Biochemical and Biophysical Research Communications 430 (2013) 1054–1059

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Phosphorylation of the zinc finger transcriptional regulator ZAT6 by MPK6 regulates *Arabidopsis* seed germination under salt and osmotic stress

Xiao-Min Liu [a], Xuan Canh Nguyen [b], Kyung Eun Kim [b], Hay Ju Han [b], Jaehyeong Yoo [a], Kyunghee Lee [b], Min Chul Kim [a,b], Dae-Jin Yun [a,b], Woo Sik Chung [a,b,*]

[a] Division of Applied Life Science (BK21 Program), Gyeongsang National University, Jinju 660-701, Republic of Korea
[b] Plant Molecular Biology and Biotechnology Research Center, Gyeongsang National University, Jinju 660-701, Republic of Korea

ARTICLE INFO

Article history:
Received 28 November 2012
Available online 17 December 2012

Keywords:
Arabidopsis
Zinc finger protein
Salt stress
Osmotic stress
Phosphorylation

ABSTRACT

C_2H_2-type zinc finger proteins (ZFPs) play diverse roles in plant response to abiotic stresses. ZAT6, an *Arabidopsis* C_2H_2-type ZFP, has been reported to regulate root development and nutrient stress responses. However, its roles in regulation of abiotic stress response are incompletely known. Here, we demonstrate that salt or osmotic stress triggers a strong increase in *ZAT6* expression in leaves. Transgenic plants overexpressing *ZAT6* showed improved seed germination under salt and osmotic stress. Intriguingly, ZAT6 interacts with a stress-responsive mitogen-activated protein kinase MPK6 *in vitro* and *in planta*. ZAT6 is phosphorylated by both recombinant and plant endogenous MPK6. Serine 8 and serine 223 in ZAT6 were identified as the sites phosphorylated by MPK6. In contrast to wild-type form of ZAT6, overexpression of phosphorylation mutant form did not display significantly enhanced salt and osmotic stress tolerance. Altogether, our results suggest that phosphorylation by MPK6 is required for the functional role of ZAT6 in seed germination under salt and osmotic stress.

1. Introduction

Plants have developed complicated signaling mechanisms to perceive external signals and adapt their cellular metabolism to various environmental stresses. Salt stress is one of major abiotic stresses that inhibits plant growth and limits crop yield through induction of ionic as well as osmotic stress [1].

Transcription factors (TFs) are important components for regulating stress-responsive genes [2]. Zinc finger proteins (ZFPs) form a relatively large family of transcriptional regulators in plants. Based on the number and the location of characteristic residues, the ZFPs proteins are classified into several types, such as C_2H_2, C_2HC, C_2HC_5, C_3HC_4, CCCH, C_4, C_4HC_3, C_6 and C_8 [3]. C_2H_2-type ZFPs typically contain 1–4 conserved QALGGH motifs in their zinc finger domains. In plants, a number of C_2H_2-type ZFPs have been reported to play crucial roles in response to abiotic stresses, including salt and osmotic stress [4].

In *Arabidopsis*, involvement of several C_2H_2-type ZFPs, AZF1, AZF2, AZF3 and ZAT10, in drought and salt stress response have been well characterized [5]. Further analysis showed that ZAT10 played a dual role in response to abiotic stresses [6]. ZAT7 was reported to positively mediate salt stress tolerance through regulation of defense responsive genes such as *WRKY70*, *AOX1*, *COR78* and *NHX*. Moreover, the ERF-associated amphiphilic repression motif is required for its biological function [7]. ZAT6 has been previously reported to regulate root development and nutrient stress responses [8,9]. A recent study showed that expression of the *ZAT6* gene was induced by salt stress. Transgenic *Arabidopsis* plants that expressed chimeric repressors derived from the ZAT6 were tolerant to salt stress [10]. However, the regulatory mechanisms underlying ZAT6-mediated salt and osmotic stress response need to be further explored.

Mitogen-activated protein kinase (MAPK) cascade functions as crucial upstream regulators to mediate abiotic stress response and tolerance. It is highly conserved among eukaryotes and comprises three kinases: a MAPK kinase kinase (MAPKKK), a MAPK kinase (MAPKK) and a MAPK. MAPKs are serine/threonine kinases able to phosphorylate a wide range of substrates, including transcription factors and/or other kinases [11]. Among 20 *Arabidopsis* MAPKs, MPK6 plays important roles in diverse stress responses [12]. In response to salt or osmotic stress, MPK6 was reported to be rapidly activated. [13]. Teige et al. (2004) showed that MKK2 directly targets and activates MPK6 in salt stress signaling [14]. Recently a MAPKKK, MKKK20, was reported to be involved in the response to osmotic stress through its regulation of MPK6 activity [15]. However, little is known about its downstream targets. ACS6 is phosphorylated by MPK6 *via* three sites and this phosphorylation

* Corresponding author at: Division of Applied Life Science (BK21 Program), Gyeongsang National University, Jinju 660-701, Republic of Korea. Fax: +82 55 759 9363.
E-mail address: chungws@gnu.ac.kr (W.S. Chung).

