

Arabidopsis SOS3 plays an important role in salt tolerance by mediating calcium-dependent microfilament reorganization

Jiamin Ye · Wenhua Zhang · Yan Guo

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Abstract

Key message SOS3 mediates calcium dependent actin filament reorganization that plays important roles in plant responses to salt stress.

Abstract *Arabidopsis* salt overly sensitive 3 (SOS3) plays an important role in plant salt tolerance by regulation of Na^+/K^+ homeostasis. Plants lacking SOS3 are hypersensitive to salt stress and this phenomenon can be partially rescued by the addition of calcium. However the mechanism underlying remains elusive. We here report that the organization of actin filaments in *sos3* mutant differs from that in wild-type plant. Under salt stress abnormal actin assembly and arrangement in *sos3* are more pronounced, which can be partially complemented by addition of external calcium or low concentration of latrunculin A, an actin monomer-sequestering agent. The effects of calcium and Lat A on actin filament organization of *sos3* mutant are accordant with their effects on *sos3* salt sensitivity under salt stress. These findings indicate that the salt-hypersensitivity of *sos3* mutant partially results from its disordered actin filaments,

and SOS3 mediated actin filament reorganization plays important roles in plant responses to salt stress.

Keywords *Arabidopsis* · Microfilament · Calcium · Salt tolerance

Abbreviations

SOS Salt overly sensitive
MF Microfilament

Introduction

Plants need to cope with a variety of environmental changes during their life cycle. Soil salinity is one of the major factors that reduce plant growth and lead to limitation of agricultural crop productivity worldwide (Apse 1999; Zhu 2001). A primary part of growth inhibition under salt stress is caused by uptaking excess Na^+ , and high accumulation of Na^+ disrupts K^+ nutrition and hinders functions of many enzymes in the cytoplasm (Xiong et al. 2002; Xiong & Zhu 2002). Plants develop subtle mechanisms to keep intracellular ionic homeostasis of Na^+/K^+ in order to adapt environmental salt stress (Zhu 2002, 2003).

In *Arabidopsis thaliana*, the salt overly sensitive (SOS) pathway is identified as an important regulatory system activated by salt stress to maintain a high K^+ and low Na^+ homeostasis for salt tolerance, and defined to have three components, SOS1, SOS2 and SOS3 (Zhu et al. 1998; Zhu 2002). SOS3, a calcium sensor with four EF-hand domains and a N-myristoylation signal peptide, perceives the increase of intracellular calcium stimulated by salt stress (Liu and Zhu 1998; Ishitani et al. 2000; Sanchez-Barrena et al. 2005), and then recruits SOS2, a Ser/Thr protein

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J. Ye · W. Zhang (✉)
State Key Laboratory of Crop Genetics and Germplasm Enhancement, College of Life Sciences, Nanjing Agricultural University, Nanjing 210095, China
e-mail: whzhang@njau.edu.cn

J. Ye · Y. Guo (✉)
State Key Laboratory of Plant Physiology and Biochemistry, College of Biological Sciences, China Agricultural University, Beijing 100094, People's Republic of China
e-mail: guoyan@cau.edu.cn

kinase, to the plasma membrane (Halfeter et al. 2000; Liu et al. 2000; Guo et al. 2001, 2004). The SOS3–SOS2 complex further activates the downstream target protein SOS1 (Qiu et al. 2002; Quintero et al. 2002). SOS1 is a plasma membrane-localized Na^+/H^+ antiporter to prevent the accumulation of Na^+ to toxic levels (Wu et al. 1996; Shi et al. 2000; Qiu et al. 2002, 2004). Genetic evidence suggests that the recessive *sos3* mutant shows hypersensitivity to salt stress, and accumulates more Na^+ and retains less K^+ than wild-type plants. The salt sensitivity of *sos3* can be partially rescued by the addition of external calcium (Liu and Zhu 1997, 1998); however, the mechanism underlying is not clear.

Plant cytoskeleton is mainly composed by microtubules (MTs) and microfilaments (MFs). The dynamics of cytoskeleton plays an important role in almost every intracellular activity and involves in responses to different environment stimuli (Olinevich and Khokhlova 2003; Wang et al. 2011a). It has been reported that salt stress affects MT organization (Shoji et al. 2006; Wang et al. 2011b). High KCl treatment inhibits the cell elongation of root cells in maize and affects the orientation of MT array from transverse to parallel in cortical cells (Blancaflor and Hasenstein 1995). Transverse orientation of MT arrays in tobacco BY-2 cells is also influenced under 150 mM NaCl treatment and turned to be random in arrangement (Dhokhshe et al. 2003). SPR1, a plant specific small MT-binding protein, genetically interacts with SOS1 and SOS2 (Furutani et al. 2000). Mutation in either SOS1 or SOS2 suppresses both MT disruption and helical growth phenotypes of *spr1*. Furthermore both *sos1* and *sos2* mutants display altered helical growth response to low dose MT-target drug (Nakajima et al. 2004; Shoji et al. 2006). These results imply that MTs involves in response to plant salt stress.

Calcium plays an important role in plant response to salt stress as a second messenger (Zhu 2001; Xiong et al. 2002), and previous studies reveal a close interaction between Ca^{2+} signals and MT cytoskeleton (Hori et al. 1994; Tsai and Hwang 1998; Allen et al. 2003; Mironov et al. 2005). Depolymerization of MTs results in an increased intracellular calcium signal, and which is suppressed by a MT stabilization drug paclitaxel (Thion et al. 1996, 1998; Wang et al. 2007). Furthermore, the reorganization of the cortical MTs and the seedling survival under salt stress are coordinated and regulated by a calcium influx (Wang et al. 2007).

