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SALT-INDUCED SYSTEMIC CA^{2+} SIGNAL REGULATES PRE-mRNA SPLICING IN *ARABIDOPSIS THALIANA*

MOHAMMED ALBAQAMI

Botany & Microbiology Department, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia
Corresponding email: mmbaqami@ksu.edu.sa

Abstract

Plants adjust their biological processes to cope with numerous environmental factors. Alternative pre-mRNA splicing is a common post-transcriptional regulatory mechanism of environmental stress tolerance, such as in response to high salt exposure. Salinity induces transcriptome-wide reprograming via regulation of pre-mRNA splicing; however, the early response signals that trigger this modification are currently unknown. Several external stimuli, including high salinity, cause intracellular calcium ion (Ca^{2+}) accumulation. Under salt stress, those ions form a specific long-distance signal that communicates responsiveness and adaptation messages between plant parts. However, while salt-induced Ca^{2+} signaling is known to mediate several adaptation events, its role in regulating pre-mRNA splicing is yet unknown. Here, evidence is presented that salt-induced long-distance Ca^{2+} signaling is involved in salt stress-regulated pre-mRNA splicing. Application of localized salt stress (NaCl) to *Arabidopsis thaliana* root tips modulates the expression and alternative splicing of several transcripts in the shoot. These transcript changes were inhibited by localized treatment of the mid-region of the root with $LaCl_3$, a plasma-membrane Ca^{2+} channel blocker, suggesting salt-triggered Ca^{2+} signaling acts as a long-distance modulator of RNA transcription at both the levels of initiation and alternative pre-mRNA splicing. Interestingly, salt-induced Ca^{2+} signaling affects several stress-related pathways, including the Salt Overly Sensitive (SOS) and abscisic acid (ABA) signaling pathways, as well as expression of stress-associated genes and RNA splicing factors. These findings indicate the salt stress-activated long-distance calcium waves are involved in the molecular regulation of RNA splicing, which may contribute to entire-plant salt stress tolerance.

Key words: Arabidopsis, RNA splicing, Abiotic stress, Salt stress, Ca^{2+} signaling.

Introduction

Plants fine-tune their growth and development based on interactions with their environment. Among the environmental factors that are essential for optimal plant development are optimum concentrations of soil ions. However, high environmental levels of sodium ions (Na^+) can lead to salt stress through increased ion accumulation and osmotic pressure, ultimately resulting in a significant reduction of plant growth and yield (Munns & Tester, 2008).

Salt stress initiates an early signaling response in plants that leads to long-term molecular and cellular adaptations. Initially, plants can sense monovalent cations, including sodium ions, by means of recently identified glycosyl inositol phosphorylceramide (GIPC) sphingolipids in the plasma membrane (Jiang *et al*., 2019), or by yet unidentified salt sensors. Upon sensing salt, the plant triggers early salt-induced signals, which consist of an increase in free cytosolic Ca^{2+}, generation of reactive oxygen species (ROS), and an upsurge of cyclic nucleotides such as cyclic guanosine 3',5'-monophosphate (cGMP) (Kiegle *et al*., 2000; Donaldson *et al*., 2004; Miller *et al*., 2010). Although there are several overlapping signals between stresses such increased osmotic pressure and high salt, analysis of calcium peak amplitude and peak oscillation patterns have confirmed a salt-specific response (Kiegle *et al*., 2000). Furthermore, the development of biosensors for detecting calcium has made it possible to trace salt stress induced Ca^{2+} fluxes throughout the whole plant (Swanson *et al*., 2011). In Arabidopsis, it has been shown that local application of Na^+ to root tips induces specific calcium waves in the roots, which then spread throughout the entire plant body to mediate salt adaptation mechanisms (Choi *et al*., 2014; Feng *et al*., 2018). This long-distance calcium signal is inhibited by application of lanthanum chloride ($LaCl_3$), a plasma-membrane Ca^{2+} channel blocker, to the mid-region of the root (Choi *et al*., 2014). In addition, it has been shown that salt-triggered systemic calcium waves are reliant on the ion channel protein TWO PORE CHANNEL 1 (TPC1) and the plasma membrane-bound NADPH oxidase RESPIRATORY BURST OXIDASE HOMOLOG D (AtRBOHD) (Choi *et al*., 2014; Evans *et al*., 2016).