0006-291X/$ - see front matter © 2012 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.bbrc.2012.12.039

promotes the accumulation of ACS protein and ethylene production [16]. Dual phosphorylation of EIN3 by both MPK3 and MPK6 was reported to modulate its activity in the ethylene signaling [17]. In addition, NIA2 phosphorylation by MPK6 is required for NO production and signal transduction in response to H_2O_2 during *Arabidopsis* root development [18].

In this study, we identified a novel target of MPK6, ZAT6, involved in the regulation of salt and osmotic stress response. ZAT6 functions as a positive regulator of seed germination under salt and osmotic stress. We further show that a stress-responsive MAPK, MPK6, interacts with and phosphorylates ZAT6. Moreover, phosphorylation of ZAT6 is essential for its positive regulation of stress tolerance.

2. Materials and methods

2.1. Plant materials and stress treatments

Arabidopsis thaliana wild-type (Columbia), *mpk6–3*, *ZAT6* and *ZAT6*^{AA} overexpressing transgenic plants were grown in a growth chamber under a 16:8-h light/dark regime at a constant temperature of 22 °C. For GUS staining assays and RT-PCR analysis, two-week-old seedlings were collected at indicated time points after salt or sorbitol treatment.

2.2. Plasmid constructs for protein expression

The full-length sequences of *MPK6* and *ZAT6* were amplified by PCR from an *Arabidopsis* cDNA library and cloned into pGEM-T easy Vector (Promega). For the expression of GST-fused proteins in bacteria, *ZAT6* was subcloned into pGEX-2T, while *MPK6* was subcloned into pGEX-5X-1 (GE Healthcare).

2.3. Pull-down assay

His-MPK6 (5 μg) proteins were pre-incubated with 10 μl of Ni–NTA agarose in 1 ml of binding buffer (50 mM Tris–HCl, pH 7.5, 200 mM NaCl, 1% Triton X-100, 0.1 mM EDTA, 0.5 mM DTT) for 2 h at 4 °C. His-MPK6 bound to the Ni–NTA agarose was incubated with purified GST-ZAT6 protein (5 μg) in 1 ml of binding buffer overnight at 4 °C. Beads were washed and boiled with 4× SDS loading buffer for 5 min. The eluted proteins were separated by electrophoresis on 10% SDS–PAGE. Proteins interacting with His-MPK6 were detected using polyclonal anti-GST antibody.

2.4. Site-directed mutagenesis

The pGEX-ZAT6 construct was used as the template for site-directed mutagenesis with the QuikChange II site-directed mutagenesis kit (Stratagene). Six individual constructs were generated with the following substitutions: ZAT6(S8A), ZAT6(S13A), ZAT6(S77A), ZAT6(T86A), ZAT6(S223A), and ZAT6(S8A/S223A). The mutations were confirmed by nucleotide sequencing before protein expression.

2.5. Kinase assay and in-gel kinase assay

The *in vitro* phosphorylation assays were performed in kinase buffer (25 mM Tris–HCl, pH 7.5, 1 mM DTT, 20 mM $MgCl_2$, 2 mM $MnCl_2$, 50 μM ATP). GST-MPK6 fusion proteins (1 μg) were mixed with GST (1 μg), MBP (1 μg), or GST-ZAT6 fusion proteins (3 μg) in a total kinase reaction volume of 20 μl. The reactions were performed as previously reported [19]. For in-gel kinase assay, protein extracts (50 μg) from treated two-week-old WT and *mpk6–3* plants were incubated at 60 °C for 10 min and separated on 10% SDS polyacrylamide gels. As substrates for the kinases, gels were embedded with 0.2 mg/ml MBP or 0.5 mg/ml GST, purified GST-ZAT6 proteins. The in-gel kinase assay was performed as described previously [20].

2.6. Mass spectrometry

GST-ZAT6 protein phosphorylated *in vitro* were in-gel digested using modified trypsin (Promega). Purification of the phosphorylated peptides was performed by using TiO_2 micro-columns,

