SbbHLH85, a bHLH member, modulates resilience to salt stress by regulating root hair growth in sorghum

Yushuang Song · Simin Li · Yi Sui · Hongxiang Zheng · Guoliang Han · Xi Sun · Wenjing Yang · Hailian Wang · Kunyang Zhuang · Fanying Kong · Qingwei Meng · Na Sui

Received: 11 March 2021 / Accepted: 29 September 2021 / Published online: 11 October 2021
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2021

Abstract

bHLH family proteins play an important role in plant stress response. However, the molecular mechanism regulating the salt response of bHLH is largely unknown. This study aimed to investigate the function and regulating mechanism of the sweet sorghum SbbHLH85 during salt stress. The results showed that SbbHLH85 was different from its homologs in other species. Also, it was a new atypical bHLH transcription factor and a key gene for root development in sweet sorghum. The overexpression of SbbHLH85 resulted in significantly increased number and length of root hairs via ABA and auxin signaling pathways, increasing the absorption of Na+. Thus, SbbHLH85 plays a negative regulatory role in the salt tolerance of sorghum. We identified a potential interaction partner of SbbHLH85, which was phosphate transporter chaperone PHF1 and modulated the distribution of phosphate, through screening a yeast two-hybrid library. Both yeast two-hybrid and BiFC experiments confirmed the interaction between SbbHLH85 and PHF1. The overexpression of SbbHLH85 led to a decrease in the expression of PHF1 as well as the content of Pi. Based on these results, we suggested that the increase in the Na+ content and the decrease in the Pi content resulted in the salt sensitivity of transgenic sorghum.

Introduction

The problem of soil salinization exists worldwide. Salt stress caused by soil salinization affects the growth, development, and harvest yield of plants; in serious cases, salt stress even leads to plant death (Asano et al. 2012; Sui et al. 2017). The most cost-effective way to use salinized soil is to develop salt-tolerant crop varieties based on the well-established knowledge of molecular mechanisms underlying plant salt resistance, thereby promoting agricultural production. Sweet sorghum (Sorghum bicolor (L.) Moench) is a crop with high sugar content and high biomass yield, which has the laudatory name of second-generation sugarcane (Sui et al. 2015). In today’s world, sweet sorghum is an important food, feed, and energy crop. Besides high biomass, sweet sorghum also has tolerance to various abiotic stresses, especially to salt (Schnippenkoetter et al. 2017). Many genes have been found to play an important role in regulating salt tolerance in sweet sorghum (Song et al. 2020; Wang et al. 2014b; Yang et al. 2018; Zheng et al. 2011). It is believed that the outcomes from studying the characteristics of these genes can be applied to improve the salt tolerance in other crops, which also is of great significance for understanding plant growth and development in saline alkali environments.

Salt stress increases the level of sodium ion (Na+) and potassium ion (K+), thus reducing the level of nutrient elements (e.g., N and P) and causing the imbalance of nutrient distribution in plants (Colla et al. 2008; Wang et al. 2020). The phosphorus content in pomelo and orange decreases significantly in response to salt stress (Ma et al. 2005). Phosphorus (P) is an essential element required for plant growth...
and development (Dalen 2012; Miao et al. 2009). Phosphorus transfer between different tissues and subcellular organelles in plants is facilitated by a series of transport proteins having phosphorus transfer activity (Deng et al. 2017). The amount of phosphotransporters on the plasma membrane directly determines the absorption and distribution efficiency of phosphorus in plants (Mudge et al. 2002). PHOSPHATE TRANSPORTER 1 (PHT1) encodes an inorganic phosphate (Pi) transporter, which is regulated by the plant-specific phosphate transport chaperone PHF1 during its transport to the plasma membrane and plays a fundamental role in the acquisition and reactivation of Pi (González et al. 2005; Shin et al. 2004).

The bHLH transcription factor proteins have a highly conserved basic/helix-loop-helix special domain, namely the bHLH domain (Li et al. 2018; Maia et al. 2012; Yao et al. 2018). bHLH transcription factors are involved in salt stress response (Chen et al. 2018, 2017; Zhou et al. 2009). The expression of both AthHLH92 and OrbHLH001 was induced by salt, and their overexpression improved salt tolerance in plants (Jiang et al. 2009; Li et al. 2010). When the bHLH transcription factor AtNIG1 was knocked out, the resulting mutant became more sensitive to salt stress in Arabidopsis. AtNIG1 regulated salt tolerance by binding specifically to E-box sequences in the promoter regions of many salt stress-related genes (Kim and Kim, 2006).

Studies showed that plant bHLH transcription factors were also involved in root hair formation (Gajewska et al. 2018; Yan et al. 2014). The root hair is a tubular protrusion formed by the extension of root-specific epidermal cells, which contains enzymes and nutrient transporters involved in nutrient absorption (Menand et al. 2007; Wei and Li 2018). Root hairs increase the contact area between plants and soil and determine the efficiency of root absorption of water and nutrients (Krasilnikoff et al. 2003). The Arabidopsis bHLH transcription factors GLABRA3 and ENHANCER OF GLABRA3 have redundant functions. The number of root hairs decreased slightly in the single mutants of these two genes, but decreased significantly in the double mutants (Bernhardt et al. 2005). The polar growth of root hairs is initially triggered by RHD6/RLS1 of the bHLH family, and then, its elongation is activated by the expression of RSL4/RLS2 (Vijayakumar et al. 2016). RSL4 is essential for root hair elongation in Arabidopsis and controls the final root hair cell size (Zhu et al. 2020). RSL2 affects reactive oxygen species (ROS) production and root hair growth (Rymen et al. 2017).

In this study, we first cloned a bHLH gene, SbbHLH85, in sweet sorghum in M-81E, which was induced by salt stress and ABA (Liu et al. 2015a). Some reports showed the involvement of the bHLH transcription factor in salt stress response and root hair formation. We first overexpressed SbbHLH85 in sorghum and Arabidopsis to investigate the molecular mechanism underlying the regulation of root hair development by SbbHLH85 in response to salt stress in sweet sorghum. The results showed that, different from its homologs in other species, the sorghum SbbHLH85 was a new atypical bHLH transcription factor and a key gene for root development in sorghum. The overexpression of SbbHLH85 in sorghum and Arabidopsis significantly increased the number and length of root hairs. However, salt resistance was significantly lower in overexpression lines. The overexpression of SbbHLH85 can influence the expression of the genes involved in ABA and auxin signal transduction (PYL and PIN3), peroxidase (PERs), root hair development, and receptor-like proteins (RLKs). In addition, SbbHLH85 interacts directly with SbPHF1, a phosphate transporter chaperone protein in sorghum, affecting the transport of Pi. Based on these results, we suggested that SbbHLH85 participated in regulating ABA and auxin signal transduction pathways and distribution of nutrients to affect the development of root hairs, thus affecting the absorption of Na⁺ and the content of ROS and mediating plant salt response.

