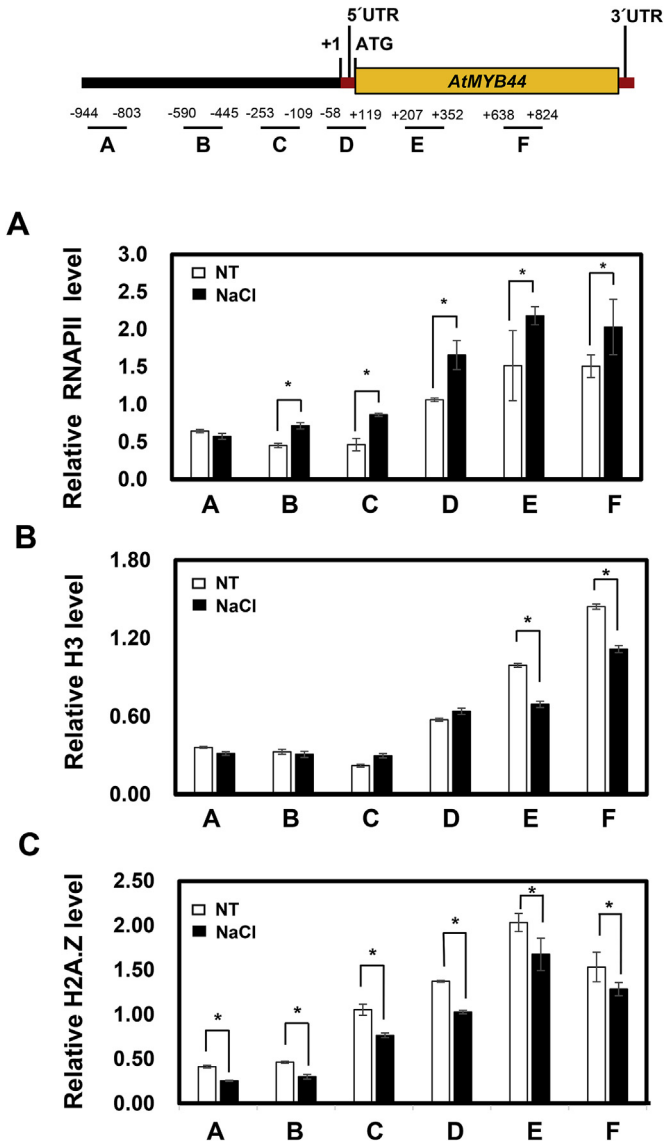

Fig. 2. Chromatin modifications at *AtMYB44* genomic regions in response to salt stress. Two-week-old wild-type (Col-0) Arabidopsis plants were treated without (NT) or with 250 mM NaCl, grown for a further 6 h, and used for chromatin immunoprecipitation (ChIP) assays using antibodies against RNAPII (A), histone 3 (B), or H2A.Z histones (C). The ChIP experiments were performed two independent times. qPCR was performed using specific primers (Supplementary Table 1). Primers specific to each region were designed from the nucleotide sequences (Supplementary Table S1), as depicted in the schematic diagram of *AtMYB44* locus (At5g67300), and used in the qPCR assays. The ChIP signal was normalized to signals from input DNA and an internal control (*AtACTIN2*). Columns marked with an asterisk indicate significant difference ($P < 0.05$). Bars represent standard error.

3.2. Nucleosome eviction on AtMYB44 in response to salt stress

In plants, nucleosome occupation has been found to influence the transcription of many genes in response to various signals such as drought stress, heat shock, and abscisic acid (ABA) treatment [15–17]. To examine nucleosome occupation on *AtMYB44* under salt stress conditions, a ChIP assay was performed using the anti-histone H3 antibody. As shown in Fig. 2B, salt stress clearly accelerated nucleosome eviction on *AtMYB44* gene-body regions (regions E and F), but not significantly from promoter (regions A through C) or TSS (region D) regions. Furthermore, salt stress also induced H2A.Z eviction from all tested regions including the promoter, TSS, and gene-body (Fig. 2C). Based on these data, we speculate that normal nucleosomes (H2A/H3) may replace H2A.Z-containing nucleosomes (H2A.Z/H3) at *AtMYB44* promoter and TSS regions, but not gene-body regions under salt stress conditions.

