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Research Article

Linker histone variant HIS1-3 and *WRKY1* oppositely regulate salt stress tolerance in Arabidopsis

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Abstract

The salt overly sensitive (SOS) pathway plays an important role in plant salt stress; however, the transcriptional regulation of the genes in this pathway is unclear. In this study, we found that Linker histone variant HIS1-3 and WRKY1 oppositely regulate the salt stress response in Arabidopsis (*Arabidopsis thaliana*) through the transcriptional regulation of *SOS* genes. The expression of *HIS1-3* was inhibited by salt stress, and the disruption of *HIS1-3* resulted in enhanced salt tolerance. Conversely, the expression of *WRKY1* was induced by salt stress, and the loss of *WRKY1* function led to increased salt sensitivity. The expression of *SOS1*, *SOS2*, and *SOS3* was repressed and induced by HIS1-3 and WRKY1, respectively, and HIS1-3 regulated the expression of *SOS1* and *SOS3* by occupying the WRKY1 binding sites on their promoters. Moreover, WRKY1 and HIS1-3 acted upstream of the SOS pathway. Together, our results indicate that *HIS1-3* and *WRKY1* oppositely modulate salt tolerance in Arabidopsis through transcriptional regulation of *SOS* genes.

Introduction

Soil salinity is one of the most important factors limiting agricultural development and crop production worldwide (Godfray et al., 2010; Mark and Peter, 2010; Agarwal et al., 2013). Salt stress can disrupt a variety of cellular and physiological processes as a result of ion toxicity, osmotic stress, and secondary oxidative stress.

To adapt to or resist salt stress, plants have evolved several strategies including osmotic adjustment (such as osmoprotectant accumulation), ionic balance (especially for Na^+/K^+ homeostasis), and anti-oxidation (antioxidant enzyme accumulation and activity enhancement to resist oxidative stress; Munns and Tester, 2008; Jaleel et al., 2009; Arbona et al., 2013; Zhang et al., 2013; Tang et al., 2015; Fu et al., 2018). Ion transporters, such as the salt overly sensitive (SOS), Na^+/H^+ antiporter (NHX), and high-affinity potassium transporter, are important mediators of the ionic balance during salt stress. In *Arabidopsis thaliana*, the SOS pathway contains three main components, including SOS3, SOS2, and SOS1, which have been identified as being involved in the exclusion of excess Na^+ ions out of the cell across the plasma membrane in the roots (Shi et al., 2000; Zhu, 2003; Martinez-Atienza et al., 2007; Munns and Tester,

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2008). SOS3 (CBL) encodes a Ca^{2+}-binding protein and senses transient increases in cytosolic Ca^{2+} concentration, which is elicited by salt stress (Liu and Zhu, 1998; Tuteja, 2007). SOS2 encodes a serine/threonine protein kinase and can be activated by SOS3 in a calcium-dependent manner (Halfter et al., 2000; Yang et al., 2009). SOS1, a plasma membrane Na^+/H^+ antiporter, can be phosphorylated and activated by the SOS2–SOS3 kinase complex directly, resulting in Na^+ efflux (Shi et al., 2000; Qiu et al., 2002). In addition, Yang et al. (2009) found that the overexpression of SOS1/SOS3 led to enhanced salt tolerance in *A. thaliana* (Yang et al., 2009). These results have extensively indicated that the expression of *SOS1*, *SOS2*, and *SOS3* plays an important role in plant salt stress; however, the transcriptional regulation of these regulatory proteins is not clear. In eukaryotes, chromatin remodeling is critical for the regulation of stress-responsive gene expression (Kim et al., 2010; Luo et al., 2012; Zhu et al., 2013). Linker histones, the main structural components of chromatin, bind to the linker DNA between nucleosome cores, thereby stabilizing higher-order chromatin structure and limiting the accessibility of DNA to the regulatory factors (Horn et al., 2002; Koop et al., 2003; Kim et al., 2010). The linker histones may control specific processes during development and respond to environmental stress by changing the expression of H1 variants (Jerzmanowski et al., 2000). Some linker histone variants are induced by drought stress, such as *HIS1-3* of Arabidopsis (*A. thaliana*; Ascenzi and Gantt, 1997), *H1.S* of tomato (*Lycopersicon esculentum*; Scippa et al., 2000, 2004), *H1-D* of wild tomato (*Lycopersicon pennellii*; Wei and O'Connell, 1996), and *H1.S* of cotton (*Gossypium herbaceum*; Trivedi et al., 2012). Ascenzi and Gantt (1997) found that the expression of *HIS1-3* was induced by drought stress and abscisic acid (ABA) treatment (Ascenzi and Gantt, 1997). They also found that *HIS1-3* was primarily expressed in the root meristem and elongation zone in drought-stressed plants (Ascenzi and Gantt, 1999). The expression of *HIS1-3* was upregulated in ABA-responsive element-binding protein AREB1△QT-overexpressing plants but downregulated in a dominant loss-of-function mutant of AREB1 (Fujita et al., 2005). These data suggest that *HIS1-3* may be a stress-inducible H1 variant. However, whether HIS1-3 is involved in the regulation of salt stress is unclear.

The WRKY transcription factors, which belong to a larger family of plant-specific regulatory proteins, are another important regulatory component of salt stress tolerance. Members of this family contain a highly conserved WRKY domain at the N-terminus and a zinc finger motif (C2H2 or C2HC) at the C-terminus. Based on their primary structure, the WRKY proteins have been categorized into three groups (I, II, and III). The WRKY proteins specifically recognize the W-box (contains a TGAC core sequence; Eulgem et al., 2000; Rushton et al., 2010). It has been proven that WRKY proteins play important roles in many plant processes, including plant development and responses to biotic and abiotic stress (Eulgem et al., 2000; Johnson et al., 2002; Ulker et al., 2007; Agarwal et al., 2011; Phukan et al., 2016). In Arabidopsis, 18 WRKY genes were induced by 150-mM NaCl (Jiang and Deyholos, 2006). The expression of *AtWRKY25* and *AtWRKY33* is induced by salt stress, and increased *AtWRKY25* or *AtWRKY33* expression can enhance salinity tolerance in transgenic plants (Jiang and Deyholos, 2009). *AtWRKY8* plays a crucial role in the response to salt stress through directly binding the promoter of Response to Desiccation 29A to modulate salinity tolerance, and the VQ motif-containing protein 9 protein acts as a repressor of *AtWRKY8* to maintain an appropriate balance of the *AtWRKY8*-mediated signaling pathway (Hu et al., 2013). In addition, many WRKY genes in other plants are also involved in the response to salt stress, such as *ZmWRKY33*, *ZmWRKY17*, *GhWRKY34*, and *OsWRKY45* (Tao et al., 2011; Li et al., 2013; Zhou et al., 2015; Cai et al., 2017). *WRKY1* (also known as zinc-responsive transcriptional activator ZAP1) was the first WRKY gene identified from Arabidopsis and belongs to the group I WRKY family (de Pater et al., 1996). *WRKY1* may be involved in the salicylic acid (SA) signaling pathway, the induction of which is partially dependent on nonexpresser of PR gene 1 (Duan et al., 2007), while *WRKY1* regulates stomatal movement in the guard cells (Qiao et al., 2016).

In this report, we showed that the expression of *HIS1-3* could be inhibited by salt stress, and the loss-of-function of *HIS1-3* enhanced salt tolerance in Arabidopsis. The HIS1-3 protein was found to act as a negative regulator in the response to salt stress through the SOS pathway. Conversely, *WRKY1* positively regulated plant salt tolerance and maintained Na^+/K^+ homeostasis via the SOS pathway. Further investigation showed that HIS1-3 and WRKY1 recognized and bound to the same W-boxes in the promoter regions of *SOS1* and *SOS3*. The functional antagonism between *HIS1-3* and *WRKY1* may be a specific mechanism by which Na^+/K^+ homeostasis is maintained in order to regulate salt tolerance.

Results

The *his1-3* mutant is tolerant to salt stress

Many studies have confirmed that *HIS1-3* plays an important role in many abiotic stresses, such as water deficit and ABA treatment. Thus, we speculated that *HIS1-3* might also respond to salt stress. We used reverse transcription-quantitative PCR (RT-qPCR) and *ProHIS1-3*::β-glucuronidase (GUS) lines to analyze the expression of *HIS1-3* under salt stress. The results showed that the expression of the *HIS1-3* gene was inhibited by salt stress (Figure 1, A and B). We also tested the expression profiles of *HIS1-3* in various tissues using RT-qPCR and histochemical analysis and found that *HIS1-3* was expressed in most of the examined tissues, with high expression in the rosette leaf and inflorescence (Supplemental Figure S1).

To further verify this, seeds of two T-DNA mutants (SAIL_799_A07 and SALK_025209C) were screened from the SALK Arabidopsis T-DNA mutant collection, named *his1-3-1*

