Histone Deacetylase Gene *SlHDA3* Involves in ABA, Drought and Salt Response in Tomato

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Research Article

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Abstract

Histone deacetylation, one of vital modifying factors of post-translation modifications, which is catalyzed by histone deacetylase. The genes of histone deacetylase(HDACs) play critical roles in various stress responses. However, detailed functions for most SlHDAC members in tomato still unknown. In this work, we found that a histone deacetylase, *SlHDA3*, involved in response to NaCl and drought abiotic stresses. The expression of *SlHDA3* was also induced significantly by NaCl, drought stress and endogenous hormone treatments. Silencing of *SlHDA3* in tomato, the RNAi transgenic plants presented depressed tolerance to drought and salt stresses compared with WT tomato. The results of sensitivity analysis indicated that the length of hypocotyl and roots in RNAi plants were more inhibited by ABA and salt stress than that of WT at post-germination stage. Worse growth status were exhibited in *SlHDA3* transgenic plants under salt and drought stress as are evaluated by a series of physiological parameters related to stress responses, such as decreased RWC, survival rate, ABA content, chlorophyll content and CAT activity, and increased MDA content and proline content. Besides, the expressions analysis of transgenic plants showed that the transcripts of genes which associated with responses to abiotic stress were down-regulated under salt-stressed conditions. To sum up, *SlHDA3* acts as a stress-responsive gene, plays a role in the positive regulation of abiotic stress tolerance, and may be one of the new members in the engineering breeding of salt- and drought-tolerant tomato.

Key Message

SlHDA3 functions as a stress-responsive gene, plays a role in the positive regulation of abiotic stress tolerance

Introduction

Abiotic stresses, such as drought and high salinity, not only affects crop growth and development but also adversely affects the yield of crop. A series of compromising related to plant growth and development are continuously resulted from stresses, which may break plant homeostasis. Usually, tomato is mainly cultivated as nutritious edible fruit and vegetable crop, which can be commercially grown worldwide. Although tomato plants have a certain degree of tolerance to various abiotic stresses, crop losses are more severe under extreme weather conditions. It is urgent and necessary to obtain the stress-tolerance crop varieties and it is profitable to cultivate stress-tolerant crops that impressionable to abiotic stress for modern agriculture areas. It is well known that the stress response is regulated by both ABA-dependent and ABA-independent regulatory systems (Fujita, et al. 2010; Tran, et al. 2004). ABA plays an important part in abiotic stress adaptation by regulating stomatal closure and by stimulating numerous stress-related genes, and then enhancing the tolerance of plants to various stresses (Nakashima, et al. 2012).

Previous researches have shown that histone deacetylase act as indispensable components during the process plant growth and development, such as the construction of leaf morphology, the elongation of

hypocotyl, the development of root, the regulation of flowering time, the fruit ripening and so on (Wang, et al. 2014). HDACs genes have been identified and functionally studied in the model plant *Arabidopsis*. Detailed functional characterization of HD2 subfamilies genes have been reported involves in the regulation of responses to abiotic stresses including ABA, salt and drought (Chen and Wu 2010; Li-Ting, et al. 2010; Luo, et al. 2012; Song and C.-P. 2005; Sridha and Wu 2006). Members of HD2 subfamilies genes, *AtHDT1*, *AtHDT2*, *AtHDT3* and *AtHDT4*, were related to ABA and salt stresses in *Arabidopsis* (Chinnusamy, et al. 2008; Yuan, et al. 2013; Zhaofen, et al. 2016).

Distinguish from HD2 subfamilies and SIR2 subfamilies, the members of RPD/HDA1 subfamilies are wide participation in diverse processes, including seed development and germination (G., et al. 2013; Georgieva, et al. 1991), root hair development (Liu, et al. 2013), leaf morphogenesis (Luo, et al. 2012; Scofield and Murray 2006), flower development (Gonzalez, et al. 2007; Tian, et al. 2003), light signaling and hypocotyls growth (Benhamed, et al. 2006; Kim, et al. 2013), ABA and salt stress response (Luo, et al. 2012), and plant cell cycle and development (Varotto, et al. 2003). *AtHDA6*, a close homolog of *SlHDA3* gene in *Arabidopsis*, involves in seed development and seed performance (G., et al. 2013; Tanaka, et al. 2008), ABA and salt stress response (Luo, et al. 2012), leaf morphogenesis (Luo, et al. 2012; Scofield and Murray 2006), circadian regulation (L., et al. 2012), flowering controlling (C.-W., et al. 2011; Gu, et al. 2011; Keqiang Wu1 and Chaikam2 2008), JA signaling (Thines, et al. 2007; Zhu, et al. 2011), light signaling (Tessadori, et al. 2009), DNA methylation (Aufsatz, et al. 2002), cold stress response and ethylene pathway (To, et al. 2011). These results suggested that histone deacetylation regulated by *HDA6* is referred in plant growth and development and abiotic stress response in *Arabidopsis*. Our previous research showed that the expression level of *SlHDA3* is significantly induced by various abiotic stresses such as NaCl, dehydration and high/low temperature, indicating that *SlHDA3* may involves in abiotic stress tolerance (Guo, et al. 2017).

Herein, we reported the functional characterization of *SlHDA3*, we generated tomato RNAi plants of *SlHDA3*, and the transgenic lines showed reduced tolerance to ABA, drought, and salt. These phenotypes were further confirmed by analysis of physiological and biochemical features and related gene expression features.

Materials And Methods

Plant materials and growth conditions

All tomato seed of WT(wild type, *Solanum lycopersicum* Mill. cv. Ailsa Craig) and homozygous T3 *SlHDA3*-RNAi transgenic lines were surface sterilized and grown in soil under sodium lights(16h days, 25 C, and 8h nights, 18 C) and 80% humidity.

Hormone treatments

35-day-old WT tomato seedlings with similar growth status were chosen for hormone treatments. The experimental group tomato plants were sprayed with 100μM ABA, 50μM GA3, 50μM IAA and 50μM SA

solution, while the control group tomato plants were sprayed with water (Fujita, et al. 2010). All seedlings were enclosed in plastic as soon as possible after spraying, the leaves were collected at 0, 1, 2, 4, 8, 12, 24 and 48h, and all the gathered samples were frozen as soon as possible in liquid nitrogen and stored at -80 °C until used for this study.

Sensitivity assays

The sensitivity assays were carry out for further research the sensitivity of *SlHDA3* seedling growth to ABA and salt stress, the germinated seeds of WT and transgenic were selected and transferred to MS medium containing ABA (0, 4 and 8 μM) and NaCl (0, 100 and 150 mM) according to their consistent germination status. Pictures were taken and the length of root and hypocotyl was measured 7 days later.

