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ORIGINAL PAPER

SlHDA5, a Tomato Histone Deacetylase Gene, Is Involved in Responding to Salt, Drought, and ABA

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Abstract

Histone deacetylation catalyzed by histone deacetylases is an important type of histone modification. Histone deacetylases affect various processes of plant development and involve in responding to hormones and biotic and abiotic stresses. Here, we report a tomato PRD3/HDA1 histone deacetylase gene, *SlHDA5*, which is expressed ubiquitously in different tissues and development stages. Expression profiles in hormone treatments showed that *SlHDA5* was induced by abscisic acid (ABA) and methyl jasmonate (MeJA). Seedlings growth of *SlHDA5*-RNAi lines were more inhibited on the medium containing salt compared with wild type (WT). Under salt stress, chlorophyll in mature leaves degraded earlier in transgenic leaves than that in WT, and transgenic plants displayed wilting earlier and more severe than WT. After drought treatment, transgenic plants wilted and dehydrated earlier than WT, which was confirmed by lower water and chlorophyll content, and higher malondialdehyde (MDA) content in transgenic plants manifesting that the tolerance of transgenic plants to drought receded. Under the treatment of ABA, root length of transgenic seedlings was more strongly repressed by contrast with WT, suggesting repression of *SlHDA5* increased seedling sensibility to ABA. Our study indicated that silencing of *SlHDA5* resulted in decreasing tolerance to salt, drought, and ABA.

Keywords Histone deacetylases · RNAi · Tomato · Salt · Drought · ABA

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Introduction

In eukaryotic cells, the basic unit of chromatin is nucleosome, which is an octamer assembled by two copies of H3, H4, H2A, and H2B and wrapped by 145–147 bp DNA (Luger et al. 1997). In the process of genetic information transmission, DNA sequences and epigenetic markers control the activation of genes together (Berger 2007). The known epigenetic events contain DNA methylation and histone modifications. Until now, the histone post-translation modifications which have been recognized are as follows: acetylation, methylation (lysines and arginines), phosphorylation, ubiquitylation, sumoylation, ADP-ribosylation, deimination, carbonylation, glycosylation, and proline isomerization (Fuchs et al. 2006; Kouzarides 2007a).

Among these histone modifications, research on acetylation is the earliest. Histone modifications often occur in the N-terminal tails. For example, histone acetylation and deacetylation appear at the N-terminal lysines 5, 8, 12, 16, 20 of H4, 9, 14, 18, 23, 36, 56 of H3, 5 of H2A and 12, and 15 of H2B (Kouzarides 2007b; Loidl 2004; Lusser et al. 2001). It is worth noting that the histone acetylation is a

dynamic process which is catalyzed by two kinds of enzymes known as histone acetyltransferases (HATs) and histone deacetylases (HDACs). Previous researches showed that HDACs widely exist in yeast, human, and plants (Taunton et al. 1996), and it was firstly isolated from plants (Sendra et al. 1988). Subsequently, more and more HDACs had been characterized and functionally studied. Based on the homology with yeast, HDACs in plants were classified into three groups: reduced potassium dependence 3/histone deacetylase 1 (RPD3/HDA1) superfamily, silent information regulator 2 (SIR2) family, and histone deacetylase 2 (HD2) family (Pandey et al. 2002; Yang and Seto 2007). In the past few years, HDACs in different species have been isolated, such as pea (Sendra et al. 1988), potato (Lagace et al. 2003), barley (Demetriou et al. 2009), *Arabidopsis* (Tian and Chen 2001), maize (Pipal et al. 2003), rice (Jang et al. 2003), tobacco (Bourque et al. 2011), tomato (Zhao et al. 2015a), and so on. So far, there are 18 *HDAC*s that have been identified from *Arabidopsis* genome, and among these genes, 12 genes belong to RPD3/HDA1 superfamily, 4 genes belong to HD2 family, and 2 genes belong to SIR2 family (Alinsug et al. 2009; Pandey et al. 2002). However, in tomato, there are only 14 HDACs, and RPD3/HDA1 superfamily, HD2 family, and SIR2 family contain nine, three, and two members, respectively (Zhao et al. 2015a).