Materials and methods

Plant materials and growth conditions

In this study, wild-type Arabidopsis (WT) was used as a control. The WT and mutant seeds were evenly seeded on 1/2 Murashige and Skoog (MS) medium with corresponding antibiotics; the composition of 1/2 MS medium was as described previously (Chen et al. 2010). The culture dish was placed at low temperature for 3 days and cultured in the tissue culture room (22 °C-16-h light/18 °C-8-h dark), and the screened positive seedlings were transferred to nutrient soil for further cultivation until the seeds matured. The harvested seeds were seeded using the same method in 1/2 MS medium with salt, and the phenotype of the plants was observed.

Sweet sorghum cultivar M-81E was used in this experiment. The seeds of sweet sorghum were cultured in the sand. Tap water was irrigated before emergence, and 1/2 Hoagland nutrient solution was added every day after emergence. When the seedlings grew to the four-leaf stage, Hoagland nutrient solution containing 0, 50, 100, 150, and 200 mM NaCl was applied to sweet sorghum under salt stress.

Cloning, bioinformatics, and expression analysis of SbbHLH85

The SbbHLH85 CDS sequence was obtained by comparing the AtRSL2 sequence on the Ensembl website (http://ensembl.gramene.org/). Online website NCBI (https://www.ncbi.
Subcellular localization of SbbHLH85 protein

The KpnI and BamHI sites were used to insert cloned SbbHLH85 CDS into pCAMBIA1300-35S-sGFP vectors to produce 35S: SbbHLH85-green fluorescent protein (GFP) constructs. These were transferred into Agrobacterium tumefaciens GV3101 and used to infect tobacco epidermal cells. GFP signal was observed using a two-photon laser scanning confocal microscope (TCS S8MP, Leica, Germany). 35S: GFP transgenic tobacco was used as a localization control for expression in the cytoplasm/nucleus. The primers are listed in Supplementary Table 1.

Generation of transgenic plants

For the overexpression of SbbHLH85 in Arabidopsis, SbbHLH85 genes were linked to pROKII vectors using the XbaI and KpnI restriction sites and transferred to A. tumefaciens GV3101 to obtain SbbHLH85-overexpressing plants by infecting WT inflorescence. The transgenic Arabidopsis plants were screened with kanamycin (50 g/mL) and verified using RT-PCR. The full-length cDNA of SbbHLH85 was inserted into the pMWB110 vector through BamHI and KpnI sites to obtain the pMWB110-SbbHLH85 vector. The pMWB110-SbbHLH85 vector was introduced into sorghum by Agrobacterium-mediated transformation. PCR, herbicide (glufosinate) treatment, and bar rapid detection kit were used to detect transgenic plants. The primers used are listed in Supplementary Table 1.

Agrobacterium-mediated genetic transformation in sorghum Tx430

This method was based on the transformation protocol in sorghum (Do et al. 2016), with some modifications. Briefly, the husks were removed 12–16 days after pollination, and the immature seeds were sterilized in 70% (v/v) ethanol for 5 min and then in 12% (v/v) bleach for 10–15 min. Finally, the seeds were rinsed three times with autoclaved water. Immature embryos, 1.0–1.5 mm in length, were isolated from immature seeds and placed in infection liquid medium (0.44 g/L Murashige–Skoog salts, 1×B5 vitamins, 68 g/L sucrose, 36 g/L glucose, 1 g/L asparagine, 1 g/L casamino acids, 0.2 g/L cysteine, 2 mg/L 2,4-dichlorophenoxyacetic acid, and 200 μM acetosyringone, pH 5.2). The vector 85-OE and 85-CR were introduced into the Agrobacterium strain EHA105 by heat shock. Positive EHA105 cells were cultured in YEB medium (5 g/L beef extract, 5 g/L peptone, 1 g/L yeast extract, 5 g/L sucrose, and 10 mM magnesium sulfate, pH 7.0) overnight until optical density at 600 nm (OD_{600 nm}) was 1.0. About 100–200 immature embryos were subjected to heat treatment (43 °C for 3 min) before inoculation with 1 mL of bacterial cells for about 5 min. After infection, embryos were transferred to the co-cultivation medium (infection medium with 8 g/L agarose). The scutellum was faced up and co-cultivated at 25 °C in the dark for 2–3 days. After co-cultivation, the embryos were subcultured on CIM (Do et al. 2016) resting medium containing 250 mg/L timentin at 28 °C in the dark for 6–7 days and then transferred to the CIM selection medium containing 50 mg/L paromycin for 85-CR or 5 mg/L bialaphos for 85-OE for about 10 days. Another 20-day selection was performed in the same medium until resistant calli were formed, and then transferred into SM medium (Do et al. 2016) (250 mg/L timentin, 50 mg/L paromycin or 5 mg/L bialaphos, and 8 g/L agarose, pH 5.7) at 28 °C under 16-h light: 8-h dark conditions for 2–3 weeks. Elongated shoots, 1–3 cm in length, were transferred to the rooting medium [half-strength Murashige–Skoog basal salt with vitamins, 30 g/L sucrose, 0.1 g/L myo-inositol, and 2.6 g/L Gelzan (gellan gum), pH 5.6] for rooting. Putative transgenic plants with healthy roots were transferred into pots before moving to the field. The primers are listed in Supplementary Table 1.

Quantification of biomass, MDA content, and Na\(^{+}\) and K\(^{+}\) contents

For measuring biomass, we first took whole plants out of the pot, washed them, weighed them, and recorded the fresh weight. Then, the plants were dried in an oven for 7 days and then weighed again to record dry weight (105 °C, 15 min and 65 °C, 7 days). Fresh weight and dry weight were measured for each treatment using five replicates (Song et al. 2019). The MDA contents were determined as described by Ma (Ma et al. 2013): The leaves of 0.2 g of each line were weighed, and 5 mL of 0.1% trichloroacetic acid was added for grinding. The homogenate was mixed with 5 mL of 0.5% thiobarbituric acid (TBA), boiled for 10 min, taken out, cooled to room temperature, and centrifuged at 3000 rpm for 15 min. The supernatant was taken, and the volume was measured. The absorbance of the solution at wavelengths 532 nm and 600 nm was determined using an ultraviolet (UV) spectrophotometer. The blank control contained 0.5% TBA solution. The Na\(^{+}\) and K\(^{+}\) contents were determined by the specific steps described by Song: Each line was treated
with 0 and 100 mM NaCl. After 10 days, 0.3 g roots were placed in 5 mL of ddH2O and boiled for 2 h. The plant residue was filtered, and the volume was made up to 10 mL. The contents of Na⁺ and K⁺ of each treated line were determined using a flame spectrophotometer (Song et al. 2020).

### Diaminobenzidine and nitroblue tetrazolium staining

*Arabidopsis* seedlings were grown for about a month and treated with 0 or 100 mM NaCl in 1/2 concentration Hoagland solution for 48 h. The rosette leaves of WT and overexpressing plants with the same growth were put in diaminobenzidine (DAB) or nitroblue tetrazolium dye solution and placed in the dark for more than 12 h. They were then put in the bleach (3:1:1 ethanol: acetic acid: glycerol) and boiled in boiling water for 10–15 min for decolorization. The color change in the blade was observed, and images were taken.