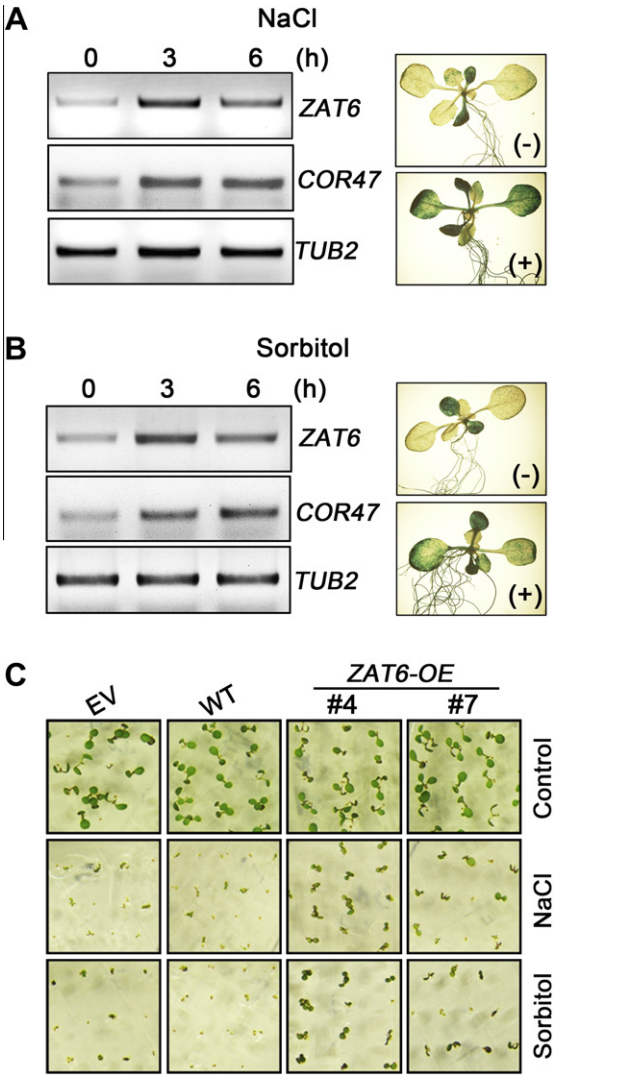

Fig. 1. Overexpression of *ZAT6* improves seed germination under salt and osmotic stress. (A) Expression pattern of *ZAT6* transcripts in response to salt stress. Total RNAs were isolated from two-week-old plants grown on MS medium treated with 250 mM NaCl. RT-PCR was performed with *ZAT6* gene-specific primers. *Tubulin2* (*TUB2*) was used as an internal control (left panel). GUS expression in the leaves of *ProZAT6::GUS* transgenic plants following 250 mM NaCl treatment (right panel). (B) Expression pattern of *ZAT6* transcripts in response to osmotic stress. Total RNAs were isolated from two-week-old plants grown on MS medium treated with 400 mM sorbitol. RT-PCR was performed with *ZAT6* gene-specific primers. *Tubulin2* (*TUB2*) was used as an internal control (left panel). GUS expression in the leaves of *ProZAT6::GUS* transgenic plants following 400 mM sorbitol treatment (right panel). (C) Seed germination of vector control (EV), wild type (WT) and transgenic plants overexpressing *ZAT6* (*ZAT6-OE*) under salt and osmotic stress. Photographs of EV, WT and *ZAT6-OE* (lines 4, 7) seedlings were grown on MS medium (control), or MS medium containing 100 mM NaCl or 250 mM sorbitol at 5 days after the end of stratification.

MALDI-TOF analysis was performed using a Voyager-DE STR mass spectrometer (PerSeptive Biosystems Inc.) as previously described [19].

2.7. GUS histochemical staining assay

Transgenic *Arabidopsis* plants harboring the *ProZAT6::GUS* construct were generated by floral dip. Two-week-old T3 seedlings exposed to 200 mM NaCl or 250 mM sorbitol for 2 h were used for GUS histochemical staining assays as previously described [21].

2.8. Firefly luciferase complementation imaging (LCI) assay

ZAT6 was cloned into pCAMBIA1300-cLUC and *MPK6* were into pCAMBIA1300-nLUC [22]. The DNA constructs were introduced into *Agrobacterium tumefaciens* strain GV3101. Three-week-old *Nicotina benthamiana* plants were used for *Agrobacterium*-mediated transformation. After 3 days, *Agrobacterium*-infiltrated leaves were sprayed with 1 mm Luciferin solution containing 0.01% Triton X-100 and kept in the dark for 5 min for detecting luminescence. LUC images were detected using a low-light cooled CCD imaging apparatus (AndoriXon; Andor).

2.9. Stress tolerant assay

Wild-type, EV, *ZAT6* and *ZAT6*^{AA} overexpressing transgenic *Arabidopsis* seeds were sterilized and placed on MS solid medium composed different concentration of NaCl or sorbitol. After incubation for one week, the percentage of seedlings that had germinated and developed green expanded cotyledons was determined.

3. Results and discussion

3.1. Overexpression of ZAT6 improves seed germination under salt and osmotic stress

Previous research showed that transcript levels of *ZAT6* were dramatically increased upon salt and osmotic stress treatment [10]. We confirmed expression patterns of the *ZAT6* gene under salt and osmotic stress by RT-PCR analysis. As shown in Fig. 1A and B (left panel), the stress-responsive marker gene *COR47* was highly induced by salt or osmotic stress treatment. The *ZAT6* transcript levels were also increased at 3 h after stress application. To precisely define the expression of *ZAT6* spatially in response to salt and osmotic stress, we examined the pattern of GUS expression in *ProZAT6::GUS* transgenic plants. *ZAT6* was expressed in cotyledons under normal condition. NaCl or sorbitol treatment triggered a strong increase in *ZAT6* expression in leaves (Fig. 1A and B, right panel). These results indicate that gene expression of *ZAT6* is strongly induced by both salt and osmotic stresses in leaves.

