



**Full Length Article**

## Inhibition of *SbABI5* Expression in Roots by Ultra-high Endogenous ABA Accumulation Results in Sorghum Sensitivity to Salt Stress

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### Abstract

In this study, we firstly investigated the germination rate, growth and dry weight of shoots and roots in two sorghum cultivars, Liaoza15 and Longza11, to analysis the physiological responses of sorghum to salt and ABA. We found that Liaoza15 had high salt and ABA tolerance, but Longza11 was hypersensitive to salt and ABA stress during germination and early seedling growth. Secondly, we found that salt-induced ABA accumulation was higher and more sustained in Longza11 than in Liaoza15. Nextly, we analyzed the salt-induced expression of *SbABIs* in the two sorghum cultivars and *AtABIs* in wild type Arabidopsis. The result showed that the change in *SbABI5* expression in the roots was the most obvious difference between the two cultivars, namely salt did not induce *SbABI5* in the roots of Longza11 but increased it by about 3-fold in the roots of Liaoza15. And the salt treatments also increased the expression of *AtABI5* in the roots, suggesting changes of *SbABI5* expression in the roots is a key step in the ABA signaling pathway to improve salt tolerance in sorghum. Finally, we found that the expression of *SbABI5* was strongly induced by relatively low concentrations of ABA rather than by extremely high concentrations of ABA. © 2016 Friends Science Publishers

**Keywords:** Salt stress; Sorghum; Abscisic acid; Roots; *SbABI5*; Ultra-high endogenous ABA

### Introduction

Salt is a significant soil component and stressor that limits plant growth and crop productivity in agricultural fields worldwide (Zhu, 2002; Chinnusamy *et al.*, 2005). Substantial research efforts have been devoted to understanding the mechanisms of salt tolerance in plants (Brocard *et al.*, 2002; Jia *et al.*, 2002; Nishiyama *et al.*, 2011; Gurmani *et al.*, 2014). Abscisic acid (ABA) is a signaling molecule that mediates the responses to salt stress (Knight and Knight, 2001; Nishiyama *et al.*, 2011), and is considered a vital signal of salt tolerance because of its rapid biosynthesis and significant accumulation in plant cells upon exposure to salt stress conditions. Plant roots are the first tissues to sense soil stress, and are the main sites of ABA biosynthesis (Chinnusamy *et al.*, 2005). Many studies have shown that environmental signals stimulate ABA biosynthesis in the roots, which is translocated to the shoot (Laurie *et al.*, 2002; Chinnusamy *et al.*, 2005; Rus *et al.*, 2001).

Many genes are involved in ABA-mediated salt tolerance in plants, including *ABIs* (ABA-insensitive)

(Lopez-Molina *et al.*, 2001; Finkelstein *et al.*, 2002; Nambara *et al.*, 2002; Gong *et al.*, 2015), and *ABFs* (ABA Responsive Element Binding Factor) (Choi *et al.*, 2000). The *ABIs* are divided into two major classes according to their biochemical functions: protein phosphatases (*ABI1* and *ABI2*) and transcription factors (*ABI3/VP1*, *ABI4*, and *ABI5*) (Giraudat *et al.*, 1992; Leung *et al.*, 1994; Meyer *et al.*, 1994; Leung *et al.*, 1997; Finkelstein *et al.*, 1998; Rodriguez *et al.*, 1998; Hu and Yu, 2014). *ABI1* and *ABI2* encode two homologous serine/threonine phosphatases of class 2C and are highly homologous to one another. *ABI3*, *ABI4* and *ABI5* are members of the B3, APETALA2- (*AP2*) and basic leucine zipper- (*bZIP*) domain families, respective (Giraudat *et al.*, 1992; Finkelstein *et al.*, 1998; Finkelstein *et al.*, 2000; Lopez-Molina *et al.*, 2000). It has been found that *AtABIs* are involved in Arabidopsis salt stress response. For example, the mutants of *ABI1*, *ABI2* *ABI4* are sensitivity to salt stress in Arabidopsis. *AtABI5* is induced salt stress in Arabidopsis (Quesada *et al.*, 2000; Brocard *et al.*, 2002; Ohta *et al.*, 2003). Some salt-tolerance regulation factors affect level of *AtABI3* transcripts (Bu *et al.*, 2009; Li *et al.*, 2013).

Sorghum (*Sorghum bicolor*) is one of the most important cereal crops in the world and is a major source of human food, animal feed, and biofuel feedstock (Bafeel 2014; Ngara and Ndimba, 2014). Many studies have attempted to unravel the complex physiological mechanisms of salt stress tolerance in sorghum, and have implicated increasing  $\text{Ca}^{2+}$  concentrations (Bernstein *et al.*, 1993), osmotic adjustment (Richardson and McCree, 1985; Anjaneyulu *et al.*, 2014), and chromosome endoreduplication (Ceccarelli *et al.*, 2006) as contributing processes. Some studies have demonstrated the involvement of the ABA mechanism in sorghum salt tolerance. For example, Monreal *et al.* (2007) showed that salt stress strongly enhances the activity of phosphoenolpyruvate carboxylase kinase (PEPCK), which is known to be controlled by ABA in sorghum leaves. Salt- and ABA-treatments were shown to cause up-regulation of mRNA encoding the glycine-rich RNA-binding protein, which is known to be modulated in sorghum by the light signal (Aneeta *et al.*, 2002). The differential gene expression profile was monitored in *Sorghum bicolor* following exposure of seedlings to high salinity, osmotic stress, or ABA; the findings indicated that 89 genes were induced >2-fold in the shoots and 84 genes were induced >2-fold in the roots by salt and ABA treatments (Buchanan *et al.*, 2005). Thus, there is a large extent of overlap in gene expression response to salt and ABA treatments. Although those studies have indicated that sorghum salt tolerance is dependent upon the ABA signaling pathway, there is a lack of molecular evidence linking ABA signal with sorghum in response to salt stress.