### Root hair experiment

The homologous genes of *SbbHLH85* are *AtRLS2* and *AtRSL4* in *Arabidopsis*. *AtRSL2* is closely related to root hair development and elongation, while *AtRSL4* is a functional redundancy gene. Therefore, we selected WT, M-81E, over-expressing (At-OX4, At-OX13, Sb-OX1, Sb-OX3, Sb-OX6, and Sb-OX7), *RSL2* mutant (*rsl2-1* and *rsl2-3*), *RSL4* mutant (*rsl4*), and *RSL2 and RSL4* double mutant (*rsl2rsl4*) lines as experimental subjects. The main root hair development and the root hair elongation were tested. Each *Arabidopsis* seed was on demand in 1/2 MS medium. The sorghum seeds were hydroponic. The development and elongation of root hairs at the root tip 5 mm from the main root of each line were observed under an electron microscope after 7 days in the lab. The microscope magnified the images by 40 times.

### RNA-seq assay

The roots of WT, overexpressing, mutant, and double mutant lines were collected and preserved in liquid nitrogen. RNA-seq and differential gene expression analysis were carried out using Illumina NovaSeq 6000 (Biomarker Technologies, Beijing, China). The transcriptome analysis of 24 samples was completed, and 156.37 Gb of clean data were obtained. The HISAT2 system was used to sequence the clean reads of each sample with the designated reference genome, and the reads were assembled by StringTie comparison. The reads on the sample were assembled and quantified by StringTie comparison after the comparison and analysis. The gene expression was analyzed based on the comparison results. StringTie used fragments per kilobase of script per million fragments mapped as an index to measure the expression level of transcripts or genes. The differentially expressed genes were identified according to their expression levels in different samples, and Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and Cluster of Orthologous Groups of proteins (KOG) databases were used for functional annotation and enrichment analysis. KEGG pathway analysis was carried out for the common differentially expressed genes in each comparison group. The heat map clustering analysis was performed for the expression level of selected genes, which was completed on the platform of BMKCloud.

### RT-PCR validation of genes related to plant salt resistance and root hair development

We extracted RNA from the root tissues of WT, At-OX13, *rsl2-3*, and *rsl2rsl4* lines, which had been growing for about 1 month, and reverse-transcribed it into cDNA, followed by RT-PCR. The primers used are listed in Supplementary Table 2. In sorghum, M-81E and Sb-OX1 were treated with 0 mM and 100 mM NaCl for 48 h. Then, RNA was extracted and transformed into cDNA, following which RT-PCR was performed. The primers used are listed in Supplementary Table 3.

### Yeast two-hybrid experiment

The CDs of *SbbHLH85* were cloned into the bait vector pGBK7T7 to obtain *SbbHLH85*-BD and then transformed into yeast strain Y2HGold (Clontech). After detecting that the gene had no self-activating activity, we constructed a yeast two-hybrid library of sorghum, and hybridized and screened it according to the matching scheme described in Clontech's matchmaker gold yeast two-hybrid user's manual. After screening, the interaction between *SbPHF1* and *SbbHLH85* was verified by the yeast two-hybrid experiment. The full-length coding sequence (CDS) of *SbPHF1* was cloned into pGADT7 to obtain *SbPHF1*-AD, and *SbbHLH85*-BD and *SbPHF1*-AD were co-transformed into Y2HGold. Then, the growth of the colony on the corresponding medium was observed. The primers used in the yeast two-hybrid experiment are listed in Supplementary Table 1.

### BiFC experiment

The CDs of *SbbHLH85* and the N-terminal of pSPYNE-35S were fused to obtain *SbbHLH85*-N-YFP, while the CDs of *SbPHF1*, and the C-terminal of pSPYNE-35S were fused to obtain *SbPHF1*-C-YFP. The obtained plasmid was introduced into *A. tumefaciens* (GV3101), and transient transformation was used to infiltrate the tobacco. The fluorescence...
was observed under a confocal laser scanning microscope (Olympus) after 48 h of normal culture. The primers of BiFC are listed in Supplementary Table 1.

Measurement of the phosphorus (Pi) content

To measure the Pi content, we first washed the sweet sorghum leaf sheaths. The surface moisture was dried, and then, 0.3 g of the sheaths were weighed and ground in a mortar. Some quartz sand and 5 mL of distilled water were added; each treatment was performed in five replicates. The homogenate was transferred to a 25-mL volumetric flask. The residue was washed in the mortar and volumed to the scale; 10 mL of the supernatant of the mixed solution was centrifuged at 6000 rpm for 15 min. The absorbance of the solution at wavelength 660 nm was determined using a UV spectrophotometer. The measured absorbance value was substituted into the standard curve to calculate the Pi content.

Statistical analysis

The statistical results were described as mean ± standard deviation. SPSS ver. 17.0 statistical software was used to analyze the data. One-way analysis of variance was used as the designated package. Different letters indicated a significant difference in the average (0.05) of the Duncan test.

Accession numbers

The sequence data in this paper can be found in TAIR, NCBI or Ensembl database. The mutant numbers are as follows: rsl2-1 (SALK_048849), rsl2-2 (SALK_048857), rsl2-3 (SALK_101872), rsl2-4 (SALK_143203). The accession numbers are as follows: SbbHLH85 (SORBI_3008G147800), SbPHF1 (SORBI_3002G060900), SbPYL4 (XM_002465362), SbPIN3 (XM_002436716), SbRSH2 (XM_021465244), SbRSL4 (XM_002464182), SbRHL1 (XM_021450304), SbPER3 (XM_002437414), SbGLO1 (XM_002466296), SbPER4 (XM_002441658), SbPER35 (XM_002466362), SbRLK1 (XM_021446241), SbRLK2 (XM_021466234), SbRLK8 (XM_002455281), AtPIN3 (AT1G70940), AtSAUR50 (AT4G34760), AtPYL6 (AT2G40330), AtPRX33 (PRX33), AtPRX37 (AT4G08770), AtRCI3 (AT1G05260), RSL4 (AT1G27740), RHL1 (AT1G48380), RHS18 (AT5G22410), RHS3 (AT1G54130), RHS15 (AT4G25220), RHS19 (AT5G67400), RHD3 (AT3G13870), RHS17 (AT4G38390), RHD6 (AT1G66470), RHS10 (AT1G70460), RHS12 (AT3G10710), CLH1 (AT1G19670), DGK5 (AT2G20900), CYP97C1 (AT3G53130), OSM34 (AT4G11650), PR4 (AT3G04720), PDC1 (AT4G33070).