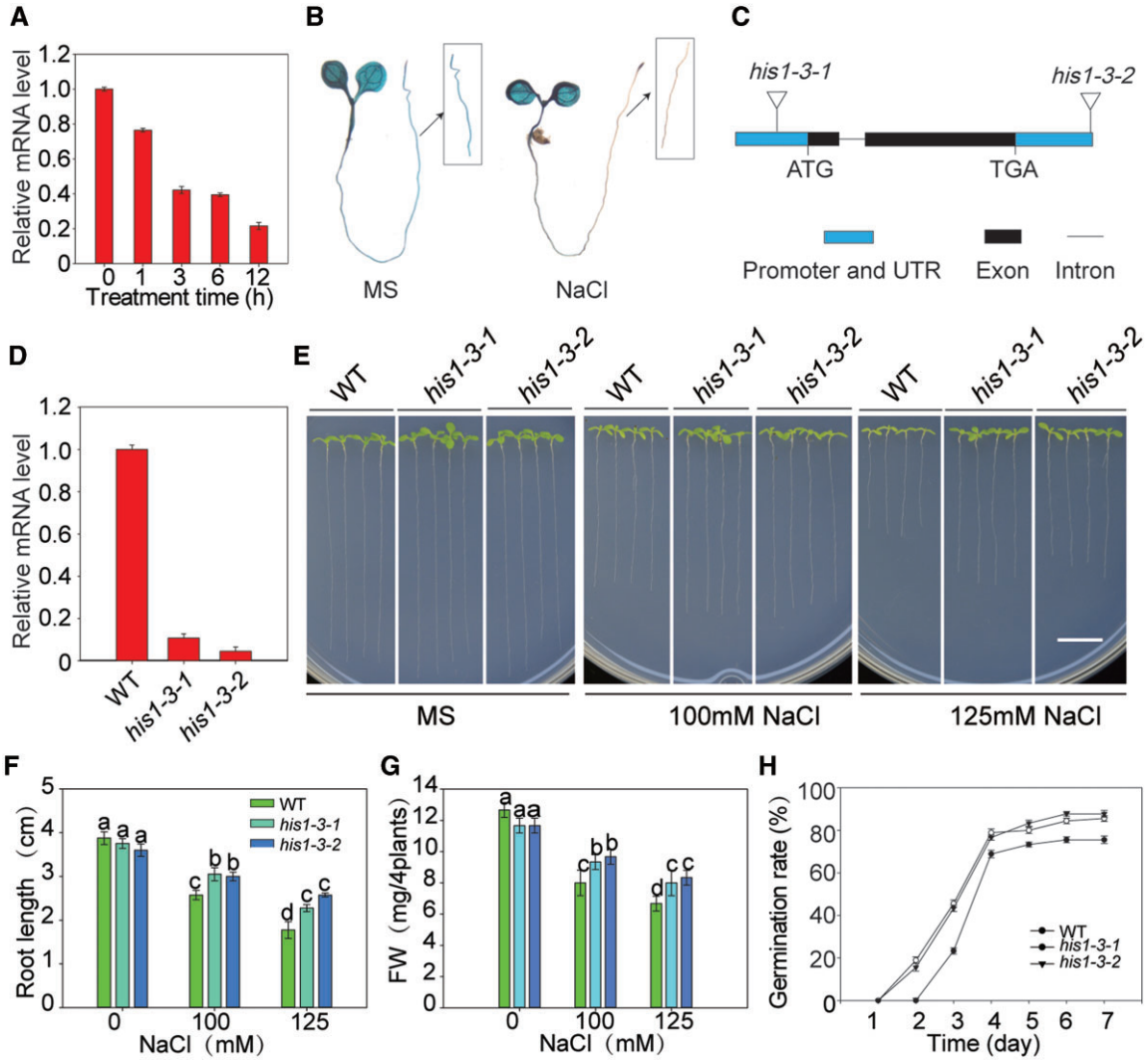

Figure 1 Loss of function of *HIS1-3* leads to enhanced salt tolerance. A, The effect of salt stress on *HIS1-3* transcript levels were analyzed by RT-qPCR, with *Actin8* as the internal control. Two-week-old WT seedlings were treated with NaCl for 0, 1, 3, 6, and 12 h. The vertical bars indicate the mean ± SE of three biological replicates. B, GUS staining of 7-d-old *ProHIS1-3*::GUS seedlings treated with NaCl. Seven-day-old seedlings were treated with NaCl (100 mM) for 0 and 6 h and then stained with X-Gluc for 12 h at 37°C. C, The location of the T-DNA insertion in the *HIS1-3* gene. Exons are shown as black boxes and introns are shown as lines. The triangle indicates the locations of the T-DNA insertion sites. D, The RT-qPCR analysis of the WT and *his1-3* mutants. The *Actin8* gene was amplified as an internal control. Similar results were obtained in at least three biological replicates. E, Salt tolerance assay of the WT and *his1-3* mutants. The seedlings were grown on MS medium for 3 d and then transferred to MS medium with or without 100 or 125 mM of NaCl for 2 weeks. Bar = 1 cm. F and G, Root length (F) and fresh weight (G) of the plants described in (E). H, Germination rate of the WT and *his1-3* mutants. The seedlings were grown on MS medium with NaCl and then counted at a fixed time every day. Data are presented as means ± SE of three replicate experiments. Statistical significance was determined by Student's *t* tests; significant differences ($P < 0.05$) are indicated by different lowercase letters.

and *his1-3-2*. The data showed dramatically decreased transcript levels in the *his1-3* mutants compared with the wild-type (WT) plants (Figure 1, C and D). The root length and fresh weight of the mutants were significantly increased compared with the WT plants (Figure 1, E–G). The seed germination rate of the mutants was higher than that in the WT under salt stress (Figure 1H). Together, these results indicated that the loss-of-function of *HIS1-3* resulted in increased salt tolerance.

To test whether the *his1-3* mutants responded specifically to sodium, the *his1-3* mutants were grown on Murashige and Skoog (MS) media containing KCl, LiCl, and mannitol. We observed that there was no morphological difference between the *his1-3* mutants and WT (Supplemental Figure S2). This result suggested that *HIS1-3* may perform a critical function in Na^+ toxicity.

Overexpression of *HIS1-3* leads to increased salt sensitivity

To further confirm the role of *HIS1-3* in salt stress, we built the 35S::*HIS1-3* construct and then transformed it into WT

plants to obtain the overexpression lines. The independent homozygous lines (OE1 and OE2) were chosen to further analyze the salt stress phenotype, and the expression of *HIS1-3* was found to be much higher than that in the WT (Figure 2A). Under salt stress, the overexpression seedlings were more sensitive than the WT (Figure 2, B–D). Furthermore, the seed germination rate of the overexpressing lines was lower than that in the WT under salt stress (Figure 2E). Under normal growth conditions, the *his1-3* mutants, *HIS1-3*-overexpressing lines, and WT exhibited similar growth and development in both the vegetative and reproductive phases (Supplemental Figure S3). These results showed that the overexpression of *HIS1-3* was responsible for the enhanced salt sensitivity.

HIS1-3 mediates Na^+/K^+ homeostasis via the SOS pathway

To study whether *HIS1-3* responds to salt stress by regulating the Na^+ balance, we analyzed the Na^+ and K^+ contents in the WT, *his1-3* mutants, and *HIS1-3*-overexpressing lines subjected to salt treatment. Compared with the WT, the Na^+ contents in the roots and the aboveground parts of the mutants were much lower, and so, the ratios of Na^+/K^+ were reduced (Figure 3, A and C). The contents of K^+ did not differ among the WT, *his1-3* mutants, and *HIS1-3*-overexpressing lines (Figure 3B). On the contrary, the Na^+ contents and the Na^+/K^+ ratio in the *HIS1-3*-overexpressing lines were higher (Figure 3, A and C). Thus, *HIS1-3* may respond to salt stress by mediating Na^+/K^+ homeostasis.

The Ca^{2+}-dependent SOS signaling pathway is an Na^+-specific signaling pathway that mainly consists of three protein components: SOS1, SOS2, and SOS3. We, therefore, examined the transcription levels of *SOS1*, *SOS2*, and *SOS3* in the WT, *his1-3* mutants, and *HIS1-3*-overexpressing lines using RT-qPCR. The results showed that the expression of *SOS1*, *SOS2*, and *SOS3* in the mutants were significantly higher than that in the WT. In contrast, the expression of these genes were significantly decreased in the *HIS1-3*-overexpressing lines (Figure 3, D–F). All the above results demonstrated that *HIS1-3*, as a negative transcriptional regulator, mediated Na^+/K^+ homeostasis through the SOS pathway.

WRKY1 positively regulates salt tolerance

The HIS1-3 protein has been found to interact with the transcription factor *WRKY1* in banana (*Musa acuminata*), which is a unique transcription factor in plants containing one or two highly conserved WRKY domains (Wang et al., 2012). A large number of studies have confirmed that WRKY

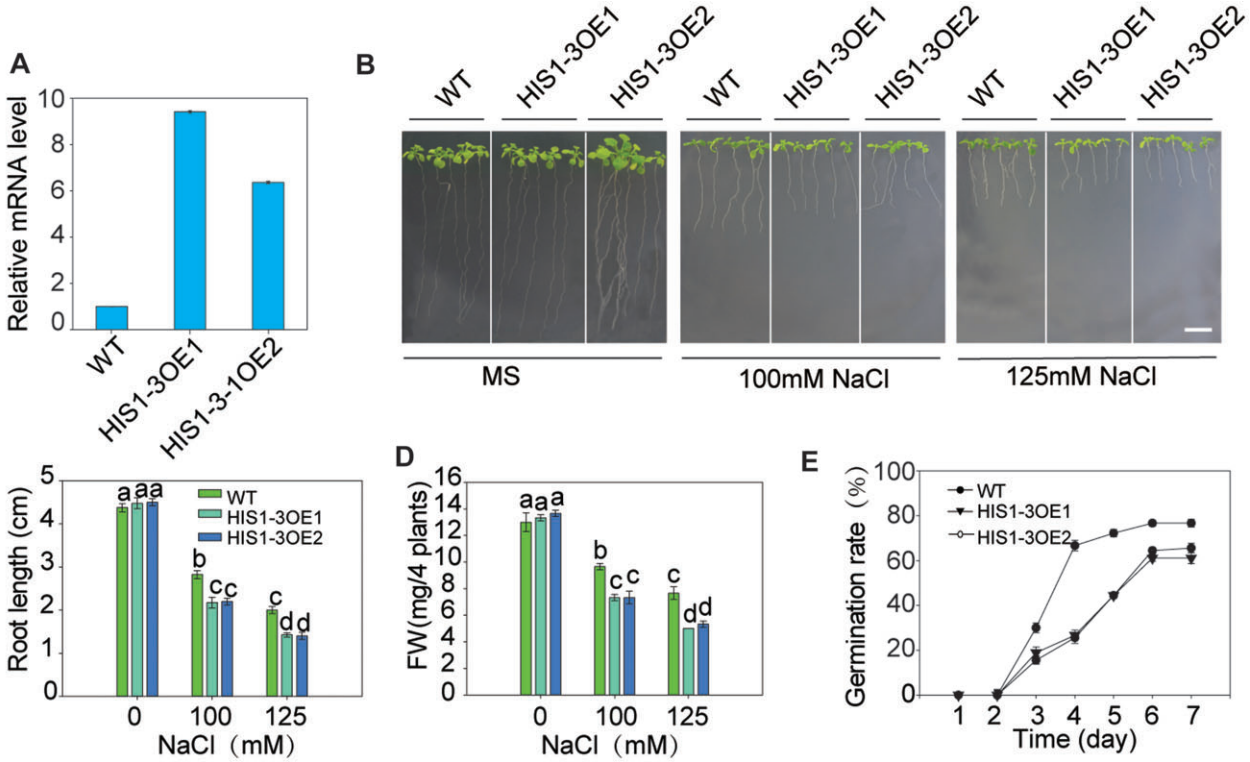

Figure 2 HIS1-3-overexpressing plants show increased salt sensitivity. A, The RT-qPCR analysis of the WT and *HIS1-3*-overexpressing plants. The *Actin8* gene was amplified as an internal control. Similar results were obtained in at least three biological replicates. B, Salt sensitivity assay of the WT and *HIS1-3*-OE seedlings. Three-day-old WT and *HIS1-3*-OE seedlings were transferred to MS medium with or without 100 mM or 125 mM of NaCl. Bar = 1 cm. C and D, Root length (C) and fresh weight (D) of the plants described in (B). E, Germination rate of the WT and *HIS1-3*-OE seedlings. The seedlings were grown on MS medium with NaCl and then counted at a fixed time every day. Data are presented as means ± SE of three replicate experiments. Statistical significance was determined by Student's *t* tests; significant differences ($P < 0.05$) are indicated by different lowercase letters.