In general, H2A.Z-containing nucleosomes located at gene-body regions wrap the DNA more tightly and thus decrease transcription [18]. Kumar and Wigge [16] proposed, in a study of thermoresponse in Arabidopsis, that the occupancy of H2A.Z-containing nucleosomes could physically disturb the binding of RNAPII, which can suppress the expression of their target genes. Recently, Sura et al. [19] showed that the gene-bodies of H2A.Z-containing nucleosomes have a strong repressive effect on the transcription of drought stress-responsive genes in Arabidopsis. Consistently, in the present study, salt stress reduced H2A.Z occupancy to accelerate RNAPII recruitment to the promoter and TSS regions resulting in induction of *AtMYB44* transcription.

3.3. Histone modifications on AtMYB44 in response to salt stress

In general, histone acetylation (such as H3 and H4 acetylation) and H3 methylation at some lysine residues (such as H3K4 and H3K36 methylation) are involved in gene activation [11,20]. In an experiment with maize, Li et al. [21] found that salt stress increased the transcript levels of cell wall modification genes in association with an increase in H3K9ac levels. In addition, Chen et al. [22] showed that salt stress could increase H3K4me3 levels on *DREB2A* and *RD29B*, and this was correlated with enhanced expression.

In the present study, however, histone methylation (H3K4me3) and acetylation (H3ac, H3K9ac and H4ac) levels on *AtMYB44* gene regions were not altered in response to salt stress (Supplementary Fig. 2). Interestingly, other than a slight increase in H3ac on *RD29A* (TSS proximal region), significant histone modifications on the two stress marker genes, *RD29A* and *COR15A*, were also not observed in our experiments (Supplementary Figs. 3 and 4).

In 2017, Dai et al. [23] conducted genome-wide mapping of nucleosome occupancy in Arabidopsis inflorescences, and reported that H2A.Z repressed gene expression by modulating nucleosome structure, and was preferentially associated with H3K4me3 at promoters. However, salt stress-induced histone modifications including H3K4me3, H3K9ac, H3ac, and H4ac on *AtMYB44* were not detected in the present study (Supplementary Fig. 2).

3.4. Binding of AtMYB44 proteins on their own promoters

AtMYB44 transcript levels were significantly increased in both wild-type and *AtMYB44*-overexpressing (*AtMYB44-OE*) transgenic plants [1] under salt stress conditions (Fig. 3A). However, we observed that overexpression of *AtMYB44* (in *AtMYB44-OE* transgenic plants) clearly reduced the accumulation of RNAPII on its TSS-proximal region (region D) under both normal growth and salt stress conditions (Fig. 3B). This result implies that AtMYB44 can act as a repressor of the transcription of its own gene.

A large-scale ChIP-sequencing experiment showed that AtMYB44 could bind to its own promoter [24]. The *AtMYB44* promoter (~1.5 kb) contains five AtMYB44-binding sites (AACnG) [25] (Supplementary Fig. 5). A DNA-primer set that targets region A of the *AtMYB44* promoter (Fig. 2), which contains two AACnG sites, (Supplementary Fig. 5), was used for ChIP-qPCR assays. ChIP-qPCR assays with *AtMYB44-GFP-OE* #28-3 transgenic plants [1] and anti-GFP antibodies confirmed that AtMYB44 did indeed bind to its own promoter, and this binding was significantly reduced under salt stress conditions (Fig. 4). This result also suggests that AtMYB44 acts as a repressor of the transcription of its own gene. It is of interest how AtMYB44 regulates its own expression in response to salt stress.

