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ORIGINAL ARTICLE

# Functional Analysis of the HD-Zip I Gene ZmHDZ1 in ABA-mediated Salt Tolerance in Rice

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**Abstract** The homeodomain-leucine zipper I (HD-Zip I) transcription factors play crucial roles in the regulation of abiotic stress responses, whereas, up to now, most of them are still functionally unknown. In present study, we identified a maize HD-Zip I gene, designing as *ZmHDZ1*, and investigated its potential roles in salt stress response. The qRT-PCR results showed that expression of *ZmHDZ1* was induced by ABA and salt stress. Transactivation assay and transient expression analyses indicated that ZmHDZ1 protein was located in the nucleus in tobacco leaf cells and had the transactivation activity in yeast. Over-expression of *ZmHDZ1* in rice reduced tolerance to salt stress, thus, led to greater accumulation of malondialdehyde (MDA) as well as higher level of relative electrolyte leakage (REL) compared to WT plants. In addition, the transgenic seedlings also increased sensitivity to exogenous ABA. Furthermore, the expression levels of four salt responsive genes were different between transgenic and WT rice under normal or NaCl treatment. These results suggested that *ZmHDZ1* function as a negative regulator in response to salt stress through the ABA-mediated signal transduction pathways.

**Keywords:** ABA, HD-Zip transcription factor, Maize, Salt stress

## Introduction

Plants commonly suffer from various environmental stresses, such as low/high temperature, drought and high salinity, which have an enormous adverse effect on plant growth and development and cause tremendous yield losses in crops all over the world. To cope with these environmental challenges, plants have evolved molecular and physiological mechanisms to respond and adapt to different abiotic stresses. In the previous study, many stress-relative genes have been identified in different plant species (Liang et al. 2014; Wang et al. 2015b). Besides, plant transcription factors (TFs), acting as important regulatory proteins, play crucial roles in response to different abiotic stress through regulating the expression of many functional genes. Several types of TFs have been identified as major factors to enhance plant tolerance to stress, such as CBF, DREB, bZIP, WRKY and MYB TFs and so on (Shinozaki et al. 2003). Additionally, an increasing number of evidence indicates that the members of homeodomain-leucine zipper (HD-Zip) family also involve in plant response to environmental stress (Harris et al. 2011).

The HD-Zip proteins are unique to plants, which contains a DNA-binding homeodomain (HD) and a protein-protein interaction leucine zipper (LZ) domain, and these proteins are divided into four subfamilies (HD-Zip I-IV) according to their phylogenic relationship and structural features (Harris et al. 2011; Zhao et al. 2011). With the development of molecular biology and biological information technology, HD-Zip proteins have been identified from numerous plants, including Arabidopsis, rice, sunflower, tomato and maize (Agalou et al. 2008; Lin et al. 2008; Zhao et al. 2011). It is believed that the HD-Zip I members are mainly involved in response to various environmental stress, and many HD-Zip I proteins have been functionally identified (Ariel et al. 2007). For example, over-expression of a sunflower HD-Zip I protein Hahb-4 enhanced Arabidopsis tolerance to drought stress (Manavella et al. 2006). Similarly, a *Medicago truncatula* HD-Zip I protein MtHB1 was induced by salinity stress and made transgenic plants better adaptive growth under salt stress (Ariel et al. 2010). Although, several HD-Zip proteins have been well characterized in different plants, the functions of most HD-Zip family members are still known.

In this study, we isolated a HD-Zip I protein ZmHDZ1

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from maize based on a previous study (Zhao et al. 2011). Expression pattern analysis showed that *ZmHDZ1* was induced by ABA and NaCl stress. Further functional investigation showed that over-expression of *ZmHDZ1* reduced tolerance to salt stress in transgenic rice, in addition, the *ZmHDZ1* over-expression plants were more sensitive to exogenous ABA. These data suggested that *ZmHDZ1* involved in the regulation of salt stress response probably through an ABA-mediated signaling pathway, which also provided a candidate for maize molecular and genetic breeding to improve stress tolerance.