We predict that ABIs may play an important role in sorghum salt stress tolerance. Thus, we identified two sorghum cultivars (Liaoza15 and Longza11), which are obviously different in salt tolerance from 50 different sorghum cultivars from Northeast China. In this study, we analyzed the responses to salt and ABA during germination and young seedling growth of Liaoza15 and Longza11. Then, we examined the ABA accumulation and expression of ABI genes in the two cultivars under salt stress conditions. Our results explore that *SbABIs* expression is related with ABA content for salt tolerance in sorghum, and *SbABI5* expression is a key step in the ABA signaling pathway for salt tolerance in sorghum, which can be inhibited by ultra-high ABA level.

## Materials and Methods

### Plant Materials and Growth Conditions

Seeds of the sorghum hybrid cultivars Liaoza15 (LA-17×LR9198) and Longza11 (403A×Hahui576) were obtained from the Liaoning Academy of Agricultural Sciences and the Heilongjiang Academy of Agricultural Sciences, respectively (Liu *et al.*, 2010; 2011). The two sorghum cultivars had been characterized as having

obviously different salt tolerances by examining the salt tolerance of 50 different sorghum cultivars from Northeast China (data not shown). Sorghum seeds were sown in Petri dishes under controlled conditions, and grown in a growth chamber at 28°C with 16 h light/8 h dark cycles to examine the germination rates. The sorghum seeds were germinated for 4 d then were transplanted to fresh plates for experimental treatments and to examine shoot length, root length, shoot dry weight, and root dry weight. Seedlings were photographed using a digital camera (Cyber-shot DSC-S85; Sony, Japan).

### Salt and ABA treatments

Two NaCl and four ABA treatments of sorghum (*sorghum bicolor* (L.)) seeds and seedlings were conducted (Table 1). For the purpose of measuring germination rates of two sorghum hybrid cultivars under salt stress and ABA treatments, sorghum seeds were sown in Petri dishes for a total of 4 d, 6 d, and 8 d. To investigate shoot length, root length, shoot dry weight, and root dry weight, sorghum seeds were germinated for 3 d, then were transferred to fresh plates for different treatments lasting 3 d and 5 d each. At least 20 seedlings were observed for each experiment, and three replications were conducted for each experiment. Statistical analyses of the numerical data were conducted by Microsoft Excel 2003 and SPSS statistical software 13.0 (SPSS Inc.).\* and \*\* indicates significant differences in comparison to WT at  $P < 0.05$  and  $P < 0.01$ , respectively (Student's *t*-test).

### ABA Analysis

After salt-inducement treatments, the samples were immediately frozen in liquid nitrogen and homogenized at ice-cold temperature. The homogenates were centrifuged for 15 min at 3000 g, and the supernatants were used for the ABA assay. ABA analyses were carried out using the competitive ELISA method described by Chen *et al.* (2006) and Lü *et al.* (2007). The antigens and IgG-horseradish peroxidase used in the ELISAs were purchased from the Phytohormones Research Institute at China Agricultural University. The experiments were repeated at least 3 times with similar results. Statistical analyses of the numerical data were conducted by Microsoft Excel 2003 and SPSS statistical software 13.0 (SPSS Inc.).\* and \*\* indicates significant differences in comparison to WT at  $P < 0.05$  and  $P < 0.01$ , respectively (Student's *t*-test).

### Quantitation of Gene Expression by RT-PCR

To assay the expression levels of *SbABI1*, *SbABI3*, *SbABI4*, *SbABI5* and *SbRAB28* genes after stress, 14-d seedlings were treated with 200 mM NaCl. Seedlings grown at 22°C and treated with distilled water were used as control. Equal amounts of leaves and roots were sampled at

0-, 3-, 6- and 12-h time points. Total RNA was extracted with the RNasy plant mini kit (Qiagen) supplemented with an on-column DNA digestion (Qiagen RNase-Free DNase set), according to the manufacturer's instructions. Total RNA (3  $\mu$ L) was reverse transcribed and subjected to the RT reaction with the Superscript II RT kit (Invitrogen) in a 25  $\mu$ L reaction volume at 42°C for 1 h. Real-time PCR reactions were performed with gene-specific primers for genes (Table 1). Expression levels of the actin gene (Table 1) served as an internal control. The real-time PCR was conducted using SYBR Premix Ex Taq™(TaKaRa) with 1  $\mu$ L of cDNA in a 10  $\mu$ L reaction volume. The amplification program was as follows: 1 cycle of 95°C for 30 s; and 40 cycles of 95°C for 15 s, 60°C for 30 s and 72°C for 30 s. At least 3 technical replicates were performed for each experiment. The expression of genes in untreated wild type at 22°C was used as control. Since there were no significant differences among the time intervals in untreated wild type, we chose 0 h for data normalization as suggested by manufacture's guide (Applied Biosystems guide for real-time qPCR). The experiments were repeated at least 3 times with similar results. Statistical analyses of the numerical data were conducted by Microsoft Excel 2003 and SPSS statistical software 13.0 (SPSS Inc.).\* and \*\* indicates significant differences in comparison to WT at  $P < 0.05$  and  $P < 0.01$ , respectively (Student's *t*-test).

