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ORIGINAL ARTICLE

A Moso bamboo gene *VQ28* confers salt tolerance to transgenic Arabidopsis plants

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Abstract

Main conclusion **Overexpression of *PeVQ28* in Arabidopsis regulated the expression of salt/ABA-responsive genes and indicated that *PeVQ28* may affect the ABA synthesis induced by stress in plants by regulating salt tolerance.**

Abstract Plant-specific VQ proteins, which contain a conserved short FxxhVQxhTG amino acid sequence motif, play an important role in abiotic stress responses, but their functions have not been previously studied in Moso bamboo (*Phyllostachys edulis*). In this study, real-time quantitative PCR analysis indicated that expression of *PeVQ28* was induced by salt and abscisic acid stresses. A subcellular localization experiment showed that *PeVQ28* was localized in the nuclei of tobacco leaf cells. Yeast two-hybrid and bimolecular fluorescence complementation analyses indicated that *PeVQ28* and *WRKY83* interactions occurred in the nucleus. The *PeVQ28*-overexpressing Arabidopsis lines showed increased resistance to salt stress and enhanced sensitivity to ABA. Compared with wild-type plants under salt stress, *PeVQ28*-transgenic plants had lower malondialdehyde and higher proline contents, which might enhance stress tolerance. Overexpression of *PeVQ28* in Arabidopsis enhanced expression of salt- and ABA-responsive genes. These results suggest that *PeVQ28* functions in the positive regulation of salt tolerance mediated by an ABA-dependent signaling pathway.

Keywords Abscisic acid · Fluorescence complementation analysis · Malondialdehyde · *PeVQ28* · Proline content · Salt · Yeast two hybrid

Abbreviations

BiFC	Bimolecular fluorescence complementation;
CAT	Catalase
MDA	Malondialdehyde
POD	Peroxidase
POR	Proline
SOD	Superoxide dismutase
RWC	Plant relative water content
VQ	Valine–glutamine motif-containing protein

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Introduction

The environment has an important impact on plant growth and development. Numerous environmental factors have negative, and even harmful, effects on plants. Such factors include biotic stresses, for example, diseases, insect pests, and weeds, and abiotic stresses, such as drought, salinity, and high and low temperatures (Ingram and Bartels 1996; Katiyaragarwal. 2006; Yamaguchi-Shinozaki and Shinozaki 2006). Among the abiotic stresses, drought, salinity, and low temperature have the greatest influence on plants (Xiong et al. 2002; Jakab et al. 2005). Plants have evolved numerous mechanisms to enable adaption to detrimental environmental changes. During these adaption periods, plants regulate their response to stress through the regulation of the osmotic

balance, synthesis of stress-associated proteins and antioxidants, and expression of characteristic genes (Shinozaki and Yamaguchi-Shinozaki 1997; Zhu 2002). In particular, valine–glutamine (VQ) motif-containing proteins are indicated to be involved in plant responses to abiotic stresses (Perruc et al. 2004; Lai et al. 2011; Hu et al. 2013b; Kim et al. 2013; Song et al. 2016; Wang et al. 2017).

The plant-specific VQ proteins are of increasing interest owing to their interactions with WRKY transcription factors (Lai et al. 2011). At present, 34, 40, 61, 18, and 74 VQ members have been identified in Arabidopsis, rice (*Oryza sativa*), maize (*Zea mays*), grape (*Vitis vinifera*), and soybean (*Glycine max*), respectively (Cheng and Chen 2012; Li et al. 2014; Wang et al. 2014, 2015b; Song et al. 2016). The VQ proteins are structurally characterized by five highly conserved amino acids in the core sequence FxxxVQx-LTG, where x represents any amino acid and VQ are the highly conserved amino acid residues (Pecher et al. 2014). Recently, it was observed that the last three amino acids in the conserved domain may be of other types besides LTG, such as FTG, ITG, LTA, and VTG (Kim et al. 2013).

As a transcriptional regulator, VQ proteins can interact with a variety of proteins to regulate several physiological and biochemical processes in plants. The WRKY transcription factors are the most important interactors with VQ proteins (Wu et al. 2017). For example, *AtVQ15* interacts with *WRKY25* and *WRKY51* to regulate plant tolerance to salt and osmotic stress (Perruc et al. 2004). In addition, transient expression in protoplasms of Arabidopsis leaves revealed that most VQ members are capable of interacting with each other (Wang et al. 2015a).

The transcriptional expression of *VQ* genes is induced or inhibited by salt, drought, low nitrogen, temperature stress, and ABA, indicating that the VQ protein family plays an important role in the regulation of plant responses to abiotic stress (Hu et al. 2013b; Wang et al. 2014, 2015b). In an Arabidopsis study (Hu et al. 2013b), *AtVQ9* (*AtMVQ10*) mutants showed a higher germination rate and superior seedling growth under NaCl treatment, whereas the reverse was true in overexpression plants (Perruc et al. 2004). These results indicate that *AtVQ9* is involved in the negative regulation of Arabidopsis resistance to NaCl stress. Perruc et al. (2004) observed that *AtVQ15* (*AtCAMBP25*) overexpression lines were highly sensitive to NaCl and osmotic stress during seed germination and seedling growth, whereas the mutant showed enhanced resistance. Thus, *AtVQ15* might negatively regulate the tolerance of Arabidopsis to NaCl and osmotic stresses. In maize and rice, *ZmVQ19* and *ZmVQ54*, and *OsVQ2*, *OsVQ16*, and *OsVQ20*, respectively, were highly expressed during drought induction (Wang et al. 2015b). In soybean, *GmVQ6* and *GmVQ53* were highly expressed in roots and stems under low-nitrogen conditions, which indicated that these genes affected growth and development (Wang et al. 2014). Collectively, these results suggest that VQ proteins play important roles in plant growth and responses to environmental stresses.

The majority of functional studies of *VQ* genes have been conducted on Arabidopsis. In our previous study, 29 VQ proteins were identified in Moso bamboo (*Phyllostachys edulis*) and were classified in seven subfamilies on the basis of phylogenetic relationships (Wang et al. 2017). As an extension of our previous research, in the present study we characterized the function of *PeVQ28*. The *PeVQ28* cDNA was isolated from Moso bamboo and transformed into Arabidopsis by *Agrobacterium*-mediated methods to obtain *PeVQ28*-overexpressing Arabidopsis plants. The present results will aid in elucidating the molecular mechanism of *PeVQ28* and provide a theoretical reference for breeding of stress resistance in Moso bamboo.