Recent studies have also indicated that the MF cytoskeleton involves in regulation of plant salt tolerance (Wang et al. 2010a; Liu et al. 2012). Salt stress induces MF assembly and bundle formation in early stage; however, longer exposure under high salt concentration leads the MF depolymerization and seedling death (Wang et al. 2010a;

Liu et al. 2012). The salt tolerance is improved by stabilization of MF with phalloidin, while it is reduced by latrunculin A/B (Lat A/B) and cytochalasin D (Cyt D) through blocking MF assembly (Wang et al. 2010a; Zhou et al. 2010). Although it has been demonstrated that the MF cytoskeleton participates in plant response to salt stress and Ca^{2+} signal functions in regulating MF dynamics (Wang et al. 2002, 2004, 2010b), there is a lack of documentation how the MF cytoskeleton and Ca^{2+} signal coordinately function in this regulatory process. Here we report that salt stress first induced MF polymerization and reorientation, and long-time salt stress at high concentration led MF depolymerized that in turn triggered plant death. The *sos3* mutant showed more rapid and dramatic MF reorganization and reorientation under salt stress comparing to that in wild-type plants. In addition calcium partially rescued the growth defect and actin damage phenotypes of *sos3* under salt stress, which can be mimicked by low concentration of Lat A treatment. These results indicate that SOS3 plays an important role to link calcium and the MF dynamics in plant salt stress response.

Materials and methods

Plant materials

Seeds of wild type (*gl1*) and the *sos3* mutant (in *gl1* background) (Liu and Zhu 1997) were sterilized with the solution that contains 20 % sodium hypochlorite and 0.1 % Triton X-100 for 10 min, washed five times in sterilized water, and then sown on Petri dishes containing Murashige and Skoog (MS) basal medium, 0.5 % (w/v) PhytoGel (Sigma-Aldrich) and 2.5 % (w/v) sucrose, pH 5.8. The plates maintained at 4 °C for 3 days and then were moved to a growth chamber at 23 °C under continual illumination. The seedlings germinated vertically for 5 days with a root length ~1.5 cm were transferred onto MS medium with different treatments, according to the requirements for growth phenotype observation. Four-day-old seedlings germinated under the same growth condition as above were moved to fresh petri dishes containing various solutions as described, and after ~24 h treatment in growth chamber the materials were applied for actin filaments assay.

Suspension cells material

Ten-day-old seedlings of wild type and the *sos3* mutant grown on MS medium plates were cut into pieces, moved onto MS basal medium, 0.3 % (w/v) PhytoGel (Sigma-Aldrich) and 2.5 % (w/v) sucrose, pH 5.8, containing 1 mg/L 2,4-D and 0.1 mg/L 6-BA, and cultured at 23 °C in the dark to induce the callus formation for 2–3 weeks.

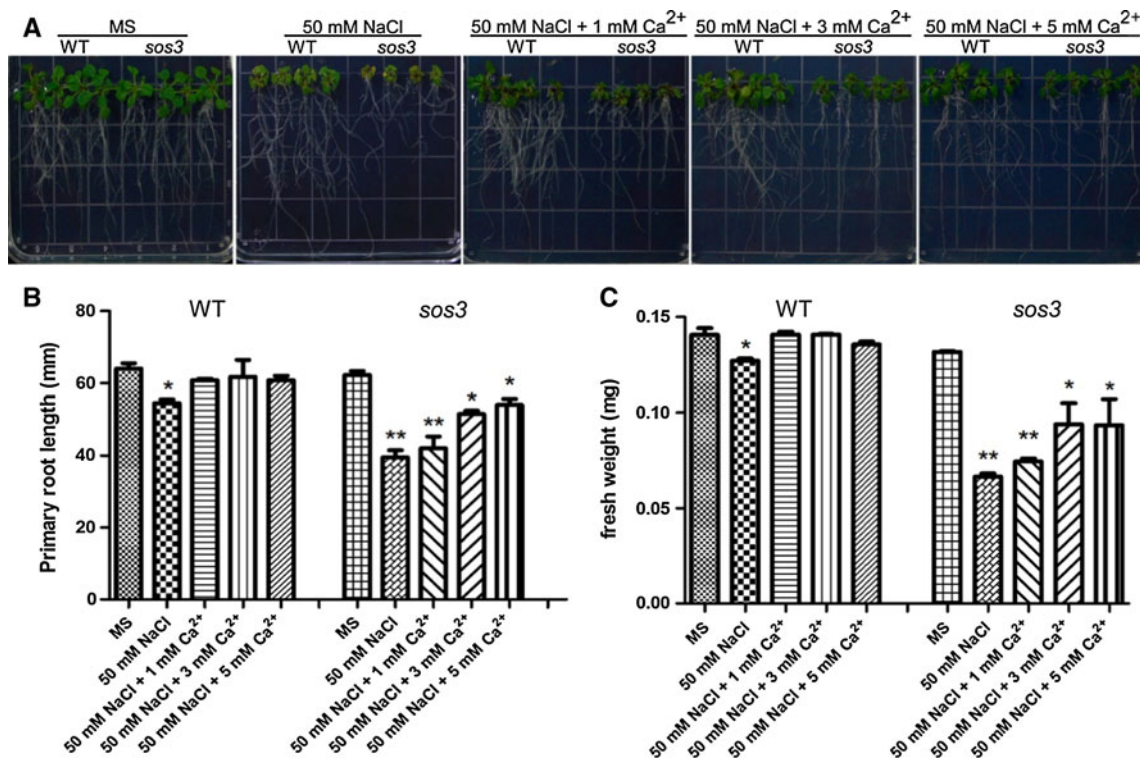


Fig. 1 *sos3* is a salt-sensitive mutant and external calcium partially rescues its phenotype. **a** Five-day-old wild-type and *sos3* seedlings were transferred onto MS medium containing 0, 50 mM NaCl, and 50 mM NaCl with 1, 3, 5 mM CaCl₂ respectively. The pictures were taken after 10 days of treatment. Primary root length (**b**), and fresh

weight (**c**) regarding to (**a**) were measured at day 10 after transfer. Error bars represent SD ($n > 15$). A Student's *t* test was used to determine statistical significance; asterisk means significant differences ($P \leq 0.05$) and remarkable significant differences ($P \leq 0.01$) are indicated by double asterisks in **b** and **c**