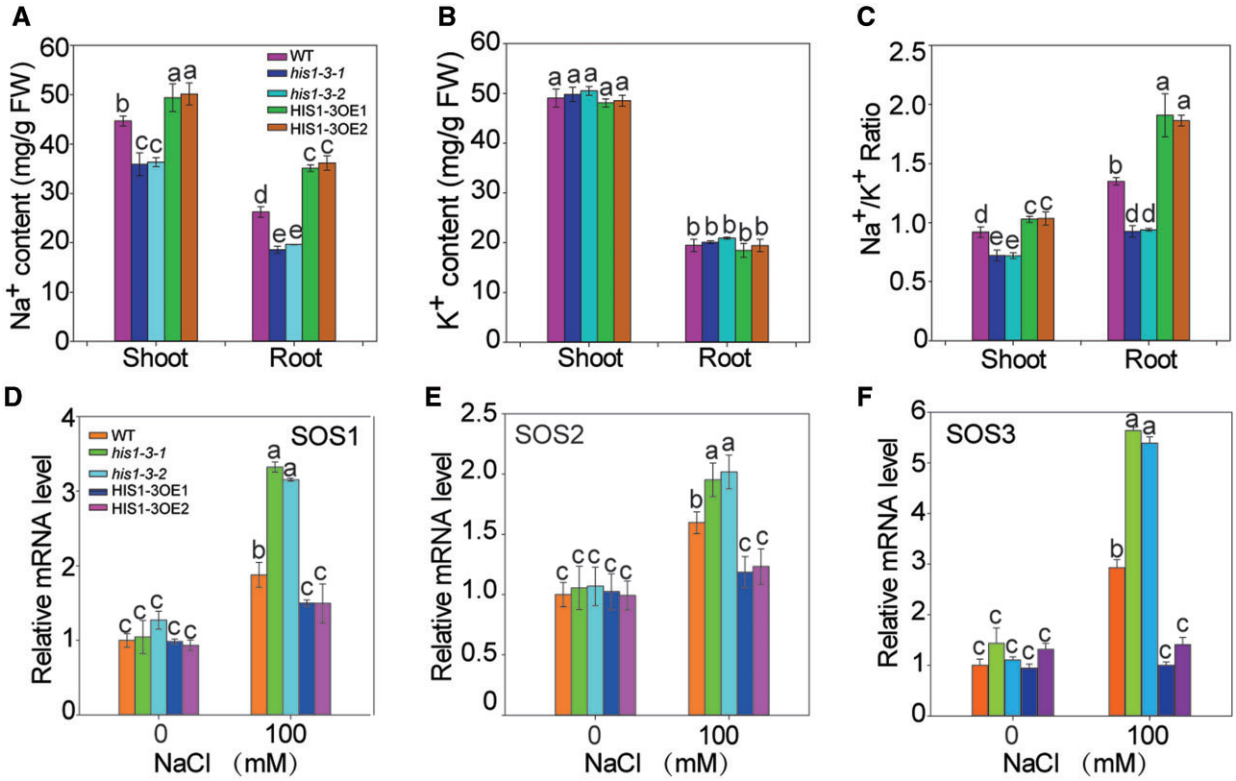

Figure 3 HIS1-3 mediates Na^+/K^+ homeostasis via the SOS pathway. A and B, Na^+ and K^+ content in the shoots and roots of WT, *his1-3* mutants, and *HIS1-3*-OE seedlings. The seedlings were treated with 100 mM of NaCl for 2 weeks, and then, the roots and shoots of these samples were collected. C, The Na^+–K^+ ratio was calculated from the Na^+ and K^+ content. D–F, Expression patterns of genes involved in the SOS pathway in the WT, *his1-3* mutants, and *HIS1-3*-OE seedlings under salt stress. Using RT-qPCR, gene expression was compared between 2-week-old seedlings of WT, *his1-3* mutants, and *HIS1-3*-OE seedlings after 0-h and 6-h treatment with 100 mM of NaCl. *Actin8* was used as the internal control. All data shown here are presented as the means ± SE of three replicate experiments. Statistical significance was determined using Student's *t* tests; significant differences ($P < 0.05$) are indicated by different lowercase letters.

transcription factors are involved in the regulation of various abiotic stresses such as low Pi, high temperature, and pathogen invasion. Therefore, we speculated that *WRKY1* may be involved in the response to salt stress. First, we tested the expression profiles of *WRKY1* under salt stress in various tissues. The results of RT-qPCR and histochemical analysis showed that the expression of *WRKY1* was induced by salt stress (Figure 4, A and B). The RT-qPCR and histochemical analysis also showed that *WRKY1* was expressed in most of the examined tissues, with high expression detected in the cauline leaf and inflorescence (Supplemental Figure S4).

We then used two independent T-DNA insertion mutants (*wrky1-1*: SALK_070989C and *wrky1-2*: SALK_136009C) and two *WRKY1* overexpression lines (*WRKY1*-OE1 and *WRKY1*-OE2) to test the salt tolerance phenotypes. We identified the T-DNA insertion site (Figure 4C), and the homozygous mutants were used for phenotype analysis. The expression of *WRKY1* was blocked in the two mutants (Figure 4D). Under salt stress, the root length and fresh weight of the mutants were significantly decreased compared with the WT plants (Figure 4, E–G). The seed germination rate of the mutants was lower than that in the WT under salt stress (Figure 4H). In contrast, *WRKY1* was highly accumulated in the *WRKY1*-OE lines and led to enhanced salt tolerance (Figure 5). We then observed the growth phenotypes of the *wrky1* mutants, *WRKY1*-overexpressing lines, and WT in the soil and found that they exhibited similar growth and development in both the vegetative and reproductive phases (Supplemental Figure S5). Together, these results indicated that *WRKY1* played a positive role in salt tolerance.

To test whether the *wrky1* mutants were also specific to the sodium response, the *wrky1* mutants were grown on MS media containing KCl, LiCl, and mannitol. No significant difference was observed between the *wrky1* mutants and WT (Supplemental Figure S6), which suggests that *WRKY1* may play an important role in Na^+ toxicity.

WRKY1-mediated salt tolerance by maintaining Na^+/K^+ homeostasis

To further examine whether *WRKY1* also regulates salt stress through the SOS pathway, we tested the contents of Na^+ and K^+ in the WT, *wrky1* mutants, and *WRKY1*-overexpressing lines. The results showed that the Na^+ content and the Na^+/K^+ ratio were much higher in the mutants and lower in the *WRKY1*-overexpressing lines (Figure 6, A–C).

The transcript levels of *SOS1*, *SOS2*, and *SOS3* in the WT, *wrky1* mutants, and *WRKY1*-overexpressing lines were then analyzed by RT-qPCR. The results showed that the