The expression of all genes in *Arabidopsis* under control conditions and salt treatments were downloaded from the publicly available microarray data set in the AtGenExpress expression atlas (<http://weigelworld.org/resources/microarray/AtGenExpress>) (Schmid *et al.*, 2005). The expression of *AtABIs* under control and salt treatments were extracted from the data set and used to calculate the ratio of gene expression in control to salt stress conditions in order to identify the expression change of *AtABIs* under salt stresses.

## Results

### Phenotype of Two Sorghum Cultivars under Salt Stress

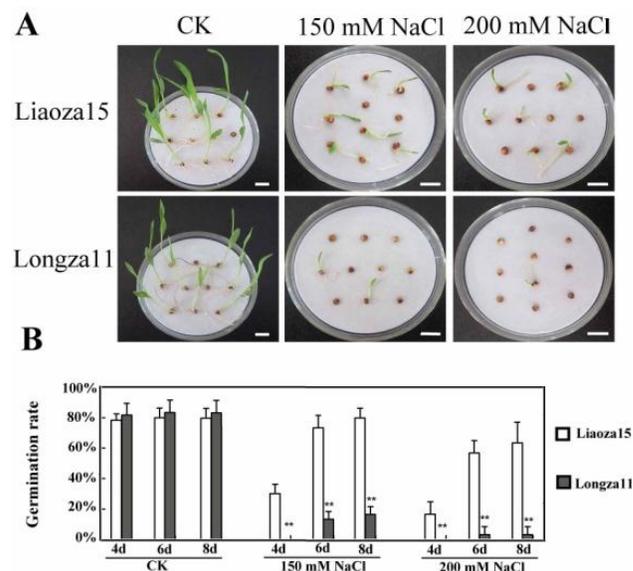
First, germination of the two cultivars was examined under salt stress. After the length of the radicles equals to the length of seeds, and the length of embryos equal to 1/2 length of seeds, the germination process of sorghum seeds are completed. The result showed that germination of the two cultivars was completed within 4 d and showed no significant differences under the control condition (Fig. 1). However, germination under salt stress was significantly different in Liaoza15 and Longza11, the germination process are delayed in salt treated Liaoza15 and Longza11 (Fig. 1). Beside of this, the germination rate of Liaoza15 was obvious higher than Longza11 in both 150 mM and 200mM treatment of NaCl (Fig. 1A). These results indicated that the salt treatments altered the *sorghum* seed germination, and Longza11 is sensitivity to salt stress in seed germination.

**Table 1:** Experimental treatments

Group No.	Treatments
1	Control (distilled water treatment)
2	150 mmol l <sup>-1</sup> NaCl
3	200 mmol l <sup>-1</sup> NaCl
4	1 mmol l <sup>-1</sup> ABA
5	5 mmol l <sup>-1</sup> ABA
6	10 mmol l <sup>-1</sup> ABA
7	20 mmol l <sup>-1</sup> ABA

**Table 2:** Sequences of primers used in this work for gene expression determination by real-time PCR after reverse transcription

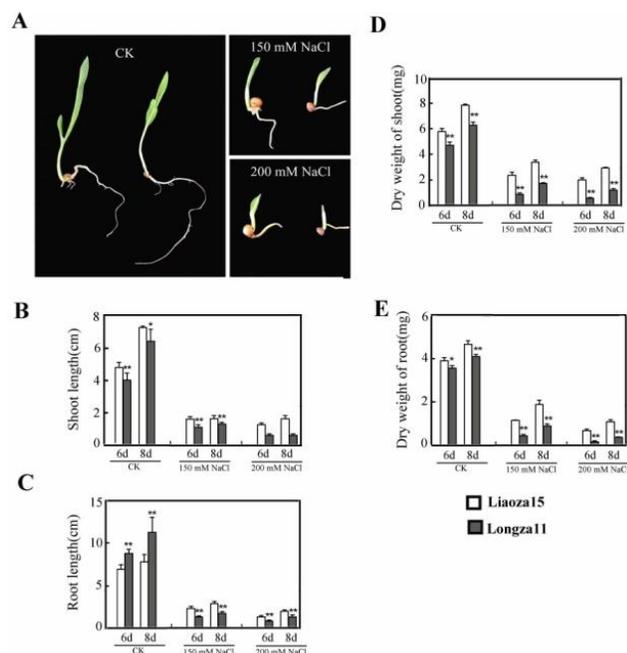
Gene name*	Forward	Reverse
<i>SbABI1</i>	cgaatgaagaggcatgcaaa	tgcgacaccattgtcttgtg
<i>SbABI3</i>	acatcgaccagatcctcgac	ccagaacgggtttgtgtga
<i>SbABI4</i>	gatcagcagcagctcttaatt	aaactcaaacgaccaagca
<i>SbABI5</i>	gcagcagacaatgctgttcc	gcagcggaggaccacaa
<i>SbRAB28</i>	gagaggcacaccaaatctc	cccttgctcattgtcaagc
<i>SbActin</i>	gccgtctctctctgtatgc	cgagcttctctctatgt