Grinded the callus under aseptic condition, and transferred them into conical flasks with 20–30 ml liquid MS basal medium, in addition of 2 mg/L 2,4-D and 0.1 mg/L 6-BA. The flasks were cultured at 24 °C on rocking incubator with 140 rpm/min for 10 days to acquire suspension cells and subcultured every 7 days to keep fresh suspension cells system.

F-Actin staining in *Arabidopsis*

F-actin staining was practiced as described by Traas et al. (1987) with some modification. In brief, suspension cells or 4-day-old seedlings with different treatments were washed with liquid MS medium for three times and then incubated in F-actin stabilizing buffer (5 mM MgSO₄, 10 mM EGTA, 100 mM PIPES, 5 % DMSO, and 0.05 % NP-40, pH 6.8) with 0.34 μM Alexa-fluor[®]-488-phalloidin for 15 or 30 min at room temperature respectively. The time of staining could be slightly modified according to the effect evaluation after scanning.

MF observations

MF organization arrangement was observed and captured the images on a Leica SP5 confocal microscope, using a

63× (Plan-Apochromat, NA1.4) oil objective lens. The samples of seedlings or suspension cells after staining were excited at 488 nm using a krypton-argon laser, and detect the emission fluorochrome signal from Alexa-488[®] with a 505–530 nm bandpass filter.

To assess the bundle formation rate of suspension cells in wild type and *sos3* mutant respectively, we initially applied fluorescence images of actin filaments from Leica SP5 confocal microscope with a 63× oil objective lens (Plan-Apochromat, NA1.4) with pinhole 1.5, detector 850. At least fifty cells per sample were analyzed, and each assessment was repeated at least three times.

F-actin cosedimentation assay

SOS3 was dialyzed for 1 h against 1× KMEI buffer (10 mM imidazole, 100 mM KCl, 1 mM MgCl₂, and 1 mM EGTA, pH 7.0). Protein concentration was determined using the BIO-RAD protein assay kit, with BSA as a standard. Actin was purified from rabbit skeletal muscle acetone powder as described in Pardee and Spudich (1982) in G buffer (5 mM Tris-HCl, pH 8.0, 0.2 mM ATP, 0.1 mM CaCl₂, 0.5 mM DTT, and 0.01 % NaN₃). Proteins were centrifuged at 400,000g for 1 h at 4 °C prior to use, mixed with 2 mM preformed F-actin, and incubated in

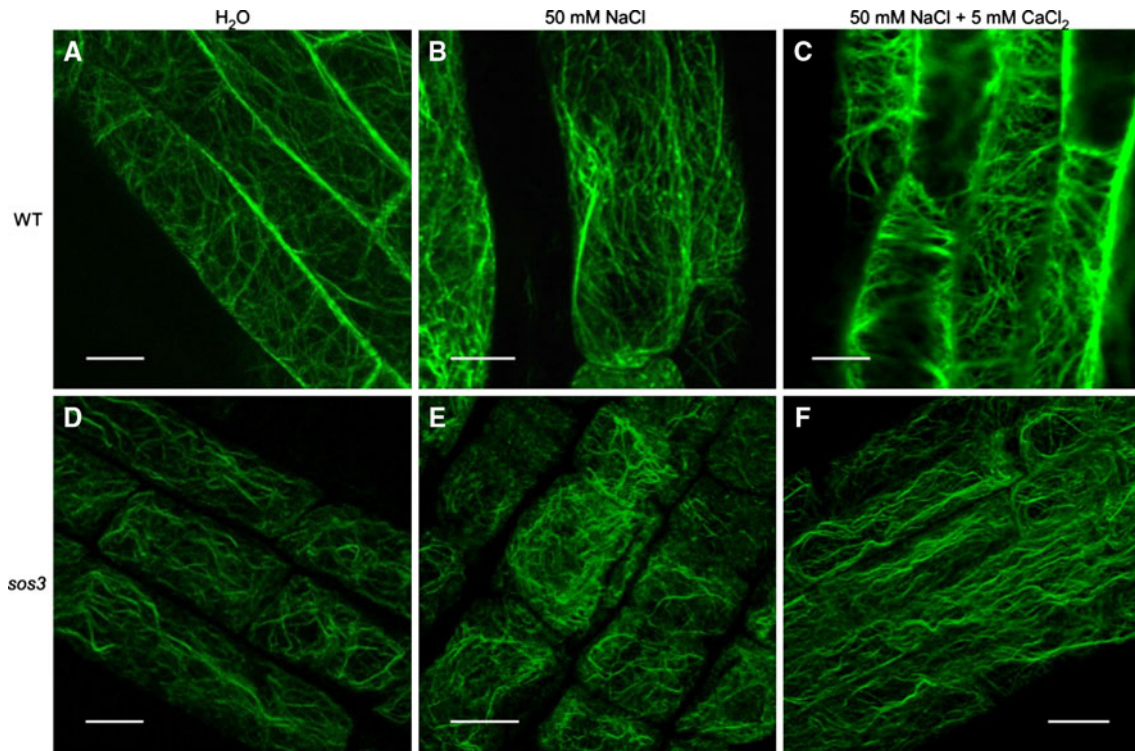


Fig. 2 Calcium involves in SOS3 regulated MF reorganization in salt stress. Four-day-old seedlings of wild type and *sos3* grown on MS medium were treated by deionized water (a) and (d), 50 mM NaCl (b) and (e), or 50 mM NaCl supplemented with 5 mM Ca^{2+} (c) and