Calcium binding proteins, such as calcineurin B–like proteins (CBLs) mediate integration of Ca^{2+} signals into an appropriate cellular response through Ca^{2+}-dependent protein phosphorylation by CBL-interacting protein kinases (CIPKs) (Van Zelm *et al*., 2020). Salt Overly Sensitive (SOS) signaling pathway is a well-explored mechanism that illustrates how plants decode salt stress-induced Ca^{2+} signals to maintain ionic balance at the level of individual cell (Manishankar *et al*., 2018). First, salt stress-triggered Ca^{2+} waves are rceived via the calcium sensor SOS3/CBL4, which then binds to and activates the CBL-interacting protein kinase SOS2/CIPK24 (Zhu, 2016). The resulting SOS2-SOS3 complex phosphorylates and initiates the Na^+/H^+ antiporter SOS1/NHX7 to maintain a consistent sodium ion concentration within the cell (Zhu, 2016). Independent studies have additionally explored the function of calcium binding proteins in the regulation of plant hormones, gene expression, and RNA stability, further illustrating how plants convert Ca^{2+} signals into salt adaptation mechanisms (Yang & Guo, 2018).

Alternative pre-mRNA splicing (AS) is a post-transcriptional processing step for regulating gene expression that also mediates transcriptome complexity and diversity. AS has a vital role for abiotic and biotic stress resistance in plants, as indicated in its modulation of drought, high/low temperatures, increased osmotic pressure, and high salinity tolerance (Laloum *et al*., 2018).

Transcriptome-wide studies have revealed marked AS modifications under salt stress, with differentially-spliced transcripts generated through various AS events such as usage of alternative 5' or 3' splice-sites (5'SS, 3'SS), or intron retention (IR) (Ding *et al*., 2014; Feng *et al*., 2015; Zhu *et al*., 2018). Additional evidence of AS importance in salt tolerance is provided by the knockout mutants of several spliceosomal proteins and splicing regulators, which exhibit hypersensitivity to high salt stress. For example, SNW/SKI-INTERACTING PROTEIN (SKIP) is a spliceosomal protein that interacts with other splicing factors such as SERINE/ARGININE-RICH SPLICING FACTOR 45 (SR45) and plays an essential role in splice site selection. Interestingly, Arabidopsis mutant lines for both *SKIP* and *SR45* display salt-sensitive phenotypes when grown in saline environment (Feng *et al*., 2015; Wang *et al*., 2012; Albaqami *et al*., 2019). Furthermore, several other studies have confirmed roles of other splicing-related proteins in salinity tolerance mechanism, such as SUPERSENSITIVE TO ABSCISIC ACID AND DROUGHT 1 (SAD1, also known as LSm5), a protein arginine methyltransferase (PRMT5, also known as SKB1), STABILIZED 1 (STA1), At U1A, and SM PROTEIN E1 (SME1) (Lee *et al*., 2006; Cui *et al*., 2014; Feng *et al*., 2015; Gu *et al*., 2018; Huertas *et al*., 2019).

Although salt-induced Ca^{2+} signals are involved in many different regulatory steps of gene expression, and salt stress is known to extensively stimulate AS changes in plants, the underlying regulatory mechanism and especially the role of Ca^{2+} signals in the modification and regulation of RNA splicing remain poorly understood. Here, evidence is presented that salt-triggered long-distance calcium signals mediate salt-regulated AS. Root tip-localized salt treatment was found to influence shoot expression and AS of selected salt-responsive genes. However, inhibition of calcium signal by localized $LaCl_3$ treatment at mid-region of the root results in preventing this changes. These results show that salt-induced calcium signals mediate the expression and AS of genes related to ion homeostasis, stress response, and RNA splicing.