Histone acetylation and deacetylation determine the activation and silence of eukaryotic genes. For example, acetylation of H3 Lys9 (H3K9) is the marker for active genes while deacetylation of H3K9 and H3K14 is the marker for silenced genes (Chen and Tian 2007; Earley et al. 2006). Furthermore, HDACs integrate histone modification and DNA methylation to regulate gene silencing (Liu et al. 2012a). Researches showed that in the process of plant development, HDACs play vital roles in various events such as the leaf morphology construction, growth of hypocotyl and root, flowering time, and fruit ripening (Wang et al. 2014). In *Arabidopsis*, RPD3/HDA1 superfamily member HDA6 and its homologous HDA19 are the most studied histone deacetylase. *HDA6* and *HDA19* redundantly function in modulating the germination process and embryonic properties after germination by repressing embryo-specific gene function (Chen et al. 2010; Chen and Wu 2010; Tanaka et al. 2008). Additionally, *HDA6* mediates heterochromatin silencing, transposable element silencing by interacting with DNA methyltransferase MET1 and histone demethylase FLD (Liu et al., 2012a; Liu et al., 2012b; To TK et al., 2011). Moreover, *HDA6* interacts with *FLC* (FLOWERING LOCUS C) to regulate flowering time in *Arabidopsis* (Yu et al. 2011). Other *Arabidopsis HAD* genes have also been proved participating in various developmental processes, such as gametophyte, embryo, and root epidermis cell development (Cigliano et al. 2013; Liu et al. 2013; Luo, et al. 2015).

Besides the important role of HDACs in plant development, they also take part in responding to hormones and biotic and abiotic stresses. *HDA6* is involved in jasmonate response, and the expression of the jasmonate responsive genes is down-regulated when *HDA6* is repressed (Wu et al. 2008). Experiments on *hda19-1*, a mutant of *HDA19*, *axe1-5*, a mutant of *HDA6*, and *HDA6* interfering plants showed that respective deletion of *HDA6* and *HDA19* increases the hypersensitivity to ABA and salt stress in *Arabidopsis* and plant deficiency in *HDA6* and *HDA19* display decreased expression of ABA and abiotic stress-responsive genes as well (Chen et al. 2010; Chen and Wu 2010). Nevertheless, HDA19 interacts with WRKY38 and WRKY62 and abolishes their activation to fine-tune plant basal defense responses (Kim et al. 2008a). In rice, overexpression of *HDA705* decreases ABA and salt stress resistance during seed germination and enhances osmotic stress resistance during the seedling stage which indicating its role in regulating seed germination and the response to abiotic stresses in rice (Zhao et al. 2016).

In tomato, SlHDA1, SlHDA3, and SlHDA4 have been proved interacting with MADS-box proteins TAG1 (TOMATO AGAMOUS1) and TM29 (TOMATO MADS BOX 29), which are involved in reproductive development, suggesting that *SlHDAC* genes may contribute to plant reproductive development (Zhao et al. 2015a). Although *HDAC*s in tomato have been identified and classified, the functional process and molecular mechanism are not very clear. Here, we focused on a tomato RPD3/HDA1 superfamily member *SlHDA5* whose homolog, *HDA2*, was expressed primarily in embryos and dry seeds in *Arabidopsis* (Hollender and Liu 2008; Schmid et al. 2005). Previous report revealed that *SlHDA5* is localized in nucleus and accumulated to a high level in flowers and fruit of 10 dpa, but decreased as fruit development and ripening (Zhao et al. 2015a). Furthermore, *SlHDA5* was induced by abiotic stress, such as high temperature, dehydration, and salt (Guo et al. 2017). To further explore the roles of *SlHDA5* in drought and salt stress response, we generated tomato plants silencing *SlHDA5* by RNA interference, and the transgenic plants showed reduced tolerance to salt, drought, and ABA. These phenotypes were further confirmed by analysis of physiological and biochemical features.

Materials and Methods

Plant Materials and Growth Conditions

Solanum lycopersicon Mill. cv. Ailsa Craig^{++} (AC^{++}) was used as wild type. All tomato seedlings used for hormone and treatments and tolerance assay were grown under greenhouse condition: 16/8 h day/night cycle, 25/18 °C day/night

temperature, 250 μmol m^{-2} s^{-1} light intensity, and 80% humidity.

Hormone Treatments

Seedlings about 35 days old that are consistent in growth status were chosen for treatment. The whole tomato plants were sprayed with 100 μM ABA, 50 μM MeJA, 50 μM auxin (IAA), and 50 μM salicylic acid (SA) (all plant hormones are manufactured by Sigma) solution respectively while seedlings for control were sprayed with distilled water (Fujita et al. 2004). Seedlings were enclosed in plastic immediately after spraying and collected leaves at 0, 1, 2, 4, 8, 12, and 24 h for further analysis. All samples were immediately frozen in liquid nitrogen and stored at − 80 °C for RNA extraction.