(f) for 24 h respectively, and then stained with Alexa-488-phalloidin, and MF fluorescence signals of root elongation epidermal cells were observed. Over ten seedlings of each line under different treatments were observed. Bar 12 μm for fluorescence images

50 ml volume of $1\times$ KMEI buffer for 20 min at 23 °C. The samples were centrifuged at 150,000g for 30 min at 4 °C. SCAB1 is a known F-actin binding protein (Zhao et al. 2011) and used as a positive control. Proteins in supernatants and pellets were analyzed by SDS-PAGE, respectively.

Results

External calcium partially rescues *sos3* salt-hypersensitive phenotype

It has been reported that addition of calcium in medium partially rescues *sos3* salt-sensitive phenotype (Liu and Zhu 1997, 1998). To determine regulatory mechanism underlying, we first repeated the published results. Five-day-old seedlings of *sos3* and wild type germinated on MS nutrient medium were transferred to MS medium with or without 50 mM NaCl. Indeed *sos3* mutant was more sensitive to salt stress than wild-type seedlings (Fig. 1a).

When 5-day-old seedlings of wild type and *sos3* were transferred to the MS medium with combination of containing 0 or 50 mM NaCl and containing additional 0, 1, 3 or 5 mM CaCl_2 and grown for 10 days, the extra 3 or 5 mM Ca^{2+} in the medium significantly increased *sos3* salt

tolerance in both root length and fresh weight (Fig. 1a–c). Furthermore, the extra Ca^{2+} in the medium did not affect the growth of wild type and *sos3* without NaCl treatment (Supplemental Fig. 1).

Mutation in SOS3 disrupts arrangement of actin filaments

A number of studies have reported that MF dynamics is involved in plant response to abiotic stresses (Hwang and Lee 2001; Olinevich and Khokhlova 2002; Wang et al. 2010a; Zhou et al. 2010; Shi et al. 2011). Salt stress leads to the damage of the integrity of MF network (Liu et al. 2012). We investigated MF dynamics in *sos3* and wild type under salt stress. Four-day-old seedlings of *sos3* and wild type germinated on MS medium were transferred to fresh Petri dishes for different treatments with deionized water, 50 mM NaCl or 50 mM NaCl supplemented with 5 mM Ca^{2+} for 24 h. MFs were stained by Alexa-488-phalloidin for 30 min and observed by a Leica SP5 confocal microscope.

The MFs in wild type were well-organized network without 50 mM NaCl treatment (Fig. 2a). MFs were slightly bundled and rearranged preferentially as long cables along longitudinal direction under 50 mM NaCl

