ANALYSES OF ABA RESPONSES GENES IN *ARABIDOPSIS THALIANA sos5* MUTANT UNDER NaCI STRESS

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Abstract: Abscisic acid (ABA) is an important phytohormone that regulates a lot of physiological, biochemical and molecular processes during plants life and response stress conditions. In this paper, *Arabidopsis thaliana salt overly sensitive* (*sos5*) mutant which known hyper sensitive to NaCI stress was used to investigate ABA regulation. Because of this, ABA depended genes determined by q-RT PCR method under 2h. 100 mM NaCI stress. Findings indicated that RD29A, RD 29B and RD22 which ABA dependent gene expressions were highly lower than *Col-gl* (wild type); however ERD1 which ABA in depended gene expression was higher than WT. Based on these results, we suggest that defect of ABA responses gene regulation might be responsible for hypersensitivity to Arabidopsis *sos5* mutant under salt stress.

Keywords: ABA, *Arabidopsis thaliana*, *sos5* mutant, salt stress

Introduction

Salinity is one of the most serious problems limiting the productivity of agricultural crops, with adverse effects on germination, plant defense system and crop yield (Munns & Tester, 2008). More than 45 million hectares of irrigated land have been damaged by salt, and 1.5 million hectares are taken out of production each year as a result of high salinity levels in the soil around the world (Munns & Tester, 2008). High salinity affects plants in several ways: water stress, ion toxicity, nutritional disorders, oxidative stress, alteration of metabolic processes, membrane disorganization, reduction of cell division and expansion, genotoxicity (Hasegawa, Bressan, Zhu, & Bohnert, 2000; Munns, 2002; Zhu, 2007). According to them, plant growth, development and survival highly reduced. Plants effected from salt stress in some different aspects such as osmotic, ionic and toxic. Meanwhile, plants have evolved several mechanisms to cope with salt stress. One of the most related defense mechanism is that produce ABA and express ABA related genes under salinity. For example, some researches showed that ABA-deficient mutants perform poorly under salinity stress (Xiong, Gong, Rock, Subramanian, Guo, Xu, et al., 2001). On the other hand, it has been shown that ABA-dependent and -independent transcription factors may also cross talk to each other in a synergistic way to amplify the response and improve stress tolerance (Shinozaki & Yamaguchi-Shinozaki, 2000).

There are some mutants characterized related to ABA and these are giving clues about ABA's role under stresses. But, we have very few ideas about Arabidopsis *sos5* mutant related to ABA. Shi et. al., (2003) has been shown firstly the *sos5* mutant of *A. thaliana* by root growth under salt stress. The root of *sos5* shows a drastic reduction of elongation growth combined with radial swelling of the elongation zone. Cell walls appear abnormally thin in *sos5*, apparently lacking the middle lamella (Shi et al., 2003).

To better understand the ABA related genetic pathway linking *sos5* mutant with salt tolerance and root growth, we exposed 2 hour 100 mM NaCI both WT and mutant. Then, we compared some ABA dependent and independent gene expression levels. We found that abscisic acid (ABA) dependent genes lower on *sos5* mutant compared to WT. We propose that Arabidopsis *sos5* mutant is hypersensitive to salt stress because of does not able to expressed ABA dependent genes enough and seem very sensitive.

Materials and Methods

Growth conditions

Arabidopsis thaliana ecotype *Col gl* wild type and the mutant (*sos5-1*) were kindly provided by Jian-Kang Zhu (University of California, Riverside, CA, USA). Growth conditions were as previously described (Blaukopf et al.,

2011). For phenotypic observation, ten seedlings were manually transferred to test media containing standard medium alone or also including the NaCI, and were examined using a dissecting microscope (Leica EZ4 HD). Stock ABA solution was prepared that was dissolved in 0.01 M NaOH.

Quantitative real-time PCR (qRT-PCR)

Samples were treated in biological triplicates. For each biological replicate, 150 seedlings were grown on a nylon mesh (20 mmmesh size; Prosep, Belgium) for 4 d and were transferred to standard medium with or without 100 mM NaCl and incubated for 120 min. Roots were removed from the seedlings, frozen in liquid nitrogen, ground in a ball mill (Retsch, Germany) for 2 min and RNA was extracted using peqGOLD Trifast (Peqlab, Germany) according to the manufacturer's instructions. The RNA concentration was measured using a Nano Drop 2000c Spectrophotometer (Thermo Scientific, USA). For each sample, 1 mg of total RNA was reverse-transcribed with oligo(dT) primers using a first-strand cDNA synthesis kit (Thermo Scientific, USA) according to the manufacturer's instructions.
Real-time PCR was performed using Solis BioDyne 5 × HOT FIREPol EvaGreen qPCR Mix Plus (no ROX) (Medibena, Austria), and a CFX96 TM Real-Time PCR Detection System (Bio-Rad, USA) was used for detection. Information on the oligonucleotides used can be found in Data Table 1. For real-time PCR, the following program was used: 95.0 8C for 15 min, 40 cycles of 95.0 8C for 10 s, 55.0 8C for 30 s, 72.0 8C for 30 s. Each biological replicatewas analysed in technical duplicates. The average technical error was 0.5 (+1) Ct values. Technical outliers were identified when the DCt between technical replicates was 2.5, and the higher Ct value was removed. The remaining technical replicates were averaged and the DCt (Ct test – CtUBQ5) was calculated.The effect of genotypes and treatments was tested by subjecting the DCt values of three biological replicates to a two-sided t-test.
For this study, the relative mRNA levels of three ABA dependent genes and one independent gene were analysed.