Phenotype analyses and evaluation of salt stress tolerance and drought tolerance assay

35-day-old tomato plants that including WT and *SlHDA3*-RNAi lines were selected for salt stress tolerance and drought tolerance assay based on their uniformity. In the experiment of salt stress tolerance assay, all the plants were irrigated with 200 mL 400 mM NaCl solution every 48h, and the leaves from treated tomato plants at 24h were gathered for stress related-gene expression analysis. As for the experiments of drought tolerance assay, all the tomato plants were irrigated with water enough and with holding water until 21days.

The survival rate, chlorophyll contents, relative water content (RWC), MDA contents, ABA concentration, proline content and CAT activity of WT and transgenic plants after treating were detected (Zhu, et al. 2018).

Total RNA extraction and quantitative RT-PCR analysis

Total RNA was extracted using RNA trizol, the extract was digested by Dnase and after that total RNA was used to reverse-transcribed to first-strand cDNA. The qRT-PCR reaction system and the analysis of qRT-PCR was performed as showed as our previous report (Guo, et al. 2017). NRT(no reverse transcription control) and NCT(no template control) were carried out for further analysis of each gene. For abiotic stress, the tomato *SlEF1⍺* gene was also performed as the internal standards (Nicot, et al. 2005).

Results

SlHDA3 transcript was increased under hormone treatments

Previous research identified that HDACs were referred to plant responses to hormone treatments and involved in the processes of plant hormone-induced growth and development (Liu, et al. 2014; Luo, et al. 2012; Ming 2015). To clarify the detailed function of *SlHDA3* in hormone treatments during plant growth and development in tomato plants, we first test whether *SlHDA3* expression was affected by hormone-induced such as ABA, GA_3, IAA and SA. Figure 1 showed that the transcript of *SlHDA3* were induced under ABA, GA_3, IAA and SA. When suffered with exogenous ABA, the expression of *SlHDA3* was obviously

induced at 4h and 8h. While suffered with exogenous GA_3, IAA and SA, the transcripts accumulated of *SlHDA3* were up-regulated continuously and peaked at 8h, 4h and 12h respectively, then declined to the lowest level at 48h.

The sensitivity to ABA was increased in *SlHDA3* RNAi seedlings

The results in our previously work showed that SlHDA3 is highly homologous to the histone deacetylase gene AtHDA19 in *Arabidopsis* (Guo, et al. 2017). The 3D structures of SlHDA3 and AtHDA6(encoded by HDA6 gene in *Arabidopsis*) were generated by utilizing the SWISS-MODEL tool (http://www.swissmodel.expasy. org; Fig. S1) and the 3D structures models of SlHDA3 and AtHDA6 were very similar, indicating that *SlHDA3* in tomato may play analogous roles to that of *AtHDA6* in *Arabidopsis*.

Based on the similarity to the *AtHDA19*, we speculated that *SlHDA3* may be involved in the regulation of ABA signaling pathway in tomato. To measure the sensitivity of *SlHDA3*-RNAi plants, the experiment of ABA treatment(0, 4 and 8μM) was performed. The results suggested that the length of roots and hypocotyl in *SlHDA3*-RNAi was relatively small deviations with WT in the absence of ABA(0μM), while there were obviously shorter than that in the control in the presence of ABA(4 and 8μM)(Fig.2). These findings demonstrate that suppress the expression of SlHDA3 in tomato may be results in increased sensitivity to ABA.

The expression of ABA biosynthesis- and signal transduction-related genes were down-regulated

Increased transcript accumulation in *SlHDA3*-RNAi when suffered with ABA treatment prompted us to test whether the expression level of ABA biosynthesis- and signal transduction-related genes were affected in *SlHDA3*-RNAi plants. The expression of *SlPYL1-SlPYL8*, eight ABA-dependent receptor genes (Danquah, et al. 2014), were detected both in WT and *SlHDA3*-RNAi plants. As it is showed in Figure 3, no obvious differences was observed in transgenic lines and WT tomato at 0h. However, various degrees of down-regulation was presented in *SlHDA3*-RNAi plants compared with WT at 4h. *SlNCED1* and *SlNCED2*，two ABA biosynthesis-related genes (Ji, et al. 2014), also were measured in WT and *SlHDA3*-RNAi plants. The transcription of *SlNCED1* and *SlNCED2* was in *SlHDA3*-RNAi plants slightly higher than that in WT at 0h but no significant difference in the statistical level. While significant difference was exhibited in WT and *SlHDA3*-RNAi plants and the expression level was down-regulated in *SlHDA3*-RNAi plants at 4h. Besides, *SlABF2* and *SlABF4*, two ABA-responsive element binding factor (ABF) genes (Chen, et al. 2016), were also obviously reduced in *SlHDA3*-RNAi plants.

Silencing of *SlHDA3* significantly decreases drought tolerance

The results in our published previously indicated that the transcript of *SlHDA3* was significantly increased under dehydration stress (Guo, et al. 2017), the effects of drought stress on WT and *SlHDA3*-RNAi tomato were conducted in soil. No significant difference in morphological phenotype was observed between WT and *SlHDA3*-RNAi plants (0d, Fig.4a). The leaves of *SlHDA3*-RNAi lines started turning to yellow and rolling, while the WT were less withered after 14 days of drought tolerance (Fig. 4b). Significant difference

was exhibited on the 21th day after drought treatment, most leaves in RNAi plants were yellow and wilting (or even dead), whereas the WT plants began changing into yellow and rolling (Fig.4c). Based on drought tolerance differences between WT and *SlHDA3*-RNAi plants, the survival rates were tested. A lower survival rate of *SlHDA3* transgenic plants than that of WT plants was observed 21 days after drought tolerance (Fig. 4d). Besides, the leaves of WT and transgenic line at 0, 14 and 21days were gathered to measure contents of total chlorophyll and RWC for further confirm this stress tolerance phenotype.The degradation of total chlorophyll in *SlHDA3* transgenic plants leaves was faster than that in WT at both 14 and 21 days after drought treatment (Fig. 4e). As shown in Fig. 4f, the decreased of RWC in *SlHDA3* transgenic plants was more faster than that in WT plants (Fig. 4f). Meanwhile, parameters of other physiological indicator were further tested including CAT activity, proline content and MDA content. CAT activity in WT leaves was higher than that in transgenic lines during the post-drought treatment. However, proline content and MDA content were significantly higher in transgenic plants than that in WT under drought treatment (Fig. 4g-i). The results indicate that *SlHDA3* is involved in tomato drought resistance.