Materials and Methods

Plant material: This study used the *Arabidopsis thaliana* (Arabidopsis, wild-type (WT)) of the Columbia-0 (Col-0) ecotype. For salt stress experiments, fully mature seeds were collected from plants cultivated at 25°C and 16 hours (h) light / 8 h dark, 120 mmol/m^2/s. Seeds were allowed to dry for ~ two weeks at room temperature and stored under a dry atmosphere.

For *In vitro* growing of Arabidopsis, seeds were surface-sterilized in 10% bleach (sodium hypochlorite) with 0.05% Tween-20 for 15 min and rinsed five times with sterile ddH_2O. Seeds were then sown on a plate of 1x Murashige and Skoog (MS) mineral salts supplemented with 1% sucrose and vitamins and 0.8% agar, micropropagation grade. For stratification, sterilized seeds were kept at 4°C for three days before being transferring to the growth chamber.

Salt stress treatment: Salt stress (NaCl) and calcium signal inhibitor ($LaCl_3$) treatments were conducted as described in (Choi *et al*., 2014). Briefly, sterilized seeds were germinated on MS basal medium for ten days at 25°C and 16 h light / 8 h dark, 120 mmol/m^2/s. Treatments were performed on ten-day-old seedlings. Before salt stress application, the site of NaCl treatment (root tips) and the site of calcium signal inhibitor treatment (upper part of the root toward the shoot, herein referenced as the mid-region of the root) were covered by a ~5-mm strip of Kimwipe, and then it is saturated with ~20 ul of MS media and allowed to acclimatize for one hour (Fig. 1). Afterwards, ~20 ul of liquid MS supplemented with 25 µM $LaCl_3$ was added to the Kimwipe strip located at the mid-region of the roots for localized Ca^{2+} signal inhibitor treatment, and left for 30 minutes (min.). Subsequently, ~20 ul of liquid MS with or without 150 mM NaCl was added to the Kimwipe strip located on the root tips for localized salt stress treatment. Plates containing treated samples were kept in the plant growth incubator for one hour before tissue sampling. Shoot samples were separated from the roots using a sharp blade and stored directly into liquid nitrogen for RNA extraction.

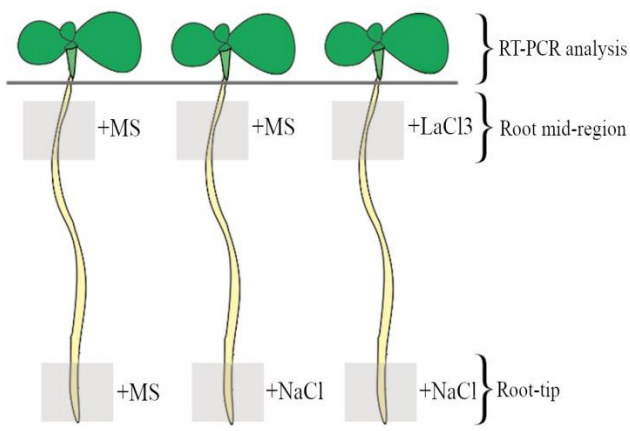

Fig. 1. Schematic diagram illustrating the salt treatment experiment. MS media treated seedling (control) on the left (+MS, +MS), salt (150 mM NaCl)-treated seedling in the middle (+MS, +NaCl), and calcium blocker pre-treated seedling on the right (+$LaCl_3$, +NaCl). Gray transparent boxes indicate the sites of NaCl and $LaCl_3$ application using ~5-mm strips of Kimwipe. The horizontal gray line across all three samples indicates the position of shoot/root separation after treatment for downstream analysis. All seedlings were ten days old and acclimatized using MS media for one hour before starting stress treatment.

Total RNA isolation and RT-PCR analysis: Total RNA was isolated from frozen samples with the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The RNA was quantified by a NanoDrop spectrophotometer, and treated with DNase I (Fermentas, Hanover, MD) according to the manufacturer's protocol to remove residual genomic DNA.