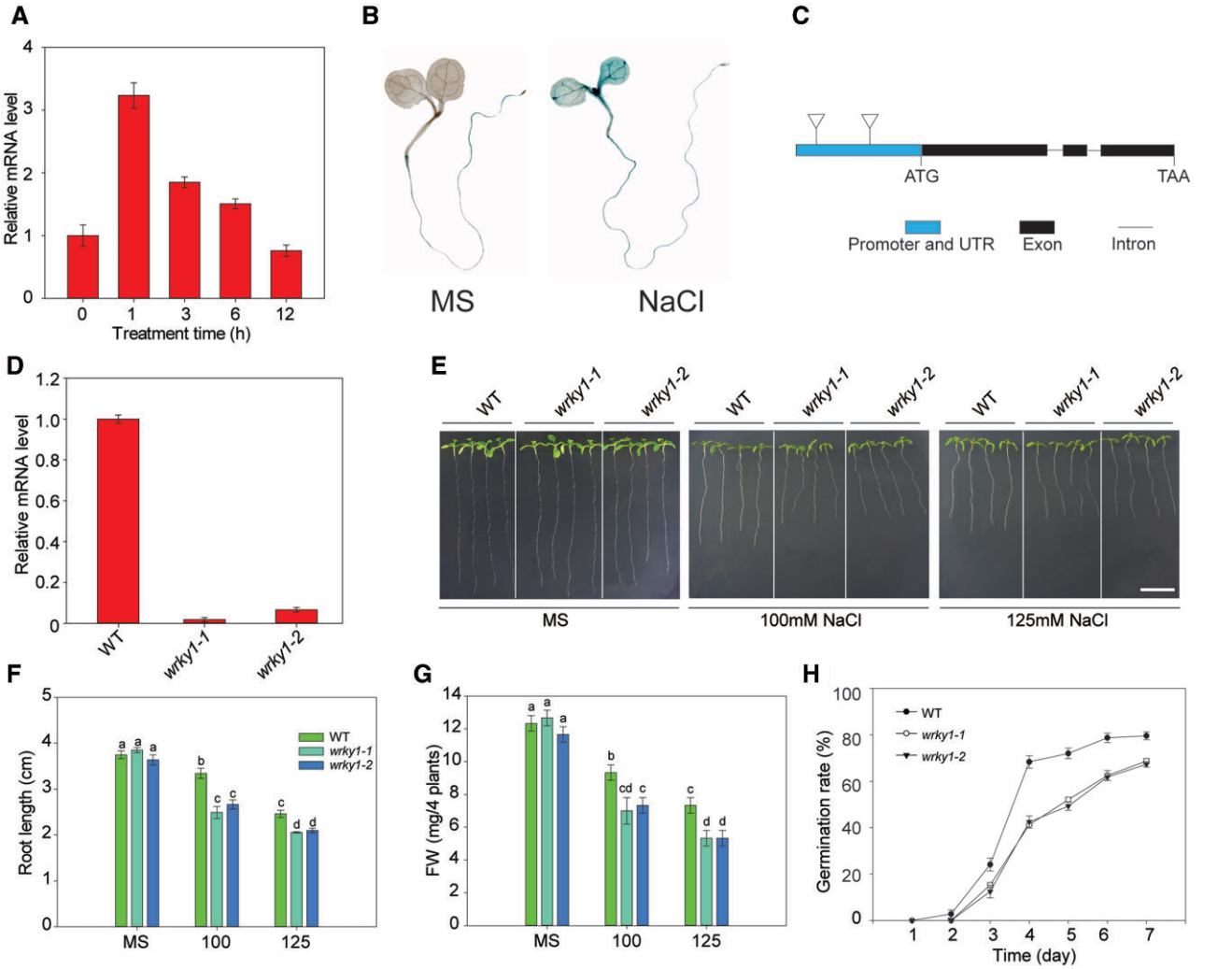

Figure 4 The *wrky1* mutants are sensitive to salt stress. A, Effect of salt stress on *WRKY1* transcript levels analyzed by RT-qPCR, with *Actin8* as the internal control. Two-week-old WT seedlings were treated with NaCl for 0, 1, 3, 6, and 12 h. The vertical bars indicate the means ± SE of three biological replicates. B, The GUS staining of 7-d-old *ProWRKY1*::GUS seedlings treated with NaCl for 6 h. The 7-d-old seedlings were treated with NaCl (100 mM) for 0 and 6 h and then stained with X-Gluc for 12 h at 37°C. C, The T-DNA insertion sites of the *WRKY1* gene. Exons are shown as black boxes, and introns are shown as lines. The triangle indicates the locations of the T-DNA insertion sites. D, Transcription analysis of the *WRKY1* gene in the *wrky1-1* and *wrky1-2* mutants by RT-qPCR. The *Actin8* gene was amplified as an internal control. Similar results were obtained in at least three biological replicates. E, The *WRKY1* loss-of-function mutation led to sensitivity to salt stress. Seedlings were grown on MS medium for 3 d and then treated with or without 100 or 125 mM of NaCl for 2 weeks. Bar = 1 cm. F and G, Measurements of the root length (F) and fresh weight (G) described in (E). H, Germination rate of the WT, *wrky1-1*, and *wrky1-2* mutants grown in MS medium with NaCl and then counted at a fixed time every day. Data are presented as means ± SE of three replicate experiments; statistical significance was determined by Student's *t* tests; significant differences ($P < 0.05$) are indicated by different lowercase letters.

expression of *SOS1*, *SOS2*, and *SOS3* in the mutants was significantly decreased compared to that in the WT but increased in the *WRKY1*-overexpressing lines (Figure 6, D–F). Overall, *WRKY1*-mediated Na^+/K^+ homeostasis positively regulates salt tolerance.

HIS1-3 and WRKY1 bind to the promoters of three *SOS* genes

To assess whether *HIS1-3* and *WRKY1* synergistically regulated the response to salt stress, we first confirmed that both were localized in the nucleus (Supplemental Figure S7, A and B). We then studied whether there was an interactive relationship between them by using yeast two-hybridization and bimolecular fluorescence complementation (BiFC), but the results showed that HIS1-3 did not interact with WRKY1 (Supplemental Figure S7, C and D). Subsequently, we explored whether *HIS1-3* and *WRKY1* synergistically affect the expression of SOS pathway genes by transient transactivation assay. Consistent with the above results, *WRKY1* activated the expression of *SOS1*, *SOS2*, and *SOS3*, whereas *HIS1-3* reduced the expression of *SOS1*, *SOS2*, and *SOS3* (Figure 7, A and B). Under the co-expression of HIS1-3 and WRKY1, the expression of *SOS1*, *SOS2*, and *SOS3* exhibited no obvious change compared with the WT (Figure 7B).

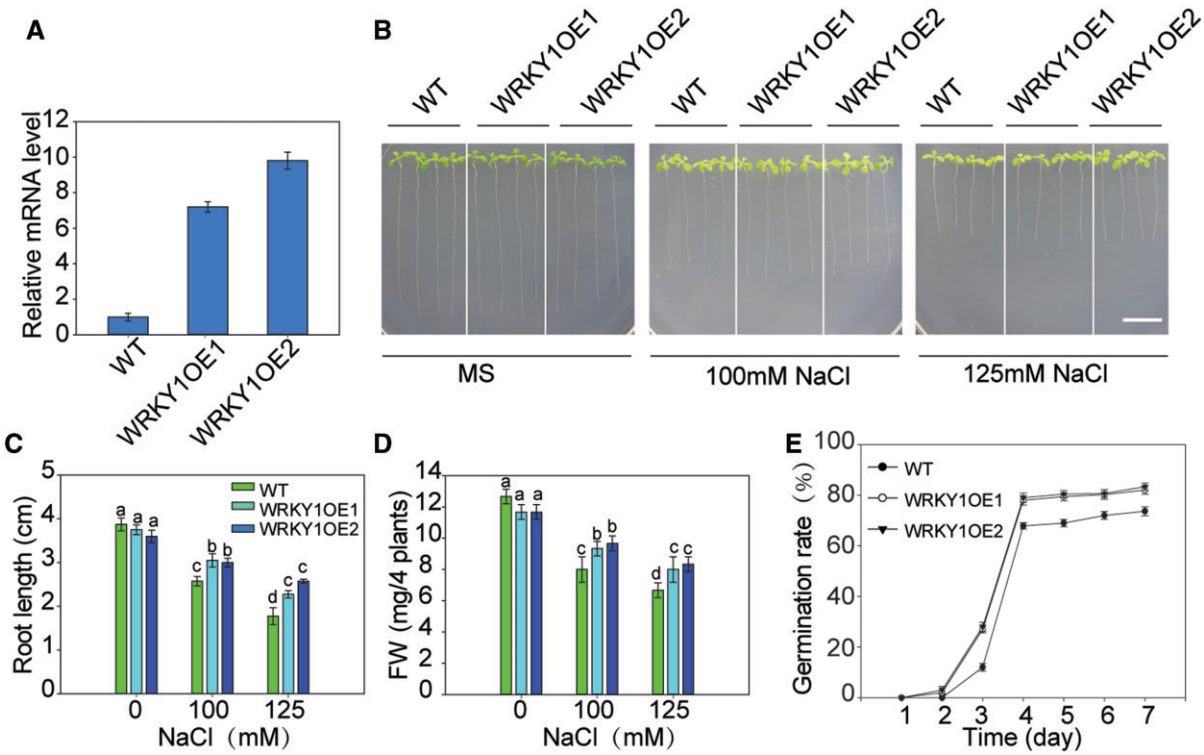

Figure 5 *WRKY1* positively regulates salt tolerance. A, The expression of *WRKY1* in the WT and *WRKY1*-overexpressing lines using RT-qPCR. *Actin8* was used as the internal control. Data are presented as the means ± SE of three replicate experiments. B, Growth of WT and *WRKY1*-overexpressing lines under salt stress. Seedlings were grown on MS medium for 3 d and then transferred to MS medium with or without 100 or 125 mM of NaCl for 2 weeks. Bar = 1 cm. C and D, Root length (C) and fresh weight (D) of plants described in (B). E, Germination rate of WT and *WRKY1*-overexpressing lines grown in MS medium with NaCl and then counted at a fixed time every day. These experiments were repeated 3 times with similar results, and the data are presented as the means ± SE. Statistical significance was determined by Student's *t* tests; significant differences ($P < 0.05$) are indicated by different lowercase letters.

These results showed that *HIS1-3* reduced the expression of *WRKY*1-activated *SOS1*, *SOS2*, and *SOS3*.

It has been reported that WRKY can regulate target gene expression by binding to the typical cis-element W-box in the promoters of these genes, and so, we analyzed the promoters of the *SOS1*, *SOS2*, and *SOS3* genes by bioinformatics and found that W-boxes existed on the promoters of these genes (Figure 7C). To determine whether the SOS genes were directly regulated by HIS1-3 and WRKY1, we performed chromatin immunoprecipitation (ChIP) experiments using *35S::HIS1-3::GFP* and *35S::WRKY1::GFP* transgenic plants. Enrichment of specific promoter fragments in the precipitate was measured using qPCR. These results showed that WRKY1 was enriched in pSOS1-1, pSOS2-3, pSOS2-4, pSOS3-1, pSOS3-2, and pSOS3-3 with the GFP antibody, and HIS1-3 was enriched in pSOS1-1, pSOS1-2, pSOS2-1, pSOS3-1, and pSOS3-3, with the binding sites on *SOS1* and *SOS3* being consistent with WRKY1 and those on *SOS2* being adjacent to WRKY1 (Figure 7, D–E). We also intended to further verify this result by yeast one-hybrid (Y1H) assay, but WRKY1 exhibited self-activation (Supplemental Figure S8). These data supported the hypothesis that HIS1-3 may suppress the expression of *SOS1* and *SOS3* by occupying the binding sites of WRKY1 on the promoters of *SOS1* and *SOS3*.

WRKY1 and HIS1-3 act upstream of the SOS pathway

SOS1, a putative plasma membrane Na^+/H^+ antiporter, is a downstream target of the SOS2–SOS3 complex. The *sos1* mutations render plants extremely sensitive to salt stress, and the overexpression of *SOS1* improves salt tolerance in *A. thaliana* (Wu et al., 1996; Shi et al., 2003). In order to genetically study the role of *HIS1-3*, *WRKY1*, and *SOS1* in regulating salt stress, we generated the double mutants *wrky1/35S::SOS1*, *his1-3/sos1-1*, and *his1-3/wrky1* (Supplemental Figure S9). Consistent with our results (Figure 4), the *wrky1* mutant was hypersensitive to salt stress and the *his1-3* mutant was tolerant to salt stress. The salt sensitivity of the *wrky1* mutant was rescued by *35S::SOS1* (Figure 8, A–C), and the increased salt tolerance of the *his1-3* mutant was eliminated by the loss-of-function of *sos1-1* (Figure 8, D–F). Meanwhile, there was no significant difference between the growth of the *his1-3wrky1* double mutant and WT under salt stress (Figure 8, G–I). This result suggested that WRKY1 and HIS1-3 acted upstream of the SOS genes and competed with each other.