Materials and methods

Experimental materials, growth conditions, and stress treatment

Seedlings of Moso bamboo (*Phyllostachys edulis* (Carrière) J.Houz.) were cultivated in a greenhouse [24 °C, 16 h/8 h (light/dark) photoperiod, and 80% relative humidity]. The Moso bamboo seeds were collected from the Tianmu Mountain National Nature Reserve in Zhejiang Province, China. The growing medium was a mixture of black soil and vermiculite (3:1, v/v) (Wu et al. 2015; Wang et al. 2016; Chen et al. 2017; Gao et al. 2017; Liu et al. 2017). For application of stress treatments, the soil mixture was irrigated with either 100 μM ABA (Kim et al. 2013) or 200 mM NaCl solutions (Zhao et al. 2012). Leaves were harvested at 0, 1, 3, 6, 12, and 24 h after treatment, respectively. In addition, untreated root (R), stem (St), young leaf (YL), leaf (L), shoot (S), and rhizome (Rh) samples were immediately frozen in liquid nitrogen and stored at − 80 °C for RNA extraction, and roots were used as comparators.

Seeds of tobacco (*Nicotiana tabacum* L.), wild-type (WT) Arabidopsis [Colombia (Col-0) ecotype], and three T_3 transgenic lines (L1, L3, and L10) were incubated in Murashige and Skoog (MS) medium at 4 °C for 3 d to undergo vernalization, then transferred to a greenhouse [24 °C, 16 h/8 h (light/dark), 80% relative humidity] for 10 d, and transplanted into square pots (12 cm diameter) containing a mixture of black soil and vermiculite (1:3, v/v). Two- to three-week-old seedlings were used for the experiments.

Isolation of *PeVQ28* and generation of Arabidopsis transgenic lines

The 513 bp coding sequence of *PeVQ28* was cloned using the gene-specific primers 5′-ATGGGGGAGTACCACAGA ATGA-3′ (forward) and 5′-TTAAGATGCGTACATTTC ACCAACC-3′ (reverse).

The SmaI and SalI restriction sites were inserted upstream and downstream, respectively, of the coding sequence of *PeVQ28*. The constructs were transformed into Arabidopsis Col-0 plants by *Agrobacterium*-mediated transformation using a previously described method (Wu et al. 2017).

RNA isolation and RT-qPCR analysis of gene expression

Total RNA was extracted as described previously (Wang et al. 2017). The *PeVQ28* cDNA was synthesized using PrimeScript™ RT Master Mix (Takara, Tokyo, Japan) in accordance with the manufacturer's instructions. The *PeVQ28* gene-specific primers and ABA-associated gene primers were designed using Primer Express 5.0 (Table S1). Real-time quantitative PCR (RT-qPCR) was performed on an ABI 7300 real-time system in accordance with a previously reported procedure (Wang et al. 2017). Data were processed following the methods of Livak and Schmittgen (Livak and Schmittgen 2001; Zhao et al. 2012). The tonoplast intrinsic protein 41 (*TIP41*) was used as an internal control (Fan et al. 2013).

Subcellular localization analysis

The coding sequence of *PeVQ28*, lacking the stop codon, was amplified by PCR using the following primers: 5′-TGC TCTAGAATGGGGGAGTACCACA-3′ (forward, XbaI site) and 5′-CGCGGATCCAGATGCGTACATTTCA-3′ (reverse, BamHI site). The amplified fragment was inserted into the pCAMBIAI1305 vector (Clontech, Beijing, China) containing the *CaMV35S* promoter and the green fluorescent protein (GFP) gene to generate the *p1305-CaMV35S-PeVQ28-GFP* fusion expression vector. The control vector was *p1305-CaMV35S-GFP*. The suspension was infiltrated into tobacco leaves using an injection method and the GFP fluorescence was observed using a confocal microscope (LSM710, Carl Zeiss, Jena, Germany) (Dai et al. 2007; Cao et al. 2016).

Yeast two-hybrid and bimolecular fluorescence complementation (BiFC) analyses

The coding regions of *PeVQ28* and *PeWRKY83* (Table S2), which contained the EcoRI and BamHI restriction enzyme sites, were amplified by PCR. The pGBKT7 and pGADT7 vectors, and the target fragments were digested with the EcoRI and BamHI restriction enzymes, recovered, and ligated to generate the recombinant plasmid vectors *pGBKT7-PeWRKY83* and *pGADT7-PeVQ28*. The recombinant plasmids of the experimental group (*pGBKT7-PeWRKY83* + *pGADT7-PeVQ28*), the positive control (*pGBKT7-T53* + *pGADT7-T*), and the negative controls (*pGADT7-PeVQ28* + *pGBKT7* and *pGBKT7-PeWRKY83* + *pGADT7*) were transformed into yeast (*Saccharomyces cerevisiae*) strain AH109. The transformed yeast cells were plated on deficient media (SD/-Leu/-Trp or SD/-Ade/-Leu/-Trp/-His) for 3–5 days to determine protein–protein interactions.

The BiFC assay was performed as previously reported (Walter et al. 2004). Briefly, *PeWRKY83* and *PeVQ28* containing the XbaI and BamHI restriction enzyme sites were enzymatically digested and inserted into the pUC-SPYNE and pUC-SPYCE vectors, recovered, and ligated to obtain the recombinant plasmid vectors *PeVQ28-nYFP* and *PeWRKY83-cYFP*.

Stress resistance of transgenic Arabidopsis

First, a seed germination experiment was performed. Plants of the Arabidopsis WT and transgenic lines were cultured under identical conditions [24 °C, 16 h/8 h (light/dark), and 80% relative humidity] and seeds were collected. The seeds were germinated on plates containing MS medium supplemented with ABA (0, 0.1, 0.5, or 1 μM) or NaCl (0, 100, or 150 mM). The germination frequency was calculated after 3–5 days.

Second, the main root length of seedlings was measured. Seeds were germinated on standard MS medium following the aforementioned method, and then seedlings were transferred to MS plates containing ABA (0, 10, 30, or 50 μM) or NaCl (0, 100, 150, or 200 mM). After growth for 7 days, the main root length of each seedling was recorded.

Finally, 3-week-old T_3 transgenic lines (L1, L3, and L10) and WT Arabidopsis seedlings planted in the same pot were treated with salt solution (50, 100, 200, or 300 mM NaCl), and the control seedlings were irrigated with water. The pots were randomly arranged in a greenhouse and grown at 24 °C, in which the lighting conditions and humidity did not change. After 10–15 d of continuous treatment, the pots were irrigated with water, and plants allowed to recover for 7 days.