**Fig. 1:** The germination rates of Liaoza15 and Longza11 cultivars under salt stress

Images were taken in the two sorghum cultivars germinated and grown for 8 days in different concentrations of NaCl treatments (A). The germination rates of the two sorghum cultivars germinated and grown for 4, 6 and 8d in different concentrations of NaCl treatments (B). Each data are representative individuals of three independent experiments with at least 20 seedlings examined for each experiment. Scale bar = 1cm. Asterisks indicate statistically significant differences among the boron treatments at the indicated times according to Student's *t*-test (\* $P < 0.05$ ; \*\* $P < 0.01$ )

Moreover, we observed the growth of young seedlings of the two sorghum cultivars under salt stress. The results showed that under the control condition, the shoots were longer and the roots were shorter in Liaoza15 than in Longza11 (Fig. 2A-C). Under the salt condition, the growth of roots and shoots were obviously inhibited in both



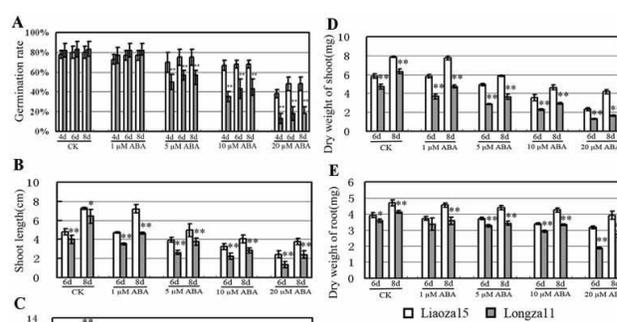
**Fig. 2:** The growth of Liaoza15 and Longza11 cultivars under salt stress

Images were taken in the two sorghum cultivars seeds germinated for 3 d then transferred to different NaCl treatments for 5d (A). Shoot length (B), root length (C), shoot dry weight (D) and root dry weight (E) were examined in the two sorghum cultivars seeds germinated for 3 d then respectively transferred to different NaCl treatments for 3 and 5d. Each data are representative individuals of three independent experiments with at least 20 seedlings examined for each experiment. Scale bar = 1cm. Asterisks indicate statistically significant differences among the boron treatments at the indicated times according to Student's *t*-test (\**P* < 0.05; \*\**P* < 0.01)

cultivars, the shoots and roots of Longza11 were both shorter than Liaoza15 (Fig. 2A-C). The dry weights of the roots and the shoots of both cultivars were obviously reduced under salt stress, compared with those of the control condition, and were higher in Liaoza15 than in Longza11 under the control and salt treatments (Fig. 2D, E), suggesting Longza11 is sensitivity to salt stress in seedling growth. The results state that compared with Liaoza15, Longza11 is hypersensitivity to salt stress.

### Phenotypes of Sorghum Cultivars under Exogenously-applied ABA

To indicate the relationship between salt sensitivity of the two sorghum cultivars and ABA signaling pathway, we examined phenotypes of the two sorghum cultivars under exogenously-applied ABA. The result showed that the germination rates of both cultivars decreased gradually following the concentrations exposed to ABA, but the reduction was consistently more significant in Longza11 than in Liaoza15 (Fig. 3A). Similarly, the lengths and the dry weights of the roots and the shoots of the two cultivars decreased gradually following the concentrations exposed to ABA, and decreased more significantly in Longza11



**Fig. 3:** The phenotypes of Liaoza15 and Longza11 cultivars under exogenously applied ABA treatments

The germination rates of the two sorghum cultivars germinated and grown for 4, 6 and 8 days in different concentrations of ABA treatments (A). Shoot length (B), root length (C), shoot dry weight (D) and root dry weight (E) were examined in the two sorghum cultivars seeds germinated for 3 d then respectively transferred to different ABA treatments for 3 and 5d. Each data are representative individuals of three independent experiments with at least 20 seedlings examined for each experiment. Asterisks indicate statistically significant differences among the boron treatments at the indicated times according to Student's *t*-test (\**P* < 0.05, \*\**P* < 0.01)

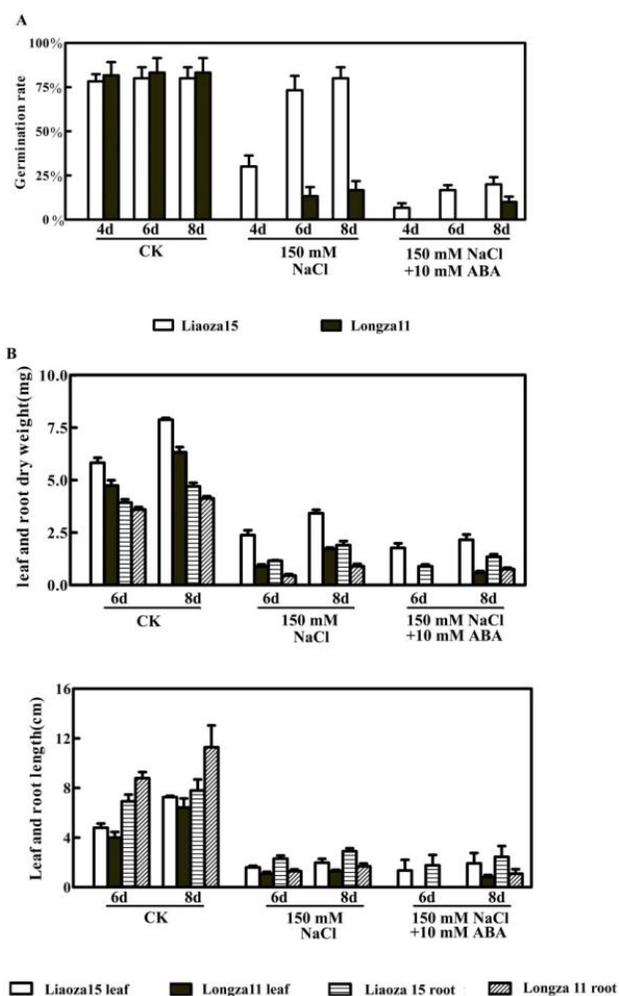
than in Liaoza15 (Fig. 3B-E). Under the control condition, the roots of Liaoza15 were shorter than those of Longza11, whereas under the ABA treatment conditions, the roots of Liaoza15 were longer than those of Longza11 (Fig. 3C). These results indicated that Liaoza15 has a higher tolerance and Longza11 has a hypersensitivity to ABA.