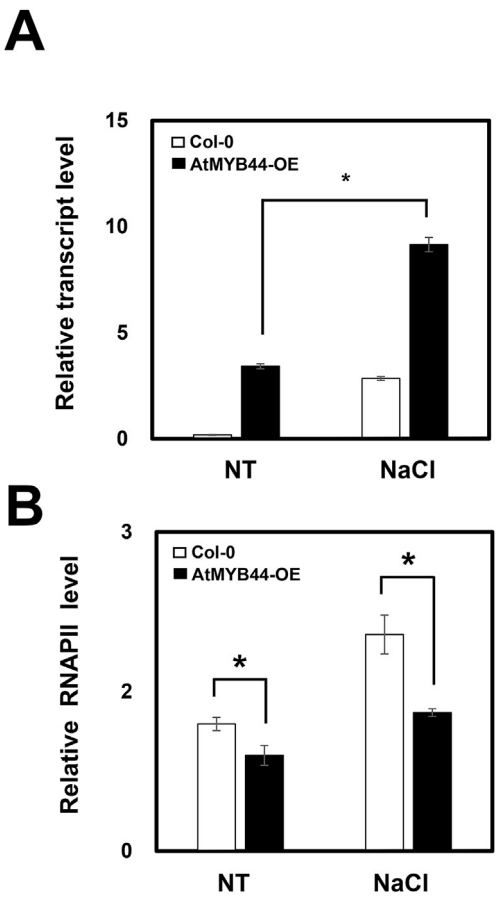

Fig. 3. Expression of *AtMYB44* in its overexpression transgenic plants. Two-week-old *AtMYB44-OE #21* plants (Jung et al., 2008) were treated without NaCl (NT) or with 250 mM NaCl for 6 h and used for ChIP and qRT-PCR assays. A, Expression level of *AtMYB44*. qRT-PCR was performed using specific primers (Supplementary Table 1). *AtACTIN2* was used as an internal control. The experiments were performed two independent times and in triplicate for each. Columns marked with an asterisk indicate significant difference ($P < 0.05$). Bars represent standard error. B, ChIP assays using antibodies against RNAPII. qPCR was performed using specific primers (Supplementary Table 1) that target the *AtMYB44* TSS proximal region (region D, Fig. 2). The ChIP signal was normalized to signals from input DNA and an internal control (*AtACTIN2*). The experiments were performed two independent times and in triplicate for each. Columns marked with an asterisk indicate significant difference ($P < 0.05$). Bars represent standard error.

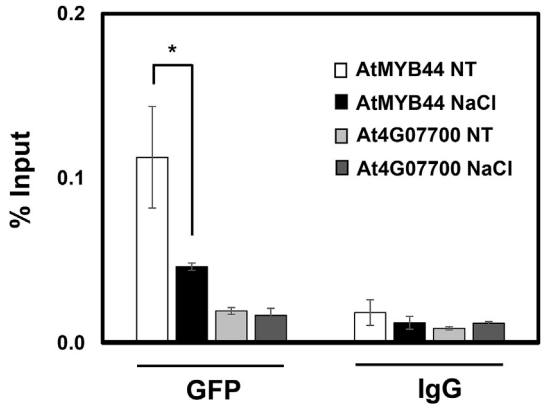

Fig. 4. Alteration of AtMYB44 binding to the *AtMYB44* promoter in response to salt stress. Two-week-old *AtMYB44-GFP #28-3* plants (Jung et al., 2008) without NaCl (NT) or with 250 mM NaCl (NaCl) for 6 h and used for ChIP assays using antibodies against green fluorescence protein (GFP). qPCR was performed using specific primers (Supplementary Table 1). *GYPSY-LIKE RETROTRANSPOSON* (At4g07700) was used as non-binding control. IgG was included as a negative control for the ChIP assay. The ChIP signal (% input) was calculated using the formula: $2^{\Delta Ct} = 2^{[Ct(ChIP) - Ct(Input)]}$. The experiments were performed two independent times. Columns marked with an asterisk indicate significant difference ($P < 0.05$). Bars represent standard error.

Using microarray and northern blot analyses, Jung et al. [1] showed that AtMYB44 could act as a repressor under salt stress conditions. Salt-induced expression of a group of *Ser/Thr protein phosphatase 2C* (*PP2C*) genes, such as *ABI1*, *ABI2*, *AtPP2CA*, *HAB1*, and *HAB2*, was significantly suppressed in *AtMYB44-OE* transgenic plants. The C-terminal catalytic domain of the AtMYB44 protein contains the amino acid sequence LSLSL [25,26], which is a well-conserved motif found in all Arabidopsis sub group 22 R2R3-MYB transcription factors including AtMYB44, AtMYB70, AtMYB73, and AtMYB77. The LxLxL motif is known as an ethylene-responsive element binding factor-associated amphiphilic repression (EAR) motif, which is proposed to play an important role in many repressors of transcription in plants [27]. These results support the idea that AtMYB44 works as a transcription repressor that actually represses its own expression.

As we mentioned above, salt-induced activation of the *AtMYB44* promoter could be due to the eviction of H2A.Z-containing nucleosomes from this chromatin region, which in turn affects the binding affinity of AtMYB44 on this region. H2A.Z occupancy at a genome locus is typically correlated with responsiveness to environmental factors [18]. The presence of H2A.Z increases the susceptibility of a promoter region to *trans*-acting factor(s) that promote or repress the transcription of a corresponding gene [16,18]. Hu et al. [28] observed, in a study of embryogenic stem cells, that H2A.Z deposition led to an abnormal nucleosome structure and increased chromatin accessibility to different activator and repressor complexes.