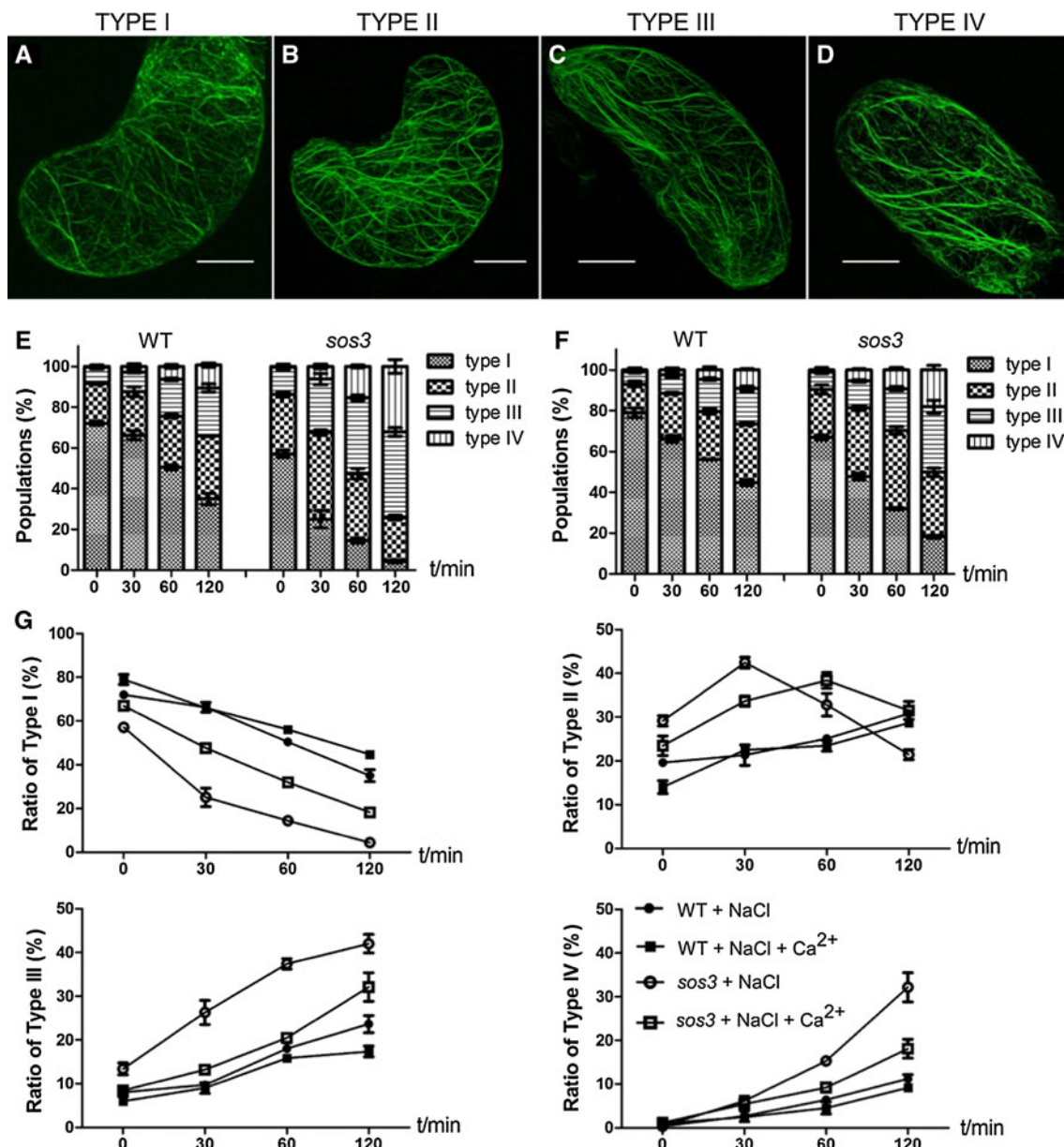


Fig. 3 Calcium functions in regulation of SOS3-dependent MF reorientation under salt stress in suspension cells. Confocal images of suspension cells after stained by Alexa-488-phalloidin. Actin organization was classified into four groups. Representative images of type I, well-organized network with fine actin filaments (a); type II, organized network with bundles formation (b); type III, long MF cables along longitudinal direction (c); type IV, chaotic organization with some fragmentation (d). Bar 10 μ m for fluorescence images. Histograms show the MF organization in suspension cells of wild

treatment (Fig. 2b), while the organization of MFs was recovered partially by the addition of 5 mM CaCl_2 in 50 mM NaCl treatment (Fig. 2c). In contrast, the MFs in *sos3* seedlings bundled as long cables along longitudinal direction even without NaCl treatment (Fig. 2d), and 50 mM NaCl treatment induced serious depolymerization of MFs that displayed fragmented and chaotic

type (WT) and *sos3* at the indicated times after 50 mM NaCl (e) or 50 mM NaCl supplemented with 5 mM Ca^{2+} treatments (f). The cells according to MF arrays were classified into four groups: type I, type II, type III and type IV. The data represent the mean \pm SD of three independent experiments; >150 cells per line were measured at the indicated times under different treatments. g Linear graphs show the ratio change of four-type cells regarding to e and f. Values are mean \pm SD

organization (Fig. 2e). Interestingly, the disordered MF organization under salt stress in *sos3* was recovered partially by the addition of 5 mM CaCl_2 , though the bundles along vertical axis were still much more obvious than that in wild type (Fig. 2f), which is consistent with the results shown in Fig. 1 that calcium partially rescued *sos3* growth phenotype under salt stress.