RNA Extraction and Quantitative Real-Time PCR

Total RNA was extracted using RNA trizol (Takara) according to the manufacturer's instructions. The first strand cDNA was synthesized by M-MLV reverse transcriptase (Promega) using 1-μg RNA as template and Oligo $(dT)_{18}$ as primer. Quantitative real-time PCR was carried out using CFX96™ Real-Time System (Bio-Rad), and the reaction system was 10 μL (5 μL 2 × SYBR Premix, 0.5 μL primers, 1 μL of cDNA, and 3.5 μL distilled water). NTC (no template control) and NRT (no reverse transcription control) were performed. Each sample was repeated for three times and standard curves were run at the same time. *SlCAC* gene (primer sequence: SlCAC-F: CCTCCGTTGTGATGTAACTGG, SlCAC-R: ATTGGTGGAAAGTAACATCATCG) and *SlEF1α* (primer sequence: SlEF1α-F: TACTGGTGGTTTTGAAGCTG, SlEF1α-R: AACTTCCTTCACGATTTCATCATA) were used as internal standard (Exposito-Rodriguez et al. 2008). The primers qSlHDA5-F (AGTGCCAAAGTTATTGCTGATTCC) and qSlHDA5-R (TTCGCCTCTGCTTTTCCCA) were used to detect the transcript level of *SlHDA5* in tissues and treated materials.

Construction of *SlHDA5* RNA Interference Vector and Plant Transformation

The *SlHDA5*-RNAi vector was constructed using pBIN19 vector. A 401-bp specific fragment of *SlHDA5* was amplified with specific primers SlHDA5-F (CGGGGTACCATCGATAGCATGTCTTTGCATAGCTACTTAA) and SlHDA5-R (CCGCTCGAGGGATCCGAGGTACGACGAGAACTTGATTG). After purifying, amplified products were digested with *Cla* I/*Bam*H I and *Kpn* I/*Xho* I and then linked into pHANNIBAL plasmid. The double-stranded RNA expression unit, containing the cauliflower mosaic virus *35S* promoter (the sense-orientated *SlHDA5* fragment (PDK intron) and the antisense-orientated *SlHDA5* fragment (*OCS* terminator)), was digested with *Sac* I and *Xba* I. Then, the unit was linked in pBIN19 and transferred into *Agrobacterium* LBA4404 strain. The final vector carried *SlHDA5*-RNAi unit was transferred into wild-type tomato by *Agrobacterium*-mediated plant transformation method (Chen et al. 2004). The positive transgenic tomato plants were selected for kanamycin and detected by PCR with NPTII-F (CTCAGAAGAACTCGTCAAGAAGG) and NPTII-R (GACTGGGCACAACAGACAATC) primers.

Salt and Drought Treatment of Transgenic Tomato

Three experiments were conducted to research the effect of *SlHDA5* on tomato salinity tolerance. Experiment 1: transgenic lines and WT seeds were sterilized and placed in culture flask with sterilized water in it. Then, the culture flask was put in constant temperature shaker which was 100 revolutions per minute and 28 °C. The germinant seeds were sowed on prepared culture flasks containing MS medium with 0 and 100 mM NaCl. A week later, picture was taken and the length of shoot and root was measured. Experiment 2: leaves of similar size, age, and position were detached from transgenic and WT plants and dipped in 300 mM NaCl for 4 days. Pictures were taken to record the phenotypes and the chlorophyll content was measured. Experiment 3: 35-day-old transgenic and WT plants were irrigated with 200 mL 400 mM NaCl solution every 3 days. Pictures were taken to record the phenotypes.

For drought treatment, 35-day-old T1 transgenic and WT plants with the similar growth status were selected and watered daily. Once drought treatment began, plants were withholding water until 30 days. All the plants were kept in a greenhouse under condition described above. Pictures were taken to record the phenotypes. Leaves were sampled at 0, 25, and 30 days after the onset of drought treatment to measure relative water content (RWC), total chlorophyll, and MDA contents.

Quantitation of RWC, Total Chlorophyll, and MDA Contents

Tomato leaves were detached from the plants and weighted (fresh weight, FW), then placed in culture dishes with water filled for 24 h. The water was removed from leaf surface using absorbent paper and weight to obtain turgid weight (TW). The leaves were placed in 50-mL centrifuge tubes and dried at 60 °C for 24 h, and then dry weight (DW) was weighted. The RWC was calculated using the following formula: RWC (%) = (FW−DW)/(TW−DW) × 100%.

For total chlorophyll measurement, leaves with the same weight were excised from control and treated plants. Samples were grinded with liquid nitrogen and extracted with 3 mL 80% aqueous acetone (*v*/*v*). The extract was kept in dark place for a night and centrifuged at 4000×*g* for 5 min. Then, the

supernatant was diluted by 80% aqueous acetone and absorbance was recorded at 645 and 663 nm. The content of chlorophyll was calculated using the following formula: Chl = (20.21 × A645 + 8.02 × A663) (Pei et al. 1997).

To measure the content of MDA, leaves of the same weight were detached respectively from control and transgenic plants. Then, the samples were grinded with liquid nitrogen and added 5 mL trichloroacetic acid (TCA), mixed, and centrifuged at 4000×*g* for 10 min. A 2-mL supernatant was removed to a new 5-mL centrifuge tube, and 2 mL distilled water was set as control; then, we diluted the extraction with 2 mL thiobarbituric acid (TBA). The mixture was incubated in boiling water for 10–15 min and then immediately cooled in ice. The absorbance in 450, 532, and 600 nm was recorded. MDA content was calculated using the following formula: MDA contents (nmol g^{-1} fresh weight) = [6.45 × (A532–A600)–0.56 × A450]/fresh weight (Lim et al. 2012).