### Phenotypes of Sorghum Cultivars under Salt and Exogenously-applied ABA

We also examined phenotypes and ABA accumulation in the two sorghum cultivars under salt stress and exogenously applied ABA. The result showed that the germination rates of both cultivars decreased in the conditions treated with salt stress and additional ABA, compared with treatments with salt stress. However, the reduction was consistently more significant in Longza11 than in Liaoza15 (Fig. 4A). Similarly, the lengths and the dry weights of the roots and the shoots of the two cultivars decreased under the treatments with salt and ABA, compared with only salt stress, and decreased more significantly in Longza11 than in Liaoza15 (Fig. 4B). These results further indicated that Liaoza15 has a higher tolerance and Longza11 has a hypersensitivity to ABA.

### ABA Accumulation in Sorghum Cultivars under Salt Stress and Exogenously-applied ABA

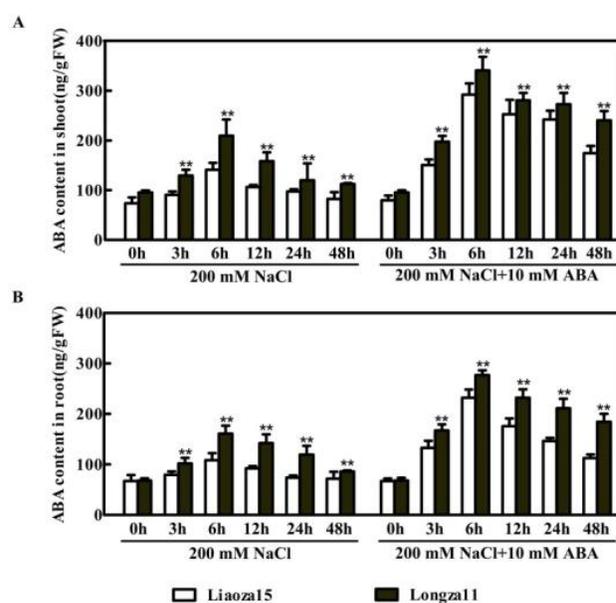
Then, we examined ABA accumulation in the two sorghum cultivars under salt stress. The results showed that ABA content in the roots and shoots showed not significant



**Fig. 4:** The phenotypes of Liaoza15 and Longza11 cultivars under salt and exogenously applied ABA treatments

The germination rates of the two sorghum cultivars germinated and grown for 4, 6 and 8d in 150 mM NaCl treatment or 150 mM NaCl and 10 mM ABA treatment different concentrations of NaCl treatments (A). Length and dry weight of shoot or length and dry weight of root were examined in the two sorghum cultivars seeds germinated for 3 d then respectively transferred to different NaCl treatments for 3 and 5d (B). Each data are representative individuals of three independent experiments with at least 20 seedlings examined for each experiment. Asterisks indicate statistically significant differences among the boron treatments at the indicated times according to Student's *t*-test (\**P* < 0.05; \*\**P* < 0.01)

differences between the two cultivars under the control condition. But under salt stress, the salt-induced ABA accumulation was obvious and that the highest ABA accumulation occurred in the 6 h treatments of both cultivars (Fig. 5A, B). Beside of this, the ABA accumulation was higher in Longza11 than in Liaoza15 (Fig. 5A, B). The ABA content after 24 h treatment was still obvious more than that of 0 h treatment in Longza11 but were not significant different after 12 h of treatment in Liaoza15 (Fig. 5A, B). In addition, the ABA accumulation was also higher in Longza11 than in Liaoza15 (Fig. 5A, B) under salt stress