With the increase of NaCl and sorbitol concentration, seed germination is delayed and inhibited more and more seriously. RNAi suppression of ZAT6 has been reported to be lethal [8]. We therefore used a gain-of-function approach to dissect the functional role of ZAT6. *Arabidopsis* transgenic plants overexpressing *ZAT6* under control of the CaMV 35S promoter were generated. Among four independent transgenic lines in which *ZAT6* was strongly expressed, we selected two homozygous lines (line 4 and line 7) for further study. When sowing on normal MS media, transgenic plants overexpressing *ZAT6* (*ZAT6-OE4* and *ZAT6-OE7*) showed a similar phenotype as WT and vector control (EV) plants (Fig. 1C, top panel). However, *ZAT6-OE* seeds germinated much earlier than

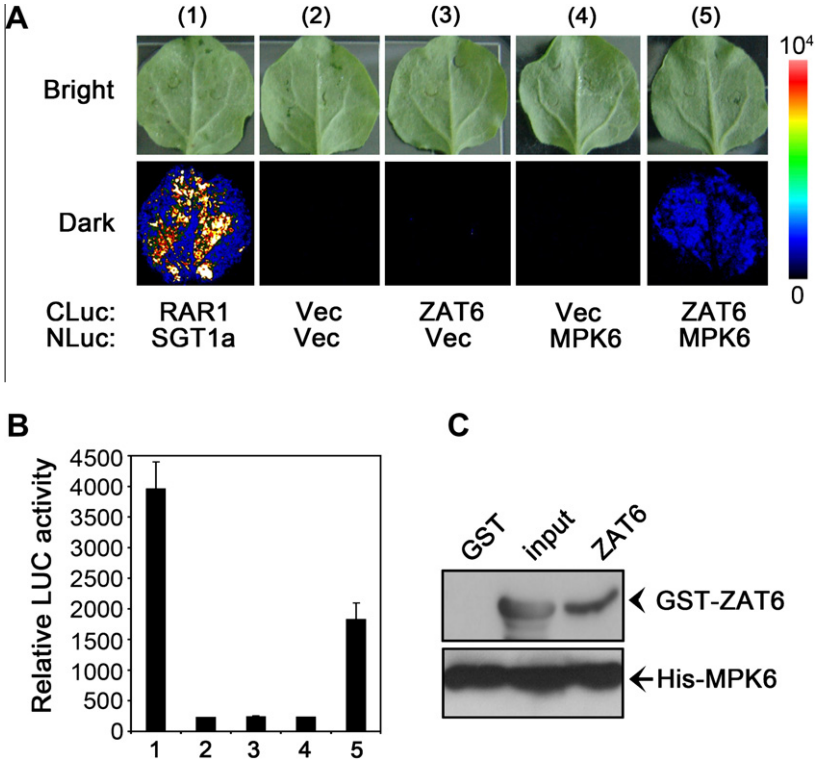

Fig. 2. ZAT6 interacts with MPK6 *in vitro* and *in planta*. (A) The firefly luciferase (LUC) images of *N. benthamiana* leaves co-infiltrated with *A. tumefaciens* containing different combination of constructs. The right pseudocolor bar shows the range of luminescence intensity in the image. CLuc-RAR1 or ZAT6, RAR1 or ZAT6 fused with *C*-terminal region of luciferase; SGT1a or MPK6-NLuc, SGT1a or MPK6 fused with *N*-terminal region of luciferase. Interaction between RAR1 and SGT1a serves as a positive control. (B) Quantification of the average LUC activity. (C) Pull-down assay for detecting direct interaction between ZAT6 and MPK6. GST protein was used as a negative control. 1 μg of GST-ZAT6 fusion protein was loaded as a positive control (input). ZAT6 and MPK6 are indicated by arrowhead and arrow, respectively.

WT and EV seeds when sowing on MS medium containing 100 mM NaCl or 250 mM sorbitol (Fig. 1C, middle and bottom panel). These data suggest that ZAT6 functions as a positive regulator of seed germination under salt and osmotic stress.

3.2. ZAT6 interacts with MPK6 in vitro and in planta

Using high-throughput yeast two-hybrid assay we isolated a stress-responsive MAPK, MPK6, as a novel ZAT6-interacting protein [19]. To confirm the direct interaction between ZAT6 and MPK6, pull-down assay was performed. The recombinant His-MPK6 were immobilized on Ni–NTA columns and incubated with the GST-ZAT6 fusion protein translated *in vitro* from *Escherichia coli*. As shown in Fig. 2C, His-MPK6 could pull-down GST-ZAT6 rather than GST protein only, suggesting that a direct interaction between ZAT6 and MPK6 *in vitro*.

To further verify the interaction between ZAT6 and MPK6 *in planta*, we performed a well-established firefly luciferase complementation imaging (LCI) assay for detecting protein–protein interaction in *N. benthamiana* [22]. We constructed several vectors, including pCAM-SGT1a-NLuc, pCAM-MPK6-NLuc, pCAM-RAR1-CLuc and pCAM-ZAT6-CLuc for LCI assays. After co-infiltration for about 48 h, we found that co-infiltration of *A. tumefaciens* containing SGT1a-NLuc/RAR1-CLuc resulted in strong LUC complementation (Fig. 2A), serving as positive control [22]. The co-infiltration of MPK6-NLuc/ZAT6-CLuc resulted in clearly detectable LUC activity, compared with that of negative controls NLuc/CLuc, MPK6-NLuc/CLuc or NLuc/ZAT6-CLuc co-infiltration which did not show LUC activity (Fig. 2A and B). This result implies that ZAT6 interacts with MPK6 *in planta*.