## Results

### Expression Patterns of ZmHDZ1

In our previous study, we had identified 55 HD-Zip genes from maize genome, which containing 17 HD-Zip I genes (Zhao et al. 2011). The expression analysis of HD-Zip I genes indicated that *ZmHDZ1* was probably involved in abiotic stress according to phylogeny and expression pattern, therefore, we chose the *ZmHDZ1* for further functional analysis. Sequence analysis showed the *ZmHDZ1* contained a 1020 bp open reading frame (ORF), encoding a protein of 339 amino acids, with conserved HD and LZ domains (Fig. S1). Generally, the expression pattern of a gene is strongly correlated with its function. Firstly, we investigated the tissue-specific expression pattern of *ZmHDZ1*. The qRT-PCR results indicated that the *ZmHDZ1* gene was constitutively expressed in all selected organs, whereas, the expression level in stem, leave and ear was relatively higher than in other tissues (Fig. 1C). As well, the expression profile of *ZmHDZ1* in response to ABA and NaCl treatments was detected. Our results showed that the *ZmHDZ1* was significantly up-regulated under ABA treatment at 1 h. However, its expression level decreased at 3, 6, and 9 h. Until the 12 h, it was significantly up-regulated again (Fig. 1A). On the contrary, under NaCl treatment, the *ZmHDZ1* was up-regulated only at 1 h, and it displayed relative low expression level at later time points (Fig. 1B). These results implied that *ZmHDZ1* might involve in abiotic stress response through the ABA signaling pathway. To examine the reliability of our experiment methods, we also detected the checked the expression patterns of two reported stress-responsive genes *ZmPSY3* and *ZmPMP3-2* (Fig. S2), and our results were in accordance with previously published results (Li et al. 2008; Fu et al. 2012).

### ZmHDZ1 Localizes in Nucleus and has Transactivation Activity

As it is known, most transcription factors play the roles as regulators in vivo and often locate in the nucleus, therefore, the subcellular localization of ZmHDZ1 was examined to

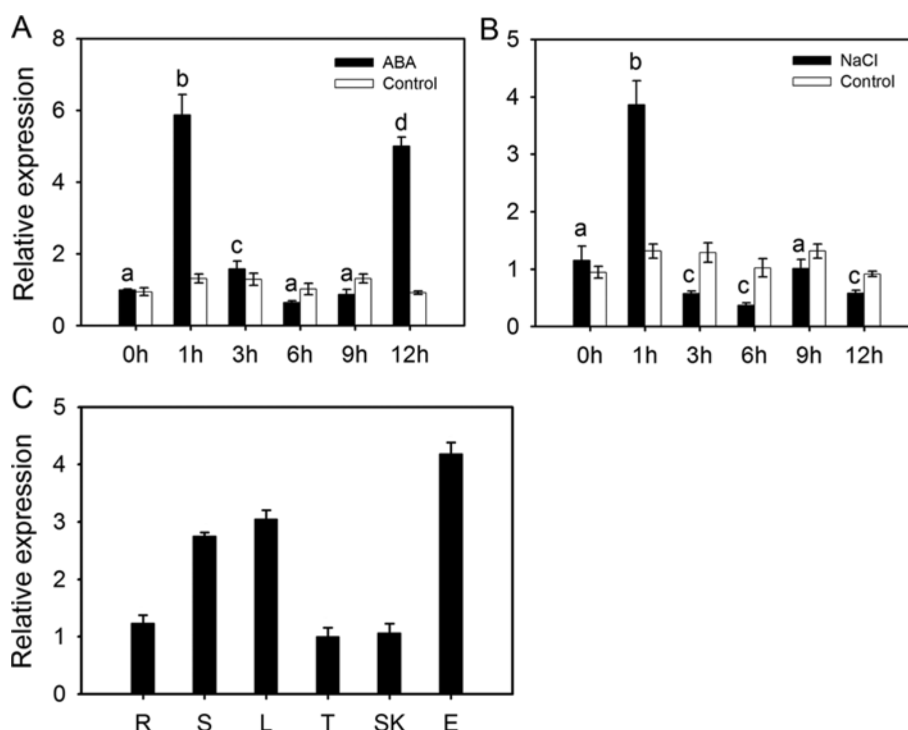


**Fig. 1.** Expression patterns of ZmHDZ1. (A, B) Expression patterns of Zmhdz1 (RT-qPCR) in maize seedlings under ABA and NaCl treatments, respectively. (C) Expression patterns of Zmhdz1 in different tissues: R, root; S, stem; L, leaf; T, tassel; SK, silk and E, ear. Different lowercase letters indicate significant differences at $p < 0.05$ (Student's t-test).