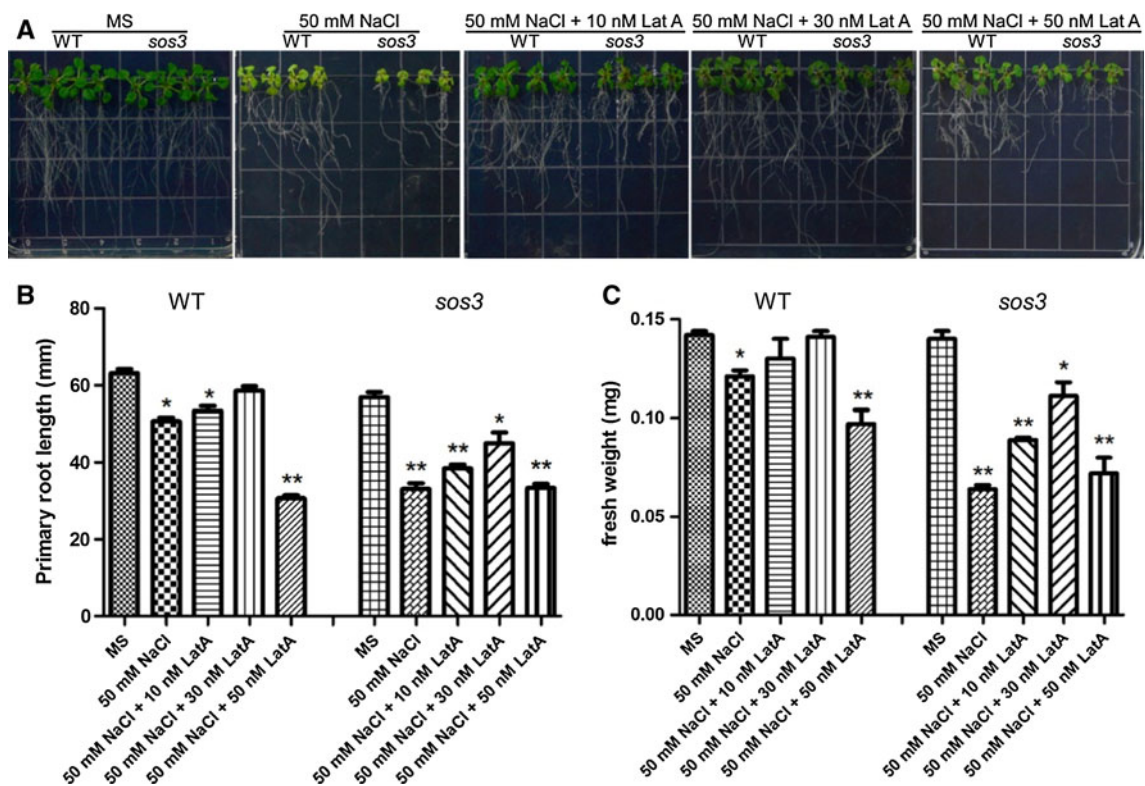


Fig. 4 Low concentration of Lat A partially rescues *sos3* salt-sensitive phenotype. **a** Five-day-old wild-type and *sos3* seedlings were transferred onto MS medium containing 0, 50 mM NaCl, and 50 mM NaCl with 10, 30, 50 nM Lat A respectively. The pictures were taken after 10 days of treatment. Primary root length (**b**), and

fresh weight (**c**) regarding to (**a**) were measured at day 10 after transfer. Error bars represent SD ($n > 15$). A Student's *t* test was used to determine statistical significance; asterisk means significant differences ($P \leq 0.05$) and remarkable significant differences ($P \leq 0.01$) are indicated by double asterisks in **b** and **c**

In order to test how MF dynamics is quickly response to salt stress in *sos3* and wild type, we generated suspension cell lines. Fresh suspension cells of wild type and *sos3* mutant grown in CIM liquid medium were treated with 50 mM NaCl at different time points, MFs were stained with Alexa-488-phalloidin and observed by the Leica SP5 confocal microscope. Consistent with the previous observation (Fig. 2), the MFs reorganized and disrupted in both wild type and *sos3* mutant along with the salt treatment. The major features of MFs along salt treatment can be grouped into four types. Under normal condition, MFs in most of the wild-type cells were well-organized network with fine filaments (Fig. 3a, type I). Under short-term salt treatment, the MFs bundled but still in a network condition (Fig. 3b, type II). After longer exposure to salt stress, the MFs reorganized into long cables along longitudinal direction (Fig. 3c, type III), and eventually the network of MFs was damaged and showed chaotic organization (Fig. 3d, type IV). The MFs were disordered more severe in *sos3* cells compared with that in wild type after salt treatment. In *sos3* mutant, more type II and III cells existed than that in wild type, and these cells rapidly increased to a higher level after 30 min NaCl treatment (Fig. 3e, g). When cells were pre-treated with 5 mM Ca^{2+} for 12 h and

then treated with 50 mM NaCl, the changes of MFs in wild-type cells were not significant (Fig. 3f, g). However the *sos3* cells with MFs in type III or IV were significantly reduced compared to the cells without 5 mM Ca^{2+} treatment (Fig. 3g). The type III cells were rapidly increased in *sos3* along with salt treatment, which was repressed nearly to a wild-type level upon with the 5 mM Ca^{2+} pre-treatment (Fig. 3f, g). However when we tested whether SOS3 binds to MFs, we did not detect the binding activity (Supplemental Figure 3).

Our results indicate that salt stress induces the bundle and disruption of MFs, this phenomenon is significantly enhanced in *sos3* mutant which is partially rescued by the presence of calcium. The results may also suggest that SOS3 plays a role in regulation of salt-induced calcium-dependent of MF reorientation and reorganization that are important for triggering plant salt tolerance.

Latrunculin A treatment mimics the function of calcium to rescue *sos3*

Our experiments imply that calcium partially rescues *sos3* salt sensitivity probably due to affecting MF reorganization. In order to test this hypothesis, we use latrunculin A