For RT-PCR, first-strand cDNA was synthesized from 1.5 µg of total RNA (DNA-free) using an oligo(dT) primer and SuperScript III (Invitrogen, Carlsbad, CA) in a 20 µl reaction conducted according to the manufacturer's protocol. Subsequently, 1 ul of cDNA was used as a template to perform polymerase chain reactions (PCR) with gene-specific primers in a final volume of 20 µl. The primers were designed using the online tool PRIMER3

(http://frodo.wi.mit.edu). Thirty cycles of PCR amplification were conducted, each consisting of 30 s denaturation at 94°C, 30 s annealing at 55°C, and elongation at 72°C for a specific time frame according to the predicted product size. Afterwards, PCR products were separated using DNA electrophoresis and the gel imaged. *Arabidopsis ACTIN-2* was used as a loading control.

Gene accession numbers: *SOS1* AT2G01980, *WRKY33* AT2G38470, *WRKY25* AT2G30250, *ABI5* AT2G36270, *SOS3* AT5G24270, *MYB33* AT5G06100, *ABF2* AT1G45249, *SR30* AT1G09140, *SOS4* AT5G37850, *SR34b* AT4G02430, and *ACT2* AT3G18780, *SOS2* AT5G35410.

Results

Salt-induced root Ca^{2+} signals enhance expression of SOS genes in the shoot: One of the major players in maintaining whole-plant ion balance under high salt stress is the SOS pathway. Components of this signaling pathway, *SOS1*, *SOS2*, and *SOS3*, show spatial and temporal differences in expression under different saline conditions (Ji *et al*., 2013). It is also worth mentioning that the activities and expression of *SOS* genes are controlled by calcium signals (Manishankar *et al*., 2018). Thus, in order to ascertain the role of salt-induced systemic Ca^{2+} signaling in pre-mRNA AS; first, the response of different *SOS*s in the shoot to localized salt treatment of the root tips was determined. Further, a plasma-membrane Ca^{2+} channel blocker ($LaCl_3$) was applied to the mid-region of the root to determine if blocking Ca^{2+} signaling inhibits Na^+-triggered changes in shoot gene expression. In this experiment, three different treatments were applied to the root tips of ten-day-old Arabidopsis seedlings using ~5-mm strips of Kimwipe to ensure precise local treatment, as explained before in the methods part and illustrated in Figure 1. Control plants were treated with MS media on both root tips and the mid-region of the root (the signal blocking region); salt-treated plants were treated with 150 mM NaCl on root tips and control MS media on the blocking region; and the last group consisted of seedlings treated first with $LaCl_3$ for 30 minutes on the blocking region and followed by 150 mM NaCl on the root tips. Before treatment, all plant samples were acclimatized with control MS media-saturated Kimwipe strips for one hour; subsequently, the $LaCl_3$ and NaCl treatments were applied as described for another one hour. Although NaCl and $LaCl_3$ treatments were applied on portions of the root, gene expression analysis was only conducted on the detached shoot as shown in (Fig. 1).

Under root tip-localized salt treatment (150 mM), shoot expression of *SOS1*, *SOS2*, and *SOS3* was elevated compared to that in MS-treated seedlings (Fig. 2). However, this induction was eliminated when the seedlings were treated with a putative calcium channel blocker (25 ul $LaCl_3$) in the mid-region of the root (Fig. 2). These findings indicate that salt-induced calcium signals progress from the root tips to the aerial parts of Arabidopsis seedlings and modulate shoot expression of *SOS* genes in response to root tip-localized ion treatment. The two differentially-expressed alternative transcripts of *SOS3* shown in Figure 2 are discussed in further detail below. Together, these results and preceding reports (Choi *et al*., 2014) confirm that salt-triggered systemic calcium waves mediate cellular responses to salinity in plants.