Results

SbbHLH85 was a salt stress–responsive gene

For preliminary characterization of the function of SbbHLH85, we studied its conserved domain, expression pattern, and subcellular localization. First, the phylogenetic tree analysis based on the protein sequence showed that SbbHLH85 was closely related to A0A2S3HF40 in Panicum hallii and A0A3L6RB2 in Panicum miliaceum. SbbHLH85 shared 33.15% and 32.18% homology with the Arabidopsis genes AtRSL2 and AtRSL4, respectively (Fig. 1a). After analyzing the domain structure, we found that all aforementioned proteins had a bHLH domain at the C end (Fig. 1b). The SbbHLH85 gene contained a 951-bp coding sequence that encoded 316 amino acids. Different from RSL2 and RSL4, the SbbHLH85 protein comprised a bHLH domain between amino acids 238 and 287, which contained a A4-R7 motif harboring an alanine at the fourth position and arginine at the seventh position (Fig. 1c and Supplementary Fig. 1). The SbbHLH85 had a unique A4-R7 motif and hence could not directly bind to the promoter of the downstream genes (Toledo-Ortiz et al. 2003). The expression pattern of SbbHLH85 was further explored by measuring the relative abundance of SbbHLH85 in the roots of M-81E under different salt stresses. RT-PCR results showed that the expression of SbbHLH85 in sorghum roots decreased with the increase in the salt concentration, dropping to the lowest level at 100 mM NaCl (Fig. 1d). The subcellular distribution of SbbHLH85 was examined by fusing SbbHLH85 with GFP. In the lower epidermal cells of tobacco, signals were detected in the nucleus (Fig. 1e). Therefore, SbbHLH85 was a bHLH transcription factor negatively induced by salt and localized in the nucleus.

SbbHLH85 actively regulated the development of root hairs

We generated SbbHLH85-overexpressing lines At-OX4 and At-OX13 controlled by the CaMV 35S promoter in Arabidopsis to explore the effect of SbbHLH85 on root hair development (Supplementary Fig. 2). We used the AtRSL2 mutants rsl2-1 and rsl2-3 and the AtRSL4 mutant rsl4 to test the effect of SbbHLH85 on root development. All the Arabidopsis mutants were purchased from the TAIR website (https://www.arabidopsis.org/). The double mutant rsl2rsl4 was provided as a gift by Professor
Hongwei Guo of Southern University of Science and Technology. Subsequently, the complementing lines Crsl2-1, Crsl2-3, Crsl4, and Crsl2rsl4 were created by expressing SbbHLH85 in rsl2-1, rsl2-3, rsl4, and rsl2rsl4, respectively. Figure 2a shows that the number and length of root hairs in the overexpressing lines were the largest and the longest, whereas the control mutants rsl2-1, rsl2-3, and rsl4 had fewer and shorter root hairs. Further, no root hairs were observed in the double mutant rsl2rsl4. Thus, SbbHLH85 could complement the root hair defect in the single and double mutants (Fig. 2a–c).

We generated SbbHLH85-overexpressing lines controlled by the maize ubiquitin promoter in Tx430 (a sorghum inbred line) background and detected the relative expression of SbbHLH85 in each line by RT-PCR to further investigate the role of SbbHLH85 during salt stress in sorghum. Compared with the wild type, SbbHLH85 was highly expressed in Sb-OX1, Sb-OX3, Sb-OX6, and Sb-OX7 (Fig. 2d). It was also found that SbbHLH85 overexpression increased the number and length of root hairs in sorghum (Fig. 2e–g).

**SbbHLH85 negatively regulated the salt stress response**

We tested each of the Arabidopsis overexpressing lines to explore the role of SbbHLH85 in regulating salt stress response in plants. We studied the effects of salt stress on plant growth, including germination rate and seedling survival. For the germinating plants, we sowed seeds of each line in 1/2 MS medium containing 0, 100, or 150 mM NaCl. The plants with overexpression grew more slowly than WT and the mutant lines in the salt medium (Fig. 3a). The germination rate and root length of plants with overexpression were higher than those in WT and mutants irrespective of the nonstressed or salt stress condition (Fig. 3b and c). Under abiotic stress, plants were exposed to ion stress, oxidative stress, and osmotic stress at the same time. Next, we compared the germination rate and root length of each line under different stress conditions. The growth of seedlings in NaCl, LiCl, and mannitol media showed different degrees of slow growth (Supplementary Fig. 3a). Among these, NaCl treatment resulted in the worst growth, the shortest taproot length, and the lowest germination rate (Supplementary Fig. 3b and 3c). We also
studied the salt tolerance of different lines in the seedling stage. Salt stress reduced the biomass of each line, but the fresh weight and dry weight of mutant lines decreased to a lesser extent (Fig. 3d and Supplementary Fig. 4a). The contents of MDA increased more in WT plants and plants with overexpression, but less in mutant lines (Supplementary Fig. 4b). The ion content analysis under salt stress showed that the Na\(^+\) content in overexpressing lines were higher than that in WT control (Fig. 3e), while the trend was opposite for the K\(^+\) content (Fig. 3f). DAB solution can react with H\(_2\)O\(_2\), which reflects the content of H\(_2\)O\(_2\) in plants. Similarly, NBT staining reflects the content of O\(_2\)\(^{-}\). Under salt stress, DAB and NBT staining of overexpressing lines were the deepest, indicating that the overexpression of SbbHLH85 caused more ROS production in plants (Supplementary Fig. 3d and e). We also transformed SbbHLH85 into the mutant lines to investigate whether SbbHLH85 could rescue the salt-sensitive phenotype. The results showed that the physiological indexes of the complemental lines Crsl2-1, Crsl2-3, Crsl4, and Crsl2hrs14 under salt stress recovered (Fig. 4; Supplementary Fig. 4c and d). Overall, these findings suggested that the ectopic overexpression of SbbHLH85 in Arabidopsis might affect the homeostasis of Na\(^+\) and K\(^+\), the content of ROS, and the degree of membrane lipid peroxidation to improve salt tolerance.

We further tested the salt tolerance of the SbbHLH85-overexpressing lines in sorghum to better understand the function of SbbHLH85 in salt stress. Under salt stress, the overexpressing lines in sorghum showed obvious weak growth, yellow leaves, and even curly symptoms (Fig. 5a). Next, we studied the biological processes of salt stress. The biomass accumulation in overexpressing lines was lower than that in M-81E (Fig. 5b and c). Under salt stress, the content of Na\(^+\) in overexpressing lines increased significantly and the content of K\(^+\) decreased (Fig. 5d and e; Supplementary Fig. 5a). Compared with the WT of M-81E, MDA content of overexpressing lines increased significantly after salt treatment (Supplementary Fig. 5b). This was consistent with the conclusion from Arabidopsis that SbbHLH85 participated in salt stress response.
Fig. 3 SbbHLH85 negatively regulated salt tolerance in *Arabidopsis*. a Phenotypic characteristics of *Arabidopsis* lines under different salt concentrations. b Germination rate statistics of *Arabidopsis* lines under different salt concentrations. c Root length statistics of *Arabidopsis* under different salt concentrations. d Biomass of WT and transgenic plants under salt stress. e and f Na$^+$ and K$^+$ contents of WT and transgenic plants under salt stress. (Data are presented as the mean ± SD of five measurements. Means with different letters are significantly different at $P < 0.05$)
Transcriptome analysis of plants with an altered SbbHLH85 level