The sensitivity to NaCl was increased in *SlHDA3*-RNAi seedlings

The transcript of *SlHDA3* was obviously increased under salt stress especially in leaves (Guo, et al. 2017) prompting us to analyses whether the sensitivity to NaCl was effected in *SlHDA3*-RNAi seedlings. The measurement of sensitivity experiment was designed for further detecting whether *SlHDA3* seedlings had differences with WT seedlings under NaCl stress. The length of hypocotyl and roots in *SlHDA3*-RNAi lines and WT seedings has no significant difference in the absence of NaCl(0μM)(Fig.5a,d-e). However, the hypocotyl and roots length of *SlHDA3*-RNAi lines was distinctly shorter than that of WT in the incubation medium (100 and 150 μM NaCl) (Fig. 5b-e). These findings suggest that the sensitivity to NaCl was increased in *SlHDA3*-RNAi lines.

Silencing of *SlHDA3* significantly decreases salt tolerance

Based on the results of induced expression of *SlHDA3* under salt stress in our published previously and the increased sensitivity to NaCl in *SlHDA3*-RNAi seedlings, related research was performed to study whether silencing of *SlHDA3* effected salt tolerance. Before NaCl stress(0d), the growth status of *SlHDA3*-RNAi plants was similarity to WT(Fig.6a). The lower leaves of *SlHDA3*-RNAi plants turning to wilt and chlorosis, while no obvious change in WT plants after 7 days of salt stress(Fig.6b). Severe chlorotic leaves and collapsed shoot tissue was observed in *SlHDA3*-RNAi plants on the 14th day after salt stress. Whereas, the leaves in WT plant exhibit wilting and degradation of chlorophyll (Fig.6c). Related physiological and biochemical indicators were measured to further illustrate the potential physiological mechanism cause of the reduced salt stress tolerance in *SlHDA3*-RNAi plants. A significant decrease survival rate of *SlHDA3*-RNAi plants than that of WT plants (Fig.6d). No obvious change were found in chlorophyll contents, RWC, CAT activity, proline content, ABA concentration and MDA content between WT and *SlHDA3*-RNAi plants under normal conditions (Fig.6e-j). 7 days and 14 days after NaCl treatment, the decrease of The chlorophyll contents, RWC and CAT activity in *SlHDA3*-RNAi plants were faster than that

in WT plants (Fig.6e-g). On the contrary, the proline content, ABA concentration and MDA content in *SlHDA3*-RNAi plants were significantly higher than that in WT plants (Fig.6h-j). These results above suggest that the transcript of *SlHDA3* confer to salt stress in *SlHDA3*-RNAi plants.

Silencing of *SlHDA3* significantly decreases the expression of stress-related genes under salt tolerance

To further comprehend the underlying mechanisms of the depressed tolerance to salt stress, 14 stress-related genes including an endochitinase gene (Gawehns 2014), a potassium channel KAT3-like gene (Nakano, et al. 2013), a peroxidase gene, a key proline (Pro) synthetase gene *SlP5CS* (Kishor, et al. 1995), two ascorbate peroxidase (APX) genes *SlAPX1* and *SlAPX2* (Najami, et al. 2008), two pathogenesis-related (PR) genes *SlPR1* and *SlPR5* (Lim, et al. 2010), a MAP kinase kinase kinase gene *SlMAPKKK11* (Wu, et al. 2014), two MYB TF genes *SlMYB46* and *SlMYB106* (Zhao, et al. 2014), a key ascorbic acid (AsA) synthetase gene *SlGME2* (Chanjuan, et al. 2011), a pathogenesis-related protein gene *SlSTH-2* (Liu, et al. 2016), and Trihelix TF gene *SlGT26* (Yu, et al. 2015), were chosen and detected for further compared the expression between WT and *SlHDA3*-RNAi plants under salt stress for 48h. Varying degrees down-regulated of these stress-related genes were presented in *SlHDA3*-RNAi plants under stressed conditions(Fig.7), indicating that *SlHDA3* is involved in regulating stress-related genes.

Discussion

Various stresses including drought and high salinity in the environment are major factors that affect the agricultural productivity. It is profitable to cultivate stress-tolerant crops that vulnerable to abiotic stress for modern agriculture areas. Functional study of histone deacetylase genes was more detailed, especially in the past few years. Histone deacetylase gene plays significant roles in plants development, bio- and abiotic stresses. Recently, it has been proved that some stress-responsive histone deacetylase genes can affect the stress tolerance of transgenic plants. The mutant and RNAi plants of *HDA6* in Arabidopsis were involved in the salt-stress signaling pathways and the growth were inhibited 14 days after NaCl treatment, and the expression level also down-regulated when treated with ABA in *HDA6*-RNAi plants (Chen and Wu 2010). Besides, drought stress-up-regulated genes have been proved related to histone modification (To and Kim 2013). The more severe the drought, the higher expression levels in RD20 and RD29A, which were regulated by histone modifications in H3K4me3 and H3K9ac on drought stress-up-regulated genes (Jong-Myong, et al. ; Kim, et al. 2008).

AtHDT3 was participated in the regulation of heating stress response and an increased sensitivity of seed germination to ABA and NaCl was displayed in the mutant of *AtHDT3* in *Arabidopsis* (Buszewicz, et al. 2016; G., et al. 2013; Luo, et al. 2012). *OsHDT701* is involved in mediating the seed germination in response to abiotic stresses (Zhao, et al. 2014). The expression assay of *SlHDA3* under various abiotic stresses showed that the transcripts of *SlHDA3* were induced significantly in both salt and dehydration treatments (Guo, et al. 2017). In this study, we further affirmed that the expression level of *SlHDA3* was markedly improved by ABA (Fig. 1), suggesting that *SlHDA3* may play an indispensable role in various environmental stress corresponding. Experiment on post-germination seeds suggested that the length of

SlHDA3 transgenic seedling hypocotyl and root was more inhibited by ABA than that of WT (Fig. 2), indicating that silencing of *SlHDA3* improved seedling ABA sensitivity in tomato. The reduced expression of ABA biosynthesis- and signal transduction-related genes in *SlHDA3* transgenic plants further substantiate *SlHDA3* is involved in ABA biosynthesis/signal transduction through regulating the expression of related genes (Fig.3).

In the experiment of dehydration treatment, a lower survival ratio and worse growth phenotype were observed in *SlHDA3* transgenic lines. The lower chlorophyll content, RWC, CAT activity and higher proline content, MDA content were shown in *SlHDA3*-RNAi plant in our work (Fig. 4). These physiological indices are consistent with the morphology change. When suffered with NaCl treatment, the post-germination seeds growth was restrained sharply and the elongation of *SlHDA3*-RNAi seedling hypocotyl and root was more inhibited by salt than that of WT. When watered with NaCl solution, *SlHDA3* transgenic plants turned wilted and yellow earlier than that of WT. Chlorophyll content, RWC, survival ratio, CAT activity and ABA concentration were reduced while MDA content and proline content were enhanced than that in WT(Figs. 5–6). In conclusion, we speculated that *SlHDA3* act as a positive regulator in answer to the osmotic stress caused by drought and salt.