Measurement of growth and stress-related parameters

The percentage survival, relative water content (RWC) (Bates et al. 1973; Zhao et al. 2014), malondialdehyde (MDA) content (Heath and Packer 1968), activities of catalase (CAT) (Rorth and Jensen 1967; Goldstein. 1968; Del Rio et al. 1977), peroxidase (POD) (Sequeira and Mineo 1966; Kochba et al. 1977), and superoxide dismutase (SOD) (Liu et al. 2012, Zhou and Prognon 2005), and proline (PRO) content (Bates et al. 1973) were determined. All enzyme activity analysis was performed using Arabidopsis leaves.

Statistical analysis

Data analyses were performed using Excel and SPSS v10.0 (SPSS, Inc., Chicago, IL, USA), and Student's *t* test was used to determine the significance of differences between means. The mean values and standard deviations (SDs) were obtained from three biological and three technical replicates, and significant differences relative to controls are indicated at $^{**}P < 0.05$ and $^{*}P < 0.01$.

Results

Identification and expression pattern analysis of *PeVQ28*

In our previous research on the expression patterns of the *VQ* genes of Moso bamboo, we noted that *PeVQ28* was induced by salt and ABA (Wang et al. 2017). Therefore, *PeVQ28* was selected for further functional analyses. Gene-specific primers were designed on the basis of the gene sequence, and total RNA extracted from the leaves was reverse-transcribed to isolate the full-length cDNA of bamboo *PeVQ28*. Sequencing demonstrated that the PCR-amplified *PeVQ28* gene fragment (accession no. PH01007611G0010) contained a 513 bp open reading frame encoding a 170-amino acid protein with a conserved FxxxVQxLTG domain (amino acids 47–57). The cDNA sequence of *PeVQ28* showed 100% sequence similarity to the sequence in the bamboo database (https://www.bamboogdb.org/). A putative nuclear localization signal (NLS) sequence was predicted to be located in the region of amino acids 79–110 (Fig. 1a). The *VQ* genes are widely involved in abiotic stress responses in plants. To explore whether *PeVQ28* is responsive to stress, the expression levels under ABA and NaCl stresses were observed using qRT-PCR (Fig. 1b). The expression of *PeVQ28* was significantly up-regulated by salt treatment and was high at a relatively early stage (after 3 h treatment). Under ABA stress, the expression of *PeVQ28* was induced at 1 h and 24 h. Thus, *PeVQ28* was induced by salt and ABA, which indicated that the gene may play key roles in response to abiotic stress and the ABA-signaling pathway.

In addition, six tissues were selected to examine the tissue-specific expression of *PeVQ28* (Fig. 1c). *PeVQ28* was expressed in all of the tested tissues and organs, including root, stem, young leaf, leaf, shoot, and rhizome. The highest expression level detected was in the leaves.

Subcellular localization of *PeVQ28*

According to the application method of the cNLS Mapper website (Conti et al. 1998; Ossareh-Nazari et al. 2001; Harel and Forbes 2004; Kosugi et al. 2008a, b, 2009), analyzing whether a particular sequence contains an NLS sequence produces three different results: (1) score of 7–9, the gene is only located in the nucleus; (2) score of 3–6, the gene is located in the nucleus and cytoplasm; and (3) score of 1 or 2, the gene is located only in the cytoplasm. We analyzed the protein sequence of *PeVQ28* using cNLS Mapper and obtained a score of 7, which indicated that an NLS sequence was present and that *PeVQ28* was localized in the nucleus (Fig. 1a). To confirm this prediction, the *PeVQ28-GFP* fusion construct (*35S::PeVQ28-GFP*) was used to examine the subcellular localization of *PeVQ28*. The GFP signal was detected in the nuclei of tobacco cells. In the control (35S::GFP), GFP signal was observed throughout the cell (Fig. 2). Thus, PeVQ28 is a nuclear-localized protein (Figs. 1a, 2).

Analysis of the interaction between *PeVQ28* and *PeWRKY83*

In Arabidopsis, the majority of AtVQ proteins interact with WRKY transcription factors. To verify whether *PeVQ28* can interact with the WRKY transcription factor *PeWRKY83,* we performed yeast two-hybrid and BiFC experiments.

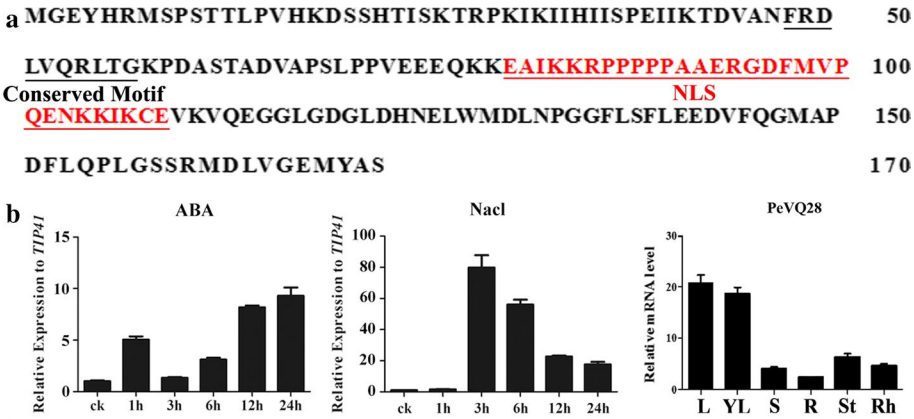


Fig. 1 Sequence analysis and expression pattern of *PeVQ28*. **a** *PeVQ28* nucleotide and deduced amino acid sequences. The VQ motif was indicated by black solid lines, and two putative NLS sequences are enclosed by red lines. **b** *PeVQ28* expression analysis (RT-qPCR) in Moso bamboo young leaf subjected to ABA and NaCl treatments, respectively. **c** Expression patterns of *PeVQ28* in various tissues: *L* leaf, *R* root, *Rh* rhizome, *S* shoot, *St* stem, *YL* young leaf