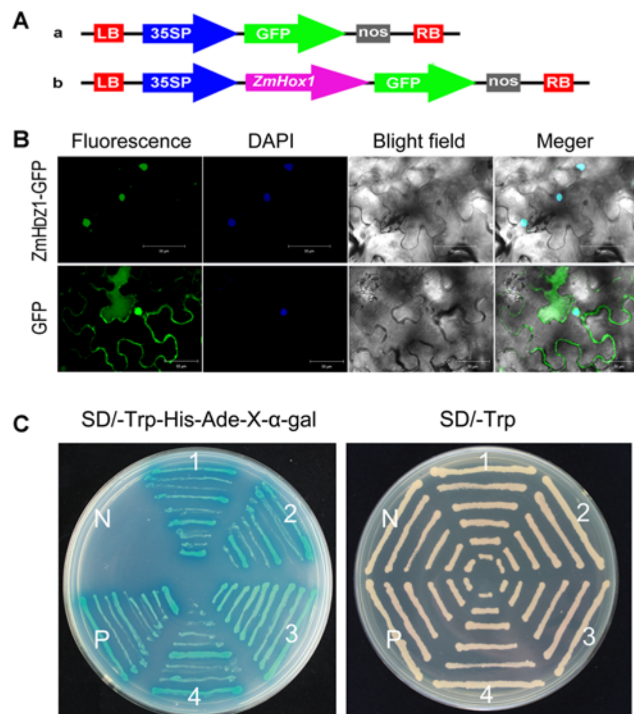


**Fig. 2.** Subcellular localization and transactivation activity assays of ZmHDZ1. (A) Schematic representation of the ZmHDZ1-GFP-fusion construct and the control empty vector (GFP) used for transient expression. (B) Fusion proteins were transiently expressed in tobacco leaves and observed under a laser scanning confocal microscope. Green color is GFP protein signal, and blue color represents DAPI stained for nucleus. Bars = 50 μm. (C) transactivation assay of Zmhdz1. 1-4, represent the cells with pGBKT7-ZmHDZ1 recombinant plasmid growing on the SD/-Trp or SD/-Trp/-His/-Ade/-X-α-gal medium, N, represents negative control, and P, stands for positive control.

verify its potential role in transcriptional regulation. The recombinant construct of the ZmHDZ1-GFP and the single GFP were transformed into tobacco leaves via *Agrobacterium* infiltrating. As observed, the green fluorescence of ZmHDZ1-GFP fusion protein was mainly located in the nucleus, which was further confirmed by DAPI staining (Fig. 2B), while the control GFP protein fluorescence was distributed throughout the whole cell. Our data predicted that the ZmHDZ1 protein was target to the nucleus.

It is evident by transactivation assay that the transformants of positive control (pGBKT7-p53 and pGADT7-SV40 large T-antigen), negative control (pGBKT7) and fusion construct pGBKT7-ZmHDZ1 grew well on the selective SD/-Trp medium (Fig. 2C), whereas, only transformants of pGBKT7-ZmHDZ1 and positive control could grow well on selective SD/-Trp/-His/-Ade/-α-gal medium and showed a-galactosidase activity (Fig. 2C). These results comfirmed that the ZmHDZ1 had transcriptional activity that could activate the reporter genes *Ade*, *His* and *LacZ* in the genome of AH109.

## *ZmHDZ1* Over-expression Makes Plants Less Tolerance to Salt Stress

To get a further research on the role of *ZmHDZ1* in response to salt stress, the *ZmHDZ1* over-expression transgenic rice was generated. The *ZmHDZ1* was expressed under the control of the CaMV 35S promoter. Semiquantitative RT-PCR results indicated that the expression level of *ZmHDZ1* gene increased in the transgenic lines (Fig. S3), and these two lines (OE-3, OE-4) were chosen for further investigation. There were no differences between WT and transgenic plants in agronomic traits under normal condition (Fig. S4). The T2 generation of transgenic seeds of *ZmHDZ1* and WT seeds were germinated and grown in the same square plates in the greenhouse. The false positive plants were confirmed and removed by histochemical analysis of GUS activity. 3-weeks-old seedlings were irrigated with 200 mM NaCl solution for 10 d for salt tolerance testing. Under normal conditions, the transgenic and WT seedlings displayed well growth vigor (Fig. 3A). After exposure to salt conditions for 10 d, all of them were inhibited in growth, while the transgenic lines showed more serious damage as compared to the WT seedlings (Fig. 3A).

To examine the degree of injury in WT and transgenic plants, relative electrolyte leakage (REL) was initially tested. As shown in Fig. 3B, the REL in WT and *ZmHDZ1* over-expression plants had no significant difference under normal growth conditions, while the REL in WT plants was significantly lower than in transgenic plants after salt stress treatment. It is reported that the malondialdehyde (MDA) would be accumulated in plants when they were exposed to abiotic stress. After salt stress, the MDA contents was increased in both WT and *ZmHDZ1* over-expression plants, meanwhile, the MDA content in transgenic plants was significantly higher than that in WT plants, while no differences was detected before salt stress between WT and transgenic plants (Fig. 3C). These findings were consistent with the phenotypic character between WT and transgenic plants after salt stress, which indicated over-expression of *ZmHDZ1* gene in rice decreased tolerance to salt stress and suggested that *ZmHDZ1* might functions as a negative regulator.