Assay for ABA Sensitivity of Transgenic Tomato Seedlings

We obtained germinating transgenic and WT seeds as described above and sown them respectively on MS medium with 0, 5, and 10 μM ABA. After a week, the phenotype was observed and root length was measured.

Results

SlHDA5 Gene Was Induced by ABA and MeJA

So far, researches have proved that HDACs respond to hormones and participate in hormone-induced development processes in other plants (Liu et al. 2014; Luo et al. 2012; Zhao et al. 2015b). To investigate the response of *SlHDA5* gene to hormones, the expression patterns of *SlHDA5* under various treatments were studied. As shown in Fig. 1a, the expression of *SlHDA5* only significantly increased at 12 h while no obvious difference was observed at other time compared with control under IAA treatment. When suffered with exogenous ABA, the expression of *SlHDA5* had no obvious change at 1–4 h, but increased clearly from 8 h and then peaked at 12 h which was about sixfold by contrast with control (Fig. 1b). However, for the treatments of exogenous SA, the expression of *SlHDA5* was slight up-regulated at 4, 8, and 12 h while at other time points, the expression remained no distinct change (Fig. 1c). When suffered with exogenous MeJA, *SlHDA5* gene was always up-regulated at 2–12 h and the peak expression about fourfold compared with control appeared at 2 h (Fig. 1d).

Repression of *SlHDA5* Increased the Sensibility to Salt

Existing research indicated that *SlHDA5* was induced by salt stress both in root and leaf (Guo et al. 2017). We selected the T1 generation of two *SlHDA5*-RNAi transgenic tomato lines with better silencing efficiency (Fig. 2), RNAi-17 and RNAi-21 for NaCl treatment. When the seedlings were growing on MS medium, the difference between WT and transgenic seedlings could barely be distinguished. When growing on MS medium with 50 mM NaCl, both WT and transgenic seedlings were inhibited, and the hypocotyl and root of transgenic seedlings were significant shorter than those of WT. When the salt concentration increased to 100 mM, seedlings were inhibited more significantly, and the difference of hypocotyl and root length between WT and RNAi seedlings was also more obvious (Fig. 3a, c, d). As shown in Fig. 3b, after soaking in 300 mM NaCl solution for 4 days, the leaves from transgenic plants became more transparent compared with WT, and the chlorophyll degradation was severer. The result of total chlorophyll content measurement was consistent with the phenotype we observed (Fig. 3e). In addition, we treated the 6-week-old plants of WT and transgenic lines with 400 mM NaCl. After a week, the leaves of transgenic plants turned wilted, and the lower leaves were yellow obviously while WT was a little wilted. At about 2 weeks after treatment, leaves of transgenic plants turned severe wilted and yellow, and the lower leaves fell off while leaves of WT just showed wilted and a little yellow (Fig. 3f). In conclusion, the *SlHDA5*-RNAi transgenic plants showed reduced salt tolerance at the seedling and whole plant stages.

Silencing of *SlHDA5* Gene Reduced the Tolerance to Drought

Previous researches also demonstrated that the expression *SlHDA5* was induced by dehydration stress (Guo et al. 2017), so we speculated that the tolerance of *SlHDA5*-RNAi transgenic plants to drought may be altered. To confirm this, the drought tolerance test was carried out. Figure 4a displayed that the lower leaves of transgenic plants turned yellow and a bit of wilted at 25 days after drought stress while the WT plants had no evident change. After 30 days, the transgenic plants were entirely yellow, wilted, and collapsed. Nevertheless, the WT plants were only a little wilted in lower leaves and the upper leaves were still green. At the same time, we sampled the leaves of transgenic and WT plants at 0, 25, and 30 days to measure RWC, contents of total chlorophyll, and MDA. Figure 4b revealed that in WT tomato leaves, RWC reduced about 15% at 25 days and 23% at 30 days, but in transgenic plants, RWC reduced by 30% at 25 days and 50% at 30 days. At 25 and 30 days after drought stress, the total chlorophyll contents of WT leaves decreased by 50 and 58%, but the chlorophyll contents of two transgenic lines'