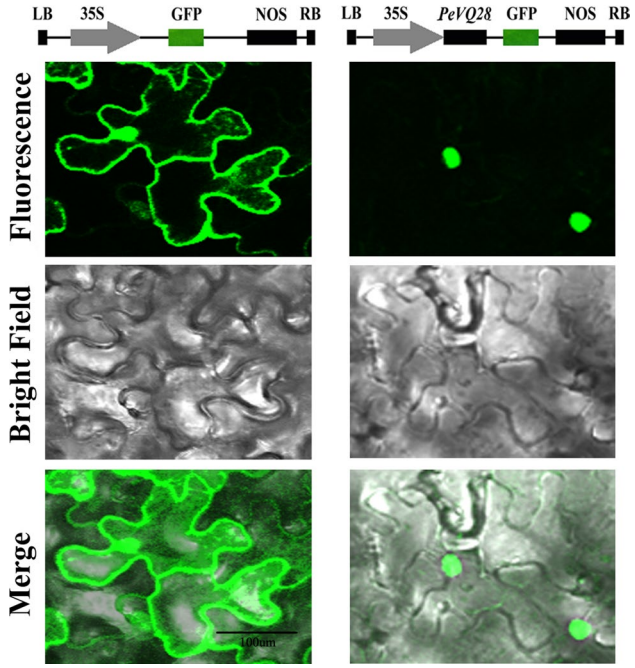


Fig. 2 Nuclear localization of *PeVQ28*. The 35S::GFP::PeVQ8 construct and the control vector 1305(35S::GFP) were transformed in *Nicotiana tabacum* leaves. The GFP signals in root cells were observed by confocal microscopy

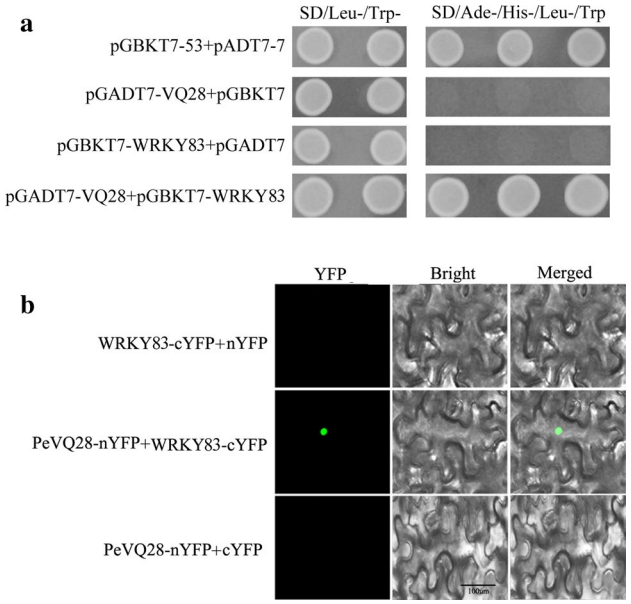


Fig. 3 Interaction of *PeVQ28* with *PeWRKY83*. **a** Interaction of *PeVQ28* with *PeWRKY83* in yeast. The bait construct (pGBKT7-PeVQ28) and the prey constructs (pGADT7-PeWRKY83) were co-transformed yeast strain AH109, then examined on SD/-Leu/-Trp and SD/-Ade/-His/-Leu/-Trp plates. Positive control, pGBKT7-53 + pGADT7-T; negative control, pGBKT-53 + pGADT7-Lam. **b** BiFC assays of *PeVQ28* interaction with *PeWRKY83* in vivo. Yellow fluorescent protein (YFP) images were detected at an approximate frequency of 8.46% (1100 of 1300 tobacco leaf epidermal cells analyzed exhibited BiFC events)

The yeast cells harboring the recombinant plasmids (*pGBKT7-PeWRKY83* + *pGADT7-PeVQ28*, *pGBKT7-T53* + *pGADT7-T*, *pGADT7-PeVQ28* + *pGBKT7*, and *pGBKT7-PeWRKY83* + *pGADT7*) grew normally on SD/-Leu/-Trp medium (Fig. 3a). However, on SD/-Ade/-Leu/-Trp/-His medium, the yeast cells of the positive control and the experimental group grew normally, whereas those of the negative control group did not grow normally. Thus, *PeVQ28* interacted with the *PeWRKY83* transcription factor.

Tobacco transformed with *PeVQ28-nYFP* + *PeWRKY83-cYFP* was observed to show YFP signal concentrated in the nucleus, whereas the control groups (*PeVQ28-nYFP* + *cYFP* and *PeWRKY83-cYFP* + *nYFP*) did not show a fluorescent signal (Fig. 3b). Thus, the BiFC assay also indicated that *PeVQ28* and the *PeWRKY83* transcription factor were capable of interacting.

Molecular analysis of *PeVQ28*-containing transgenic Arabidopsis

To verify the effects of *PeVQ28* on plant stress tolerance, *PeVQ28* was transformed into the WT Arabidopsis (Col-0) under the control of the *CaMV35S* promoter (Fig. S1a). A total of 30 independent transgenic plants was obtained, and the expression level of *PeVQ28* was determined by β-glucuronidase assay (GUS) and RT-qPCR in eight randomly selected lines (Fig. S1a, c). *PeVQ28* was not expressed in WT plants, but was expressed in transgenic lines. According to the RT-qPCR analysis, the expression levels were greater in the L1, L3, L8, and L10 lines, but those of the first three lines were more similar (Fig. S1d), so they were used in the subsequent stress-resistance experiments.

Resistance of *PeVQ28*-overexpressing Arabidopsis plants to salt stress

We first performed a seed germination experiment on MS plates supplemented with different concentrations of NaCl. On the 0 mM NaCl MS plate, no differences were observed among the germination frequencies of the three transgenic lines (L1, L3, and L8) and that of WT seeds. In contrast, under high-salt-stress conditions induced by 100 or 150 mM NaCl treatment, the germination frequency of the WT and transgenic lines was significantly inhibited, but that of the WT was significantly lower than the latter (Fig. 4a, b and Table S3).

Next, main root length assays were conducted on MS plates in the presence of 0 or 150 mM NaCl. On the 0 mM NaCl MS plate, no significant differences were observed among the growth rates of the three transgenic strains (L1,

L3, and L8) and that of the WT. Under the 150 mM NaCl treatment, the transgenic plants exhibit less severe growth inhibition than that of the WT (Fig. 4c), and the main root lengths of the overexpression lines (3.9–4.1 cm) showed less inhibition than that of the WT (2.1 cm) (Fig. 4d).