We analyzed the RNA-seq data of WT, overexpressing, mutant, and double mutant lines under control and NaCl treatments to reveal the molecular mechanism of SbbHLH85 regulating plant response to salt stress and root hair development. We selected 22 representative genes for RT-PCR verification to verify the accuracy of transcriptome, and found that the transcriptome data were of high quality (Supplementary Fig. 6b). In the RNA-seq experiment, we used 48-h NaCl treatment and three biological replicates. Under salt stress, 597 differentially expressed genes (DEGs) were found in WT, 244 DEGs in overexpressing lines, 204 DEGs in single mutants, and 278 DGEs in double mutants. We intersected these DGEs and got 156 DGEs (Supplementary Fig. 6a). Then, we used hierarchical clustering and correlation analysis to analyze the expression patterns of 156 DEGs. The GO analysis showed that these DEGs were involved in biological process, molecular function, and cellular component; of these, they are mainly involved in biological process (Supplementary Fig. 7). The KEGG and KOG analyses showed that these DEGs were mainly related to phenylalanine metabolism, hormone signal transduction, and secondary metabolite metabolism (Supplementary Fig. 6c and 8). After further analysis of the genes in these pathways, we found that they were mainly involved in auxin signaling pathway (AtPIN3 and AtSAUR50), ABA signaling pathway (AtPYL6), and also the production of peroxidase (PER) (AtPRX33, AtPRX37, AtRC13, and AT4G08780) and receptor-like kinases (RLK) (AT4G00970 and AT4G04570) (Fig. 6a) and development of root hairs (Fig. 6b). This was consistent with our previous conclusion that SbbHLH85 participated in salt stress and affected the development of root hair.

SbbHLH85 had a unique A4-R7 motif and hence could not directly bind to the promoter of the downstream genes. We identified all sorghum genes homologous to the Arabidopsis ones to further study the molecular mechanism of SbbHLH85 regulating salt response and root hair development of sorghum. These genes were annotated as auxin signal, ABA signal, root hair development, PER, and RLK. This study identified one ABA signal transduction gene (SbPYL4), one auxin signal transduction gene (SbPIN3), two root hair development–related genes (SbRSH2 and SbRHL1), four PER genes (SbPER3, SbGLO1, SbPER4, and SbPER35), and three RLK-related genes (SbRLK1, SbRLK2, and SbRLK8) in sorghum (Fig. 6c). This was consistent with the previous findings, indicating that SbbHLH85 was involved in root hair development and salt tolerance of sorghum.
SbbHLH85 interacted with SbPHF1 and regulated Pi accumulation to participate in salt tolerance

Increasing lines of evidence showed that the bHLH proteins acted by forming protein complexes with other interacting proteins (Abe et al. 2003; Oh et al. 2007). We used a yeast two-hybrid system to screen for interactors so as to find the potential chaperone of the bHLH85 protein. First, the BD domain of pGBK7 and bHLH85 was fused as the bait. After, we proved that bHLH85 had no self-activating activity (Fig. 7a); the cDNA library containing the prey protein insert fused with GAL4-AD was used to co-transform the yeast cells with SbbHLH85-BD. Three colonies were positive for X-a-gal and ABA. Among these candidates, only the binding of SbPHF1 and SbbHLH85 was stable. SbPHF1 encodes a SEC12-like protein and is homologous to AtPHF1. As a chaperone of phosphate transporter PHT1, SbPHF1 helps transport PHT1 to the plasma membrane. The SbPHF1-AD vector was co-transformed into the Y2H competent state with the SbbHLH85-BD vector to confirm their interaction in yeast. As shown in Fig. 7b, the lines in the experimental group grew normally on the medium, which was mixed with X-a-gal but lacked isoleucine and tryptophan (SD/-L/-T/X), and the colony turned blue. Similarly, the lines in the experimental group grew normally on the medium mixed with X-a-gal and ABA but lacking isoleucine, tryptophan, histidine, and adenine (SD/-L/-T/-H/-Ade/X/A), and the colony turned blue. This finding showed that SbbHLH85 had a strong interaction with SbPHF1 (Fig. 7b).

We then used a BiFC system to verify the aforementioned observation so as to determine whether the interactions also existed in plant cells. The A. tumefaciens lines with SbPHF1-C-YFP and SbbHLH85-N-YFP were mixed and transfected into tobacco leaves. At the same time, the empty carrier was combined with each fusion structure and injected into tobacco leaves. After incubation for 2 days, a YFP signal was observed under a fluorescence microscope. The co-transformed samples showed YFP fluorescence in the nucleus, while none of the control samples showed any YFP signal (Fig. 7c). This demonstrated that SbbHLH85...
and SbPHF1 were co-localized and interacted in the plant nucleus.

We examined the expression of PHF1 and PHT1 and the content of Pi in SbbHLH85-overexpressing and WT sorghum to investigate the effect of the interaction between SbbHLH85 and SbPHF1. The results showed that the overexpression of SbbHLH85 led to a decrease in the expression of PHF1 and PHT1 as well as the content of Pi (Fig. 7d–f).

**Discussion**

How plants find a balance between environmental stress and plant growth is a new and important research topic (Monlau et al. 2015). Many indirect findings have confirmed that the bHLH proteins are involved in salt tolerance in plants (Babitha et al. 2015; Long et al. 2010; Waseem et al. 2019). The overexpression of SbbHLH85 is proved to increase the salt sensitivity of sorghum. The possible mechanism is that SbbHLH85 and SbPHF1 interaction affects the development of root hair through ABA and auxin signal transduction pathways, and hence the distribution of nutrients in plants, thus regulating the salt tolerance of sorghum. This observation enriched the regulation network of the salt stress response, and might be of great significance for improving crop productivity under adverse environmental conditions.
SbbHLH85, different from its homologs in other species, was a new atypical bHLH transcription factor

The bHLH transcription factors belong to the second-largest family of transcription factors in plants, named so for its bHLH domain (Herbst and Kolligs 2008). The family plays a key role in plant growth and abiotic stress (Jiang et al. 2009). In animals, bHLH transcription factors can be divided into six categories: A–F (Wang et al. 2010). The most bHLH transcription factors in plants belong to class B. Only 11% of plant bHLH proteins have a conserved motif: A4-R7, which is not found in animals (Sailsbery and Dean 2012). With the development of molecular biology, an increasing number of bHLH transcription factors have been found, especially the identification of new atypical bHLH transcription factors, which makes this family more diverse. We analyzed the protein sequence of SbbHLH85 and bHLH transcription factors in other species and found that RSL2 and RSL4 were the most closely related genes in Arabidopsis. However, different from RSL2 and RSL4, SbbHLH85 had an A4-R7 motif as shown in Supplementary Fig. 1, indicating that SbbHLH85 was a new atypical bHLH transcription factor. On the one hand, it enriched...
the diversity of the bHLH family in sorghum. On the other hand, it indicated that *SbbHLH85* might play a unique role in promoting salt tolerance and growth of sorghum, which might be of great significance for crop improvement.

**SbbHLH85 affected plant salt tolerance and root hair growth through ABA and auxin signal transduction pathways**

ABA and auxin signal transduction pathways play an important role in regulating plant salt tolerance (Huang et al. 2012; Min et al. 2015; Sun et al. 2016) and root hair growth (Guo et al. 2018, 2019; Sun et al. 2019; Van et al. 2017). For example, *PYR/PYL* act as ABA receptors and bind to PP2C family members represented by *ABI2* and *ABI1* to regulate the phosphorylation of downstream protein kinases and thus initiate the ABA signal transduction pathway to respond to salt stress (Aleman et al. 2016; Fujii et al. 2009; Nishimura et al. 2009; Raghavendra et al. 2010; Umezawa et al. 2009; Wang et al. 2014a, 2017). The PINFORMED (PIN) protein family in the growth hormone regulation pathway is involved in regulating root growth and salt tolerance (Ganguly et al. 2012; Harrison and Masson 2008; Lewis et al. 2011; Liu et al. 2015b; Lv et al. 2018; Schlicht et al. 2008; Sun et al. 2008). In this study, the expression of ABA and auxin pathway genes, peroxidase, receptor-like protein kinase, and root hair development–related genes were changed by *SbbHLH85* overexpression (Fig. 6a–c).