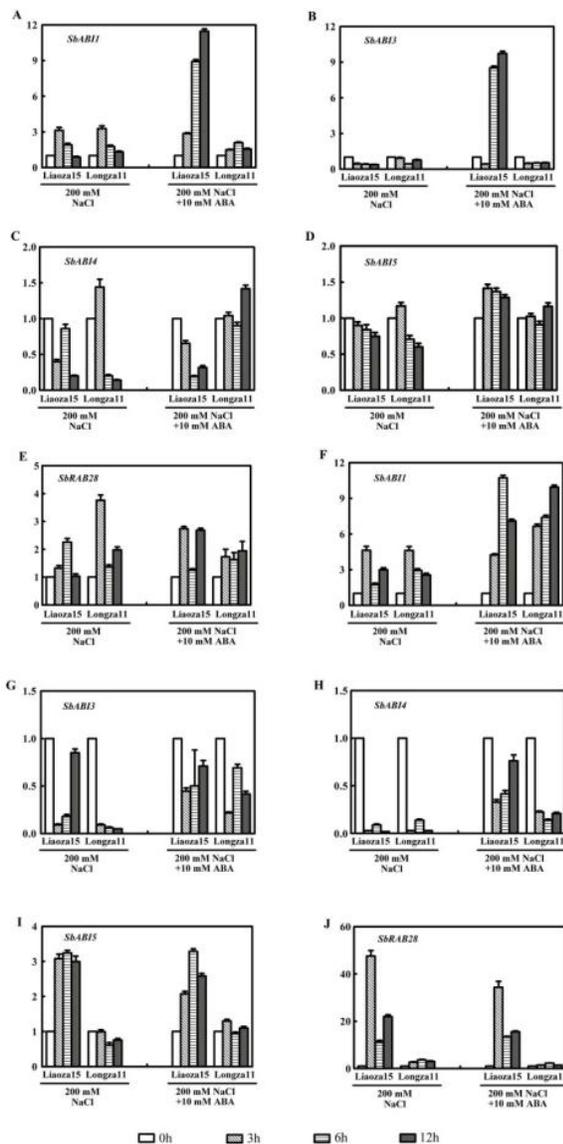
**Fig. 5:** The ABA accumulation of Liaoza15 and Longza11 cultivars under salt stress or salt and exogenously applied ABA treatments

The ABA contents in the shoots (A) and roots (B) of the two cultivars were examined in 200 mM NaCl or 200 mM NaCl and 10 mM ABA at 0, 3, 6, 12, 24, and 48 h. Asterisks indicate statistically significant differences among the boron treatments at the indicated times according to Student's *t*-test (\**P* < 0.05; \*\**P* < 0.01)

treatments and under salt and applied ABA treatments. The result state that compared with Liaoza15, Longza11 has a much higher ABA accumulation, which should be the main reason of the ABA sensitivity of Longza11.

#### Expression of ABA-regulated Genes in Sorghum Cultivars and Arabidopsis under Salt Stress and Exogenously-applied ABA

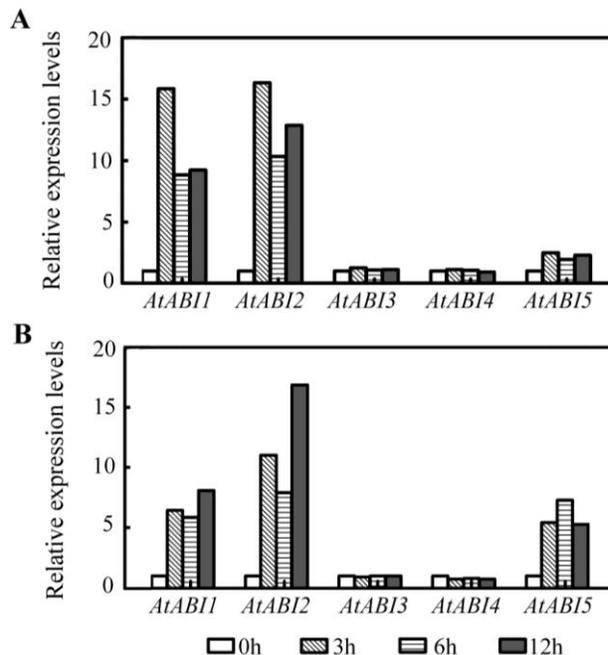
We next tested the expression of the following ABA-inducible genes in the two sorghum cultivars: *SbAB11*, *SbAB13*, *SbAB14*, *SbAB15* and *SbRAB28*. The results showed that under salt stress, *SbAB11* was induced in the shoots and roots in two cultivars, and the expression of *SbAB13* and *SbAB14*, only except *SbAB14* expression in Longza11 in the 3 h treatment, decreased in the shoots and roots in two cultivars. *SbAB15* showed no significant change in the shoots for any salt stress condition. However, salt did not induce *SbAB15* in the roots of Longza11 but increased it by about 3-fold in the roots of Liaoza15 (Fig. 6I). *SbRAB28* increased at 3 h and 6 h in the shoots of Longza11 and Liaoza15, respectively (Fig. 6J) and it highly induced in the roots by salt treatments. The expression of *SbRAB28* had more increase in the roots of Liaoza15 than in that of Longza11. The similar pattern of the expression of the genes was found under salt stress and applied ABA.



**Fig. 6:** The expression of ABA-regulated genes in the two sorghum cultivars under salt stress or salt and exogenously applied ABA treatments

The expression of *SbABI1*, *SbABI3*, *SbABI4*, *SbABI5* and *RAB28* in the shoots (A, B, C, D, E) and the roots (F, G, H, I, J) of the two cultivars were examined in 200 mM NaCl or 200 mM NaCl and 10 mM ABA at 0, 3, 6, and 12 h. The results are the means±SE (standard error) of at least three replicates

The results indicate that the expression of *SbABI5* and *SbrAB28* in the roots is obvious difference between the two cultivars, suggesting the two gene responses in the roots are the key step to cause the two sorghum cultivars in response to salt and ABA treatments. For further prove whether the expression of *ABI5* affects plant salt tolerance, we also analyzed the salt-induced expression of *AtABIs* in *Arabidopsis* using the AtGenExpress visualization tool. Results showed that the salt treatments increased the