The enhancement of tolerance to abiotic stresses are mainly due to sustaining and markedly induced transcripts of related abiotic stress-response genes (2007). The results in our study indicating that those six genes which we selected were significantly down-regulated in *SlHDA3*-RNAi transgenic plants. In this study, the expression of a series of biotic and abiotic stress-related genes were significantly down-regulated in *SlHDA3*-RNAi plants including endochitinase gene (Gawehns 2014), potassium channel KAT3-like gene (Nakano, et al. 2013), peroxidase gene, *SlP5CS* gene (Kishor, et al. 1995), *SlAPX1* and *SlAPX2* genes (Najami, et al. 2008), *SlPR1* and *SlPR5* genes (Lim, et al. 2010), *SlMAPKKK11* gene (Wu, et al. 2014), *SlMYB46* and *SlMYB106* gene (Zhao, et al. 2014), *SlGME2* gene (Chanjuan, et al. 2011), *SlSTH-2* gene (Liu, et al. 2016), and *SlGT26* gene (Yu, et al. 2015), indicating that *SlHDA3* not only play an important role the adaptation to abiotic in tomato, but also mediates signaling pathways of response to biotic and abiotic stress though modulating transcripts of related abiotic stress-response genes, including NaCl, drought and the pathogen, which further confirm our results.

In conclusion, the data indicated in our work not only elucidate the important role of *SlHDA3* in salt stress and drought stress tolerance, but also provide a foundation for further research on the application of histone deacetylase genes in the signal transduction pathway of salt stress and drought stress. However, how to realize the regulation of drought stress and salt stress by *SlHDA3* and the detailed regulation mechanism needs to be further studied.

Declarations

Author Contributions

All the work was accomplished by JG.

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Declarations

The authors declare that they have no competing interests.

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Figures

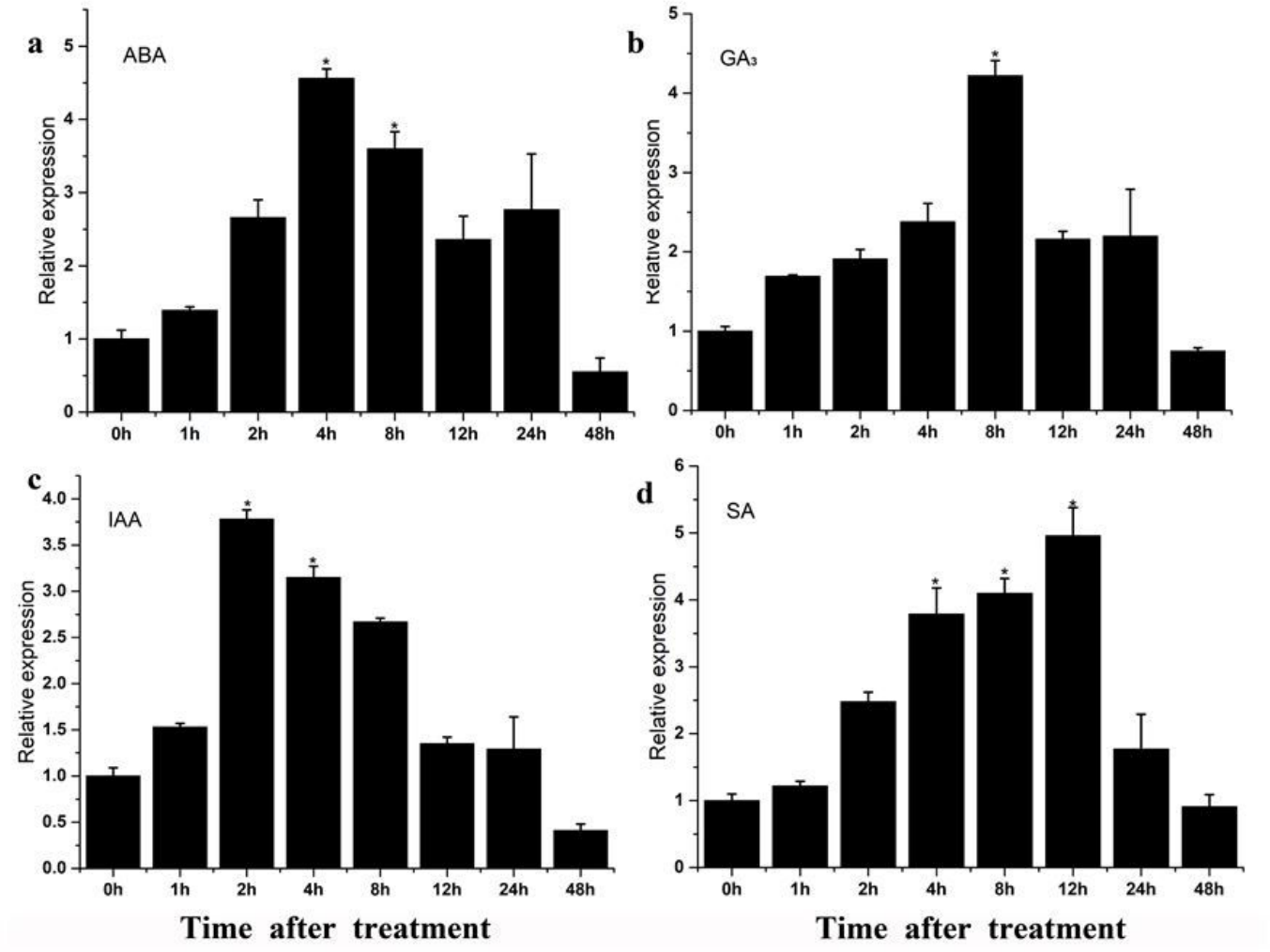

Figure 1

Expression profiles of SlHDA3 gene under hormone treatments. Gene expression was detected by qRT-PCR using total RNA from leaves of WT plants. The relative expression levels were normalized to 1 in control plants (0 h). Bars represent the mean of three biological replicates ± SE.