The homolog RSL4/RSL2 of *SbbHLH85* in *Arabidopsis* is related to the development of root hairs; its expression is also regulated by exogenous hormones and environmental changes (Guo et al. 2018, 2019; Menand et al. 2007). Auxin can activate RSL4 expression and control ROS-related genes, including three peroxidases (*PER1*, *PER44*, and *PER73*) (Mangano et al. 2017). Similarly, RSL2 can inhibit the growth-promoting effect of auxin by inhibiting ROS produced by peroxides (PERs) (Kwon et al. 2015; Mangano et al. 2017; Wanapu and Shinmyo 1996). In this study, both *Arabidopsis* and sorghum overexpressing *SbbHLH85* showed a decrease in the ROS content and a phenotype of root hair elongation.

**SbbHLH85 negatively regulated plant salt resistance by affecting plant nutrient distribution**

Under salt stress, the uptake of K⁺ by root cells decreases and the absorption of Na⁺ increases, leading to the imbalance of ion homeostasis (Song et al. 2020). Recent studies have shown that plants can protect themselves by reducing the length and density of root hair and the absorption area of excessive Na⁺ when they sense stress signals (Lv et al. 2018; Wang et al. 2008).

Salt stress can also cause an imbalance of nutrient distribution in plants, including phosphorus (P) (Yang et al. 2003). P is a key element of many biomolecules (nucleic acids, ATP, and phospholipids) in many metabolic pathways; it is one of the important nutrients needed for plant growth and development (Marschner et al. 2005). Phosphate transporters encoded by the phosphate transporter family (PHT1) genes are important proteins for plant acquisition and transport of phosphates (Shin et al. 2004). PHT1:5 is strongly induced in the root, leading to the changes in Pi mobilization between plant roots/ground. Compared with WT, PHT1:5 overexpression enhances root hair formation (Nagarajan 2010).

The transport of the PHT1 protein to the plasma membrane is regulated by plant-specific companion protein PHF1. The *PHF1* gene encodes a plant-specific protein related to the SEC12 protein structure, which is located in the endoplasmic reticulum and is strongly expressed in plant root cuticle, root hair, and its cortical cells (Bayle et al. 2011). In 2005, a mutant of PHF1 was isolated in *Arabidopsis*. Studies on the mutant showed that the mutation of PHF1 reduced the accumulation of *PHT1:1* transporter in the plasma membrane, destroyed the transport of Pi, and reduced the accumulation of Pi. In this study, *SbbHLH85* interacted strongly with *SbPHF1*. Our result suggested that the interaction between *SbbHLH85* and *SbPHF1* might destroy the transportation and accumulation of Pi in plants under salt stress and aggravate the
uneven distribution of Pi caused by high salinity, which greatly reduced the salt resistance of sorghum.

In conclusion, \textit{SbbHLH85}, different from \textit{RSL2} and \textit{RSL4} in \textit{Arabidopsis}, is a necessary gene for root development in sorghum. We then showed the molecular mechanism of \textit{SbbHLH85} regulating the salt response of sorghum by directly interacting with the specific companion protein PHF1, which can affect the distribution of Pi. \textit{SbbHLH85} participates in the ABA and auxin pathway mediated root hair development by affecting the expression of ABA and auxin pathway genes. The increase in the number and length of root hairs can promote the absorption of Na+. The increased Na+ absorption and the decreased Pi content can ultimately result in the salt-sensitive phenotype of sorghum (Fig. 8). In future, it will be an effective measure to improve the salt tolerance of crops by properly reducing the number and length of root hairs in the presence of stress.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00122-021-03960-6.

**Acknowledgements** This research was supported by financial support from the National Key R&D Program of China (2018YFD1000700, 2018YFD1000704, 2019YFD1002703), the National Natural Science Research Foundation of China (31871538, U1906204), Shandong Province Key Research and Development Program (2019GSF107079), the Development Plan for Youth Innovation Team of Shandong Provincial (2019KJE012), the Science and Technology Demonstration Project of “Bohai Granary” of Shandong Province (2019BHL002). We would like to thank Professor Hongwei Guo of Southern University of Science and Technology for providing the double mutant \textit{rsl2rsl4}.

**Author contribution statement** NS and YS planned and designed the research; YS, SL, YS, HZ, WY and XS performed experiments; NS, YS, GH, HW, KZ and FK collected data and carried out all analyses; YS, HZ and NS wrote the paper. NS and QM revised the paper. All authors read and approved the final manuscript.

**Declarations**

**Conflict of interest** The authors declare no competing interests.

**References**

Abe H, Urao T, Ito T, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2003) \textit{Arabidopsis} \textit{AtMYC2} (bHLH) and \textit{AtMYB2} (MYB) function as transcriptional activators in abscisic acid signaling. Plant Cell 15:63–78

Alemán F, Yazaki J, Lee M, Takahashi Y, Kim Ay, Li ZX, Kinoshita T et al (2016) An ABA-increased interaction of the PYL6 ABA receptor with MYC2 transcription factor: a putative link of ABA and JA signaling. Entitite Reports 6:28941

Asano T, Hayashi N, Kobayashi M, Aoki N, Miyao A, Mitsuhashi I, Ichikawa H et al (2012) A rice calcium-dependent protein kinase OsCPK12 oppositely modulates salt-stress tolerance and blast disease resistance. Plant J 69:26–36

Babitha KC, Vemanna RS, Nataraja KN, Udayakumar M (2015) Overexpression of EcbHLH57 transcription factor from Eleusine coracana L. in tobacco confers tolerance to salt, oxidative and drought stress. PLoS ONE 10:e0137098

Bayle V, Arrighi JF, Creff A, Nespolous C, Vialaret J, Rossignol M, Gonzalez E et al (2011) \textit{Arabidopsis thaliana} high-affinity phosphate transporters exhibit multiple levels of posttranslational regulation. Plant Cell 23:1523–1535

Bernhardt C, Zhao MZ, Gonzalez A, Lloyd A, Schiebelbein J (2005) The bHLH genes GL3 and EGL3 participate in an intercellular regulatory circuit that controls cell patterning in the \textit{Arabidopsis} root epidermis. Development 132:291–298

Chen AH, Yang JL, Niu YD, Yang CP, Liu GF, Yu CY, Li CH (2010) High-frequency somatic embryogenesis from germinated zygotenic embryos of Schisandra chinensis and evaluation of the effects of medium strength, sucrose, GA3, and BA on somatic embryo development. Plant Cell Tissue Organ Culture (PCTOC) 102:357–364

Chen HC, Hsieh-Feng V, Liao PC, Cheng WH, Liu LY, Yang YW, Lai MH et al (2017) The function of OshBHLH068 is partially redundant with its homolog, AtBHLH112, in the regulation of the salt stress response but has opposite functions to control flowering in \textit{Arabidopsis}. Plant Mol Biol 94:531–548

Chen, H.C., Cheng, W.H., Hong, C.Y., Chang, Y.S. and Chang, M.C. (2018) The transcription factor OshBHLH035 mediates seed germination and enables seedling recovery from salt stress through ABA-dependent and ABA-independent pathways, respectively. Rice 11

Colla G, Rouphael Y, Cardarelli M, Tullio M, Rivera CM, Rea E (2008) Alleviation of salt stress by arbuscular mycorrhizal in zucchini plants grown at low and high phosphorus concentrations. Biol Fert Soils 44:501–509

Dalen, M.S., (2012) Understanding phosphorus dynamics of two alluvial soils grown with corn at different phosphorus rates. Louisiana State University.