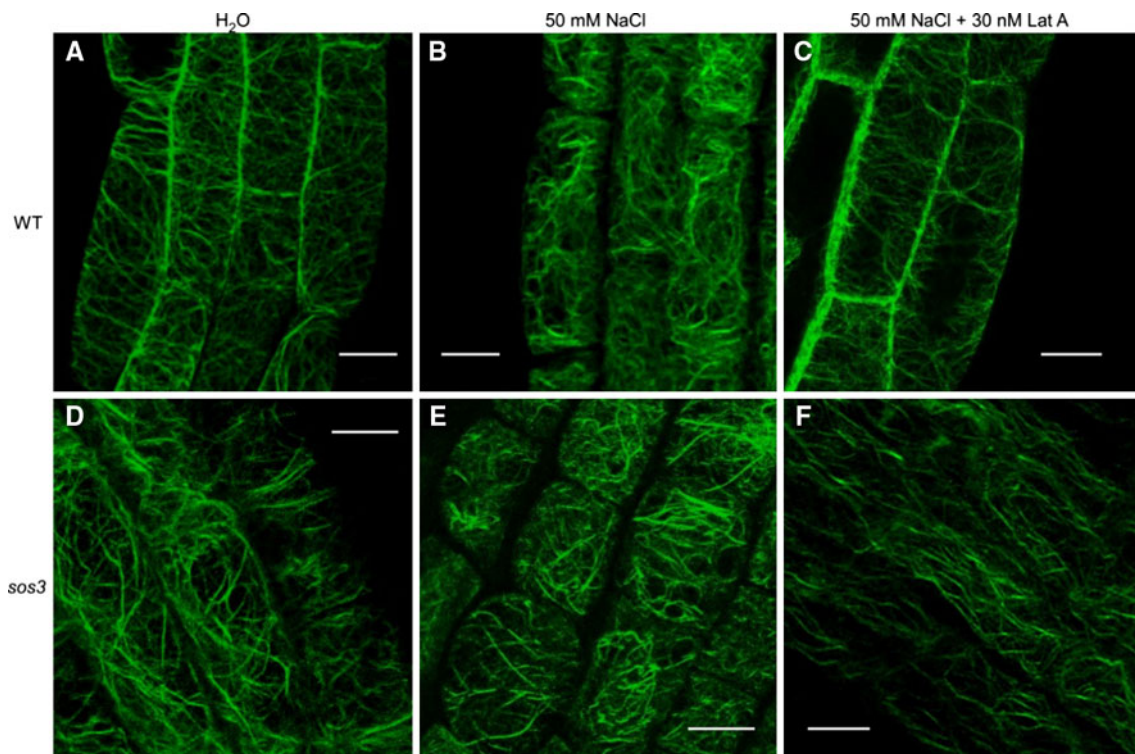


Fig. 5 Lat A mimics calcium in regulation of SOS3-dependent MF reorientation under salt stress. Four-day-old seedlings of wild type and *sos3* grown on MS medium were treated by deionized water (a, d), 50 mM NaCl (b, e), or 50 mM NaCl supplemented with 30 nM

Lat A (c, f) for 24 h respectively, and then stained with Alexa-488-phalloidin. MF fluorescence signals of root elongation epidermal cells were observed. Over ten seedlings of each line under different treatments were observed. Bar 12 μ m for fluorescence images

(Lat A), a drug depolymerizing cytoplasmic actin by binding to and sequestering actin monomers, to treat suspension cells and seedlings of *sos3* and wild type. Five-day-old seedlings of *sos3* and wild type were transferred to MS medium containing 50 mM NaCl with various concentration of Lat A and cultured for 10 days. 10 or 30 nM Lat A treatment partially rescued *sos3* salt sensitivity as the calcium treatment did, however 50 nM Lat A enhanced the salt sensitivity of both wild type and *sos3*. The root length and fresh weight were increased ~ 13.1 and ~ 23.4 % respectively with the addition of 10 nM Lat A. The treatment of 30 nM Lat A had even more significant positive effect on the *sos3* growth under salt stress. The root length was 45.0 ± 2.9 mm, increasing about 36.0 % than that without Lat A treatment, and the increase of fresh weight was about 57.8 % (Fig. 4a–c).

As a control, we found that 10 and 30 nM Lat A did not have remarkable influence on the growth of *sos3* and wild-type plants including root length and fresh weight on MS medium, while 50 nM Lat A obviously inhibited plant growth (Supplemental Figure 2). Thirty nM Lat A were then used to further test. Four-day-old seedlings of *sos3* and wild type were treated by 50 mM NaCl with or without 30 nM Lat A for 24 h and MF organization was monitored by Alexa-488-phalloidin staining. Consistent with our

previous results, 50 mM NaCl treatment altered MF reorganization in wild type and disrupted MFs in *sos3* mutant (Fig. 5b, e). However, with the presence of 30 nM Lat A in 50 mM NaCl medium decreased the MF bundles in wild type and maintained MF organization in *sos3* mutant as treated by calcium (Fig. 5c, f). In suspension cells, low concentration of 30 nM Lat A treatment had a similar effect on MFs as calcium treatment under salt stress (Fig. 6a, b). The increase of the type III cells under salt stress were significantly reduced by Lat A treatment in *sos3* cell line (Fig. 6c), suggesting that low concentration of Lat A mimics the function of calcium that rescues *sos3* mutant under salt stress and calcium rescues *sos3* mutant salt sensitive phenotype by regulation of MF reorganization.

Discussion

The plant cytoskeleton is comprised of MT and MF network, and the dynamics of them is involved in multiple cellular processes and responses to various environmental stimuli (Schmit and Lambert 1988; Blancaflor and Hasenstein 1995; Szymanski et al. 1999; Olinevich and Khokhlova 2003; Higaki et al. 2007; Zhou et al. 2010; Shi et al. 2011; Wang et al. 2011a; Liu et al. 2012). Previous