3.3. Phosphorylation of ZAT6 by both recombinant and native plant MPK6

MAPK phosphorylates its substrates on serine or threonine residue following by a proline residue (Ser/Thr-Pro motif). Apart from two DNA-binding zinc finger domains, a nuclear-localization signal (NLS) and an EAR motif, amino acid sequence analysis of ZAT6 revealed five putative phosphorylation sites (Ser8, Ser13, Ser77, Thr86, and Ser223) (Fig. 3A). On the basis of this prediction and indentified interaction, we hypothesized that ZAT6 might be a direct target of MPK6. To test this hypothesis, we performed an *in vitro* phosphorylation assay. The recombinant GST-ZAT6 protein was used as substrate. GST and MBP protein were used as a negative and positive control, respectively. As shown in Fig. 3B, besides its autophosphorylation, recombinant MPK6 could strongly phosphorylates ZAT6 but not GST protein. These data demonstrate that MPK6 is responsible for ZAT6 phosphorylation.

In addition to recombinant MPK6, we also analyzed phosphorylation of ZAT6 by the native MPK6. In this assay, recombinant GST-ZAT6 protein was embedded in an SDS–PAGE gel. Phosphorylation of the embedded ZAT6 was determined using total protein extracts

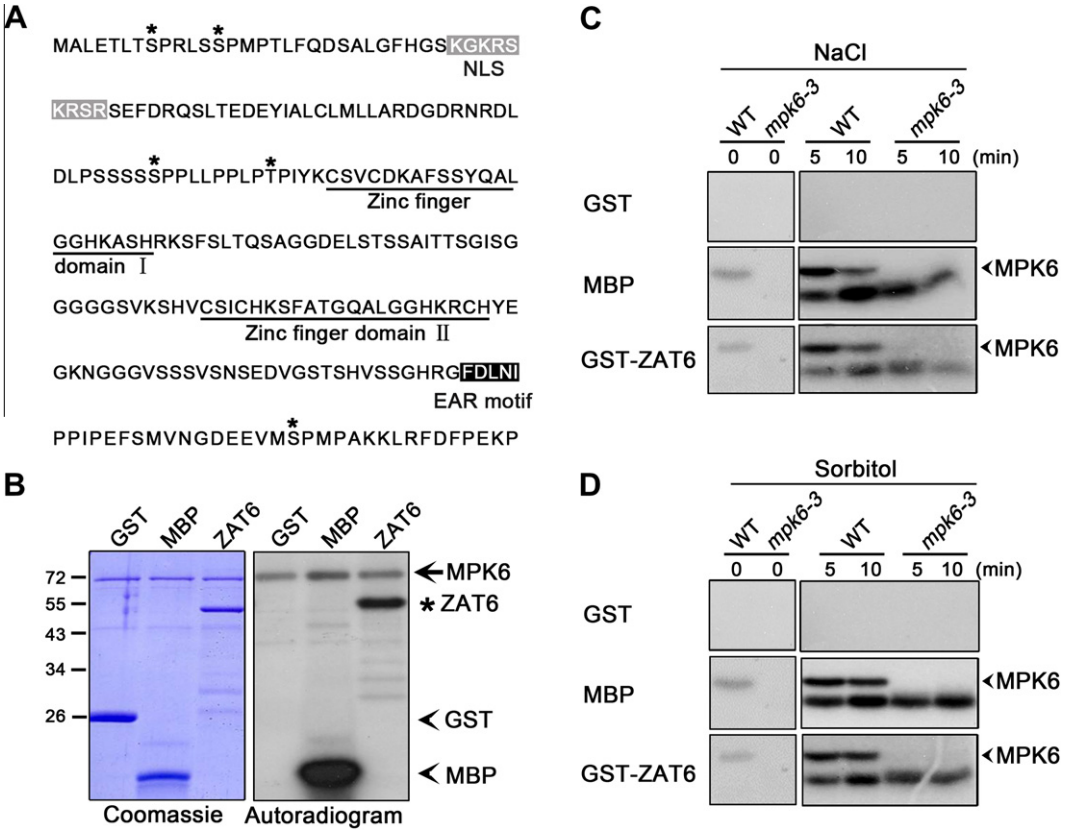

Fig. 3. ZAT6 is phosphorylated by both recombinant and native plant MPK6. (A) Amino acid sequence of ZAT6. White residues in gray and black background indicate the nuclear localization signal (NLS) and the ERF-associated amphiphilic repression (EAR) motif, respectively. Two zinc finger domains are underlined. Serine or threonine residues marked by asterisks indicate putative phosphorylation sites. (B) *In vitro* phosphorylation of ZAT6 by recombinant MPK6. GST and MBP marked by arrowheads were used as a negative and positive control, respectively. The phosphorylation was detected by autoradiography (Autoradiogram). The left panels show the proteins stained by Coomassie Brilliant Blue (Coomassie). The autophosphorylated MPK6 and phosphorylated ZAT6 are indicated by arrow and asterisk, respectively. (C and D) Phosphorylation of ZAT6 by salt and osmotic stress-activated MPK6 from *Arabidopsis* plants. WT and *mpk6–3* plants were grown on MS medium and treated with 250 mM NaCl (C) or 400 mM sorbitol (D) for the times indicated. In-gel kinase activity assays were performed using 50 μg protein extracts from treated plants. GST-ZAT6 was used as a kinase substrate. GST and MBP were used as a negative and positive control, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Phosphopeptides isolated by TiO_2 chromatography from tryptic digestion of ZAT6 phosphorylated by MPK6.