Deng MJ, Wang F, Mao CZ (2017) Plant phosphate transporters and its molecular regulation mechanism. Plant Physiol J 53:377–387

Do PT, Lee H, Moorkkan M, Folk WR, Zhang ZJ (2016) Rapid and efficient agrobacterium-mediated transformation of sorghum (Sorghum bicolor) employing standard binary vectors and bar gene as a selectable marker. Plant Cell Rep 35:2065–2076

Fujii H, Chinnusamy V, Rodrigues A, Rubio S, Antoni R, Park SY, Cutler SR et al (2009) In vitro reconstitution of an abscisic acid signalling pathway. Nature 462:660–664

Gajewksa, P., Janiak, A., Kwasniewski, M., Kedziorski, P. and Szarejko, I. (2018) Forward genetics approach reveals a mutation in bHLH transcription factor-encoding gene as the best candidate for the root hairless phenotype in barley. Front Plant Sci. 9

Ganguly A, Lee SH, Cho HT (2012) Functional identification of the phosphorylation sites of Arabidopsis PIN-FORMED3 for its subcellular localization and biological role. Plant J 71:810–823

González E, Solano R, Rubio V, Leyva A, Paz-Ares J (2005) Phosphate transporter traffic facilitator1 is a plant-specific SEC12-related protein that enables the endoplasmic reticulum exit of a high-affinity phosphate transporter in \textit{Arabidopsis}. Plant Cell 17:3500–3512

Guo Y, Jiang QY, Hu Z, Sun XJ, Fan SJ, Zhang H (2018) Function of the auxin-responsive gene TaSAUR75 under salt and drought stress. Crop J 6:181–190

Guo Y, Xu CB, Sun XJ, Zheng H, Fan SJ, Jiang QY, Zhang H (2019) TaSAUR78 enhances multiple abiotic stress tolerance by regulating the interacting gene TaVDAC1. J Integ Agric 18:2682–2690

Harrison BR, Masson PH (2008) ARL2, ARG1 and PIN3 define a gravity signal transduction pathway in root statocytes. Plant J 53:380–392
Herbst A, Kolligs FT (2008) A conserved domain in the transcription factor IFTP-2B attenuates its activity. Biochem Bioph Res Co 370:327–331

Huang GT, Ma SL, Bai LP, Zhang L, Ma H, Jia P, Liu J et al (2012) Signal transduction during cold, salt, and drought stresses in plants. Mol Biol Rep 39:969–987

Jiang YQ, Yang B, Deyholos MK (2009) Functional characterization of the Arabidopsis bHLH92 transcription factor in abiotic stress. Mol Genet Genomics 282:503–516

Kim JY, Kim HY (2006) Functional analysis of a calcium-binding transcription factor involved in plant salt stress signaling. Febs Lett 580:5251–5256

Krasilnikoff G, Gahoonia T, Nielsen NE (2003) Variation in phosphorus uptake efficiency by genotypes of cowpea (Vigna unguiculata) due to differences in root and root hair length and induced rhizosphere processes. Plant Soil 251:83–91

Kwon T, Sparks JA, Nakashima J, Allen SN, Tang YH, Blancaflor EB (2008) A conserved domain in the transcription factor ITF-2B attenuates its activity. Biochem Bioph Res Co 370:327–331

Maia AM, Silva JHD, Mencalha AL, Abdelhay E (2012) Computational modeling of the bHLH domain of the transcription factor TWIST1 and R118C, S144R and K145E mutants. BMC Bioinform 13:184

Mangano S, Denita-Juarez SP, Choi HS, Marzol E, Hwang Y, Ranocha P, Velasquez SM et al (2017) Molecular link between auxin and ROS-mediated polar growth. P Natl Acad Sci USA 114:5289–5294

Marschner P, Solaiman Z, Rengel Z (2005) Growth, phosphorus uptake, and rhizosphere microbial-community composition of a phosphorus-efficient wheat cultivar in soils differing in pH. J Plant Nutr Soil Sci 168:343–351

Menand B, Yi KK, Jouannic S, Hoffmann L, Ryan E, Linstedt P, Schaefer DG et al (2007) An ancient mechanism controls the development of cells with a rooting function in land plants. Science 316:1477–1480

Miao HY, Zhao JF, Li XJ, Sun ZH, Lu WJ, Gu JT, Guo CJ et al (2009) Cloning and expression of wheat transcription factor gene TaWRKY72-b and its effect on phosphorus use efficiency in transgenic tobacco plants. Acta Agron Sin 35:2029–2036

Min JH, Chung JS, Lee KH, Kim CS (2015) The consens-4 transcription factor, AtCOL4, positively regulates abiotic stress tolerance through an abscisic acid-dependent manner in Arabidopsis. J Integr Plant Biol 57:313–324

Montani F, Sambusiti C, Ficara E, Aboulkas A, Barakat A, Carriere H (2015) New opportunities for agricultural digestate valorization: current situation and perspectives. Energ Environ Sci 8:2600–2621

Mudge SR, Rae AL, Diattof E, Smith FW (2002) Expression analysis suggests novel roles for members of the Ph1 family of phosphate transporters in Arabidopsis. Plant J 31:341–353

Nagarajan, V.K., (2010). Dissecting the roles of MYB-related transcription factor PRF1 and high-affinity Pi transporter PhIt1.5 in pathways regulating phosphate mobilization in Arabidopsis. Purdue University.

Nishimura N, Hitomi K, Arvai AS, Rambo RP, Hitomi C, Cutler SR, Schroeder JJ et al (2009) Structural mechanism of abscisic acid binding and signaling by dimeric PYR1. Science 326:1373–1379

Oh E, Yamaguchi S, Hu JH, Yusuke J, Jung B, Paik I, Lee HS et al (2007) PLS, a phytochrome-interacting bHLH protein, regulates gibberellin responsiveness by binding directly to the GAI and RGA promoters in Arabidopsis seeds. Plant Cell 19:1192–1208

Raghavendra AS, Goungunta VK, Christmann A, Grill E (2010) ABA perception and signalling. Trends Plant Sci 15:395–401

Rymen B, Kawaamura A, Schaefer S, Breuer C, Sugimoto K (2017) ABA suppresses root hair growth via OBP4 transcriptional-regulator repression of the RSL2 promoter. Plant Physiol 173:1945–2016

Salibeyri, J.K., and Dean, R.A. (2012). Accurate discrimination of bHLH domains in plants, animals, and fungi using biologically meaningful sites. BMC Evol. Biol. 12.