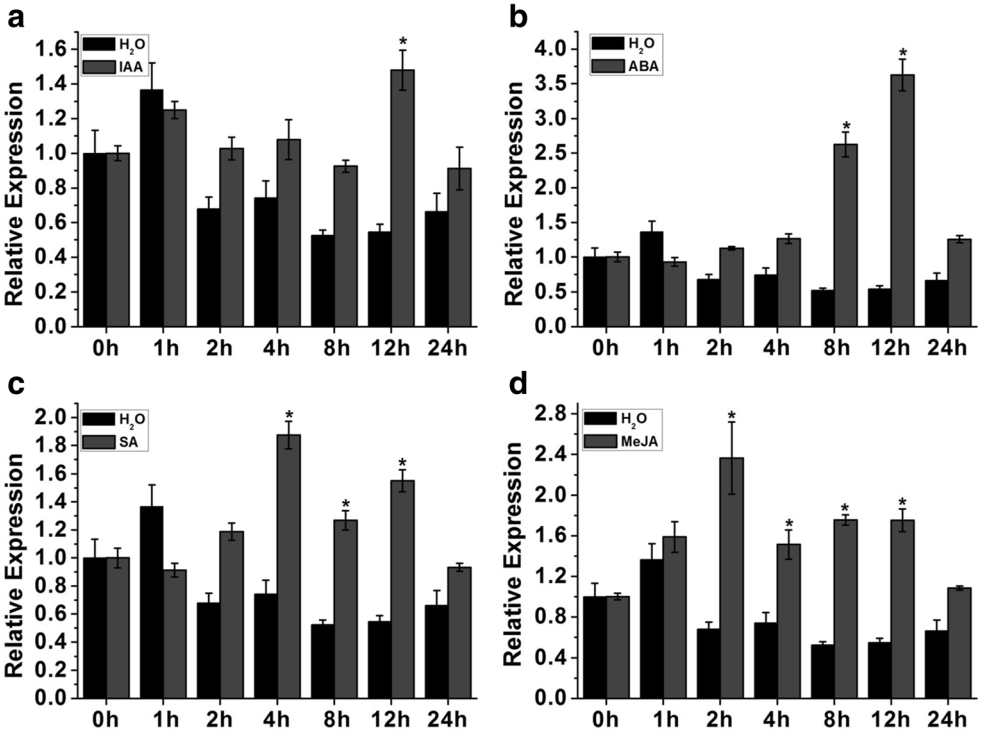

Fig. 1 Detection of *SlHDA5* under treatment of hormones by qPCR. Expression profile of *SlHDA5* in WT leaf treating with IAA (**a**), ABA (**b**), SA (**c**), and MeJA (**d**). Seedlings about 35-day-old were treated by hormones. Each sample was repeated for three times. The asterisks indicate significant differences between the treated and contrast seedlings ($P < 0.05$)

leaves were lessened respectively by 55 and 77% at 25 days and 73 and 91% at 30 days (Fig. 4c). Figure 4d displayed that after 25 and 30 days since onset of drought stress, the contents of MDA in WT plants increased by 0.75- and 1.5-fold while in two transgenic lines, the uplift amounts were respectively 1.4-fold, 2.8- and 1.4-fold, and 3.4-fold. We concluded that silencing of *SlHDA5* reduced the RWC and total chlorophyll contents, increased the MDA content, and accelerated the drying of transgenic plants under drought stress. Overall, silencing *SlHDA5* led to increasing sensibility to drought in tomato.

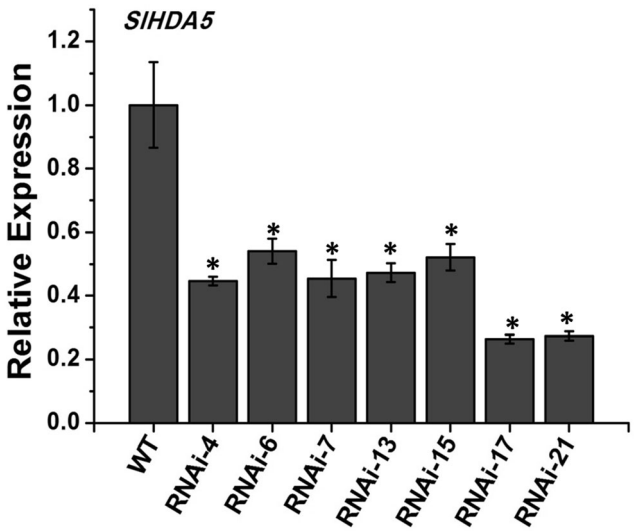

Fig. 2 Detection of *SlHDA5* in WT and transgenic lines by qPCR. Each sample was repeated for three times. The asterisks indicate significant differences ($P < 0.05$)

Tomato Seedlings Lacking of *SlHDA5* Were More Sensitive to ABA

Detection of *SlHDA5* gene expression in hormone treatment implied that *SlHDA5* was induced by ABA (Fig. 1b). So we designed experiment to verify whether *SlHDA5*-RNAi transgenic lines had difference in response to ABA with WT plants. As shown in Fig. 5a, seedlings of transgenic and WT had no obvious difference grew on MS medium, but after adding 5 μM ABA, seedlings were inhibited and the root length of transgenic seedlings was short than that of WT. When growing in the condition of 10 μM ABA, the difference of root length between transgenic and WT seedlings was more pronounced (Fig. 5b). In summary, compared with WT, the *SlHDA5*-RNAi seedlings were more sensitive to ABA.