Peptide sequence	$(M+H)^+$ (expected)	$(M+H)^+$ (measured)	Number of phosphate group	Number of Met oxidation
MALETLT**S**PR	1358.6022	1359.4434	1	1
MALETLT**S**PR	1342.6072	1343.4440	1	0

Potential phosphorylation sites by MPK6 are bold and underlined.

from WT and *mpk6–3* seedlings treated with NaCl or sorbitol, which strongly activates MPK6 and an unknown kinase. As shown in Fig. 3C and D, identical kinase activity patterns were observed when ZAT6 and MBP were used as the substrates. The MPK6 identity was confirmed by the loss of MPK6 band in *mpk6–3*. All these results above suggest that ZAT6 is phosphorylated by both recombinant and native plant MPK6, serving as a direct target of MPK6.

Next, we identified the MPK6 phosphorylation sites in ZAT6 by using TiO_2 chromatography in combination with MALDI-TOF mass spectrometry. A phosphopeptide derived from phosphorylated ZAT6 was enriched (Table 1). This phosphopeptide contains one putative phosphorylation site, serine 8 (Ser8). To demonstrate whether Ser8 is the real phosphorylation site of ZAT6, site-directed mutagenesis was performed along with additional phosphorylation assays. As shown in Fig. 4A, MPK6 was able to phosphorylate ZAT6. Whereas, when the Ser8 residue was mutated to Alanine (S8A), the protein phosphorylation by MPK6 was largely impaired other than completely abolished, indicating that Ser8 in ZAT6 protein is an important but not the only one site for MPK6-targeted phosphorylation. To determine the other sites responsible for ZAT6 phosphorylation by MPK6, we mutated Ser13, Ser77, T86 or Ser223 residue to Alanine (S13A, S77A, T86A, and S223A) and examined their phosphorylation state. phosphorylation of S223A rather than S13A, S77A or T86A protein was weaker than that of ZAT6 protein by MPK6. Therefore, both Ser8 and Ser223 were mutated to Alanine (S8A/S223A). Surprisingly, the double mutant ZAT6 protein was almost forbidden to be phosphorylated by MPK6 (Fig. 4A). Collectively, these results demonstrate that two sites, Ser8 and Ser223, are mainly responsible for ZAT6 phosphorylation by MPK6.