Numerous stress genes respond to root-localized salt treatment: In addition to the SOS pathway, salinity has been shown to influence several other stress response pathways in plants. Abscisic acid (ABA) is a plant hormone that shows essential functions in stress tolerance mechanisms. ABA-dependent transcription factors such as BASIC LEUCINE ZIPPER (bZIP) and the WRKY transcription factors are members of a large protein family established to be a master mediator of abiotic stress responses, including to high salinity (Phukan *et al*., 2016; Skubacz *et al*., 2016). It has been also shown that salt stress regulates the expression of Arabidopsis *WRKY33* through an ABA signaling-dependent mechanism that is independent of the SOS pathway (Jiang & Deyholos, 2009). Therefore, the systemic shoot response of two selected *WRKY* transcription factors (*WRKY33* and *WRKY35*) and one of the fondamental factors of ABA signaling (*ABI5*) to the Ca^{2+} signals induced by root-localized salt treatment was examined. RT-PCR analysis showed that salt treatment enhances shoot expression of *WRKY33*, *WRKY25*, and *ABI5* relative to that in untreated controls (Fig. 3). Interestingly, and similar to the previous *SOS* experiment, this induction of gene expression was inhibited in samples that were pre-treated with $LaCl_3$ to block the Ca^{2+} signal (Fig. 3). All together, these results indicate that salt-triggered whole plant calcium signals control the functions of several genes that are rlated to salt adaptation mechanisms spanning the entire plant body.

Root-to-shoot Ca^{2+} signals regulate pre-mRNA splicing: Based on the results and reported findings described above, it is clear that localized salt-triggered long-distance calcium signals regulate the response of many essential salt stress-related genes. However, whether this signal influences salt-regulated AS remains an unanswered question in the field of plant pre-mRNA splicing. It is worth mentioning that alteration of AS in response to calcium-moderated stimuli has been well-established in metazoa (Xie, 2008). To determine the presence of a like mechanism in Arabidopsis, several genes that are identified to be alternatively spliced under various stimuli, including environmental stresses, were selected and their splicing modifications in response to localized salt treatment of root tips and the associated Ca^{2+} signal examined. For each selected gene, primer pairs were designed to amplify the region predicted to be alternatively spliced (Fig. 4).

The first gene selected for evaluation, *MYB TRANSCRIPTION FACTOR 33* (*MYB33*), belongs to the MYB family of transcription factors, which is involved in abiotic stress tolerance in plants (Ambawat *et al*., 2013). Based on existing Arabidopsis alternative splicing annotations, *MYB33* is predicted to be alternatively spliced in its 5' UTR. Under normal growth conditions (MS media without NaCl supplementation), *MYB33* is alternatively spliced to generate three transcripts (I, II and III) (Fig. 4). These three spliced mRNAs exhibit differential abundance in response to root-localized salt treatment (NaCl, 150 mM), with isoform II being increased more than the other isoforms. Interestingly, the change in abundance was unaffected when the seedling was pre-treated with a calcium channel blocker ($LaCl_3$) at the mid-region of the root located more toward the shoot. These results indicate that the salt-triggered Ca^{2+} signal mediates a change of *MYB33* splicing in shoots.