Discussion

Various stresses in the natural environment affect the agricultural economical characters and crop production. To obtain the stress-tolerance crop varieties is always one of the main breeding goals. Recent decades, the research of epigenetics was more detailed. Increasing enzymes modulating the reactions of DNA and histone modification were identified affecting the tolerance to biotic and abiotic stresses, which provides a method for resistance breeding. In *Arabidopsis HDA6* mutant and *HDA6* RNA interference plants, the salt-stress signaling pathways were inhibited in a 2-week-old plant treating with NaCl

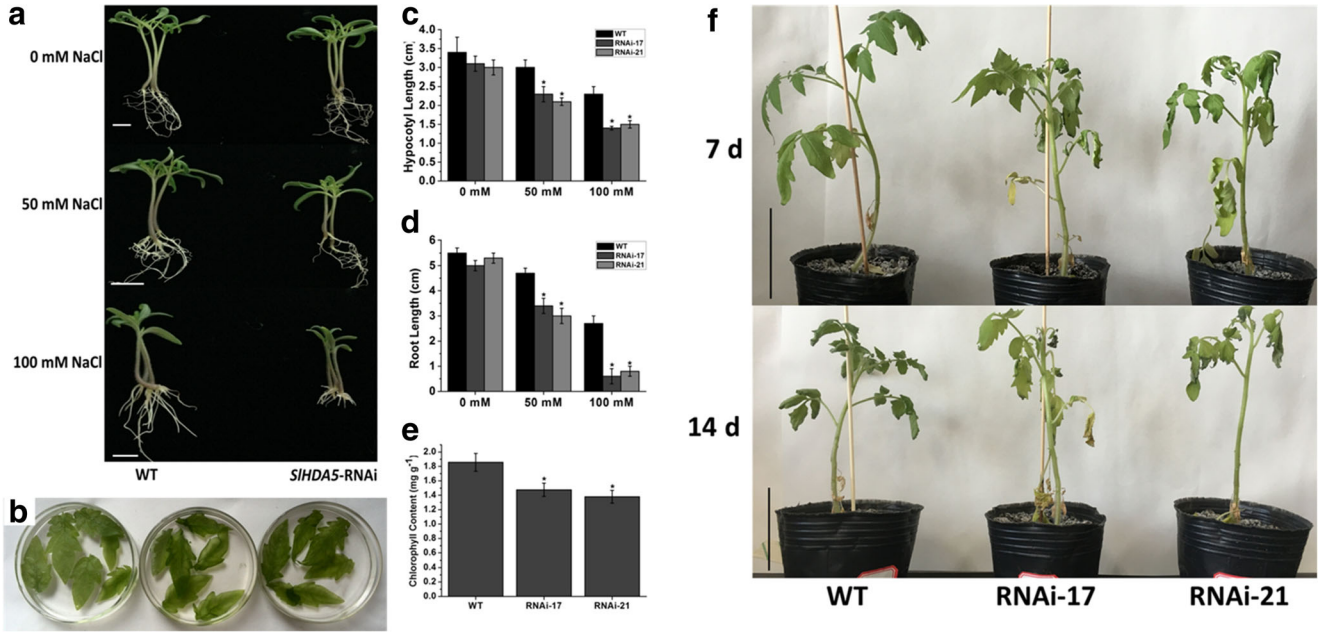

Fig. 3 Phenotype of WT and *SlHDA5-RNAi* seedlings, leaves, and plants under salt treatment. **a** WT and transgenic seedlings treating with 0, 50, and 100 mM NaCl. Scale bars = 1 cm. Hypocotyl length (**c**) and root length (**d**) of seedlings. **b** Leaves soaking with 300 mM NaCl for 4 days and chlorophyll content (**e**). **f** WT and transgenic plants treated with 400 mM NaCl for 7 and 14 days. Scale bars = 10 cm. Data are the mean from three independent replicates with three biological repeats. Asterisks indicate significant difference from WT ($P < 0.05$)

solution (Chen et al. 2010). *hda19-1*, a mutant of *HDA19*, showed lower seed germination than wild type under the treatment of 200 mM NaCl (Chen and Wu 2010).