Schlicht M, Samajová O, Schachtsschabel D, Mancuso S, Menzel D, Sambusiti C, Ficara E, Aboulkas A, Barakat A, Carriere H (2015) New opportunities for agricultural digestate valorization: current situation and perspectives. Energ Environ Sci 8:2600–2621

Schlicht M, Samajová O, Schachtsschabel D, Mancuso S, Menzel D, Sambusiti C, Ficara E, Aboulkas A, Barakat A, Carriere H (2015) New opportunities for agricultural digestate valorization: current situation and perspectives. Energ Environ Sci 8:2600–2621

Shin H, Shin HS, Dewbre GR, Harrison MJ (2004) Phosphate transport in Arabidopsis: Phl1:1 and Phl1:4 play a major role in phosphate acquisition from both low- and high-phosphate environments. Plant J 31:977–986

Song JS, Li JL, Liu ML, Meng Z, Liu KC, Sui N (2019) Nitrogen increases drought tolerance in maize seedlings. Funct Plant Biol 46:350–359

Song JS, Li JL, Sui N, Han GL, Zhang Y, Guo SJ, Sui N (2020) The sweet sorghum SlWRKY75 is negatively involved in salt response by regulating ion homeostasis. Plant Mol Biol 102:603–614

Song JS, Li JL, Sui N, Han GL, Zhang Y, Guo SJ, Sui N (2020) The sweet sorghum SlWRKY75 is negatively involved in salt response by regulating ion homeostasis. Plant Mol Biol 102:603–614

Sun FF, Zhang WS, Hu HZ, Li B, Wang YN, Zhao YK, Li KX et al (2008) Salt modulates gravity signaling pathway to regulate...
growth direction of primary roots in Arabidopsis. Plant Physiol 146:178–188
Sun L, Wang C, Zhou YF, Ruan YY, Gong X, Zhang J, Huang RD (2016) Inhibition of SbABI5 Expression in Roots by ultra-high endogenous ABA accumulation results in sorghum sensitivity to salt stress. Int J Agric Biol 18:146
Sun HW, Guo XL, Xu FG, Wu DX, Zhang XH, Lou MM, Luo FF et al (2019) Overexpression of OsPIN2 regulates root growth and formation in response to phosphate deficiency in rice. Int J Mol Sci 20:5144
Toledo-Ortiz G, Haq E, Quail PH (2003) The arabidopsis basic/helix-loop-helix transcription factor family. Plant Cell 15:1749–1770
Umezawa T, Sugiyama N, Mizoguchi M, Hayashi S, Myouga F, Yama-guchi-Shinozaki K, Ishihama Y et al (2009) Type 2C protein phosphatases directly regulate abscisic acid-activated protein kinases in Arabidopsis. P Natl Acad Sci USA 106:17588–17593
Van MH, Van DADJ, Stortenbeker N, Angenent GC, Bemer M (2017) Divergent regulation of Arabidopsis SAUR genes: a focus on the SAUR10-clade. BMC Plant Biol 17:245
Vijayakumar P, Datta S, Dolan L (2016) Root hair defective six-like4 (RSL4) promotes root hair elongation by transcriptionally regulating the expression of genes required for cell growth. New Phytol 212:944–953
Wanapu C, Shinmyo A (1996) Cis-regulatory elements of the per-oxidase gene in Arabidopsis thaliana involved in root-specific expression and responsiveness to high-salt stress. Ann Ny Acad Sci 782:107–114
Wang YN, Zhang WS, Li KX, Sun FF, Han CY, Wang YK, Li X (2008) Salt-induced plasticity of root hair development is caused by ion disequilibrium in Arabidopsis thaliana. J Plant Res 121:87–96
Wang Y, Yao Q, Chen KP (2010) Progress of studies on family members and functions of animal bHLH transcription factors. Hereditas 32:307–330
Wang, H., Lin, J., Li, X.G., Wang, Z.H., Chang, Y.H. (2014a). Molecular cloning of PhPYL4 gene and expression analysis of PhPYL4 and PhNCED2 in Pyrus betulaefolia under salt stress. J Fruit sci. 6.
Wang TT, Ren ZJ, Liu ZQ, Feng X, Guo RQ, Li BG, Li LG et al (2014b) SbHKT1;4, a member of the high-affinity potassium transporter gene family from Sorghum bicolor, functions to maintain optimal Na+/K+ balance under Na+ stress. J Integr Plant Biol 56:315–332
Wang T, Li CX, Wu ZH, Jia YC, Wang H, Sun SY, Mao CZ et al (2017) Abscisic acid regulates auxin homeostasis in rice root tips to promote root hair elongation. Front Plant Sci 8:1121
Wang WL, Wang WQ, Wu YZ, Li QX, Zhang GQ, Shi RR, Yang JJ et al (2020) The involvement of wheat U-box E3 ubiquitin ligase TaPUB1 in salt stress tolerance. J Integr Plant Biol 62:631–651
Waseem M, Rong XY, Li ZG (2019) Dissecting the role of a basic helix-loop-helix transcription factor, SbHLH22, under salt and drought stresses in transgenic Solanum lycopersicum L. Front Plant Sci 10:734
Wei Z, Li J (2018) Receptor-like protein kinases: key regulators controlling root hair development in Arabidopsis thaliana. J Integr Plant Biol 60:841–850
Yan A, Wu MJ, Zhao YQ, Zhang AD, Liu BH, Schiefelbein J, Gan YB (2014) Involvement of C2H2 zinc finger proteins in the regulation of epidermal cell fate determination in Arabidopsis. J Integr Plant Biol 56:1112–1117
Yang T, Yan CL, Liang J, Li YH, Tang HH (2003) The nutrients elements distribution in Casuarina equisetifolia seedlings under salt stress. Subtropical Plant Ence 32:1–4
Yang Z, Zheng HX, Wei XC, Song J, Wang BS, Sui N (2018) Transcriptome analysis of sweet sorghum inbred lines differing in salt tolerance provides novel insights into salt exclusion by roots. Plant Soil 430:423–439
Yao XN, Cai YR, Yu DQ, Liang G (2018) bHLH104 confers tolerance to cadmium stress in Arabidopsis thaliana. J Integr Plant Biol 60:691–702
Zheng LY, Guo XS, He B, Sun LJ, Peng Y, Dong SS, Liu TF et al (2011) Genome-wide patterns of genetic variation in sweet and grain sorghum (Sorghum bicolor). Genome Biol 12:147–157
Zhou J, Li F, Wang JL, Ma Y, Chong K, Xu YY (2009) Basic helix-loop-helix transcription factor from wild rice (OrbHLH2) improves tolerance to salt- and osmotic stress in Arabidopsis. J Plant Physiol 166:1296–1306
Zhu SR, Javier MP, José ME, Yu F (2020) Autocrine regulation of root hair size by the RALF-FERONIA-RSL4 signaling pathway. New Phytol 227:45–49

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.