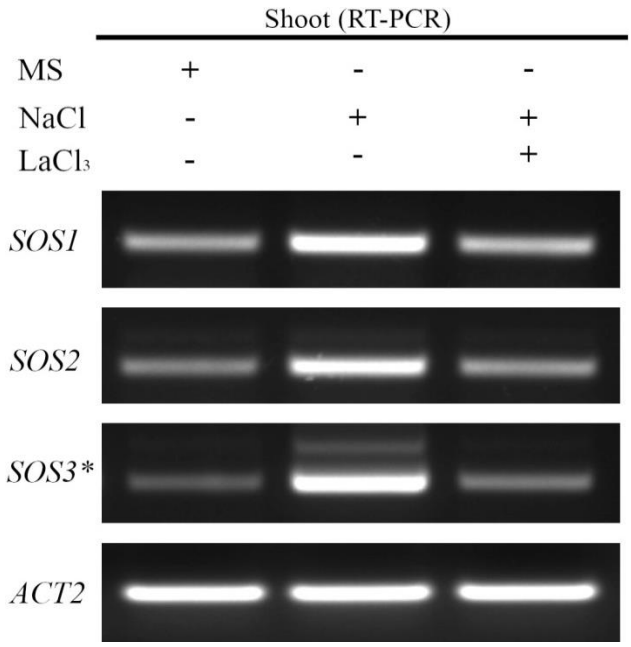

Fig. 2. Analysis of *SOS* gene expression changes in response to root-localized salt treatment. Total RNA was isolated from the shoot portion of ten-day-old Arabidopsis seedlings. Control seedlings were treated with MS media only; salt treatment consisted of MS media supplemented with 150 mM NaCl; calcium channel blocker seedlings were pre-treated with $LaCl_3$ at the mid-region of the root as described above. Electrophoresis gel image shows differential expression of *SOS1*, *SOS2*, and *SOS3* between the three treatments. Gene-specific primers were used to amplify cDNA that had been synthesized from total RNA using oligo (dT). *ACT2* was used as an internal control.* Changes in *SOS3* isoform abundance are detailed in Figure 4.

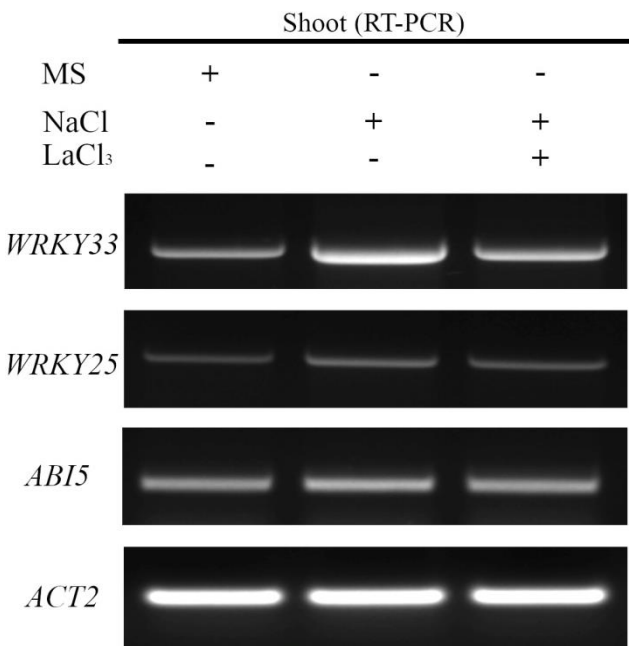

Fig. 3. Analysis by RT-PCR of expression changes in stress-responsive genes in response to root-localized salt treatment. This gel electrophoresis image illustrates the expression changes of *WRKY33*, *WRKY25*, and *ABI5*; genes were amplified using specific primers for each of the three different treatments described above (an MS media treated control as well as NaCl treatment with and without the Ca^{2+} channel blocker $LaCl_3$). *ACT2* was used as an internal control.