## Increased ABA Sensitivity of Over-expressing *ZmHDZ1* Transgenic Plants

The expression analysis indicated that *ZmHDZ1* was induced under ABA treatment (Fig. 1C), therefore, we speculated that this gene had potency to play roles in ABA signaling pathway. In order to examine whether the *ZmHDZ1* over-expression plants can response to ABA, We tested ABA sensitivity assay with exogenous ABA. The transgenic and WT seeds were germinated and grown on MS media

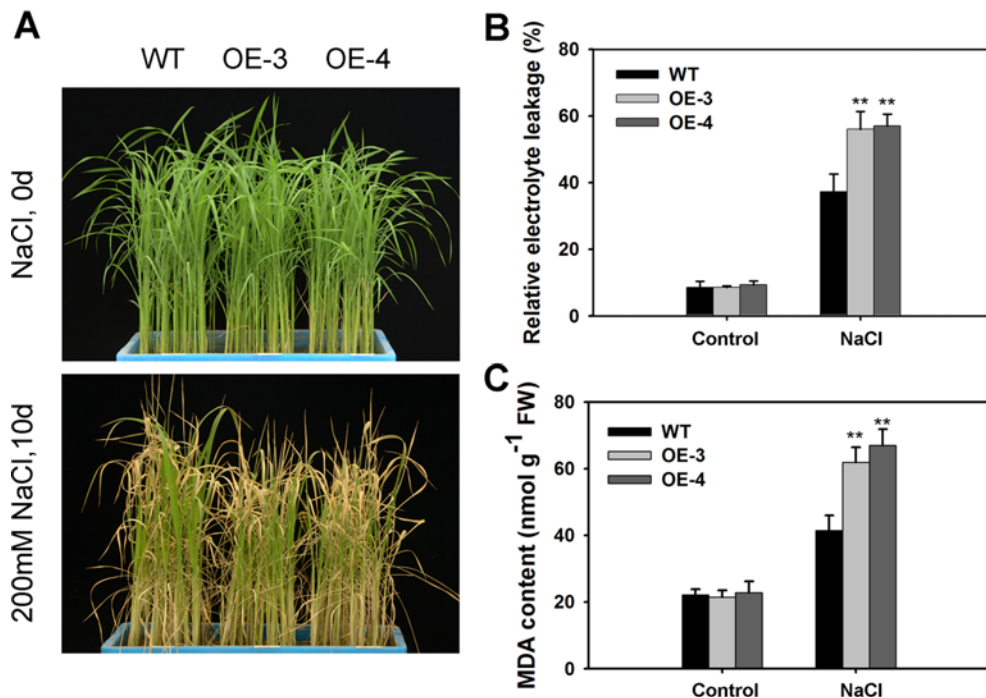


**Fig. 3.** Salt tolerance analysis for WT and ZmHDZ1 over-expression plants. (A) Character of WT and transgenic plants before and after 10 d-salt treatment. (B, C) Relative electrolytic leakage (REL) and malonaldehyde (MDA) content were measured in WT and transgenic plants after salt treatment. Data are means ± SD of three replicates. ** indicates significant differences between WT and transgenic plants at $P < 0.01$.

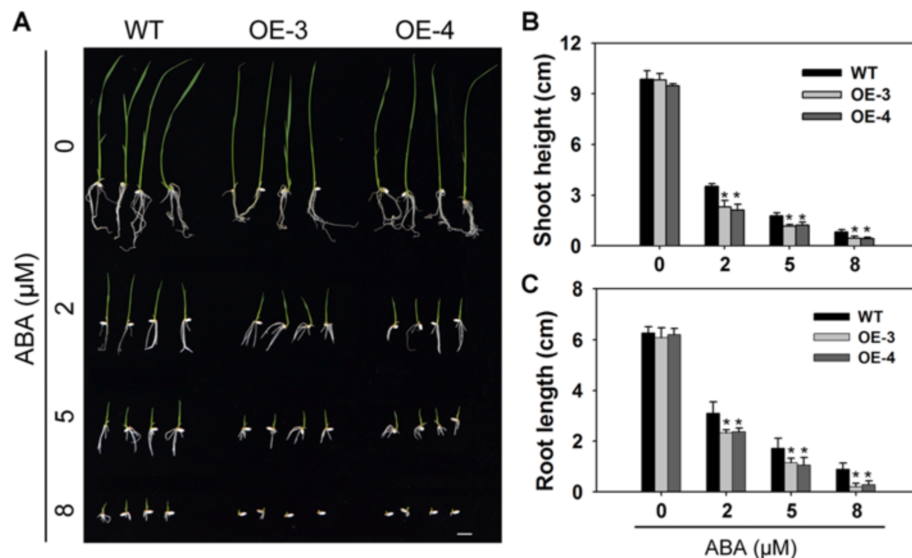


**Fig. 4.** ABA-sensitivity assay of ZmHDZ1 transgenic plants. (A) The growth phenotype of WT and transgenic plants grew in half-strength MS solid medium supplemented with 0, 2, 5 and 8 μM ABA for 7 d. (B, C) Measure the shoot height and root length of WT and Zmhdz1 transgenic seedlings after ABA treatment for 7 d. Data are means ± SD (n=20), ** $P < 0.01$, (Student's t-test).