The resistance to salt stress of overexpression plants grown in soil was examined. The phenotype of plants in the 50 mM NaCl treatment was not significantly different (Fig. 5a). The stress-induced phenotype was evident after treatment with 100, 200, and 300 mM NaCl. On the basis of the phenotype assessment, we analyzed the growth and selected stress-related parameters of WT and transgenic plants in response to treatment with 200 mM NaCl. After 15 d of treatment with 200 mM NaCl, the majority of WT plants had withered, whereas the transgenic lines grew well (Fig. 5a). The percentage survival of the transgenic plants (43.58–47.66%) was notably higher than that of the WT plants (33.89%) (Fig. 5b). In response to treatment with 200 mM NaCl, the RWC, PRO content, and activities of SOD, POD, and CAT in the WT plants were lower than those in the *PeVQ28*-overexpressing plants (Fig. 5c–g). No significant differences in MDA content between the transgenic and WT plants were observed before stress; but after salt stress treatment, the MDA content in transgenic plants was significantly lower than that in WT plants (Fig. 5h). In summary, under high-salinity conditions, *PeVQ28*-overexpressing plants showed stronger salt-stress resistance than that of WT plants.

Sensitivity of *PeVQ28*-overexpressing Arabidopsis plants to ABA

The enhanced tolerance of *PeVQ28*-overexpressing plants to salt stress motivated us to evaluate whether *PeVQ28* had an effect on ABA signaling. We first examined the ABA sensitivity of *PeVQ28*-transgenic plants in a seed germination experiment. Under the control condition, no significant difference was observed in the germination frequencies of WT and transgenic seeds (Fig. 6a). In response to supplementation with 0.5 or 1.0 μM ABA, the germination frequencies of WT and transgenic seeds decreased, but the germination frequency of transgenic seeds was less strongly reduced than that of the WT seeds (Fig. 6b).

The root growth of WT and *PeVQ28*-overexpressing seedlings was determined under treatment with 0 or 30 μM ABA. No significant difference in main root length between WT and *PeVQ28*-overexpressing seedlings was observed under the control condition; however, in response to 30-μM ABA treatment, the growth of *PeVQ28*-transgenic seedlings was restrained more severely than that of WT seedlings

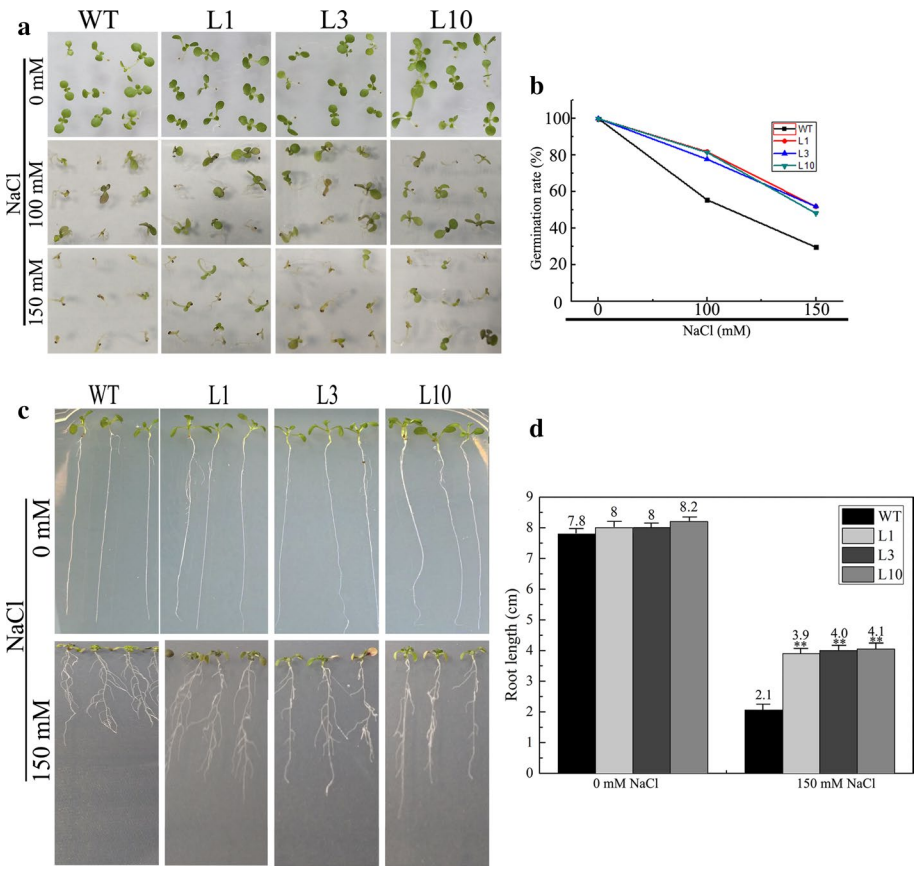


Fig. 4 Germination and phenotypes of *PeVQ28* in transgenic Arabidopsis under salt tolerance. **a** Germination performance of *PeVQ28*-overexpression and WT seeds on MS medium containing 0, 100, or 150 mM NaCl. **b** Calculation of the germination rates of transgenic and WT seeds. **c** Effect of salt stress on root length of transgenic and WT plants. **d** Calculation of the root length of transgenic and WT plants. Values are means ± SE ($n = 10$). *$P < 0.05$, *t* test; **$P < 0.01$, *t* test

(Fig. 6c, d). Thus, overexpression of *PeVQ28* increased the ABA sensitivity of Arabidopsis seedlings.

We also determined the ABA content of WT and *PeVQ28*-overexpressing plants. The content of ABA in *PeVQ28*-overexpressing plants was higher than that in WT plants (Fig. 6e).

Expression of ABA-related genes in *PeVQ28*-overexpressing Arabidopsis plants

To investigate the molecular mechanism of the *PeVQ28*-mediated response to stress conditions, we analyzed the expression levels of nine marker genes (*AtAAO3*, *AtNCED2*, *AtRD29A*, *AtRD29B*, *AtABF1*, *AtNCED3*, *AtABI1*, *AtP5CS1*, and *AtPP2CA*) in *PeVQ28*-overexpressing Arabidopsis lines. In response to 5 d of salt treatment, ABA biosynthesis genes were activated in WT and *PeVQ28*-overexpressing lines, whereas the expression levels of *AtAAO3*, *AtNCED2*, *AtRD29A*, *AtRD29B*, *AtABF1*, and *AtNCED3* in transgenic plants were significantly higher than those in WT plants (Fig. 7). In addition, *AtP5CS1*, *AtABI1*, and *AtPP2CA* showed higher expression levels in transgenic plants than in WT plants (Fig. 7). These results suggested that *PeVQ28* overexpression also induced ABA synthesis in transgenic plants under salt-stress conditions, thereby leading to increased expression levels of ABA-signaling and ABA-related genes.