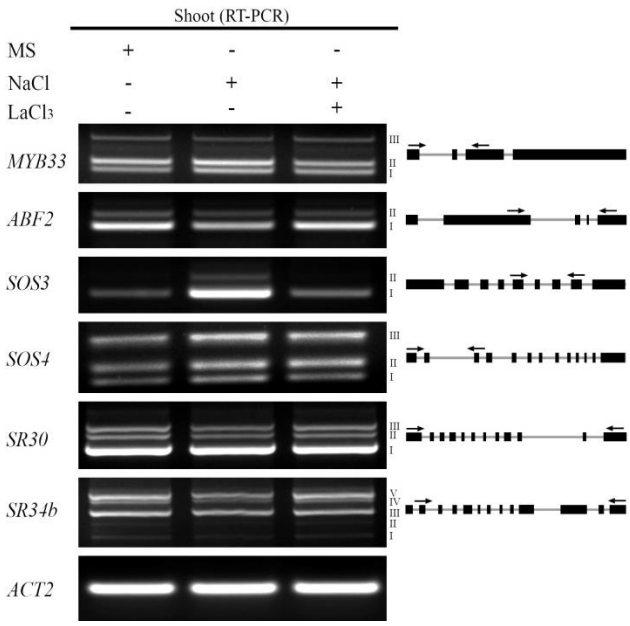

Fig. 4. Analysis of shoot-specific splicing changes in response to root-localized NaCl treatment. Electrophoresis gel image shows the changes in AS of selected genes (*MYB33*, *ABF2*, *SOS3*, *SOS4*, *SR30*, *SR34b*) across the three different treatments (control, NaCl, and NaCl with $LaCl_3$ pre-treatment). cDNA was synthesized from total RNA using oligo(dT). Gene-specific primers were designed targeting the predicted alternatively-spliced regions illustrated by arrows in the gene structure diagrams (on the right). *ACT2* was used as an internal control.

The second selected gene, *ABSCISIC ACID RESPONSIVE ELEMENTS-BINDING FACTOR 2* (*ABF2*), is an essential transcription factor in ABA-dependent stress regulation of gene expression (Lee *et al*., 2010). *ABF2* is alternatively spliced under ABA and salinity treatment. Interestingly, this change in splicing of *ABF2* is defective in mutants of splicing genes like *SHK1 KINASE BINDING PROTEIN* (*SKB1*) (Zhang *et al*., 2011). The present study determined that under normal growth conditions, *ABF2* generates two splicing isoforms (I and II), with both of these transcripts showing reduced expression under root tip-localized salt treatment (Fig. 4). As noted earlier with *ABI5*, the splicing changes of *ABF2* are eliminated upon pretreatment of seedlings with $LaCl_3$, the salt-treated seedlings instead exhibiting expression patterns akin to that of seedlings treated with MS media. These results suggest that salt-induced calcium signaling influences the splicing of *ABF2* and by extension expression of genes involved in salt stress-responsive pathways, including the ABA pathway.

As discussed above, the SOS pathway is a critical component in the mechanism of salt stress tolerance and some *SOS* transcripts are alternatively spliced (Albaqami *et al*., 2019). Thus, the splicing changes of *SOS3* and *SOS4* expressed in the shoot in response to root salt treatment was investigated. *SOS3* is alternatively spliced to generate two isoforms (I and II), with expression of both transcripts induced in the shoot by root-localized NaCl treatment; the two isoforms do not appear under normal growth conditions (Fig. 4). Furthermore, blocking the calcium wave that is generated upon salt stress inhibited the splicing changes in shoot expressed *SOS3*

with transcript levels comparable to that in shoots of untreated seedlings. In a similar way, *SOS4* undergoes regulated splicing changes, generating three isoforms (I, II, and III) under normal conditions. Root-localized salt treatment increased shoot expression of all three isoforms (Fig. 4). Interestingly, pre-treatment with $LaCl_3$ did not eliminate the salt-induced up-regulation of these isoforms. These results indicate that one or more additional signaling pathways are involved in the modulation of *SOS* splicing changes in response to salt stress, specifically for induction of the three *SOS4* isoforms.

Serine/arginine-rich (SR) proteins are well-characterized as essential splicing regulators and play fundamental roles in abiotic stress responses (Morton *et al*., 2019). Several *SRs* are alternatively spliced under different abiotic stresses, including salt stress (Palusa *et al*., 2007). Among those differentially spliced in response to salt stress are *SR30* and *SR34b*, whose shoot splicing patterns were examined in response to root-localized NaCl treatment. Under normal growth conditions, *SR30* generates three spliced forms (I, II and III) and *SR34b* generates five (I, II, III, IV and V) (Fig. 4). Both of these genes show different patterns of isoform abundance under salt treatment, and both were inhibited by $LaCl_3$ pre-treatment, with isoform expression patterns being returned to that of the control sample. Interestingly, *SR30* isoform I, which is predicted to be the functional isoform, shows enhanced expression under salt treatment as compared to the other predicted nonfunctional isoforms (II & III). Preferential induction of functional splicing isoforms in response to the salt-triggered calcium signal may explain the transcriptome-wide splicing changes observed under salt stress.