supplementary with 0, 2, 5 and 8 μM ABA. As shown in Fig. 4A, the transgenic and WT seedlings showed no difference in growth on normal MS media (Fig. 4A), whereas, they both exhibited growth inhibition on MS media containing different ABA concentrations, and this growth inhibition rate was directly proportional to ABA concentration level (Fig. 4A). In addition, the data of shoot height and root length of ABA treated seedlings showed that shoot height and root length of *ZmHDZ1* over-expression plants were significantly lower than WT seedlings on MS media with ABA (4B, 4C). It was accordance with their observed phenotype and indicated the more severely inhibition level in *ZmHDZ1* over-expression seedlings in comparison to WT seedlings (Fig. 4). These findings suggested that over-expression of the

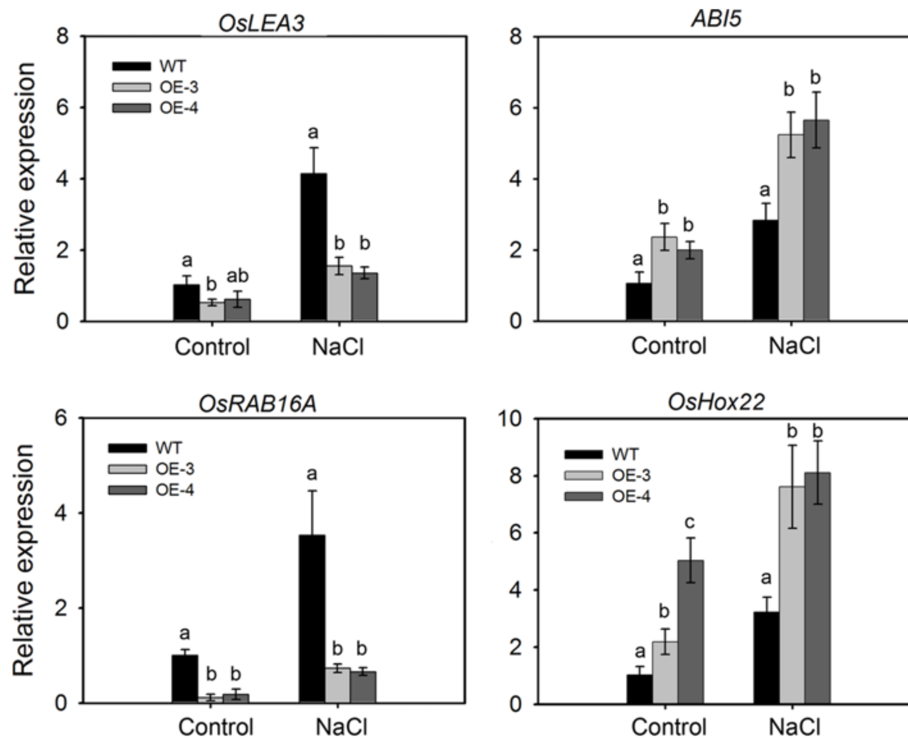


**Fig. 5.** Expression of salt-related genes in WT and ZmHDZ1 transgenic plants leaves. WT and *ZmHDZ1* over-expression plants were grown under normal conditions for 3 weeks and then irrigated with 200 mM NaCl solution. Leaves were sampled 8 h after treatment. All data represent the mean of three biological replicates and error bars indicates SD. Different lowercase letters indicate significant differences at $p < 0.05$ (Student's t-test).

*ZmHDZ1* in rice enhanced the ABA sensitivity.

Expression of Salt Stress-related Genes in Transgenic Rice Plants

To further confirm the roles of *ZmHDZ1* in response to salt stress, the expression of four salt stress-related genes was detected in WT and transgenic rice plants. Previous studies had demonstrated that the *OsHox22* and *ABI5* had the negative regulatory roles in response to salt stress (Zou et al. 2008; Zhang et al. 2012), and the *OsLEA3* and *OsRAB16A* were important downstream abiotic stress responsive genes and widely used as marker genes (Duan and Cai 2012; Li et al. 2016). The results showed that all of them were up-regulated in both WT and *ZmHDZ1* over-expression plants under salt stress treatment (Fig. 5). In addition, the expression level of *OsHox22* and *ABI5* was higher in transgenic plants than in WT after salt stress treatment, whereas, the *OsLEA3* and *OsRAB16A* displayed a lower transcript level in *ZmHDZ1* over-expression lines than in WT plants (Fig. 5). Additionally, the *OsABI5* was an ABA responsive gene and an important regulator in ABA signal transduction pathway (Zou et al. 2008). As observed in Fig. 5, under normal condition, the transcripts of *OsABI5* in transgenic lines was more abundant than that in the WT. These results further indicated the negative roles of *ZmHDZ1* in ABA signal transduction pathway.