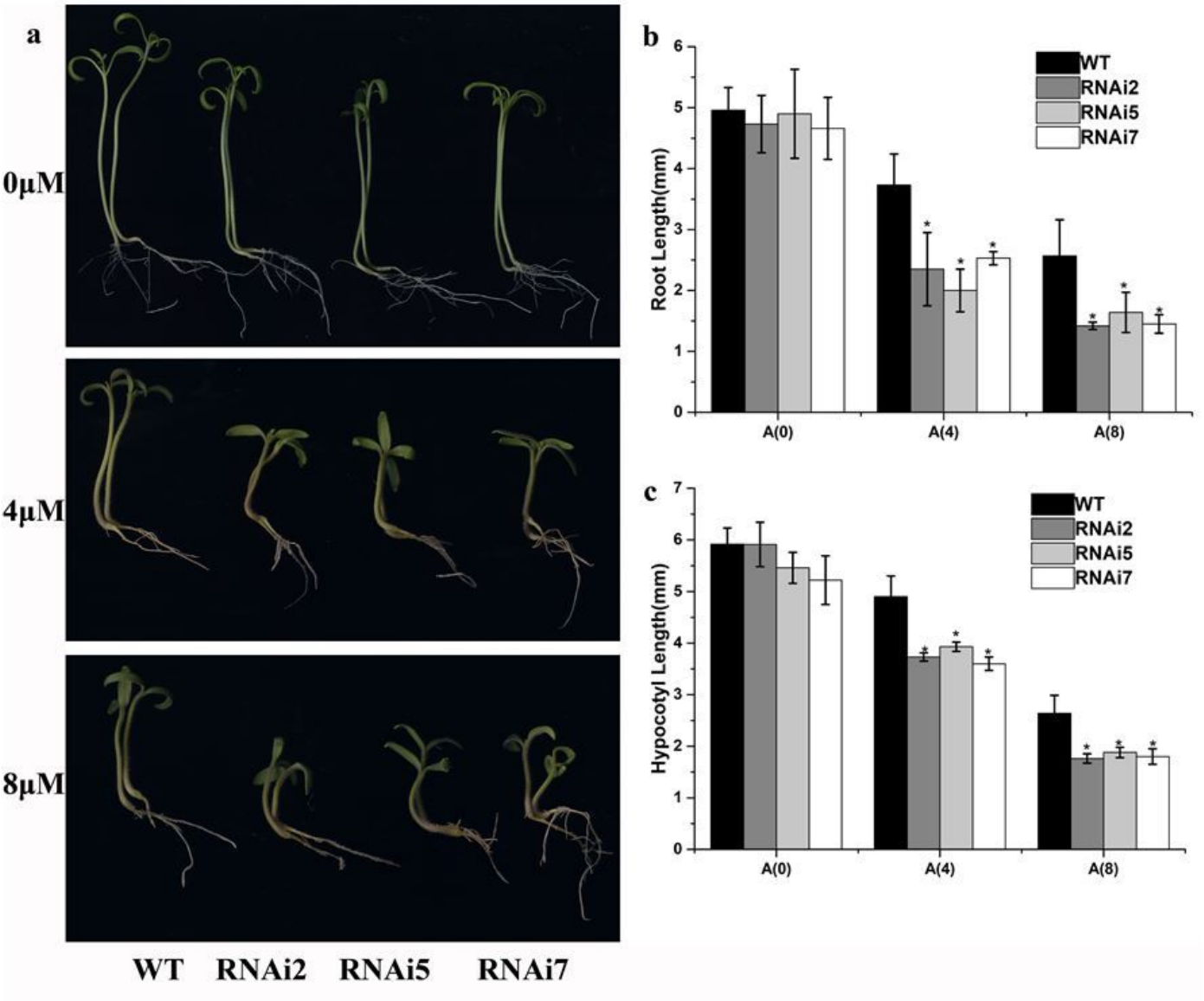

Figure 2

The sensitivity of SlHDA3-RNAi transgenic lines seedlings to ABA. a, b and c Growth phenotype of SlHDA3-RNAi transgenic lines seedlings on medium containing ABA (0, 4 and 8 μM). d and e Root and hypocotyl length of WT and transgenic seedlings. Values represent the means ± SE (n = 3). Asterisks indicate a significant difference from WT ($p < 0.05$).