In spite of the repressive activity of AtMYB44 proteins, its gene transcript levels were significantly increased in both wild-type and *AtMYB44-OE* transgenic plants under salt stress conditions (Fig. 3A). It is possible that salt stress recruits other activating transcription factor(s) that compete with AtMYB44 to target this gene promoter to regulate its expression. Song et al. [24] showed that the *AtMYB44* promoter is also a target of many ABA-responsive element-binding factors (ABFs) such as ABF1, ABF3, and ABF4. Arabidopsis ABFs can increase plant tolerance to abiotic stresses such as drought conditions [29]. In addition, ABF3-binding sites including TCACGttt and ACACGgtt [30] are located on the *AtMYB44* promoter (~1.5 kb), and two of them overlap at region A (Supplementary Fig. 5). Based on these results, we speculate that salt stress promotes the eviction of H2A.Z-containing nucleosomes. This results in the release of repressors (AtMYB44) from the *AtMYB44* promoter region, which is consequently occupied by other salt stress responsive-activators (such as ABFs) that activate *AtMYB44* gene transcription.

Conflicts of interest

We have no conflicts of interest to declare.

Acknowledgments

This work was supported by the National Research Foundation of Korea (grant number 2016R1A2B4012248).

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.bbrc.2018.04.048.

References

[1] C. Jung, J.S. Seo, S.W. Han, Y.J. Koo, C.H. Kim, S.I. Song, B.H. Nahm, Y.D. Choi, J.J. Cheong, Overexpression of *AtMYB44* enhances stomatal closure to confer abiotic stress tolerance in transgenic Arabidopsis, Plant Physiol. 146 (2008) 623–635.

[2] J.S. Seo, H.B. Sohn, K. Noh, C. Jung, J.H. An, C.M. Donovan, D.A. Somers, D.I. Kim, S.C. Jeong, C.G. Kim, H.M. Kim, S.H. Lee, Y.D. Choi, T.W. Moon, C.H. Kim, J.J. Cheong, Expression of the *Arabidopsis AtMYB44* gene confers drought/salt-stress tolerance in transgenic soybean, Mol. Breed. 29 (2012) 601–608.

[3] K. Struhl, E. Segal, Determinants of nucleosome positioning, Nat. Struct. Mol. Biol. 20 (2013) 267–273.

[4] T. Zhang, W. Zhang, J. Jiang, Genome-wide nucleosome occupancy and positioning and their impact on gene expression and evolution in plants, Plant Physiol. 168 (2015) 1406–1416.

[5] R.B. Deal, S. Henikoff, Histone variants and modifications in plant gene regulation, Curr. Opin. Plant Biol. 14 (2011) 116–122.

[6] T.K. To, J.M. Kim, Epigenetic regulation of gene responsiveness in *Arabidopsis*, Front. Plant Sci. 4 (2014) 58.

[7] C.M. Weber, S. Henikoff, Histone variants: dynamic punctuation in transcription, Genes Dev. 28 (2014) 672–682.

[8] B.R. Cairns, The logic of chromatin architecture and remodeling at promoters, Nature 461 (2009) 193–198.

[9] C. Yamamuro, J.K. Zhu, Z. Yang, Epigenetic modifications and plant hormone action, Mol. Plant 9 (2016) 57–70.

[10] Z. Du, H. Li, Q. Wei, et al., Genome-wide analysis of histone modifications: H3K4me2, H3K4me3, H3K9ac, and H3K27ac in *Oryza sativa* L. Japonica, Mol. Plant 6 (2013) 1463–1472.

[11] J.M. Kim, T. Sasaki, M. Ueda, K. Sako, M. Seki, Chromatin changes in response to drought, salinity, heat, and cold stresses in plants, Front. Plant Sci. 6 (2015) 114.

[12] D.B. Duncan, Multiple range and multiple F tests, Biometrics 11 (1955) 1–42.

[13] D. Karolchik, R. Baertsch, M. Diekhans, et al., The UCSC genome browser database, Nucleic Acids Res. 31 (2003) 51–54.

[14] C. Tyner, G.P. Barber, J. Casper, et al., The UCSC genome browser database: 2017 update, Nucleic Acids Res. 45 (2017) D626–D634.

[15] J.M. Kim, T.K. To, J. Ishida, T. Morosawa, M. Kawashima, A. Matsui, T. Toyoda, H. Kimura, K. Shinozaki, M. Seki, Alterations of lysine modifications on the histone H3 N-tail under drought stress conditions in *Arabidopsis thaliana*, Plant Cell Physiol. 49 (2008) 1580–1588.