## Discussion

HD-Zip transcription factors are plant-specific protein family that has significant part in plant growth and development. In the recent years, an increasing research suggest that HD-Zip I proteins are mainly involved in the regulation of abiotic stress responses in different plants (Olsson et al. 2004; Manavella et al. 2006; Cabello and Chan 2012; Zhang et al. 2012). Since 55 HD-Zip members were identified from maize genome through genome-wide analysis, two HD-Zip I proteins (*ZmHDZ10* and *ZmHDZ4*) had been functionally demonstrated to involve in abiotic stress response (Zhao et al. 2014; Wu et al. 2016). Over-expression of *ZmHDZ4* enhanced rice tolerance to drought stress, while heterologous expression of *ZmHDZ10* in both rice and Arabidopsis could increase salt and drought tolerance. Moreover, these both genes participated in the ABA-independent signaling pathway. However, there are still majority of maize HD-Zip I proteins remain functionally unknown to date. In this study, we identified a novel HD-Zip I gene *ZmHDZ1* from maize and investigated its potential roles in response to salt in transgenic

rice.

Generally, the physiological function of a protein is link with its intracellular location. And most of the transcription factors have two fundamental characteristics, nucleus localization and transcriptional activity. Transient expression of ZmHDZ1-GFP fusion protein in the leaves of tobacco showed that ZmHDZ1 is localized exclusively in the nucleus (Fig. 2B). Besides, the analysis of transactivation activity revealed the ZmHDZ1 had transcriptional activity and could activate the reporter genes in yeast (Fig. 2C). These results further proved the role of ZmHDZ1 in transcriptional regulation in the nucleus by activating the expression of relative function genes.

One of the most significant finding in our research was that over-expression of *ZmHDZ1* in transgenic rice plants decreased their tolerance to salt stress (Fig. 3), while no difference was observed between transgenic and WT plants under drought stress (data not shown), implying that *ZmHDZ1* is probably acting as a negative regulator in response to salt stress, which was opposite to *ZmHDZ10* and *ZmHDZ4* that acted as the positive regulator (Zhao et al. 2014; Wu et al. 2016). It has been previously studied that several HD-Zip members also function as negative regulators that involved in adverse environmental stress response in plants. For example, over-expression of *OsHox22*, a rice HD-Zip I gene, decreased the transgenic rice tolerance to drought and salt stress, while the *oshox22* mutant plants enhanced tolerance to these environmental stresses, which indicated that the *OsHox22* played the roles as negative regulators in rice (Zhang et al. 2012). As well as *MtHB2*, encoding a HD-Zip transcription factor, also function as a negative regulator that involved in the regulation of abiotic stress response (Song et al. 2012). These findings suggested that the HD-Zip members in plants might possess different metabolic network in regulation of stress response, however, the complex regulatory mechanism still unknown.

During the process of evolution, plants have evolved a series of complex responsive mechanisms to adapt to various environment stresses. It is reported that when plants are exposed to abiotic stress conditions, reactive oxygen species (e.g. $H_2O_2$) will accumulate and often cause lipid peroxidation and oxidative damage (Xiong et al. 2001; Mittler et al. 2004). Malondialdehyde (MDA) is one of the most important products of membrane lipid peroxidation, which can further aggravate membrane damage. Therefore, MDA content has become a common indicator to determine the extent of lipid peroxidation (Mittler 2002). In this study, we found that the contents of MDA in *ZmHDZ1* transgenic plants were markedly higher than that in WT plants under salt stress (Fig. 3C), which indicated that the *ZmHDZ1* over-expression plants were more susceptible to oxidative stress generated by salt stress. Moreover, in line with the more accumulation of MDA, the REL level of *ZmHDZ1* over-expression plants was also higher than WT plants (Fig. 3B), suggesting that the cell membrane of transgenic plants suffering more severe damage under salt stress.

Additionally, the expression of some salt-related genes such as *OsLEA3*, *OsRAB16A*, *OsHox22* and *ABI5* was affected by over-expression of *OsHDZ1* in rice. Generally, the *OsLEA3* and *OsRAB16A* has high expression level in stress tolerance plants, whereas, they show less abundant transcript level in transgenic rice plants than that in WT plants under normal and salt stress conditions (Fig. 5). On the contrary, two negative regulators *OsHox22* and *ABI5* in transgenic rice plants had more abundant accumulation than that in WT plants (Fig. 5). From our results, we can interfere that *ZmHDZ1* may mediate activation or inhibition of stress-related genes accompanied with other stress-responsive regulators. These data further demonstrate that over-expression of *ZmHDZ1* reduces tolerance to salt stress in rice.