Subsequently, we detected the content of Na^+ and K^+ in the *his1-3/sos1* and *wrky1/35S:SOS1* plants. The result showed that the Na^+ content in the roots and the aboveground parts of the *his1-3/sos1* mutant was much higher than that

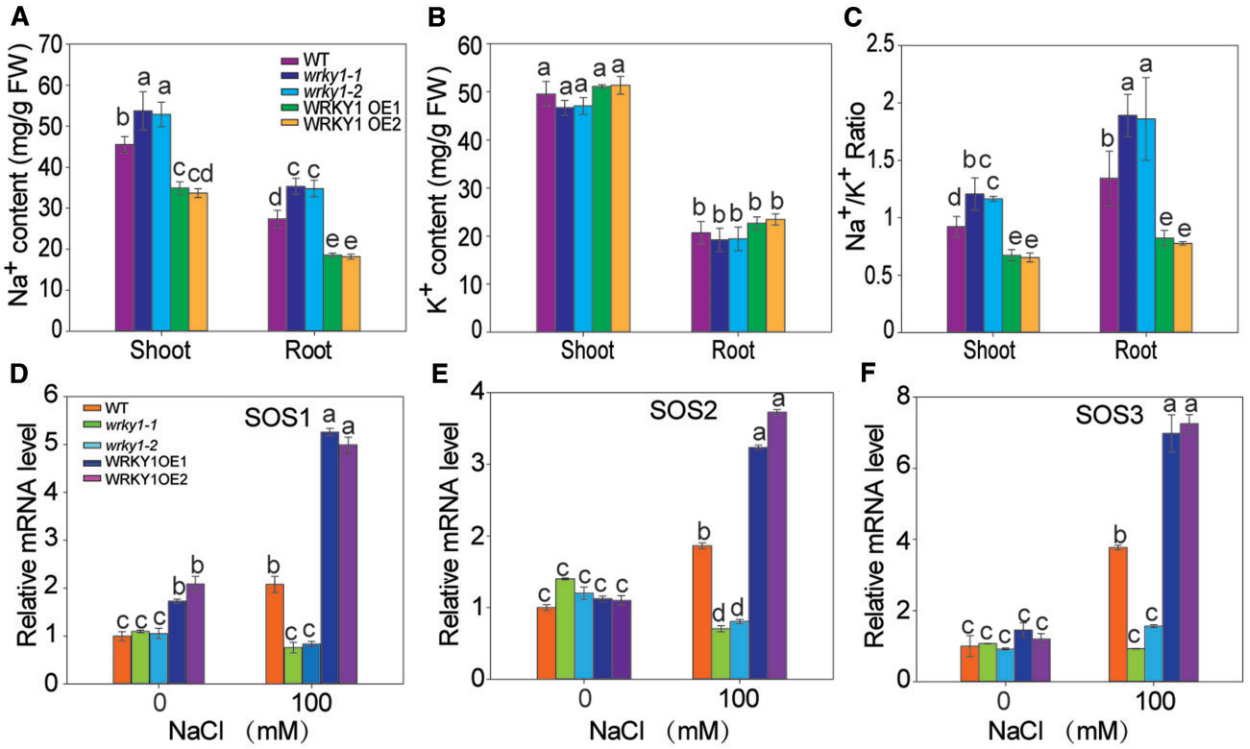

Figure 6 WRKY1-mediated salt tolerance by mediating Na^+/K^+ homeostasis. A–B, The Na^+ and K^+ content in the shoots and roots of WT, *wrky1* mutants, and *WRKY1*-OE seedlings. The seedlings were treated with 100 mM of NaCl for 2 weeks. The roots and shoots of these samples were collected. C, The Na^+–K^+ ratio of WT, *wrky1* mutants, and *WRKY1*-OE seedlings was calculated from the Na^+ and K^+ content. D–F, Transcript levels of *SOS1*, *SOS2*, and *SOS3* in the WT, *wrky1* mutants, and *WRKY1*-OE seedlings under salt stress. Gene expression was compared by RT-qPCR among 2-week-old seedlings of WT, *wrky1* mutants, and *WRKY1*-OE seedlings after 0- and 6-h treatment with 100 mM of NaCl. *Actin8* was used as the internal control. All data shown here are presented as the means ± SE of three replicate experiments. Statistical significance was determined using Student's *t* tests; significant differences ($P < 0.05$) are indicated by different lowercase letters.

of the WT (Figure 9A). On the contrary, the Na^+ contents in the *wrky1/35S:SOS1* plants were lower. The content of K^+ did not differ among the WT, *his1-3/sos1* mutant, and *wrky1/35S:SOS1* plants (Figure 9B). The result suggested that *WRKY1* and *HIS1-3* may respond to salt stress through SOS pathway-dependent Na^+/K^+ homeostasis.

Discussion

HIS1-3 and *WRKY1* play opposing roles in salt stress

The Arabidopsis genome encodes three H1 variants (*HIS1-1*, *HIS1-2*, and *HIS1-3*), and *HIS1-3* encodes a linker histone protein that is an abundant component of chromatin fibers. The quantitation of stoichiometry of linker histone in chromatin may play important roles in a variety of physiological processes (Jerzmanowski, 2007). Previous studies found that *HIS1-3* plays an important role in drought-responsive gene expression mediated by chromatin remodeling. However, the role of *HIS1-3* in salt stress is far less known. In our study, we showed that the expression of *HIS1-3* was inhibited by salt stress and the loss-of-function of *HIS1-3* led to increased salt tolerance (Figure 1). Moreover, the overexpression of *HIS1-3* enhanced salt sensitivity in Arabidopsis (Figure 2). Thus, *HIS1-3* is involved in salt stress as a negative factor.

Many studies have proved that WRKY transcription factors regulate the response to multiple biotic or abiotic stresses in plants (Rushton et al., 2010; Agarwal et al., 2011). *WRKY1* was the first member of this family to be isolated, and it was found to be inducible by SA and drought (de Pater et al., 1996; Duan et al., 2007; Qiao et al., 2016). In this study, *WRKY1* was significantly induced by salt stress, which was similar to that of *AtWRKY25* and *AtWRKY33* in Arabidopsis (Jiang and Deyholos, 2009), *ZmWRKY17/33* in maize (*Zea mays L.*; Li et al., 2013; Cai et al., 2017), and *CmWRKY17* in *Chrysanthemum morifolium* (Li et al., 2015). Phenotypic analysis showed that the *wrky1* mutants exhibited salt sensitivity, and the overexpression lines exhibited salt tolerance (Figures 4 and 5). These data suggested that *WRKY1* was able to mediate the salt stress response as a positive factor. Thus, *HIS1-3* and *WRKY1* have opposing effects on plant tolerance to salt stress.

HIS1-3 and *WRKY1* respond to salt stress via the SOS pathway

Intracellular Na^+ accumulation leads to ionic toxicity, resulting in plant growth cessation or cell death (Zhu, 2003). Thus, the maintenance of ion homeostasis is a major determinant of salt tolerance, such as by increasing Na^+ extrusion. Herein, compared to the WT, the Na^+ content was