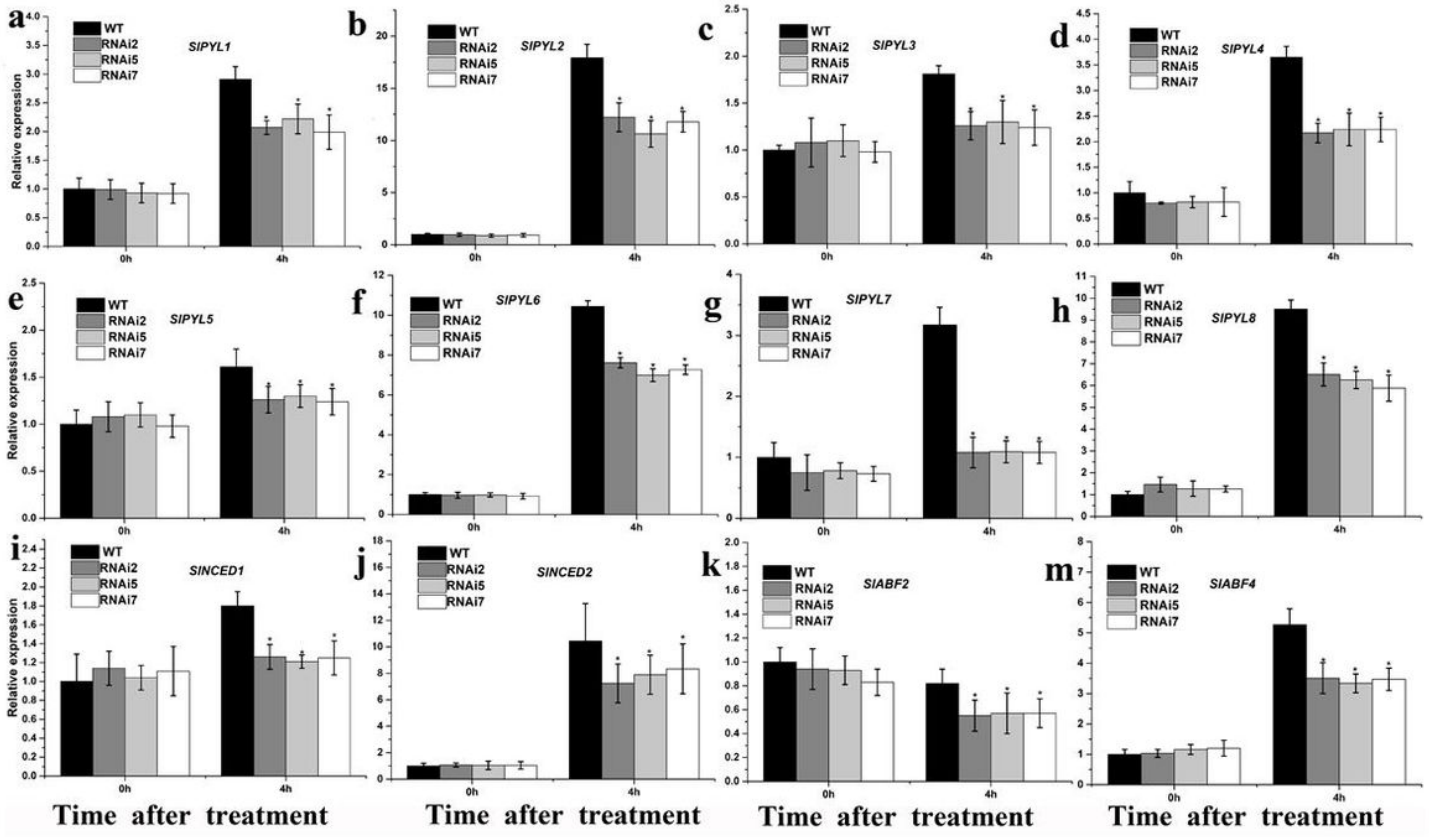

Figure 3

Comparisons of relative mRNA transcript levels of ABA biosynthesis- and signal transduction-related genes in wild-type and SlHDA3-RNAi transgenic plants under control and salt-stressed conditions. 35-day-old plants were sprayed with 100 μM ABA, leaves samples were harvested after 0, and 4 h under ABA. Values represent mean ± SD (n = 3). Asterisks above each column indicate a significant difference($p < 0.05$) between WT and transgenic lines. Values represent mean ± SD (n = 3). Asterisks above each column indicate a significant difference($p < 0.05$) between WT and transgenic lines.

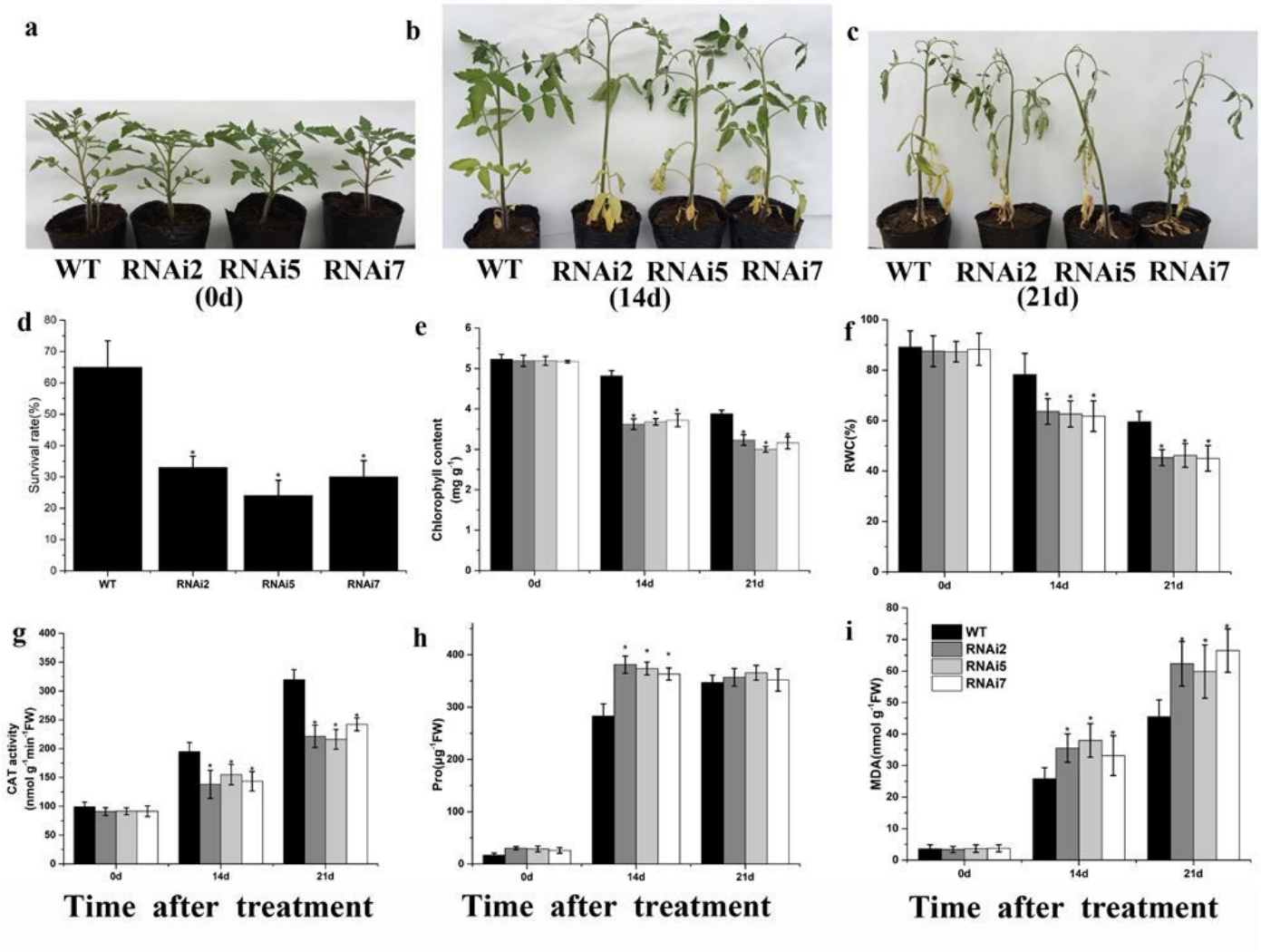

Figure 4

The appearance of WT and SlHDA3-RNAi transgenic tomato plants during drought stress. The water withholding assay was carried out with 35-day-old plants for up to 21 days. a and b Phenotypes of transgenic tomato plants and WT control at 14 and 21 days after initiation of drought assay. Representative transgenic plants, as well as WT plants were shown at the indicated time points. c Survival rate of transgenic tomato plants and WT control at 21 days and 5 days of recovery. Bars represent the mean of three biological replicates of 30 plants each. d-e Comparisons of survival rate(d), chlorophyll content(e) and leaf relative water content (f) of transgenic lines and WT plants at 0, 14 and 21 days after drought treatment. g CAT activity h Pro content and i MDA content. Representative transgenic plants, as well as WT plants were shown at the indicated time points. Values represent mean ± SD (n = 3). The data represent mean ± SD of about 10 leaves from each of three replicates. Asterisks indicate a significant difference from WT ($p < 0.05$).