Previous studies have demonstrated that the ABA signaling plays essential roles in response to various environmental stresses (e.g. drought, salt and high temperature stresses) in different plants (Finkelstein and Lynch 2000; Xiong et al. 2001; Zhang et al. 2006). And all these stress-responsive genes can be divided into two groups, including ABA-dependent and ABA-independent group (Zhu 2002). In current study, the inducible expression analysis showed that *ZmHDZ1* was up-regulated under exogenous ABA treatment (Fig. 1A), and the ABA sensitivity tests indicated that the *ZmHDZ1* over-expression seedlings were more sensitive to exogenous ABA than WT seedlings (Fig. 4). Similar results observed in other HD-Zip I proteins, such as ZmHDZ4, ZmHDZ10, Athb-7, Athb12 and OsHox22 (Valdés et al. 2012; Zhang et al. 2012; Zhao et al. 2014; Wu et al. 2016). Likewise, the *ABI5* was reported to play a major role in ABA signal pathway in rice (Zou et al. 2008). The qRT-PCR results showed that the expression level of *ABI5* in *ZmHDZ1* over-expression rice plants was higher as compared to WT plants under normal condition (Fig. 5). One possible explanation was that the *ZmHDZ1* involved in ABA signal transduction pathway and over-expression of *ZmHDZ1* in rice affected the expression of ABA-related genes. These results strongly indicate that *ZmHDZ1* involves in salt stress response through participating in the ABA-dependent signal transduction pathways.

In conclusion, we identified a novel maize HD-Zip I transcription factor *ZmHDZ1* that is induced by exogenous ABA and NaCl stresses, targeted to nucleus and had transactivation activity. Over-expression of *ZmHDZ1* reduced tolerance to salt stress in rice and led to the greater accumulation of MDA, as well as the higher level of REL. Additionally, the transgenic seedlings were more sensitive to exogenous ABA. Therefore, we proposed that the *ZmHDZ1* was functioned as a negative regulator in response to salt stress through involving in the ABA signaling pathways. We

also summarized a simple working model of *ZmHDZ1* (Fig. S5). When plants suffer from salt stress, the ABA content will be rise, which prompts the expression of *ZmHDZ1* and other ABA-responsive genes. Then, the ZmHDZ1 protein starts or inhibits the expression of salt-responsive genes, which makes the plants more sensitive to salt stress.

## Materials and Methods

### Plant Materials and Stress Treatments

Maize seedlings (*Zea mays* L. inbred line B73) grew in mixed soil (soil/vermiculite/perlite as the volume proportion of 4:1:1) in greenhouse environment under 16/8 h light/night photoperiod at 28-30ºC and relative humidity 60% ± 5% for 3 weeks. For abscisic acid (ABA) treatment, 3-weeks old seedling leaves were sprayed with 100 μM ABA solution. For salt stress, seedlings at the same stage were irrigated with 200 mM NaCl solution. The treated seedling leaves were sampled at 0, 1, 3, 6, 9 and 12 h after treatment. Different tissues at different growth stages of maize were collected for tissue-special expression analysis, including roots, stems, leaves, tassels, corn silks and ears. Three biological replicates were performed for each experiment, and the sample of each replicate from three different plants, respectively. Samples were immediately frozen in liquid nitrogen and stored at -80ºC freezer for further laboratory experiments.

The Zhonghua 11 (lowland japonica) was employed for transgenic analyses. For salt treatments, Wild-type (WT) and T2 *ZmHDZ1*-overexpressing rice seeds were grown in square plates at 28ºC under 16/8 h light/night photoperiod for 3 weeks. Then, 200 mM NaCl solution was used to irrigate the plants for 10 d, after that the malondialdehyde (MDA) content and relative electrolytic leakage of WT and transgenic seedlings were measured as described by Zhao et al (Zhao et al. 2014). For MDA content, 0.5 g (W) leaf samples were ground to fine powder, 5 ml ($V_1$) of 0.05 mol. $L^{-1}$ phosphate buffer added, and then centrifuged at 4500 r/min for 10 min. Extracted 2 ml ($V_2$) of the supernatant and added 3 ml of 0.5% thiobarbituric acid (in 5% trichloroacetic acid) up to total volume 5 ml (V). Next, the reaction mixture was heated in boiling water for 10 minute and quickly cooled. After centrifugation at 4500 r/min for 10 minute, the absorbance of the supernatant at 532 and 600 nm was determined with water as control. The concentration of MDA was calculated by the following formula: MDA Content (mol. $L^{-1}$) = $(OD_{532}–OD_{600}) \times V1 \times V/1.55 \times 10^{-1} \times W \times V2$. For relative electrolyte leakage, leaf segments were soaked and then shaken in 10 ml deionized water at 28°C for 5 h. The conductivities (C1) of the solutions were measured with the help of a DDS-11A conductivity detector. After that the leaf segments were heated in boiling water for 20 min. The conductivities (C2) of the resulting solutions were determined with the same detector after being thoroughly cooled to room temperature. The C1/C2 × 100% values were calculated and employed to evaluate relative electrolyte leakage. This test was performed three biological replicates.

For ABA treatment, rice seeds of wild-type (WT) and transgenic lines were surface-sterilized with NaClO solution, and then germinated on half-strength MS solid medium supplemented with 0, 2, 5 and 8 μM ABA. Seedlings were grown for 7 d at 28ºC with a 16/8 h light/dark photoperiod.