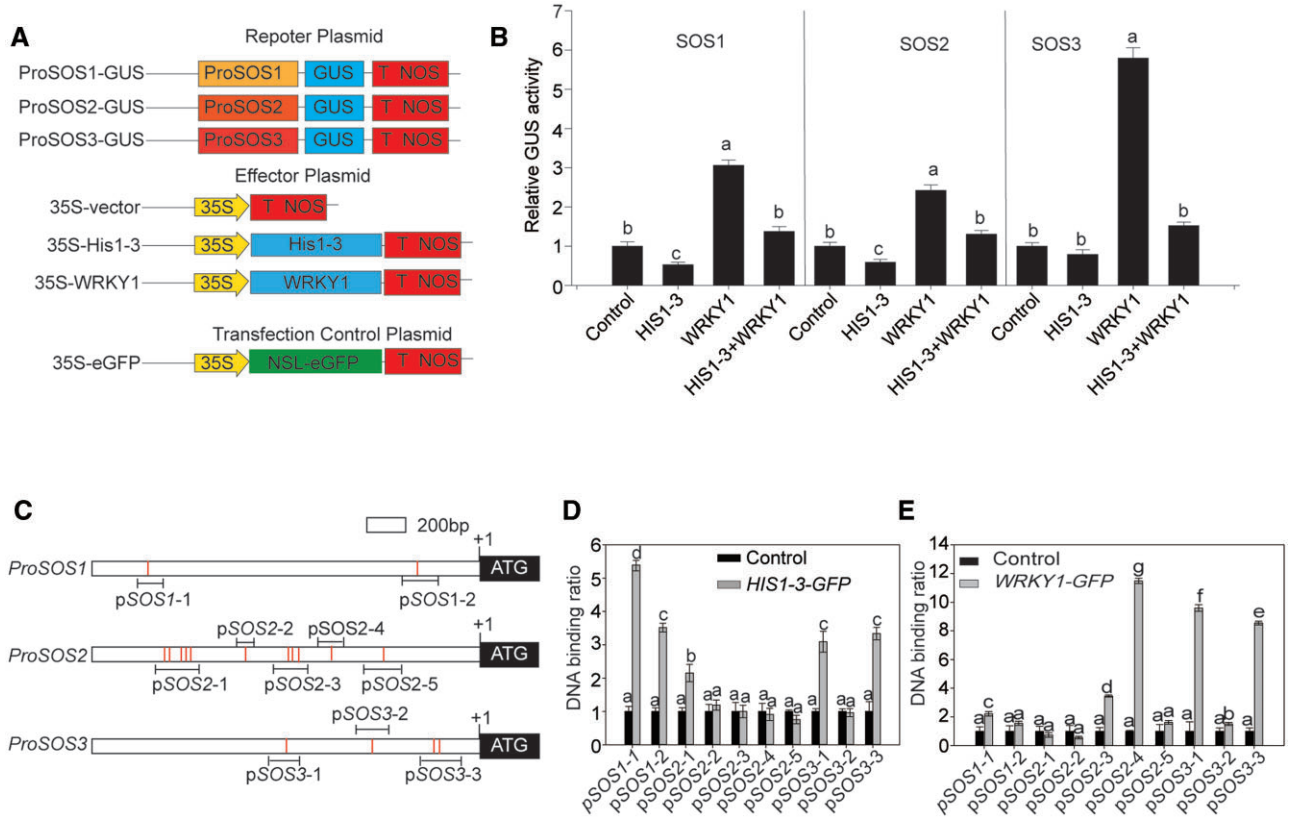

Figure 7 HIS1-3 and WRKY1 regulate three *SOS* genes by binding to their promoters. A, Schematics of all constructs used for transient expression assays in *N. benthamiana* leaves. The *SOS1*, *SOS2*, or *SOS3* promoter was fused to the GUS reporter gene. *35S::HIS1-3* and *35S::WRKY1* acted as an effector. The 35S promoter was fused to the *GFP* gene as an internal control. B, Relative GUS activity showed the expressions of *ProSOS1*, *ProSOS2*, or *ProSOS3* after co-expression with HIS1-3 and/or WRKY1. The constructs of Pro*SOS1*:GUS, Pro*SOS2*:GUS, or Pro*SOS3*:GUS were co-transformed into *N. benthamiana* epidermal cells with 35S::*HIS1-3* or 35S::*WRKY1*, and GUS activity was assessed at 48 h after injection using a MUG assay. The 35S-empty vector was used as an effector plasmid control. C, Schematic diagram of the *SOS1*, *SOS2*, and *SOS3* promoters showing the W-box present in different regions. Bars indicate the W-box (TGAC); lines beneath the bars represent the sequences for ChIP assays. D and E, The direct binding of HIS1-3 and WRKY1 to the W-box of the *SOS1*, *SOS2*, and *SOS3* promoters using ChIP-qPCR assay. The DNA binding ratio of the promoter regions of *SOS1*, *SOS2*, and *SOS3* was confirmed by qPCR using the ChIP products from the WT HIS1-3-GFP or WRKY1-GFP. Input DNAs were used as the internal control. All data shown here are presented as the means ± SE of three replicate experiments. Statistical significance was determined using Student's *t* tests; significant differences ($P < 0.05$) are indicated by different lowercase letters.

much lower in the *his1-3* mutants and higher in the *HIS1-3*-overexpressing lines (Figure 3). However, compared to the WT, the Na^+ content was much higher in the *WRKY1*-overexpressing lines and lower in the *wrky1* mutants (Figure 6). These results indicated that both *HIS1-3* and *WRKY1* may maintain Na^+ balance in cells, thereby enhancing plant salt tolerance.

The Ca^{2+}-dependent SOS pathway is an important regulatory system activated by salt stress. When *SOS3* is activated by Ca^{2+}, the concentration of which increased under salt stress, the SOS3 protein binds to SOS2 to release the SOS2 kinase domain for substrate access. SOS2 interacts with SOS3, and then, the SOS3/SOS2 protein kinase complex activates SOS1. *SOS1*, a PM Na^+/H^+ antiporter, exports Na^+ from cells (Shi et al., 2000; Qiu et al., 2002; Quintero et al., 2011). SOS2 is a key regulator in the SOS pathway and transmits the stress signal downstream through phosphorylation (Halfter et al., 2000; Guo et al., 2001). The SOS pathway is not activated when plants are not subjected to salt stress, but the molecular mechanisms underlying this process remain poorly understood. Kim et al. (2013) reported that the GI protein represses SOS2 activity in the absence of salt stress, and salt stress causes the degradation of the GI protein (Kim et al., 2013). The SOS2 kinase activity is repressed by the 14-3-3 protein, and salt stress reduces the interaction between 14-3-3 and SOS2, leading to the release of SOS2 activity (Zhou et al., 2014). In our study, the loss-of-function of *HIS1-3* led to the increased expression of *SOS1*, *SOS2*, and *SOS3*, and the overexpression of *HIS1-3* repressed the expression of these genes (Figure 3). Furthermore, the GUS activity assay showed that HIS1-3 repressed the expression of *SOS1*, *SOS2*, and *SOS3* (Figure 7B). Linker histone H1, unlike core histones (H2A, H2B, H3, and H4), regulates specific gene expression but not global transcription (Shen and Gorovsky, 1996; Jerzmanowski et al., 2000). Thus, our results indicated that HIS1-3 suppressed

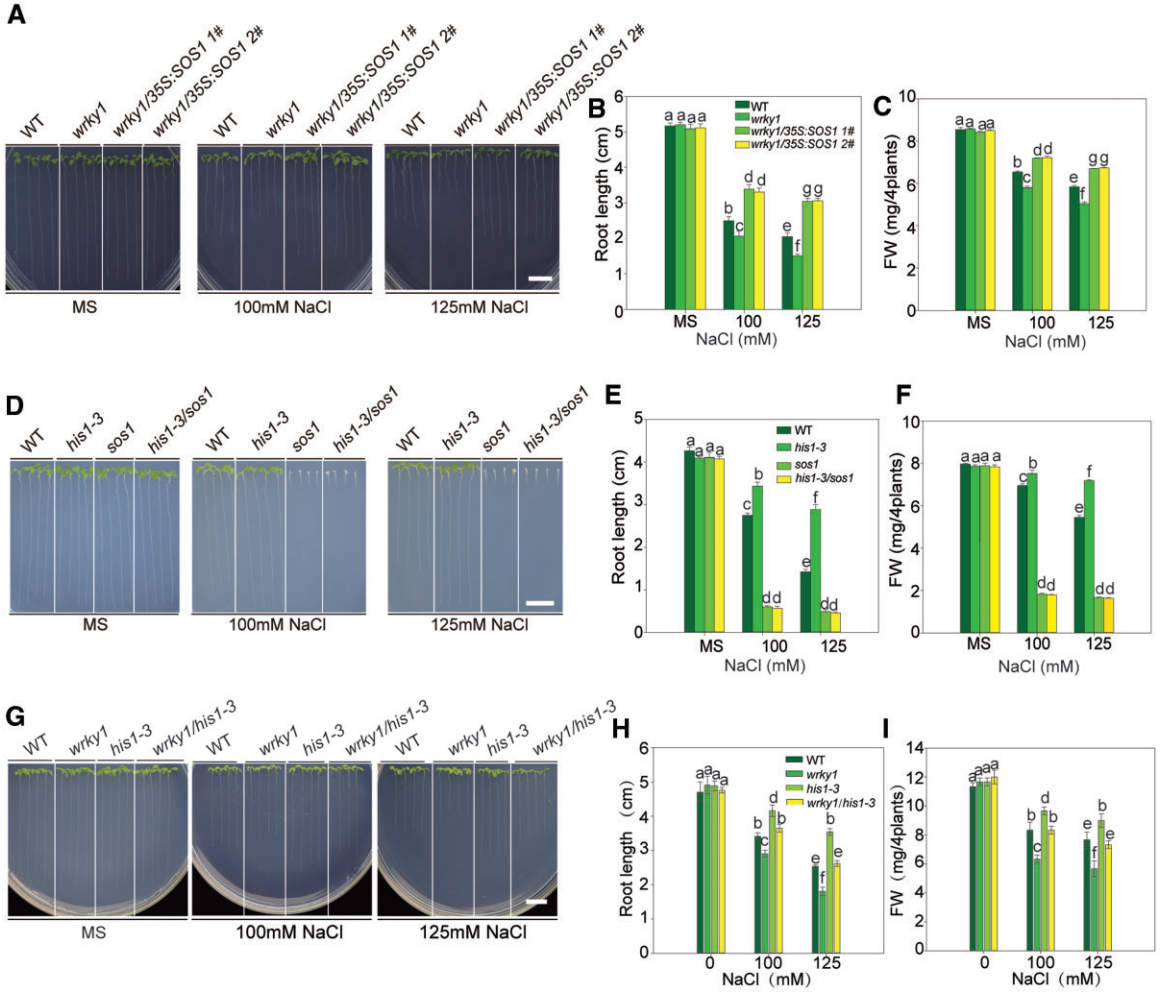

Figure 8 Genetic interaction of HIS1-3 and WRKY1 with SOS1. A, Growth of the WT, *wrky1*, and *wrky1/35S:SOS1* lines under salt stress. Seedlings were grown on MS medium for 3 d and then transferred to MS medium with or without 100 or 125 mM of NaCl for 2 weeks. Bar = 1 cm. B and C, Root length (B) and fresh weight (C) of the plants described in (A). D, Growth of the WT, *his1-3*, *sos1*, and *his1-3/sos1* double mutants under salt stress. Seedlings were grown on MS medium for 3 d and then transferred to MS medium with or without 100 or 125 mM of NaCl for 2 weeks. Bar = 1 cm. E and F, Root length (E) and fresh weight (F) of the plants described in (D). G, Growth of WT, *wrky1*, *his1-3*, and *wrky1/his1-3* lines under salt stress. Seedlings were grown on MS medium for 3 d and then transferred to MS medium with or without 100 or 125 mM of NaCl for 2 weeks. Bar = 1 cm. H and I, Root length (G) and fresh weight (I) of the plants described in (G). These experiments were repeated 3 times with similar results, and data are presented as the means ± SE. Statistical significance was determined by Student's *t* tests; significant differences ($P < 0.05$) are indicated by different lowercase letters.

the expression of *SOS1*, *SOS2*, and *SOS3* under conditions of no stress in Arabidopsis, and salt stress reduced *HIS1-3* gene expression, thereby inducing *SOS* gene expression.