[16] S.V. Kumar, P.A. Wigge, H2A.Z-containing nucleosomes mediate the thermosensory response in *Arabidopsis*, Cell 140 (2010) 136–147.

[17] S.K. Han, Y. Sang, A. Rodrigues, BIOL425 F2010, M.F. Wu, P.L. Rodriguez, D. Wagner D, The SWI2/SNF2 chromatin remodeling ATPase BRAHMA represses abscisic acid responses in the absence of the stress Stimulus in *Arabidopsis*, Plant Cell 24 (2012) 4892–4906.

[18] D. Coleman-Derr, D. Zilberman, Deposition of histone variant H2A.Z within gene bodies regulates responsive genes, PLoS Genet. 8 (2012) 10.

[19] W. Sura, M. Kabza, W.M. Karlowski, T. Bieluszewski, M. Kus-Slowinska, L. Paweloszek, J. Sadowski, P.A. Ziolkowski, Dual role of the histone variant H2A.Z in transcriptional regulation of stress-response genes, Plant Cell 29 (2017) 791–807.

[20] L.Y. Yuan, X.C. Liu, M. Luo, S.G. Yang, K.Q. Wu, Involvement of histone modifications in plant abiotic stress responses, J. Integr. Plant Biol. 55 (2013) 892–901.

[21] H. Li, S.H. Yan, L. Zhao, J.J. Tan, Q. Zhang, F. Gao, P. Wang, H.L. Hou, L.J. Li, Histone acetylation associated up-regulation of the cell wall related genes is involved in salt stress induced maize root swelling, BMC Plant Biol. 14 (2014) 105.

[22] L.T. Chen, M. Luo, Y.Y. Wang, K. Wu, Involvement of *Arabidopsis* histone deacetylase HDA6 in ABA and salt stress response, J. Exp. Bot. 61 (2010) 3345–3353.

[23] X. Dai, Y. Bai, L. Zhao, X. Dou, Y. Liu, L. Wang, Y. Li, W. Li, Y. Hui, X. Huang, Z. Wang, Y. Qin Y, H2A.Z represses gene expression by modulating promoter nucleosome structure and enhancer histone modifications in Arabidopsis, Mol. Plant 10 (2017) 1274–1292.

[24] L. Song, S.C. Huang, A. Wise, R. Castanon, J.R. Nery, H. Chen, M. Watanabe, J. Thomas, Z. Bar-Joseph, J.R. Ecker, A transcription factor hierarchy defines an environmental stress response network, Science 354 (2016) aag1550, https://doi.org/10.1126/science.aag1550.

[25] C. Jung, Y.K. Kim, N.I. Oh, J.S. Shim, J.S. Seo, Y.D. Choi, B.H. Nahm, J.J. Cheong, Quadruple 9-mer-based protein binding microarray analysis confirms AACnG as the consensus nucleotide sequence sufficient for the specific binding of AtMYB44, Mol. Cell 34 (2012) 531–537.

[26] H. Persak, A. Pitzschke, Dominant repression by Arabidopsis transcription factor MYB44 causes oxidative damage and hypersensitivity to abiotic stress, Int. J. Mol. Sci. 15 (2014) 2517–2537.

[27] S. Kagale, K. Rozwadowski, EAR motif-mediated transcriptional repression in plants an underlying mechanism for epigenetic regulation of gene expression, Epigenetics 6 (2011) 141–146.

[28] G. Hu, K. Cui, D. Northrup, C. Liu, C. Wang, Q. Tang, K. Ge, D. Levens, C. Crane-Robinson, K. Zhao, H2A.Z facilitates access of active and repressive complexes to chromatin in embryonic stem cell self-renewal and differentiation, Cell Stem Cell 12 (2013) 180–192.

[29] T. Yoshida, Y. Fujita, H. Sayama, S. Kidokoro, K. Maruyama, J. Mizoi, K. Shinozaki, K. Yamaguchi-Shinozaki, AREB1, AREB2, and ABF3 are master transcription factors that cooperatively regulate ABRE-dependent ABA signaling involved in drought stress tolerance and require ABA for full activation, Plant J. 61 (2010) 672–685.

[30] M.T. Weirauch, A. Yang, M. Albu, et al., Determination and inference of eukaryotic transcription factor sequence specificity, Cell 158 (2014) 1431–1443.