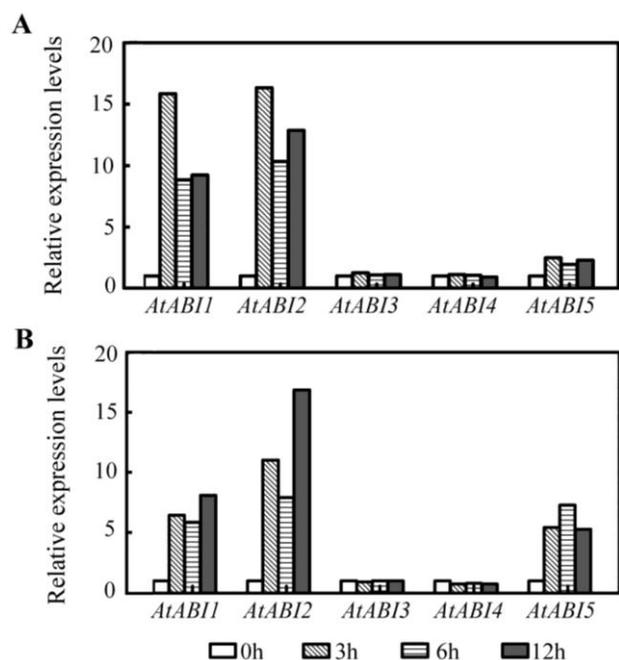
**Fig. 7:** The expression of *AtABIs* genes in *Arabidopsis* under salt stress

The expression of *AtABI1*, *AtABI2*, *AtABI3*, *AtABI4* and *AtABI5* in the shoots (A) and the roots (B) of the two cultivars were examined in 200 mM NaCl at 0, 3, 6 and 12 h by the AtGenExpress visualization tool

expression of *AtABI1*, *AtABI2*, and *AtABI5* (Fig. 7A, B). In addition, the salt-induced expression of *AtABI5* was higher in the roots than in the shoots (Fig. 7A, B). The expression of *AtABI3* and *AtABI4* showed no obvious change under any of the salt stress conditions (Fig. 7A, B). The result further states that *ABI5* are a key responsive gene in plant salt tolerance.

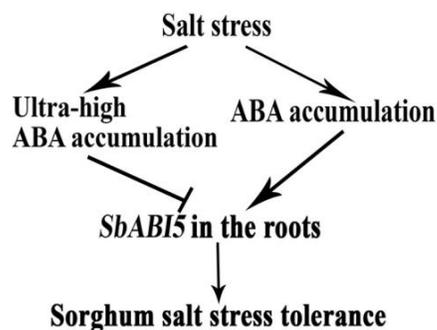
#### Expression of *SbABI5* in the Roots of Sorghum Cultivars under Exogenously-applied ABA

To definite the relationship between expression of *SbABI5* in the roots and the ABA accumulation in the two sorghum cultivars, we analyzed that the expression of *SbABI5* in the roots of two sorghum cultivars under different concentration of exogenous ABA treatments. The results showed that low concentration of ABA strongly induced expression of the *SbABI5* gene in the roots of the two cultivars (Fig. 8). Treatment with high concentration of ABA led to no obvious accumulation of *SbABI5* transcript at any of the times (Fig. 8). The results indicate that salt did not induce *SbABI5* in the roots of Longza11 is produced by the high endogenous ABA level. Thus appropriate ABA accumulation induces the expression of *SbABI5* in the roots to enhance sorghum salt tolerance. On the contrary, ultra-high ABA accumulation inhibits the expression of *SbABI5* in the roots, resulting in sorghum sensitivity to salt stress (Fig. 9).



**Fig. 8:** The expression of *SbABI5* in the roots of two sorghum cultivars under ABA treatments

The expression of *SbABI5* in the roots of the two cultivars was examined in 20  $\mu$ M and 100  $\mu$ M ABA at 0, 3, 6, and 12 h. The results are the means  $\pm$  SE (standard error) of at least three replicates



**Fig. 9:** The working model of the ABA signaling pathway in sorghum salt stress tolerance

Salt induced-ABA accumulation increases the expression of *SbABI5* in the roots, resulting in sorghum tolerance salt stress. On the contrary, salt induced-ultra-high ABA accumulation inhibits the expression of *SbABI5* in the roots, resulting in sorghum sensitivity to salt stress

## Discussion

In this study, we used two sorghum cultivars, which have different salt-tolerances, to evaluate their physiological responses to ABA and differential expression of *ABIs* induced by salt stress. The results showed that Liaozha15 has high salt and ABA tolerance and Longza11 is hypersensitive to salt and ABA stress during germination and early seedling growth (Fig. 1, 2 and 3). In addition, obvious

differences in ABA content and ABA-related gene expression were observed in both of the two cultivars (Fig. 4 and 5). Plants under salt stress are known to rely on at least two signal transduction pathways: ABA-independent and ABA-dependent (Knight and Knight, 2001; Kim *et al.*, 2003). Thus, the two sorghum cultivars generated salt-tolerance mechanisms through an ABA-dependent signal transduction process.