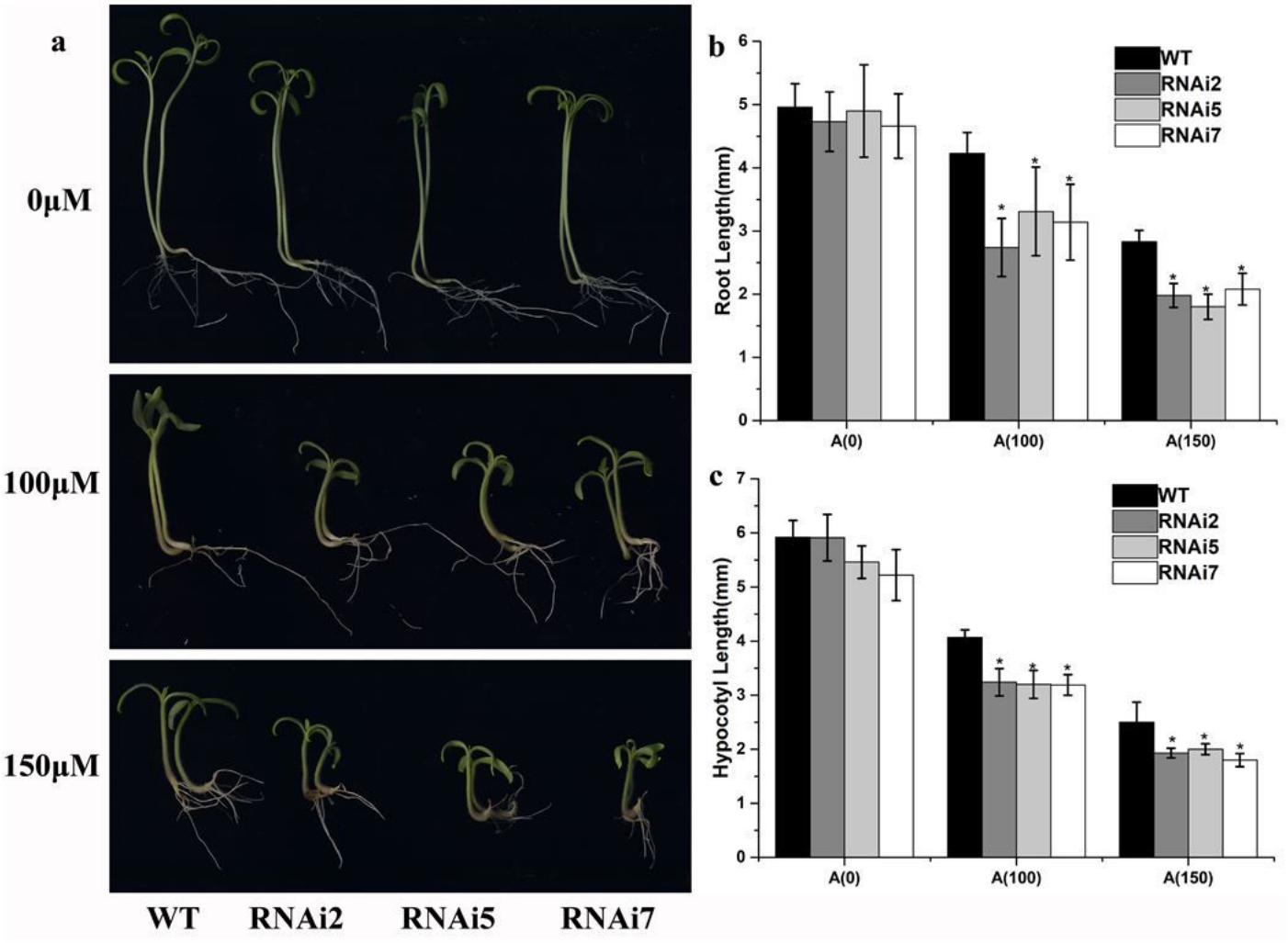

Figure 5

The sensitivity of SlHDA3-RNAi transgenic lines seedlings to NaCl. a, b and c Growth phenotype of SlHDA3-RNAi transgenic lines seedlings on medium containing NaCl (0, 100 and 150 mM). d and e Root and hypocotyl length of WT and transgenic seedlings. Values represent the means ± SE (n = 3). Asterisks indicate a significant difference from WT ($p < 0.05$).