### Subcellular Localization and Transactivation Assay of ZmHDZ1

Full-length coding sequence of *ZmHDZ1* lacking its termination codon was cloned with the gene-specific primers F2 and R2 (Table S1) and ligated into the *Spe*I and *Sma*I sites of the pCAMBIA1305-GFP vector (pCAMBIA1305-GFP was modified from pCAMBIA1305 vector) (Wang et al. 2015a). The fusion vector 35S:*ZmHDZ1*-GFP was transiently expressed in the leaves of *Nicotiana benthamiana* via Agrobacterium-mediated infiltration, and the alone pCAMBIA1305-GFP vector was set as control (Fig. 2A). After 36-48 h, the infected leaf tissues were sampled and observed under a Zeiss LSM700 (Zeiss, Jena, Germany) confocal microscope and the DNA dye 4, 6-diamidino-2-phenylindole (DAPI) was used to visualize the nucleus.

To test the transactivation of ZmHDZ1, The yeast strain AH109 *Saccharomyces cerevisiae* and the vector pGBKT7 were employed (Clontech). The full-length sequence of *ZmHDZ1* was amplified from plasmid cDNA with the primers F3 and R3 (Table S1), and inserted into *Eco*RI and *Bam*HI restriction digestion sites of the pGBKT7 vector. According to the manufacturer's protocol, the fusion expression vector pGBKT7-ZmHDZ1 was introduced into the yeast AH109 strain. The empty vector pGBKT7 was set as the negative control, and the pGBKT7-p53 and pGADT7-SV40 large T-antigen were co-transformed into the yeast AH109 strain as the positive control. The positive colonies were transferred to both SD/-Trp and SD/-Trp-His/-Ade/-X-α-gal medium and grown at 30ºC in a constant temperature incubator for 2-5 d.

### Generation of Transgenic Plants

To generate the transgenic rice plants of *ZmHDZ1*, full-length encoding sequence of *ZmHDZ1* was cloned using gene-specific premiers F1 and R1 (Table S1), and inserted into *Bam*HI and *Xba*I restriction digestion sites of the p1301a vector (p1301a was modified from pCAMBIA1301 vector. A 35S promoter is inserted into pCAMBIA1301 vector with *Eco*RI and *Sac*I restriction enzymes and a terminator is ligated into the *Sal*I and *Hind*III sites of the pCAMBIA1301 vector). The resulting vector p1301a-ZmHDZ1 was transformed into rice Zhonghua 11 via the infection of *Agrobacterium tumefaciens* strain EHA105. Positive transgenic plants were identified by histochemical analysis of GUS activity and PCR amplification of the hygromycin B gene.

### RNA Extraction, Semiquantitative and Quantitative RT-PCR (qRT-PCR) Analysis

Total RNA was isolated from maize and rice frozen tissue samples using TRizol reagent (TaKaRa).

According to the manufacturer's protocol, reverse transcription was performed using the PrimeScript ™RT reagent Kit with gDNA Eraser (TaKaRa). To detect the transcript level of *ZmHDZ1* in transgenic rice, semiquantitative RT-PCR was conducted with premiers F4 and R4, and the *OsActin1* was used as an internal control. An ABI 7300 Real-Time system (Applied Biosystems) was employed to perform the qRT-PCR to analyze the expression profiles of *ZmHDZ1* in different tissues and under ABA or NaCl stress treatments. The maize *Actin* gene was used as the internal control. The gene-specific primers used in this study were listed in Table S1.

### Statistical Analysis

The SPSS v19 software (http://www.spss.com.cn/) was employed to perform statistical analysis in our study. Significant differences were determined at $p<0.05$ with Student's t-test.

## Acknowledgements

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## Author's Contributions

Qianqian wang, Kangyong Zha and Yang Zhao conceived and designed this research. Qianqian Wang and Kangyong Zha performed the experiment. Qianqian Wang, Wenbo Chai, Yu Wang and Bin Liu analyzed the data. Haiyang Jiang, Beijiu Cheng and Yang Zhao contributed reagents/materials/analysis tools. Qianqian wang and Kangyong Zha wrote the manuscript. All authors read and approved the manuscript.

## Supporting Information

**Fig. S1.** Sequence analysis of Zmhdz1. The homeodomain (HD) and Zip motifs are marked by black and blue lines, respectively. The red boxes indicate conserved leucine residues.
**Fig. S2.** Expression patterns of maize stress responsive genes under ABA and NaCl treatments.
**Fig. S3.** The expression analysis of *ZmHDZ1* in WT and ZmHDZ1 transgenic rice.
**Fig. S4.** The phenotypes of WT and transgenic rice in normal condition.
**Fig. S5.** Working model of the *ZmHDZ1*-mediated regulatory cascade triggered by NaCl stress in rice.
**Table S1.** The primers used in this study. The underlines indicate the sequences of restriction enzyme cutting sites.

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