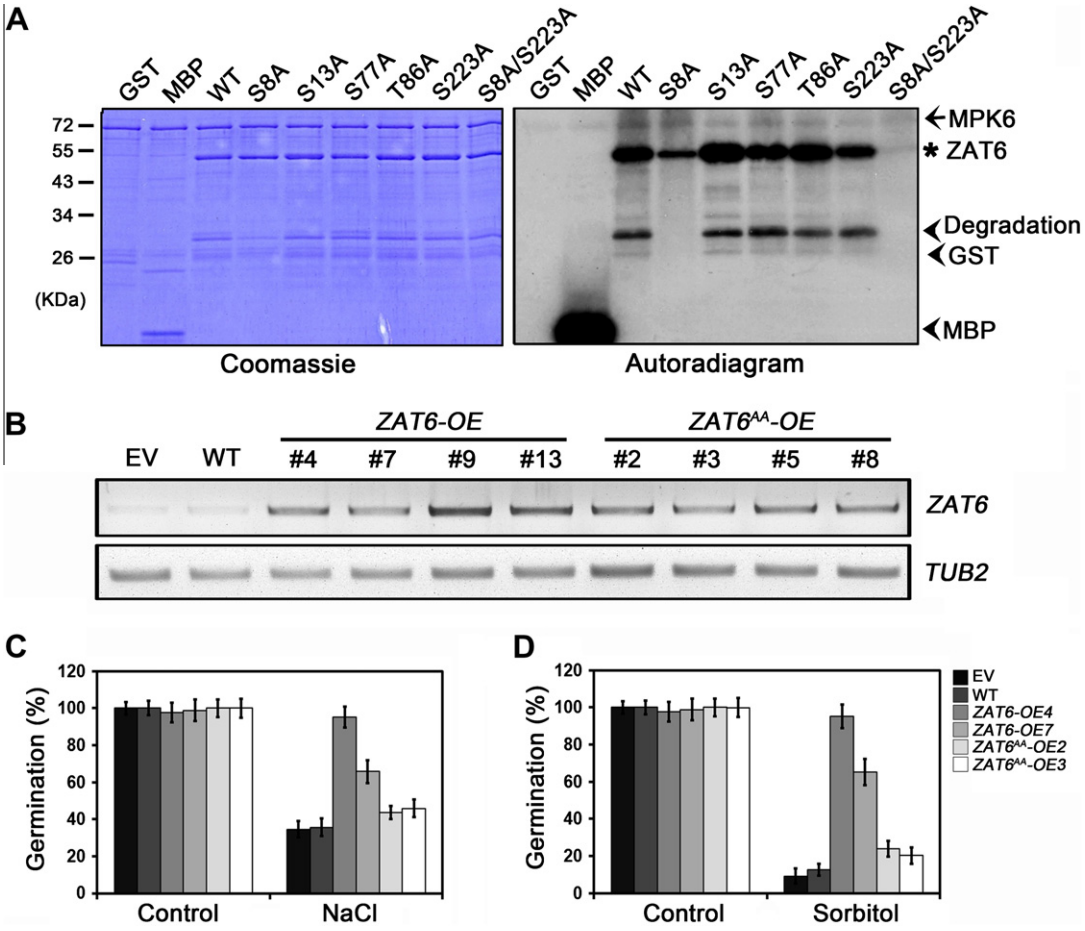

Fig. 4. Seed germination of *ZAT6-OE* and *ZAT6^{AA}-OE* plants under salt and osmotic stress. (A) Recombinant GST-tagged MPK6 was used to phosphorylate WT, S8A, S13A, S77A, T86A, S223A or double mutant S8A/S223A form of ZAT6 protein in the presence of [γ-$_{32}$P]ATP. After electrophoresis, the phosphorylated proteins were visualized by autoradiography (Autoradiogram). The assay input was stained by Coomassie Brilliant Blue (Coomassie). GST and MBP marked by arrowhead were used as a negative and positive control, respectively. The autophosphorylated MPK6 and phosphorylated ZAT6 are indicated by arrow and asterisk, respectively. (B) RT-PCR analysis of *ZAT6* expression in EV, WT, transgenic plants overexpressing *ZAT6* (*ZAT6-OE*) and *ZAT6^{AA}* (*ZAT6^{AA}-OE*). *Tubulin2* (*TUB2*) was used as an internal control. (C) Seed germination of EV, WT, *ZAT6-OE* and *ZAT6^{AA}-OE* plants grown on MS medium containing 0 or 100 mM NaCl. (D) Seed germination of EV, WT, *ZAT6-OE* and *ZAT6^{AA}-OE* plants grown on MS medium containing 0 or 250 mM sorbitol. Stress tolerant assays were performed as described in Section 2. Data are the mean ± SE of three independent replicates. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.4. Overexpression of $ZAT6^{AA}$ lose the ability to significantly improve seed germination under salt and osmotic stress

We showed that overexpression of *ZAT6* led to enhanced tolerance to salt and osmotic stress during seed germination. In order to examine whether ZAT6 phosphorylation by MPK6 plays a role in salt and osmotic stress tolerance, we generated transgenic plants expressing 35S::$ZAT6^{AA}$. Using RT-PCR analysis, two $ZAT6^{AA}$-*OE* lines (line 2 and line 3) harboring the similar expression levels of *ZAT6* as *ZAT6-OE4* and *ZAT6-OE7* lines were selected for phenotypic test (Fig. 4B). When sowing on normal MS medium, the *ZAT6-OE* and $ZAT6^{AA}$-*OE* plants showed a similar phenotype as WT and EV plants. However, the *ZAT6-OE* rather than $ZAT6^{AA}$-*OE* seeds germinated much earlier than the control seeds when sowing on MS medium containing 100 mM NaCl. After 5 days, approximately 30% of WT and EV seeds germinated, while up to 90% (line 4) and 60% (line 7) of *ZAT6-OE* seeds germinated. Surprisingly, only about 40% of $ZAT6^{AA}$-*OE* seeds germinated in the presence of 100 mM NaCl (Fig. 4C). *ZAT6-OE* seeds also germinated much earlier than $ZAT6^{AA}$-*OE* seeds on MS medium containing 250 mM sorbitol (Fig. 4D). Accordingly, in contrast to wild-type form of ZAT6, overexpression of phosphorylation mutant form ($ZAT6^{AA}$) lose the ability to significantly improve seed germination under salt and osmotic stress, indicating that MPK6-mediated phosphorylation might be required for ZAT6-regulated seed germination under salt and osmotic stress.

In conclusion, our study provides evidence showing that the transcriptional regulator ZAT6 is a novel target of the stress-responsive MAPK, MPK6. ZAT6 functions as a positive regulator of seed germination under salt and osmotic stress. Furthermore, MPK6-mediated phosphorylation of ZAT6 plays an important role in its regulation of seed germination under salt and osmotic stress.

Acknowledgments

This work was supported by grants from the World Class University Program (R32-10148) and the Basic Science Research Program (2010-0010607) of the National Research Foundation (NRF) funded by MOEST, and partly by a grant from the Next-Generation BioGreen 21 Program; (#PJ008173) funded by the Rural Development Administration, Republic of Korea. XML and JY were supported by scholarships from the BK21 program funded by the MOEST in Korea.