However, many genes have been found to induce the expression of SOS genes, such as *FcWRKY40* and *GhWRKY34* (Zhou et al., 2015; Dai et al., 2018). Herein, WRKY1 induced the expression of three SOS genes (Figure 7B). The transcript abundances of three *SOS* genes were increased in the *WRKY1*-overexpressing lines but decreased in the *wrky1* mutants, indicating that *WRKY1*-mediated salt tolerance depends on its positive regulation of the *SOS* genes. Our GUS activity analysis further confirmed that the expression of *SOS1*, *SOS2*, and *SOS3* was inhibited when HIS1-3 and WRKY1 were co-expressed in *Nicotiana benthamiana* (Figure 7B). One possible explanation for the different roles of *HIS1-3* and *WRKY1* in regulating the SOS pathway is that HIS1-3 binds to *SOS1*, *SOS2*, and *SOS3* more avidly than *WRKY1* in the absence of salt stress. In order to prove that specific competition at the promoter regions of the SOS1 and SOS3 genes existed between HIS1-3 and WRKY1, we also performed a reciprocal competitive electrophoretic mobility shift assay (EMSA). The result showed that there was no specific competition at the promoter regions of the SOS1 and SOS3 genes between HIS1-3 and WRKY1. This may indicate that the two genes do not respond to salt stress by competing for the same sites of the SOS1 and SOS3 genes. One possible mechanism is that HIS1-3 binds to the linker DNA between nucleosome cores and occupies

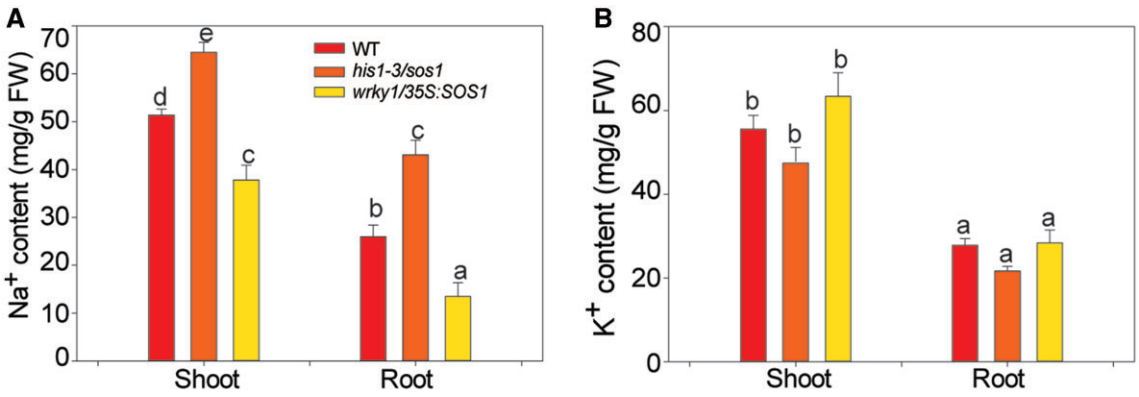

Figure 9 The Na^+ and K^+ content in the shoots and roots of WT, *his1-3/sos1*, and *wrky1/35S:SOS1* mutants. A, The Na^+ content in the shoots and roots of the WT, *his1-3/sos1*, and *wrky1/35S:SOS1* mutants. B, The K^+ content in the shoots and roots of the WT, *his1-3/sos1*, and *wrky1/35S:SOS1* mutants. The seedlings were treated with 100 mM of NaCl for 2 weeks. The roots and shoots of these samples were collected. All data shown here are presented as the means ± SE of three replicate experiments. Statistical significance was determined using Student's *t* tests; significant differences ($P < 0.05$) are indicated by different lowercase letters.

the binding site at the promoter regions of the SOS1 and SOS3 genes, thereby limiting the accessibility of WRKY1 to the SOS1 and SOS3 genes (Supplemental Figure S10).

In addition, owing to WRKY transcription factors being able to specifically recognize and bind W-box elements in the promoter regions of the target genes, we analyzed the promoter sequences of *SOS1*, *SOS2*, and *SOS3* (Figure 7C). The ChIP-qPCR data showed that HIS1-3 and WRKY1 could bind to the same region in the promoters of *SOS1* and *SOS3* and to the adjacent region in the promoter of *SOS2* (Figure 7D). Further genetic analysis indicated that the phenotypes of *his1-3/sos1-1* and *wrky1/35S::SOS1* were similar to the mutant and *SOS1* overexpression lines, and the growth of the double mutant *his1-3/wrky1* did not differ significantly from the WT under salt stress (Figure 8). The Na^+ contents were consistent with the phenotypes (Figure 9). Taken together, these results suggest that *HIS1-3* and *WRKY1* regulate salt stress via a Na^+ extrusion mechanism by directly activating the expression of SOS genes, and *HIS1-3* and *WRKY1* act upstream of the SOS genes.

In summary, we propose the possible action mode for *HIS1-3* and *WRKY1* in regulating salt stress in Arabidopsis (Figure 10). Under normal physiological conditions, the HIS1-3 protein binds to the W-boxes of the *SOS1*, *SOS2*, and *SOS3* promoter sequences by occupying the binding sites of WRKY1 on their promoters, thereby inhibiting the expression of these genes. Under salt stress, HIS1-3 protein expression decreased, resulting in the W-boxes of the *SOS1*, *SOS2*, and *SOS3* promoters becoming exposed. Subsequently, the WRKY1 protein was directly combined with the W-boxes to activate gene expression, thereby regulating the Na^+/K^+ balance in the plant in order to respond to salt stress.

Materials and methods

Plant materials, growth conditions, and treatments

The plant materials used in this study included Arabidopsis (*A. thaliana*) WT (Col-0), *his1-3* mutants (*his1-3-1*, SAIL-799-A07; *his1-3-2*, SALK-025209), and *wrky1* mutants (*wrky1-1*, SALK-070989C; *wrky1-2*, SALK-136009C). The seeds of the mutants were obtained from the Arabidopsis Biological Resource Centre. The T-DNA insertion sites were confirmed by PCR amplification using gene-specific primers and a left border T-DNA primer (Supplemental Table S1). The *sos1-1* mutant contains a 14-bp deletion that causes a frameshift, and the seeds were provided by Professor Jiankang Zhu (Shi et al., 2000). The Arabidopsis seeds were vernalized in the dark at 4°C for 2 d and then grown in a growth chamber maintained at 22°C with 16 h of daylight.

For the salt tolerance tests, seeds of the WT and the mutants or transgenic lines were germinated on MS medium for 3 d and then transferred to MS medium with or without NaCl (100 mM, 125 mM) for 2 weeks. The plants were then sampled for root length and fresh weight determination. To determine the germination rate, seeds of the WT and the mutants or transgenic lines were germinated on MS medium containing NaCl (100 mM), and the germination of the seeds was counted at a fixed time every day from the second day of sowing until 7 d later.

Vector construction and generation of transgenic plants

To generate *HIS1-3*-overexpressing and *WRKY1*-overexpressing plants, the sequences of *HIS1-3* and *WRKY1* were amplified with the specific primers (the primers are presented in Supplemental Table S1) and then cloned into pXB94 (pART27 with expanded restriction sites, 35S promoter and GFP reporter) or pBI121, respectively (named *35S::HIS1-3*, *35S::HIS1-3::GFP*, *35S::WRKY1*, and *35S::WRKY1::GFP*). These constructs were introduced into the *Agrobacterium tumefaciens* GV3101 strain and then transformed into the WT using the floral-dip method (Clough and Bent, 1998). All transgenic lines used in this study were T3 homozygous plants with a single-copy insertion.

The construct for the overexpression of *SOS1* was generated as described by Shi et al. (2003) and then transformed

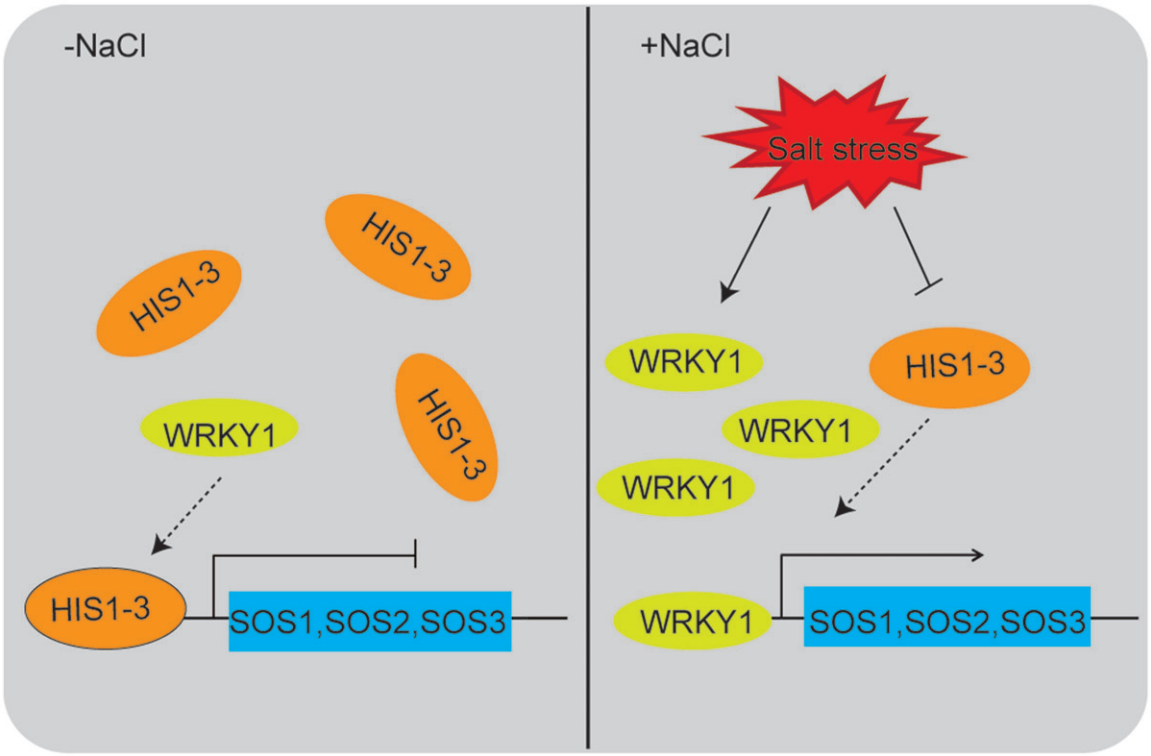

Figure 10 A working model for the regulation of salt stress in Arabidopsis by HIS1-3 and WRKY1. HIS1-3 is a negative regulator under salt stress. It inhibits the expression of *SOS1*, *SOS2*, and *SOS3* by binding to the W-boxes of the *SOS1*, *SOS2*, and *SOS3* promoter sequences under conditions of no stress, which are also the binding sites of WRKY1 on these genes. Once plants are subjected to salt stress, the expression of HIS1-3 is decreased, leading to the exposure of the W-boxes of *SOS1*, *SOS2*, and *SOS3*. Subsequently, the WRKY1 protein activates the expression of these genes and regulates the Na^+/K^+ balance in the plant in order to respond to salt stress.

into the *wrky1-1* mutant (named *wrky1/35S::SOS1*). The *his1-3/sos1* and *his1-3/wrky1* double mutants were obtained by crossing *sos1* or *wrky1* to *his1-3*. All homozygous lines of the double mutant were selected for further study.