But in tomato, the roles of *HDA* genes play in controlling tolerance to abiotic stresses have not been studied. Former work on the expression of *SlHDA5* under the treatment of various abiotic stresses showed that expression of *SlHDA5* was induced distinctly under the treatments of salt and dehydration, indicating that *SlHDA5* may play a role in responding to salt and drought (Guo et al. 2017). Experiment on post-germination seeds indicated that the elongation of transgenic seedling hypocotyl and root was more inhibited by salt than that of WT. Chlorophyll of *SlHDA5*-RNAi detached leaf degraded faster than that of WT. When treated with NaCl solution, 6-week-old transgenic plants turned wilted and yellow earlier than WT. These results mean that the photosynthetic capacity of transgenic plants decreased faster than that of WT with the existence of salt stress. Taken these results together, we concluded that repression of *SlHDA5* impaired the tolerance to salt stress in multiple development stage in tomato.

The transcriptional responsiveness of drought stress-up-regulated genes was found to be correlated with changes in histone modification (To TK and Kim, 2014). Under strong drought conditions, the histone modifications H3K4me3 and H3K9ac on drought stress-up-regulated genes, such as *RD20* and *RD29A*, were more highly enriched than under moderate drought conditions, and the nucleosome loss in the same region of *RD29A* under strong drought conditions was more than that under moderate drought (Kim et al. 2012; Kim et al. 2008b). Overexpression of *Arabidopsis* HD2-type HDAC, *HD2C*, plants showed enhanced tolerance to drought (Sridha and Wu 2006). In this work, we examined the tolerance of RNAi plants to drought, and the results indicated that when treated with drought, RNAi plants showed desiccation symptoms such as leaf rolling and wilting earlier than WT. MDA is a decomposition product of poly-unsaturated fatty acid hydroperoxides in osmotic stress (Heath and Packer 1968). The content of MDA manifests the damage of membrane. RWC is also an index to evaluate the damage caused by osmotic stress. In our work, *SlHDA5*-RNAi plant had lower chlorophyll content, RWC, and higher MDA content. These physiological indices are consistent with the morphology change. Overall, we deduced that *SlHDA5* was a positive regulator in responding to the osmotic stress caused by drought and salt.

The *Arabidopsis HDA6* mutant, axe1-5, and *HDA6* RNA interference plants displayed down-regulated expression of ABA-responsive genes when treated with ABA (Chen et al. 2010). The seed germination of *hda19-1* was lower than that of wild type under 2 μM ABA, and the ABA synthesis genes were decreasing, suggesting the increasing sensitivity to ABA with deletion of *HDA19* (Chen and Wu 2010). Members of histone deacetylases HD2 family have also been proved to regulate ABA responses. When *HD2C* was over-expressed, the transgenic plants showed higher germination rate and longer root length by contrast with WT under the treatment of ABA (Sridha and Wu 2006). In this study, the expression profile showed that *SlHDA5* was induced by ABA. To sum