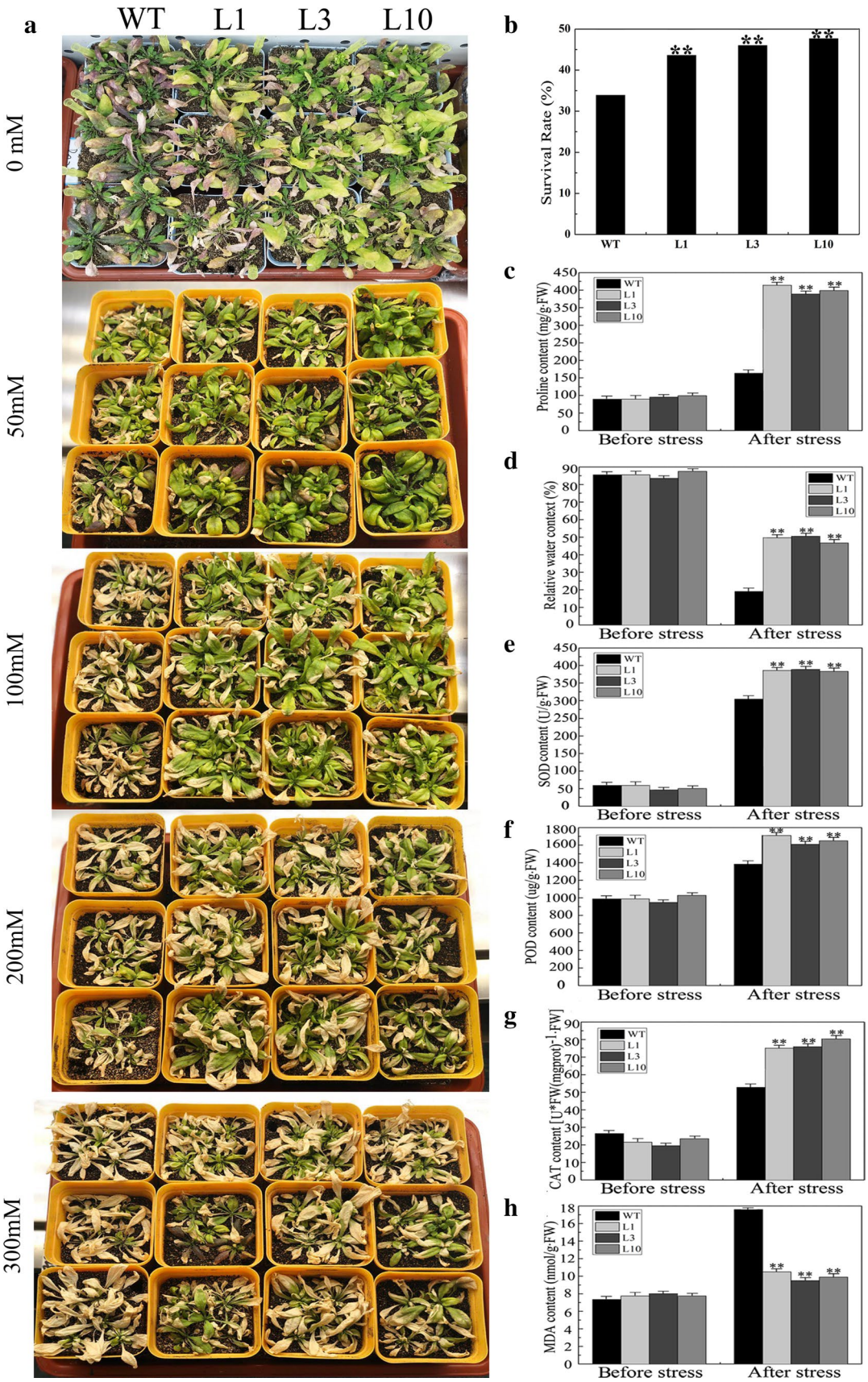
Discussion

The VQ proteins are plant-specific proteins. In our previous research, we identified 29 *VQ* genes in the Moso bamboo genomic database and classified them into seven subfamilies (I–VII) on the basis of evolutionary relationships (Wang et al. 2017). The genome-wide bioinformatics and stress-induced expression patterns of the *VQ* gene family have been analyzed in a number of species, whereas functional studies have focused on Arabidopsis *VQ* genes, such as *AtVQ9* (*AtMVQ10*) and *AtVQ15* (*AtCAMBP25*) (Perruc et al. 2004; Hu et al. 2013b). No functional analyses have been reported for *PeVQ* genes. Based on the results of previous bioinformatics research in combination with the literature on the *VQ* gene family, we selected the potential abiotic stress-related gene *PeVQ28* for functional analysis. The expression level of *PeVQ28* in response to different stress treatments was analyzed (Fig. 1b). *PeVQ28* was up-regulated under high-salt and ABA stress conditions. The expression level was 80-fold higher under high-salt conditions, indicating that the gene was likely induced by an adversity-triggered stress-response mechanism. We extracted six tissue samples (R, St, YL, L, S and Rh) of Moso bamboo for RT-PCR experiments. The results showed that *PeVQ28* was highest expressed in L, followed by YL. It shows that *PeVQ28* was highly expressed in L, so the materials used in subsequent experiments were all L.

First, the localization of the PeVQ28 protein was analyzed by transiently transforming the constructed *p1305-CaMV35S-PeVQ28-GFP* fusion expression vector into tobacco leaves, and was shown to localize in the nucleus (Fig. 2). Second, the VQ protein family can interact with WRKY transcription factors in Arabidopsis (Hu et al. 2013a). To verify the interaction between a VQ protein and a WRKY transcription factor in Moso bamboo, yeast two-hybrid and BiFC assays were performed (Fig. 3). The PeVQ28 protein, both in vivo and in vitro, could interact with WRKY transcription factors and form a complex localized in the nucleus. The *VQ*-assisted WRKY transcription factor is involved in the regulation of plant growth and development, and response to abiotic and biotic stresses, and provided a pointer for exploration of the function of *PeVQ28* (Perruc et al. 2004; Wang et al. 2015a; Wu et al. 2017).

Overexpression of a gene is a direct and effective method to examine the biological function of the gene (Hao et al. 2011; He et al. 2011; Ge et al. 2017). As expected, transgenic Arabidopsis plants were significantly more tolerant to salt stress (Fig. 4). In addition, the sensitivity of *PeVQ28*-overexpressing plants to exogenous ABA was significantly increased during the germination and seedling stages (Fig. 6). Thus, *PeVQ28* may play an important role in ABA-dependent signaling and promote different resistance levels to different stresses. The present research showed that *PeVQ28* might act as a positive regulator of ABA-dependent signaling pathways to improve plant resistance to salt stress.