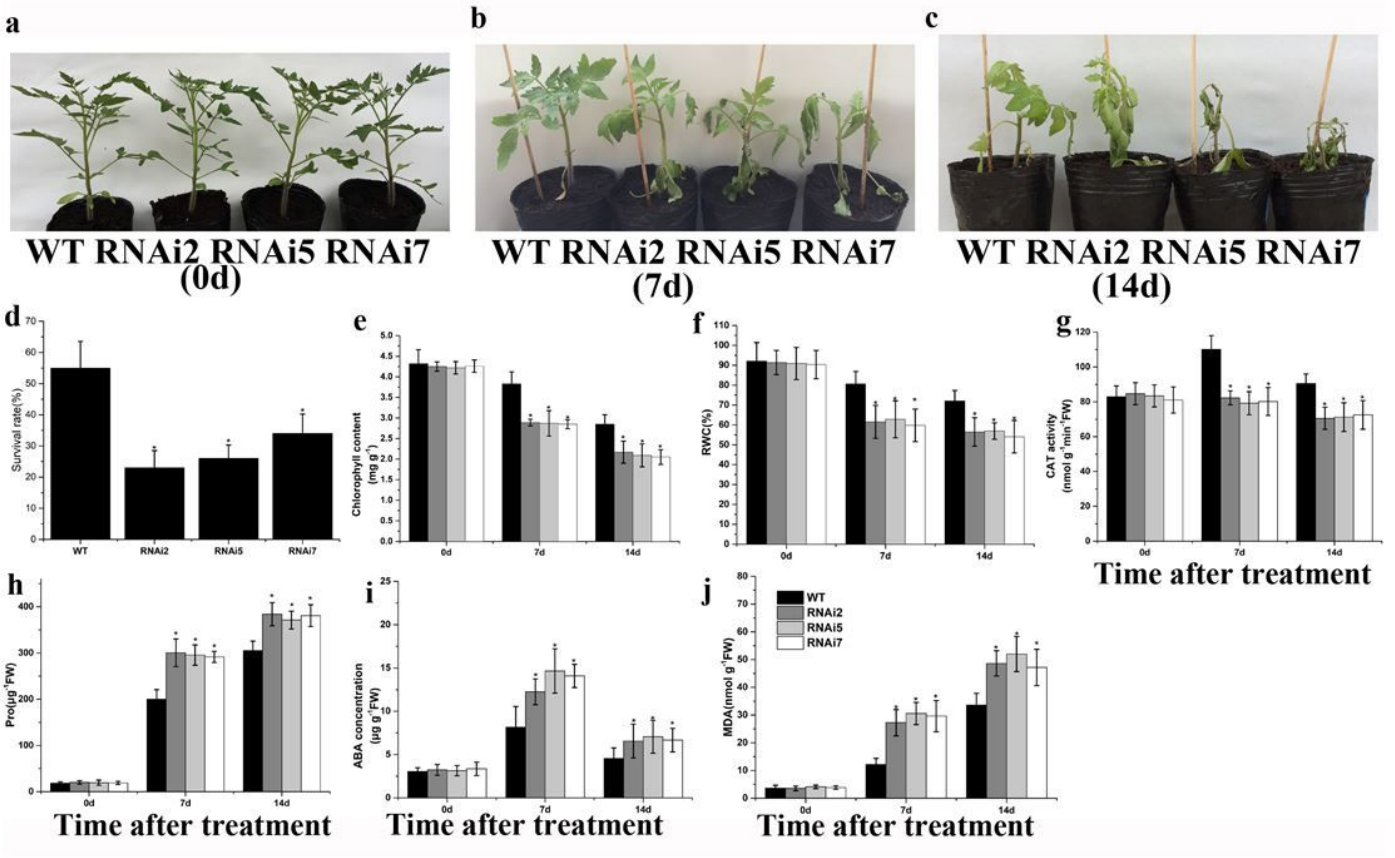

Figure 6

The appearance of WT and SlHDA3-RNAi transgenic tomato plants during salt stress. The salt stress assay was carried out with 35-day-old plants for up to 14 days. a, b and c Phenotypes of transgenic tomato plants and WT control at 0, 7 and 14 days after initiation of salt assay. Representative transgenic plants, as well as WT plants were shown at the indicated time points. d Survival rate of transgenic tomato plants and WT control at 14 days and 5 days of recovery. Bars represent the mean of three biological replicates of 30 plants each. e and f Comparisons of chlorophyll content (e) and leaf relative water content (f) of transgenic lines and WT plants at 0, 7 and 14 days after NaCl treatment. g CAT activity h Pro content i ABA concentration and j MDA content. Representative transgenic plants, as well as WT plants were shown at the indicated time points. Values represent the means ± SE (n = 3). Asterisks indicate a significant difference from WT ($p < 0.05$).

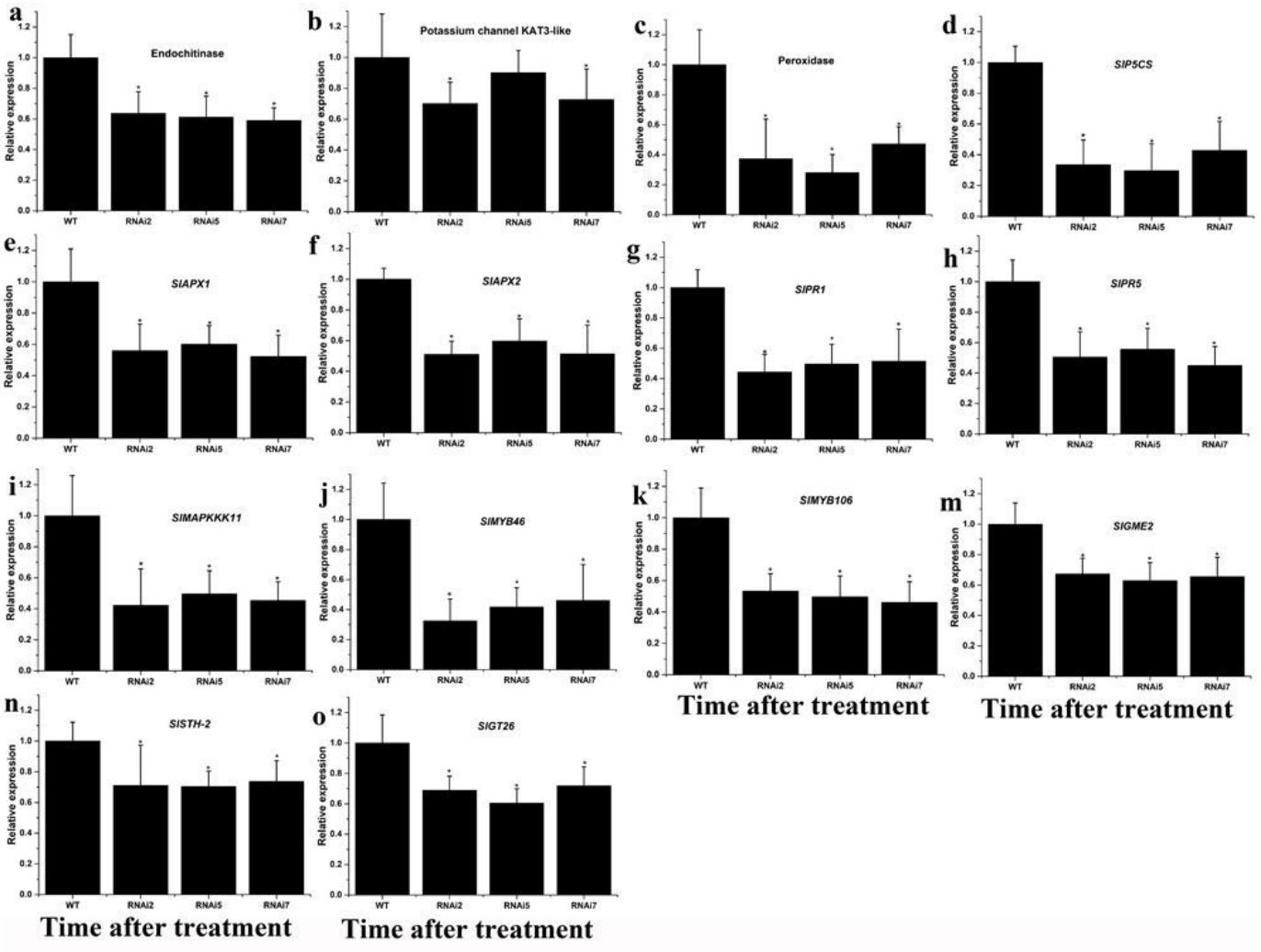

Figure 7

Comparisons of relative mRNA transcript levels of stress-related genes in wild-type and SlHDA3-RNAi transgenic plants under control and salt-stressed conditions. 35-day-old plants were treated with 400 mM NaCl, and leaves samples were harvested after 0 and 48 h under salt stress. Seedlings harvested before salt stress were used as controls. Values represent mean ± SD (n = 3). Asterisks above each column indicate a significant difference($p < 0.05$) between WT and transgenic lines.

Supplementary Files

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- SupplementaryFigureS1.doc