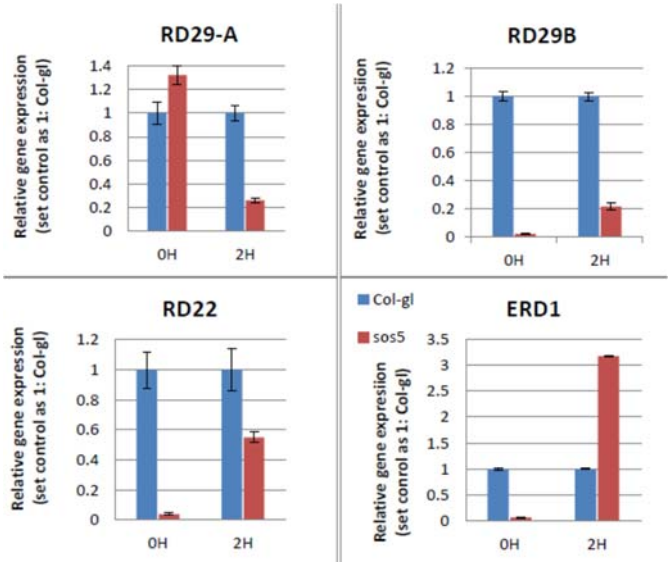
Results and Discussion

The mechanisms of genetic control of salt tolerance in plants have not yet fully understood. Because, salt stress sensed by different sensors. For instance, when plants exposed to salt stress, cell wall, cell surface and vacuole affected immediately and then triggered genes that over come to this case. One of the significant sensors that cell surface proteoglycans have been implicated in many aspects of plant growth and development, but genetic evidence supporting their function has been lacking. Shi et. al., (2003), reported in their paper that the Salt Overly Sensitive5 (SOS5) gene encodes a putative cell surface adhesion protein and is required for normal cell expansion. They isolated *sos5* mutant in a screen for Arabidopsis salt-hypersensitive mutants. According to them, under salt stress, the root tips of *sos5*mutant plants swell and root growth is arrested. The root-swelling phenotype is caused by abnormal expansion of epidermal, cortical, and endodermal cells. So, it means SOS5 gene is responsible for plant growth and development. Even under salt stress, the roots of the wild-type plants exhibit highly organized and defined cell shapes. Cell wall architecture, cytoskeleton, wall–membrane interactions and interaction between neighboring cells play a vital role in the control of cell expansion (Darley et. al., 2001). When this gene function knock out, in response to salt stress, the root tips of *sos5* plants swell and the root growth and elongation was arrested. We hypothesized that the certain phenotypic abnormalities of *sos5* related to ABA regulation.
According to our qRT-PCR results (**Figure 1.**), *At*-RD29A, *At*-RD29B and *At*-RD22 which are ABA dependent genes were highly expressed in WT plants, compare to *sos5* mutants. On the other hand, when we checked *At*-ERD1 which is ABA independent gene was higher in *sos5* mutants than WT under 100 mM NaCI. These results suggested that SOS5 gene have a synergistic function with ABA gene regulation. If there is no SOS5, in other word *sos5* mutant plants, there is a problem with ABA regulation and plants do not able to handle with salt stress affects. Based on this, Seifert et., al., (2014) pointed out that application of ABA suppresses the non-redundant role of *At*-SOS5 in the salt response and Salt oversensitivity in *At-sos5* is suppressed by the exogenous ABA applications. All tested ABA-dependent genes displayed a significant repression in *At-sos5*, suggesting that the effect of *At*-SOS5 on stress signaling might be specific for ABA.

Table 1: Oligonucleotides used for qRT-PCR.

1-) RD29A: At5g52310.1	RD29A-F: AAGTTACTGATCCCACCAAAGAAGAAAC
	RD29A-R: TTTCCTCCCAACGGAGCTCCTAAAC
2-) RD29B: At5g52300.1	RD29B-F: TCCGGTTTACGAAAAAGTCAAAGAAAC
	RD29B-R: AATCCGAAAACCCCATAGTCCCAAC
3-) RD22: At5g25610	RD22-F: ACGTCAGGGCTGTTTCCACTGAGGTG
	RD22-R: TAGTAGCTGAACCACACAACATGAG
4-) ERD1: At5g51070	ERD1-F: ACTTGGAAGGGGTGAACTTCAGTG
	ERD1-R: AGGTCCCACCAGTATAGGCTCATCG

Figure 1. Expression level of ABA related genes during under 100 mM NaCI. Error bars on each point indicate ±SE from three independent replicates.



Conclusion

The predicted lipid-anchored glycoprotein *At*-SOS5 positively regulates cell wall biosynthesis and root growth by modulating ABA signalling. Taken together, we suggest that *At*-SOS5 and ABA signalling act in genetic synergy, leading to suppression of At-sos5 by ABA. Overall, we conclude that *At*-SOS5 regulates normal root growth via an ABA-depedent signalling pathway that might be upstream of cell wall biosynthesis.

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