Plants have evolved diverse mechanisms to adapt to changes in the environment. It is well known that plants suffering from abiotic stress often accumulate reactive oxygen species leading to lipid peroxidation and oxidative stress (Xiong et al. 2002; Mittler et al. 2004). The content of MDA is a parameter often used as an indicator of reactive oxygen species stress in plant cells. Under salt stress, the MDA content of WT plants was higher than that of *PeVQ28*-transgenic plants, which showed that overexpression of *PeVQ28* may lead to increased resistance to oxidative stress induced by salt stress (Fig. 5h). Many studies have shown that accumulation of free PRO is an important mechanism in response to abiotic stress, because it can stabilize subcellular structures by regulating intracellular osmotic potential to prevent damage (Liu and Zhu 1997; Armengaud et al. 2004; Xiang et al. 2007). The present data indicated that *PeVQ28*-overexpressing plants accumulate significantly higher quantities of PRO than that of WT under stress conditions (Fig. 5c). The increase in PRO content may explain the higher osmotic pressure, which leads to lower water potential and the more efficient conservation of water by plants (Song et al. 2011). Consistent with this, leaves of *PeVQ28*-overexpressing


a
WT
L1
L3
L10
0 mM
50mM
100mM
200mM
300mM
b
Survival Rate (%)
WT
L1
L3
L10
c
Proline content (mg/g·FW)
Before stress
After stress
d
Relative water context (%)
e
SOD content (U/g·FW)
f
POD content (ug/g·FW)
g
CAT content [U*FW/(mgprot)-1·FW]
h
MDA content (nmol/g·FW)

◂**Fig. 5** Salt stress of *PeVQ28* in transgenic Arabidopsis plants. **a** Performance of WT and transgenic plants before and after salt treatment with 200 mM NaCl. **b** Survival rates of WT and transgenic plants after recovery for 7 d following salt stress. PRO (**c**), RWC (**d**), SOD (**e**), POD (**f**), CAT (**g**) and MDA content (**h**) were measured in WT and transgenic plants after salt treatment. Values are means ± SE ($n = 3$). *$P < 0.05$, *t* test; **$P < 0.01$, *t* test

plants showed a higher RWC than that of WT plants (Fig. 5d), which indicated that the transformants showed increased ability to conserve water under stress conditions. Given that PRO, CAT, SOD, and POD can be used, to a certain extent, as antioxidants to decrease oxidative damage under stress, increased PRO content and CAT, SOD, and POD activities in transgenic plants may result in lower MDA contents under stress conditions (Fig. 5c–h) (May 2008). As a result, the enhanced PRO content of *PeVQ28*-overexpressing plants may be a primary mechanism to improve salt-stress resistance.

Abscisic acid can inhibit seed germination, seedling growth, and plant development. Under a variety of stresses, ABA-dependent signaling pathways lead to ABA accumulation (Iuchi et al. 2001; Tan et al. 2003; Koiwai et al. 2004; Nambara and Marion-Poll 2005). In plants, the overexpression of certain genes induces the expression of stress-responsive genes, which in turn result in increased resistance to diverse stresses. Under non-stress conditions, *AtAAO3*, *AtNCED2*, *AtRD29A*, *AtRD29B*, *AtABF1*, and *AtNCED3* showed similarly low expression levels in *PeVQ28*-overexpressing and WT plants. However, under salt stress, the expression levels of these six genes in the overexpression plants were higher than those in WT plants (Fig. 7), indicating that *PeVQ28* plays a role in stress-induced ABA biosynthesis. In addition, the expression levels of the ABA-signaling genes *ABI1*, *AtP5CS1*, and *AtPP2CA* were examined in overexpression and WT plants under non-stress and salt-stress conditions (Fig. 7) (Hirayama and Shinozaki 2007). *AtABI1* and *AtPP2CA* encode protein phosphatase 2C, which plays a major role in ABA-mediated signaling networks associated with stress responses. Under salt stress, the expression levels of *ABI1* and *AtPP2CA* in *PeVQ28*-overexpressing plants were higher than those in WT plants. In addition, the induced expression of *AtP5CS1* may lead to the accumulation of PRO in transgenic plants, thus contributing to the enhanced tolerance of *PeVQ28*-transgenic plants to stress conditions. Thus, the increased resistance of *PeVQ28*-overexpressing plants to salt stress may result, in part, from the enhanced expression of these genes.

Protein–protein interactions are the basis for all metabolic activities in cells. VQ proteins participate in interactions with other proteins (Hu et al. 2013a, b; Wu et al. 2017). For example, *AtVQ14* interacts with *AtWRKY10* to form a complex protein that affects seed size (Wang et al. 2010), and *AtVQ22* interacts with *AtWRKY28* to negatively regulate jasmonic acid-mediated disease-related signaling pathways (Hu et al. 2013a). In the present research, we tested the interaction between *PeVQ28* and *PeWRKY83* using a yeast two-hybrid assay (Fig. 3a), which revealed that *PeVQ28* is able to interact with *PeWRKY83*. This result was verified using a BiFC analysis (Fig. 3b). Thus, based on this research and previous surveys, we conclude that proteins encoded by the *VQ* family in Moso bamboo participate in protein–protein interactions.

Author contribution statement XRC and YJW conceived the study, performed the main experimental analyses, and drafted the manuscript. RX carried out the software analyses and generated the figures and tables. YMG processed the experimental data and drafted the manuscript. HWY reviewed the project and edited the manuscript. YX conceived and guided the experiments, coordinated the project, and drafted the manuscript. All authors read and approved the final manuscript.