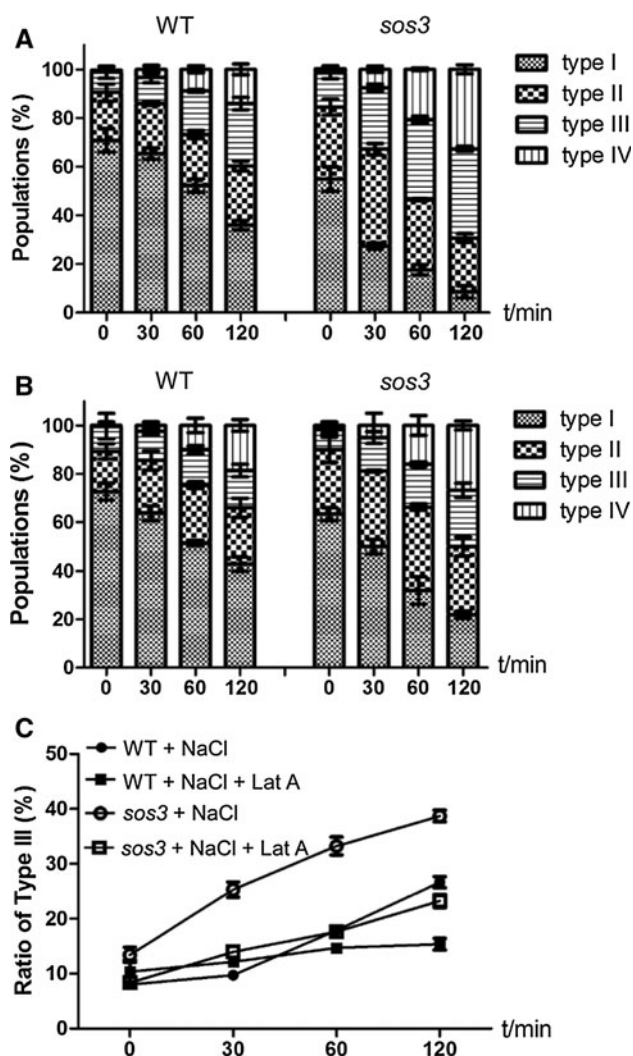


Fig. 6 Lat A mimics calcium in regulation of MF reorganization under salt stress in suspension cells. *Histograms* show the MF organization in suspension cells of wild type (WT) and *sos3* at the indicated times after 50 mM NaCl (a) or 50 mM NaCl supplemented with 30 nM Lat A treatments (b). The cells according to MF arrays were classified into four groups: type I, type II, type III and type IV, as description in Fig. 3. The data represent the mean \pm SD of three independent experiments; >150 cells per line were measured at the indicated times under different treatments. c Linear graphs show the ratio change of type III in suspension cells of wild type (WT) and *sos3* regarding to a and b. Values are mean \pm SD

studies indicate that salt stress affects both of MF and MT reorganization and the dynamics of MFs and MTs play critical roles in plant response to various environmental changes (Barrero et al. 2002; Mathur et al. 2003; Smith and Oppenheimer 2005; Shoji et al. 2006; Higaki et al. 2007; Wang et al. 2007, b; Papuga et al. 2010;).

The changes of MF arrays triggered by several signaling molecules and membrane-localized proteins have been demonstrated, such as Ca^{2+} , ABA and phospholipase D, which are involved in response to salt stress (Eun et al. 2001; Hwang and Lee 2001; Chen et al. 2003; Dhonukshe

et al. 2003; Olinevich and Khokhlova 2003). These observations imply that the dynamics of MF plays an important role in plant cell signal transduction and is involved in various responses coping with environmental changes including salt stress. Recent experiments demonstrate that the salt treatment can induce MF polymerization and bundle formation, however, when seedlings exposed to long-term of high salt treatment, MFs are depolymerized and subsequently disrupted (Wang et al. 2010a; Liu et al. 2012). These results indicate that depolymerization of MFs deprives the plant ability to resist salt stress; on the other hand, the actin polymerization is an important process during plants respond to salt stress.

SOS3 is identified to encode a calcium-binding protein, senses the change of intracellular calcium concentration and transduces the signal to downstream targets such as SOS2 (Liu and Zhu 1998; Halfter et al. 2000; Ishitani et al. 2000). It has been proposed that plant lacking SOS3 fails to activate SOS2 protein kinase that leads to less capable of activating a Na^+/H^+ antiporter SOS1, resulting in over accumulation of intracellular Na^+ (Shi et al. 2000; Qiu et al. 2002, 2004).

Our studies found that the MF reorganization responded to salt stress in *sos3* mutant is correlated with its salt-hypersensitive phenotype. The MFs in *sos3* mutant bundled under normal growth condition, and NaCl treatment further induced MF reorganization in both root cells and suspension cells of *sos3*. However, the damage of MF reorganization in *sos3* mutant was quicker and more severe than that in wild type under NaCl stress. When *sos3* plants were pre-treated with calcium, the MF reorganization was delayed and abnormal arrangement of MFs was reduced, demonstrating that the calcium involves in the regulation of MF network dynamics and SOS3 plays a role in this process under salt stress. These results imply that MF is closely related to SOS pathway which is possibly linked by calcium signal.

Previous observations demonstrate that various abiotic stresses stimulate transient increases of cytosolic Ca^{2+} concentration, which triggers downstream responses (Xiong et al. 2002; Zhu 2003; Clapham 2007). Here, we found that *sos3* salt-hypersensitivity may be partially resulted from abnormal MF organization that is associated with calcium. In our experiments, the addition of calcium to growth medium effectively suppressed the abnormal MFs in *sos3* mutant under salt stress. External calcium treatment triggers internal calcium increase (Clapham 2007; Tang et al. 2007). SOS3 is a calcium sensor, and is not a MF binding protein. It is not understood the relationship among SOS3, Ca^{2+} signal and MF dynamics under salt stress. However, we found that a trace concentration of Lat A partially mimicked the effect of calcium to enhance *sos3* salt-tolerance, suggesting that plant lacking

SOS3 may change cytosol calcium concentration that in turn affects MF organization.

Cytoskeleton dynamics regulates calcium influx through plasma membrane ligand- and voltage-gated channels (Johnson and Byerly 1993; Baumann 2001; Chen et al. 2001; Wang et al. 2004; Mironov et al. 2005). In cultured hippocampal neuron cells, the polymerization and depolymerization of MFs can modulate calcium release mediated by IP3 (inositol trisphosphate) and ryanodine-sensitive ER (endoplasmic reticulum) stores (Wang et al. 2002). In plant cells, disruption of MFs triggers mitochondrial Ca^{2+} release to the cytoplasm and results in consequent $[\text{Ca}^{2+}]_{\text{cyt}}$ changes (Lecourieux et al. 2002; Wang et al. 2010b). The MF bundles are induced by early salt stress, and the MFs depolymerize after high-level and long-term salt treatment (Wang et al. 2010a; Liu et al. 2012). These results demonstrate that MF dynamics promotes the plant salt tolerance. Based on our observations and previous studies, a possibility is proposed that the salt-induced increase of cytosolic calcium influx may function at an early stage in plant response to salt stress, and consequently triggers downstream responses to salt stress, including the dynamics of MFs. In *sos3* mutant, sensing of the initial Ca^{2+} influx is attenuated, which may affect the calcium-dependent MF reorganization.

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