References

[1] J.K. Zhu, Salt and drought stress signal transduction in plants, Annu. Rev. Plant Biol. 53 (2002) 247–273.
[2] K. Nakashima, Y. Ito, K. Yamaguchi-Shinozaki, Transcriptional regulatory networks in response to abiotic stresses in Arabidopsis and grasses, Plant Physiol. 149 (2009) 88–95.
[3] A. Kielbowicz-Matuk, Involvement of plant C(2)H(2)-type zinc finger transcription factors in stress responses, Plant Sci. 185–186 (2012) 78–85.
[4] S. Ciftci-Yilmaz, R. Mittler, The zinc finger network of plants, Cell. Mol. Life Sci. 65 (2008) 1150–1160.
[5] H. Sakamoto, T. Araki, T. Meshi, M. Iwabuchi, Expression of a subset of the Arabidopsis Cys2/His2-type zinc-finger protein gene family under water stress, Gene 248 (2000) 23–32.
[6] R. Mittler, Y. Kim, L. Song, J. Coutu, A. Coutu, S. Ciftci-Yilmaz, H. Lee, B. Stevenson, J.K. Zhu, Gain- and loss-of-function mutations in Zat10 enhance the tolerance of plants to abiotic stress, FEBS Lett. 580 (2006) 6537–6542.
[7] S. Ciftci-Yilmaz, M.R. Morsy, L. Song, A. Coutu, B.A. Krizek, M.W. Lewis, D. Warren, J. Cushman, E.L. Connolly, R. Mittler, The EAR-motif of the Cys2/His2-type zinc finger protein Zat7 plays a key role in the defense response of Arabidopsis to salinity stress, J. Biol. Chem. 282 (2007) 9260–9268.
[8] B.N. Devaiah, V.K. Nagarajan, K.G. Raghothama, Phosphate homeostasis and root development in Arabidopsis are synchronized by the zinc finger transcription factor ZAT6, Plant Physiol. 145 (2007) 147–159.
[9] J.T. Vogel, D.G. Zarka, H.A. Van Buskirk, S.G. Fowler, M.F. Thomashow, Roles of the CBF2 and ZAT12 transcription factors in configuring the low temperature transcriptome of Arabidopsis, Plant J. 41 (2005) 195–211.
[10] T. Mito, M. Seki, K. Shinozaki, M. Ohme-Takagi, K. Matsui, Generation of chimeric repressors that confer salt tolerance in Arabidopsis and rice, Plant Biotechnol. J. 9 (2011) 736–746.
[11] J. Colcombet, H. Hirt, Arabidopsis MAPKs: a complex signalling network involved in multiple biological processes, Biochem. J. 413 (2008) 217–226.
[12] M. Group, Mitogen-activated protein kinase cascades in plants: a new nomenclature, Trends Plant Sci. 7 (2002) 301–308.
[13] Kazuya Ichimura, Tsuyoshi Mizoguchi, Riichiro Yoshida, Takashi Yuasa, K. Shinozaki, Various abiotic stresses rapidly activate Arabidopsis MAP kinases ATMPK4 and ATMPK6, Plant J. 24 (2000) 655–665.
[14] M. Teige, E. Scheikl, T. Eulgem, R. Doczi, K. Ichimura, K. Shinozaki, J.L. Dangl, H. Hirt, The MKK2 pathway mediates cold and salt stress signaling in Arabidopsis, Mol. Cell 15 (2004) 141–152.
[15] J.M. Kim, D.H. Woo, S.H. Kim, S.Y. Lee, H.Y. Park, H.Y. Seok, W.S. Chung, Y.H. Moon, Arabidopsis MKKK20 is involved in osmotic stress response via regulation of MPK6 activity, Plant Cell Rep. 31 (2012) 217–224.
[16] Y. Liu, S. Zhang, Phosphorylation of 1-aminocyclopropane-1-carboxylic acid synthase by MPK6, a stress-responsive mitogen-activated protein kinase, induces ethylene biosynthesis in Arabidopsis, Plant Cell 16 (2004) 3386–3399.
[17] S.D. Yoo, Y.H. Cho, G. Tena, Y. Xiong, J. Sheen, Dual control of nuclear EIN3 by bifurcate MAPK cascades in C_2H_4 signalling, Nature 451 (2008) 789–795.
[18] P. Wang, Y. Du, Y. Li, D. Ren, C.P. Song, Hydrogen peroxide-mediated activation of MAP kinase 6 modulates nitric oxide biosynthesis and signal transduction in Arabidopsis, Plant Cell 22 (2010) 2981–2998.
[19] X.C. Nguyen, S.H. Kim, K. Lee, K.E. Kim, X.M. Liu, H.J. Han, M.H. Hoang, S.W. Lee, J.C. Hong, Y.H. Moon, W.S. Chung, Identification of a C_2H_2-type zinc finger transcription factor (ZAT10) from Arabidopsis as a substrate of MAP kinase, Plant Cell Rep. 31 (2012) 737–745.
[20] X.M. Liu, K.E. Kim, K.C. Kim, X.C. Nguyen, H.J. Han, M.S. Jung, H.S. Kim, S.H. Kim, H.C. Park, D.J. Yun, W.S. Chung, Cadmium activates Arabidopsis MPK3 and MPK6 via accumulation of reactive oxygen species, Phytochemistry 71 (2010) 614–618.
[21] W. Choi, D. Baek, D.H. Oh, J. Park, H. Hong, W.Y. Kim, H.J. Bohnert, R.A. Bressan, H.C. Park, D.J. Yun, NKS1, Na(+)- and K(+)-sensitive 1, regulates ion homeostasis in an SOS-independent pathway in Arabidopsis, Phytochemistry 72 (2011) 330–336.
[22] H. Chen, Y. Zou, Y. Shang, H. Lin, Y. Wang, R. Cai, X. Tang, J.M. Zhou, Firefly luciferase complementation imaging assay for protein–protein interactions in plants, Plant Physiol. 146 (2008) 368–376.