RNA extraction and RT-qPCR analysis

Six-week-old WT seedlings were used for analyzing the expression of *HIS1-3* and *WRKY1* in the different tissues. Treated and nontreated 2-week-old seedlings of the mutants and overexpression lines were used for analyzing the expression of *HIS1-3*, *WRKY1*, *SOS1*, *SOS2*, and *SOS3*. The total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and reverse transcribed with PrimeScript Reverse Transcriptase. The qPCR reaction was performed according to the instructions provided for the Bio-Rad iCycleriQ system (Bio-Rad Laboratories, Hercules, CA, USA) and using platinum SYBR Green qPCR SuperMix-UDG (Invitrogen). The PCR amplifications for each sample were quantified at least in triplicate and normalized using *Actin8* as an internal control. The primers used are listed in Supplemental Table S1.

GUS staining

The full-length promoters of *HIS1-3* and *WRKY1* were amplified by PCR from Arabidopsis genomic DNA and then cloned into the transformation vector pXB93 (pART27 with expanded restriction sites and the GUS reporter) using specific primers (Supplemental Table S1). The plasmids of *ProHIS1-3::GUS* and *ProWRKY1::GUS* were induced into the *Agrobacterium* strain GV3101 and transformed into Arabidopsis. Homozygous transgenic lines were germinated on MS medium for 7 d and then treated with 100-mM NaCl for 6 h. All samples were collected and stained with 5-bromo4-chloro-3-indoyl-β-D-glucuronide (X-Gluc) in the dark at 37°C for 12 h (Fan et al., 2013). The samples were bleached in 75% (v/v) alcohol for 2–6 d to remove chlorophyll and then photographed under a Volume microscope (Zeiss Axio Zoom.V16, Germany).

Analysis of Na^+ and K^+ content

Seeds of the WT, mutants, and overexpression lines were germinated on MS medium with 100-mM NaCl for 2 weeks, and then, the roots and aboveground parts were collected separately to determine the ion content. The contents of Na^+ and K^+ in the roots and aboveground parts were detected by atomic absorption spectrometry (Solar 316 M6; Thermo Fisher Scientific, Waltham, MA, USA).

Transient expression assay

The full-length promoters of *SOS1*, *SOS2*, and *SOS3* were amplified by PCR from Arabidopsis genomic DNA and then cloned into the transformation vector pXB93 (pART27 with

expanded restriction sites and GUS reporter) using specific primers (Supplemental Table S1). Transient expression assays were performed as described (Sparkes et al., 2006; Chen et al., 2009). The *A. tumefaciens* cells were collected by centrifugation and suspended to an optical density (600 nm) of 0.1 using a solution containing 50 mM of 2-(N-morpholino)ethanesulfonic acid, 5 g/L of D-Glc, 2 mM of Na_3PO_4, and 0.1 mM of acetosyringone. The constructs of Pro*SOS1*:GUS, Pro*SOS2*:GUS, or Pro*SOS3*:GUS were then co-transformed into *N. benthamiana* epidermal cells with 35S::*HIS1-3* or 35S::*WRKY1* using a needle-free syringe. The GUS activity was assessed at 48 h after injection and quantified by the fluorometric 4-methylumbelliferyl-β-D-glucuronide (MUG) assay, as described by Xu et al. (2006).

ChIP

The ChIP experiments were performed as described in published protocols (Kaufmann et al., 2010) with an anti-GFP antibody (Abmart, Shanghai, China). In brief, 3 g of 10-d-old *35S:WRKY1::GFP* seedlings, *35S:HIS1-3::GFP* seedlings, and *35S::GFP* seedlings were collected and cross-linked with 35 mL of MC buffer (1 M of Na_3PO_4, 5 M of NaCl, 0.1 M of sucrose), to which 1 mL of 37% (v/v) formaldehyde was added, and the samples were placed under vacuum for 30 min. A total of 4 mL of 1.25-M Gly was added to stop the cross-linking. After rinsing the seedlings with MC buffer, the tissues were ground with liquid nitrogen and resuspended in 15 mL of extraction buffer I (1 M of Na_3PO_4, 5 M of NaCl, 1 M of hexylene glycol, 10 mM of β-mercaptoethanol, and 1× protease inhibitor) and then filtered through a nylon mesh. The filtrate was centrifuged at 1,000*g* at 4°C for 20 min. The pellet was resuspended in 5 mL of extraction buffer II (1 M of Na_3PO_4, 5 M of NaCl, 1 M of hexylene glycol, 10 mM of β-mercaptoethanol, 20 mM of $MgCl_2$, 0.5% (v/v) Triton X-100, and 1× protease inhibitor) and centrifuged at 1,000*g* and 4°C for 20 min. The pellet was resuspended in 5 mL of extraction buffer III (1 M of Na_3PO_4, 5 M of NaCl, 10 mM of β-mercaptoethanol, and 1× protease inhibitor) and loaded on top of an equal amount of clean extraction buffer III, and then centrifuged at 1000*g* for 10 min. The crude nuclear pellet was resuspended and sonicated with ultrasonic cell disruption. The sonicated chromatin was centrifuged, and the insoluble pellet was discarded. Then, the samples were divided into three parts: one part was used for DNA, and the other two parts were incubated with an anti-GFP antibody. One microliter of anti-GFP-specific monoclonal antibody was added to the chromatin solution and incubated for 1 h at 4°C. The immunocomplexes were extracted by incubating with 40 μL of 50% (v/v) protein A–Sepharose beads for 1 h at 4°C. After several washes, the immunocomplex was eluted twice from the beads with 100 μL of elution buffer (0.1 M of glycine, 5 M of NaCl, 0.05% (v/v) Tween-20) and then reversed cross-linked. All proteins were removed. The relative concentrations of the DNA fragments were analyzed by qPCR with the different primers listed in Supplemental Table S1.

BiFC

The full length CDS of *HIS1-3* were amplified and inserted into the plant binary vector pBSPYCE to generate HIS1-3-CYFP; the full-length CDS of *WRKY1* were amplified and inserted into the plant binary vector pBSPYNE to generate WRKY1-NYFP. BiFC assay was carried out via the *Agrobacterium*-mediated transient expression of WRKY1-NYFP with HIS-CYFP in *N. benthamiana* leaves as described previously (Walter et al., 2004). Two 4-week-old *N. benthamiana* leaves were injected with *Agrobacterium* GV3101strains containing individual BiFC construct pairs and a binary plasmid expressing the p19 protein to suppress gene silencing. After 4′,6-diamidino-2-phenylindole staining, the epidermal cell layers were examined using confocal microscope. The acquire YFP signals using the LSM 710, 488 nm was used for excitation and fluorescence was detected at a 410–550 nm range.

Yeast two-hybrid assays

Arabidopsis *WRKY 1* was cloned into the pGADT7 vector to generate AD-WRKY1, whereas HIS1-3 cDNA was cloned into the pGBKT7 vector to generate BD-HIS1-3. The corresponding combinations were transformed into yeast (AH109), respectively. The mated yeast cells were selected on SD–Leu–Trp–His plates which contain 20-mM 3-Amino-1,2,4-triazole.

Y1H assays

To generate AD-HIS1-3 and AD-WRKY1, the full-length CDS of *HIS1-3* and *WRKY1* were inserted into pJG4-5 vector (Clontech, Mountain View, CA, USA). The Y1H assay was performed according to the Yeast Protocols Handbook (Clontech). Briefly, the AD fusion constructs were co-transformed with various LacZ reporter plasmids into yeast strain EGY48. Transformants were grown on SD/Trp-Uradropout plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside for blue color development.

EMSA

The full-length CDS of *HIS1-3* and *WRKY1* were cloned into the pMAL-c2 vector and MBP-HIS1-3 and MBP-WRKY1 fusion protein were expressed in *Escherichia coli Rosseta2* strain. Free probes containing W-box motifs were synthesized with biotin label at the 5′–end by Sangon Biotech (Sangon Biotech, Shanghai, China). EMSA was performed using a LightShift EMSA Optimization and Control Kit (Thermo Fisher Scientific, Waltham, MA, USA). The results were detected using a CCD camera system (Image Quant LAS 4000).

Statistical analyses

The experiment used a completely random design. The above indicators were repeated for 3 times. The data were analyzed by SPSS version l9.0. Tukey's multiple comparison method was used for difference significance analysis, and the significance level was 0.05.

Accession numbers

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession numbers HIS1-3 (*At2g18050*), WRKY1 (*At2g 04880*), SOS1 (*At2g 01980*), SOS2 (*At5g 35410*), and SOS3 (*At5g 24270*).

Supplemental data

The following materials are available in the online version of this article.

Supplemental Figure S1. Expression profile of the *HIS1-3* gene.

Supplemental Figure S2. Responses of the *his1-3* mutants to KCl, LiCl, and mannitol.

Supplemental Figure S3. Phenotypes of the WT, *his1-3* mutants, and HIS1-3-OE lines in soil-filled pots.

Supplemental Figure S4. Tissue-specific expression of *WRKY1*.

Supplemental Figure S5. Phenotypes of the WT, *wrky1* mutants, and WRKY1-OE lines in soil-filled pots.

Supplemental Figure S6. Phenotype test of the WT and *wrky1* mutant seedlings in response to KCl, LiCl, and mannitol.

Supplemental Figure S7. The relationship between HIS1-3 and WRKY1.

Supplemental Figure S8. WRKY1 exhibits self-activation by Y1H assay.

Supplemental Figure S9. Identification of transgenic plants.

Supplemental Figure S10. No specific competition at the promoter regions of *SOS3* genes between HIS1-3 and WRKY1 by reciprocal competitive EMSA.

Supplemental Table S1. Primers used for cloning, qPCR, and ChIP assay.

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Conflict of interest statement. The authors declare no conflict of interest.

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