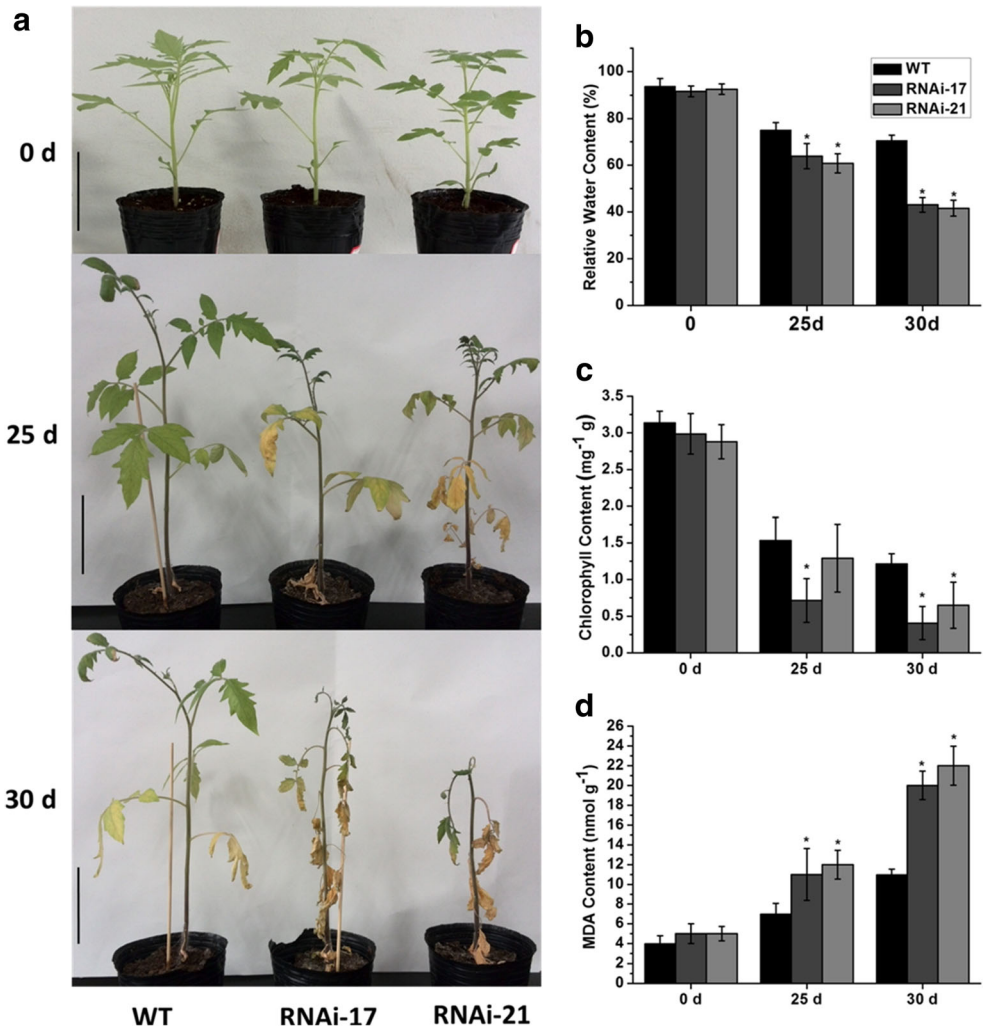

Fig. 4 **a** Phenotype of WT and transgenic plants at 25 and 30 days since the drought stress initiating. Scale bars = 10 cm. Relative water content (**b**), chlorophyll content (**c**), and MDA content (**d**) were also measured at the same time. Asterisks indicate significant difference from WT ($P < 0.05$)

up, the reported histone deacetylase genes are almost positively related to ABA sensitivity. Experiment of *SlHDA5*-RNAi seedlings revealed that the development of transgenic seedlings was repressed, which root was shorter than that of WT with the treatment of ABA, suggesting that repression of *SlHDA5* improved seedling sensitivity in tomato. These results were consistent with the former research that histone deacetylases positively influence the stress to salt, drought, and ABA.

In addition, we displayed that *SlHDA5* was also remarkably induced by MeJA, which plays a crucial role in the signaling pathways involved in responding to biotic stress such as wounding and pathogen attack (Benedetti et al. 1998). Researches demonstrated that overexpression of *HDA19*

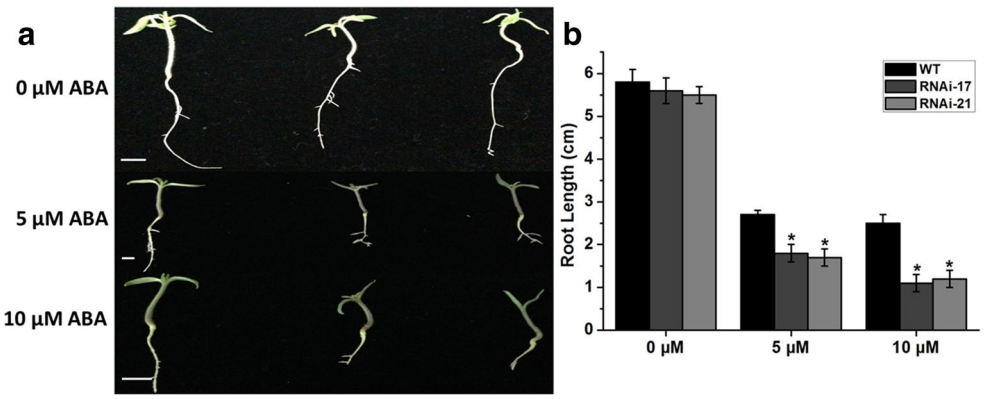

Fig. 5 Phenotype (**a**) and root length (**b**) of WT and transgenic seedlings grown on MS medium contain 0, 5, and 10 μM ABA for 7 days. Asterisks indicate significant difference from WT ($P < 0.05$). Scale bars = 1 cm

enhanced the resistant to pathogen *Alternaria brassicicola* in *Arabidopsis* and up-regulated the expression of *PATHOGENSIS-RELATED* genes, *Basic Chitinase* and *β-1*, and *3-glucanase* which are regulated by jasmonic acid (Zhou et al. 2005). Therefore, we speculated that *SlHDA5* may involve in other biotic and abiotic stresses, such as pathogenic bacteria.

Histone deacetylase was multifunctional in plant development and resisting adverse effects from environment. Some molecular mechanisms of histone deacetylase taking part in development process and stress-responding have been clarified in *Arabidopsis*. However, in other species, research on histone deacetylases is little. Here, we revealed the molecular characters of a tomato histone deacetylase gene, *SlHDA5*. Expression profiles under the treatment of hormones were also investigated. Besides, we obtained *SlHDA5*-RNAi transgenic plants and seeds. Further experiments showed that the tolerance of transgenic tomato to drought and salt stress were decreased, and the sensitivity of seedlings to ABA was increased. These results provide significant basis for breeding. But the molecular mechanisms of processes were not very clear. To elucidate the mechanism, it remains to be identified the genes that are regulated by *SlHDA5* via histone deacetylation.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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