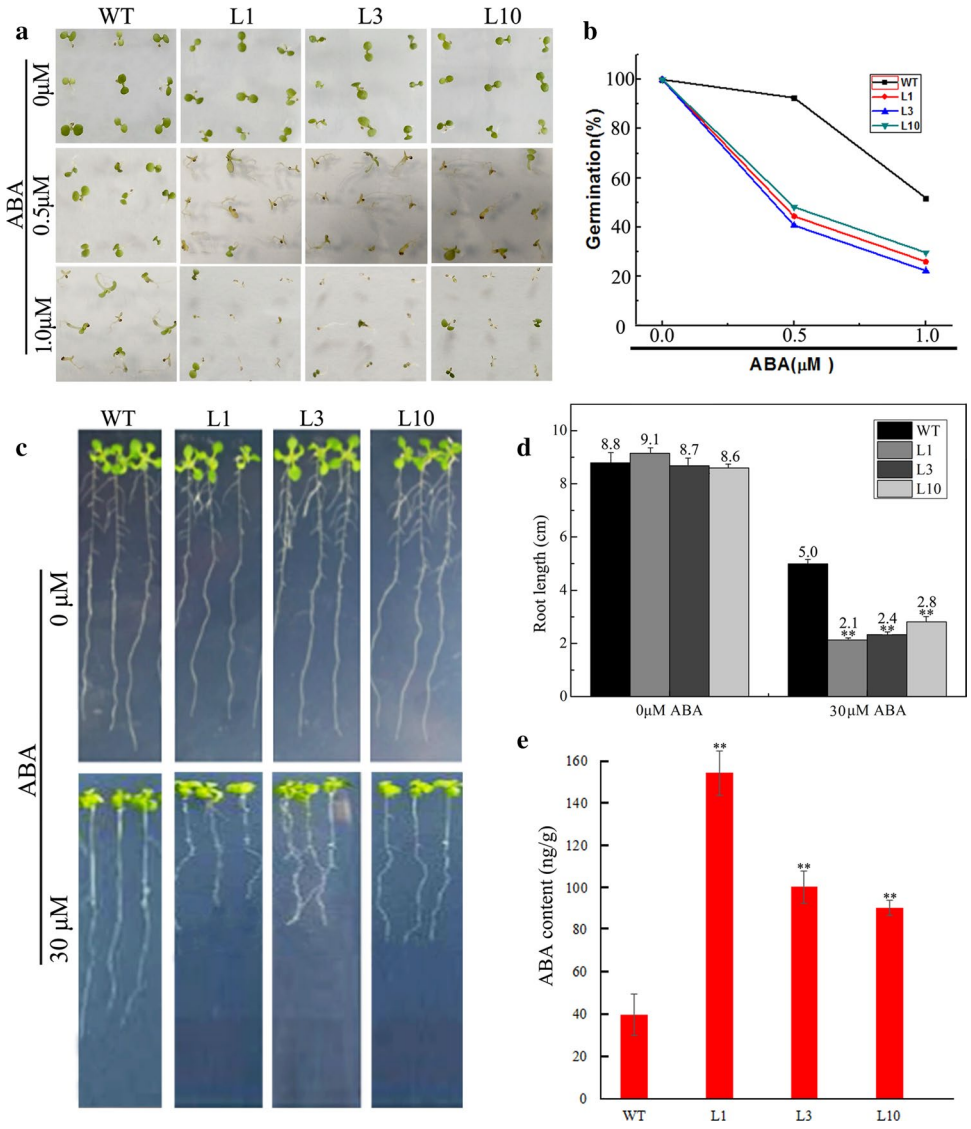


Fig. 6 ABA sensitivity of *PeVQ28* transgenic Arabidopsis. **a** Germination performance of *PeVQ28*-overexpression and WT seeds on MS medium containing 0, 0.5, or 1.0 μM ABA. **b** Calculation of the germination rates of transgenic and WT seeds. **c** Effect of salt stress on root length of transgenic and WT plants. **d** Calculation of the root length of transgenic and WT plants. Values are means ± SE ($n = 10$). *$P < 0.05$, *t* test; **$P < 0.01$, *t* test

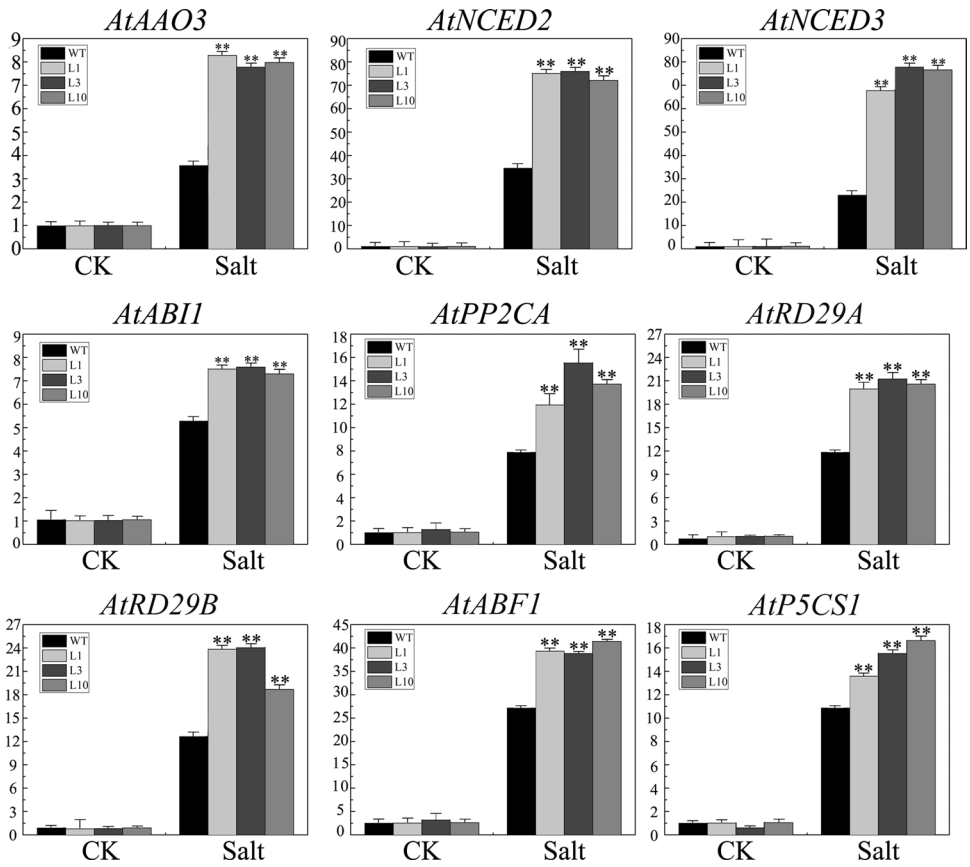


Fig. 7 Relative expression levels of stress/ABA-responsive genes in transgenic Arabidopsis plants under normal salt stress. Leaves of WT and transgenic Arabidopsis plants were sampled after salt stress. *Y*-axis: relative expression levels; *X*-axis: the time course of stress treatments. Mean values ± SE ($n = 6$)

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