Discussion

AS is a common phenomenon in all eukaryotes, and a clear central modulator of gene expression in several ways. In plants, most protein-coding genes are differentially spliced, thereby increasing transcriptome and proteome complexity. Abiotic stresses, including high salinity, widely reprogram the plant transcriptome via as a mechanism for mediating stress tolerance. Although several signaling pathways have been well-characterized in plants as being induced under salt stress, such as Ca^{2+}, ROS, and other ion signaling, their impact on AS is not well understood. In this work, it is shown that a salt stress-activated long-distance calcium wave modulates both expression and AS of *SOS* genes, abiotic stress responsive genes, and *SR* genes.

Salt stress-induced calcium waves influence systematic gene expression at both transcriptional and post-transcriptional levels; however, the possibility of other signaling components being involved in this action also cannot be ruled out. It is been well-documented that high salinity induces not only Ca^{2+} as an early signal but also K^+, pH, phospholipids, ROS, and protein kinases (Van Zelm *et al*., 2020). One or more such signals may be involved in the case of *SOS4* (Fig. 4), where the calcium signal inhibitor $LaCl_3$ did not completely block the effect of salt stress on splicing changes. Likewise, salt stress-induced calcium fluxes may have numerous impacts on gene expression, especially on changes in splicing during the stress response.

Selection of splice sites is mediated via combinations of RNA binding proteins, such as members of the SR and hnRN protein families. The cycle of phosphorylation and dephosphorylation of splicing regulators has been linked to spliceosome assembly and splice site recognition (Zhou & Fu, 2013). Further, spliceosomal protein kinases are vital in the modulation and control of splicing mechanisms via phosphorylation (Kanno *et al*., 2018). Meanwhile, salt-induced Ca^{2+} signals are perceived and decoded by numerous calcium binding proteins and calcium sensors, including calmodulin (CaM), Ca^{2+}-dependent protein kinases (CDPKs), and the SOS3-like Ca^{2+}-binding protein/calcineurin B-like protein (SCaBP/CBL) (Latz *et al*., 2013; Yang *et al*., 2019). The calcium sensors bind to and selectively activate/inactivate their cellular targets, such as protein kinases, in order to control specific stress response mechanisms. Thus, spliceosomal protein kinases may be a target of a calcium-activated downstream pathway that controls splicing changes under salt stress.

Splicing regulators themselves are regulated via salt-induced calcium signals, with emerging evidence indicating that splicing factors like SR proteins are major components of stress tolerance in plants. The two *SRs* examined here, *SR30* and *SR34B*, have also been shown to be regulated by salt stress and other abiotic stresses. In addition, RNA binding proteins are known to be regulated in a calcium-dependent manner; one example is CLEAVAGE AND POLYADENYLATION SPECIFICITY FACTOR 30 (CPSF30), which is bound by calmodulin to regulate its RNA binding activity (Delaney *et al*., 2006). In animal systems, calcium signals have long been known to regulate splicing factors, including SRs and calcium-regulated AS (Xie, 2008). Combined with the present study, these data suggest that, in addition to unknown pathways, salt stress-regulated splicing may be mediated through the regulation of SR protein activities by calcium binding proteins.

Conclusion

While salt-regulated RNA splicing has been extensively characterized in plants, the upstream signaling pathways responsible for splicing changes under salt stress remain unclear. Thus, as shown here, salt-induced systematic calcium waves modulate gene expression both during transcription initiation and alternative splicing. Interestingly, root-localized salt treatment induces a long-distance calcium wave that regulates different stress-responsive pathways. It is tempting to predict that such alteration of RNA transcripts contributes to salt stress tolerance in plants, although additional studies are needed to uncover the potential functions of specific spliced isoforms.

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