We compared the initial and overall salt-induced ABA accumulation in the two cultivars and found that the most significant difference was that ABA accumulation was ultra-high in the salt-sensitive cultivar Longza11 (Fig. 4). Although less information is available for the negative response mechanism. Pandey *et al.* (2005) found that the *Arabidopsis* ABR1 mutant was sensitive to salt and ABA and that norflurazon (NF, an inhibitor for ABA biosynthesis) restored the germination rate of the mutant seeds, which was obviously decreased by NaCl. These findings suggested that the main reason for mutant sensitivity to salt and ABA was high or/and sustained ABA accumulation. Thus, we believe that ultra-high and sustained ABA accumulation is the main reason for sorghum sensitivity to salt and ABA and that instantaneous and appropriate ABA accumulation may be a more effective response mechanism of salt stress tolerance in sorghum.

Many studies have shown that ABI proteins are both required and sufficient for ABA signaling, and that some ABIs are involved in ABA-mediated salt stress tolerance in plants (Uno *et al.*, 2000; Brocard *et al.*, 2002). For example, the plants carrying *ABI4* mutant alleles display increased tolerance to osmotic and salt stress during germination and early seedling growth and *ABI5* expression is induced by salt treatments (Quesada *et al.*, 2000; Uno *et al.*, 2000; Brocard *et al.*, 2002; Reeves *et al.*, 2011). In the current study, we found that expression of *SbABI3* and *SbABI4* obviously decreased and that expression of *SbABI1* obviously increased in the roots and shoots of both cultivars (Fig. 5). The increasing expression of *AtABI1* and *AtABI2* also occurred in *Arabidopsis* under salt stress, as shown by the data in the AtGenExpress expression database. A previous study showed that *AtABI1* and *AtABI2* are associated with salt tolerance in *Arabidopsis thaliana* (Ohta *et al.*, 2003). Thus, *ABI1* and *ABI2* may be involved in a common salt-tolerance mechanism shared between sorghum and *Arabidopsis*. Because *ABI1* acts early in the ABA signaling pathway and stimulates all known signaling responses, such as increases in the free cytosolic calcium concentration ( $[Ca^{2+}]_{cyt}$ ), and the reactive oxygen species (ROS) production (Allen *et al.*, 1999; Murata *et al.*, 2001; Hubbard *et al.*, 2011), *ABI1* may be the factor that stimulates some of the signaling responses under salt stress in sorghum.

Comparison of ABA accumulation in root and leaf tissues under salt stress conditions has revealed that the roots are more sensitive to salt-induced ABA accumulation than the shoots, suggesting that roots are more important

than shoots for the ABA signaling mechanism of salt stress tolerance in plants (Jia *et al.*, 2002). Because of obvious changes in the roots of our cultivars in the salt and ABA treatment conditions (Fig. 1 and 2), it is likely that the roots of our cultivars may contain the key factors and mechanism of salt tolerance. Remarkably, *AtABIs* are involved in Arabidopsis response salt stress (Quesada *et al.*, 2000; Brocard *et al.*, 2002; Ohta *et al.*, 2003; Bu *et al.*, 2009; Li *et al.*, 2013) but when we examined the expression of *SbABI1*, *SbABI3*, *SbABI4* and *SbABI5* in the two cultivars under salt stress, only *SbABI5* expression in the roots was the most obvious change between the two cultivars, and the high expression of *SbRAB28* and salt-induced *SbABI5* occurred jointly in the roots of salt-tolerant cultivars (Fig. 6). *RAB28* is a downstream ABA response gene (Nieva *et al.*, 2005). In Arabidopsis, *AtABI5* overexpression can induce *RABI8* and some downstream stress-response genes (Brocard *et al.*, 2002). Thus, here the high expression of *SbRAB28* can be induced by *SbABI5* and the expression of *SbRAB28* may be sufficient to promote salt stress tolerance. *AtABI5* is induced by salt stress in *Arabidopsis* (Fig. 7). In addition, *AtABI5* was induced by salt in *Arabidopsis* and the expression of *AtABI5* was higher in the roots than in shoots (Fig. 7). Thus, we speculate that *SbABI5* in the roots is the key factor mediating salt tolerance in sorghum.

Our results also showed that *SbABI5* expression was increased by exposure to low concentration of exogenously applied ABA, while exposure to ultra-high concentration of ABA did not obviously induce the *SbABI5* in the roots of either of the two cultivars (Fig. 8). Integrating with the sensitivity phenotype (Fig. 1, 2 and 3) and in ultra-high ABA accumulation in Longza11 (Fig. 4), this finding further supports the conclusion that *SbABI5* in the roots is the key factor mediating salt tolerance in sorghum, which can be inhibited by ultra-high ABA accumulate. *SbABI5* can improve salt tolerance in plants not only by inducing salt-related genes, such as *SbRAB28*, but also by regulating plant growth, based on the fact that *ABI5* has been shown to play a key role in arresting growth caused by ABA in the young seedling stage (Lopez-Molina *et al.*, 2001; Brocard *et al.*, 2002). The role of salt-induced ABA accumulation is to induce ABA-related gene expression, which then induces some salt-related genes to improve salt tolerance in plants (Zhu 2002; Chinnusamy *et al.*, 2005). It is possible that the weakening of salt tolerance in plants caused by ultra-high and sustained ABA accumulation may be a result of no or insufficient inducement of ABA-related genes.

## Conclusion

In conclusion, the increase expression of *SbABI5* gene in the roots is benefit for sorghum salt tolerance, the *SbABI5* gene expression can be induced by a moderate ABA accumulation rather than an ultra-high ABA accumulation. In future, we will investigate how *SbABI5* modulates gene

transcription to regulate the sorghum growth and improve salt tolerance.

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